Biological pretreatment and dry digestion processes for biogas production

Regina Jijoho Patinvoh
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Cover photo: Europe’s largest dry-AD, in Italy (Kompogas® plant)
Picture source: Hitachi Zosen Inova
Biogas technology has been used quite extensively to generate renewable energy from organic wastes while also recycling nutrients in the wastes and reducing harmful emissions. However, the challenges of low biogas yield from recalcitrant and inhibitory solid wastes together with high construction and operation costs of bioreactors have impeded optimal performance through this process. Additionally, solid organic wastes with total solids (TS) contents greater than 20 % are produced daily in enormous amounts; treating these wastes in conventional wet anaerobic digestion processes has required the addition of water, leading to large reactor volumes, high energy costs for heating, and costly dewatering processes for the digestate residue. In this study, the challenges mentioned above were addressed by using biological pretreatment and dry anaerobic digestion processes. Pretreatment, non-pretreatment strategies, biogas bioreactors design were also studied since this will aid optimizing its economy.

The suitability of a novel textile bioreactor for biogas production was accessed in dry digestion process (dry-AD) treating manure bedded with straw (22 – 30 %TS of feedstock). The 90-L textile bioreactor was robust and simple to operate; it can be accessed easily by developing countries where required expertise may not be available. Methane yield from the manure with straw was 290 NmolCH\textsubscript{4}/gVS using acclimatised bacteria; the digestate residue was affirmed suitable as bio-fertiliser. The efficiency of continuous plug flow reactor for dry anaerobic digestion of manure bedded with straw was also investigated at 22 %TS. Organic loading rates up to 4.2 gVS/L/d with retention time of 40 days gave better process stability.

Recalcitrant structure of chicken feather wastes was altered using Bacillus sp. C4 (Bacillus pumilus) and this pretreatment improved methane yield by 124 % compared to the untreated. Considering the fact that easily degraded feedstocks are not highly available and some problems associated with pretreatments: dry anaerobic co-digestion of citrus wastes with chicken feathers, wheat straw and manure bedded with straw, was investigated at 20 %TS in batch process. The best mixing ratio enhanced methane yield by 14 % compared to the expected yield from individual fractions. Process performance at different organic loading rates (OLR) was then investigated in continuous plug flow reactors at 21 %TS and 32 %TS of feedstock. Stability of the process decline as OLR was increased to 3.8 gVS/l/d resulting in high total volatile fatty acids (VFA), VFA/alkalinity ratio and reduction in methane yield.

**Keywords:** Solid wastes; Dry anaerobic digestion; Textile bioreactor; Plug flow bioreactor; Digestate; Process stability; Pretreatment; Co-digestion; Mesophilic
I dedicate this thesis to the Almighty God and the blessed memories of my beloved late father and mother: Mr Akotonayon Godonu and Mrs Bernice Godonu whom I painfully lost during the course of this study.
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This thesis is based on the results presented in the following articles:


STATEMENT OF CONTRIBUTIONS

My contributions to each of the publications follow:

**Paper I:** Responsible for part of the idea, performed all experimental work, analysed the data, wrote the manuscript, and executed its revision

**Paper II:** Responsible for part of the idea, performed all experimental work, analysed the data, wrote the manuscript, and executed its revision

**Paper III:** Responsible for part of the idea, the literature survey, and the data collection, wrote the major part and revision of the manuscript

**Paper IV:** Responsible for part of the idea, performed all the experimental work and the data analysis, wrote and revised the manuscript, except for the economic evaluation aspect of the work

**Paper V:** Responsible for part of the idea, the literature survey, and the data collection, wrote the major part of the manuscript

**Paper VI:** Responsible for part of the idea, performed all the experimental work, analysed the data, and wrote the manuscript
I started my academic career as a graduate assistant at Lagos State University (LASU), Nigeria. I obtained a Master’s of Science degree in chemical engineering at the University of Lagos and Lagos State University, and my focus since that time has been to provide solutions to environmental, health, and energy challenges in Nigeria. I decided to pursue my doctorate degree to enhance my career goals, and my sincere desire to pursue this degree abroad (Sweden) came from an aspiration to become more skilful in carrying out research in my field and providing solutions to some of the challenges facing my beloved country, Nigeria.

At the University of Borås, Sweden, I decided to focus on biogas production from solid wastes because this technology can generate energy while also reducing harmful emissions that affect human health and the ecosystem. As a doctoral student, my first task was to carry out a literature review on dry anaerobic digestion (dry-AD) of solid wastes for biogas production, after which I gave a short presentation providing a general overview of dry-AD processes, previous research findings, and research gaps.

I started my first experimental work, with a former Ph.D. student (Dr. Maryam M. Kabir), on biogas production from manure with straw in a textile bioreactor and dry anaerobic digestion of lignocellulosic and protein wastes in a batch process.

In the latter part of my first year, I began experimental work using a synthetic medium for biogas production. Thereafter, we started a study on the use of chicken feather for biogas production (Paper I). Chicken feathers are readily available solid waste, and more than 90% of chicken feather is keratin (insoluble protein), which has great potential for biogas production if converted to soluble protein. This potential motivated our study into the use of chicken feathers for biogas production, but because chicken feathers are recalcitrant, we needed to perform a pretreatment prior to biogas production. We focused on a biological pretreatment method that would be sustainable and cost effective. Additionally, we carried out a general study on pretreatment and non-pretreatment strategies to overcome the challenges associated with the use of recalcitrant and inhibitory feedstock for biogas production (Paper III).

During my second and third years, I focused on dry digestion of solid wastes using novel bioreactors. Plug flow reactors had been designed and developed for continuous dry digestion
processes. I began a preliminary experiment with the newly developed plug flow reactor and thereafter started a study on continuous dry fermentation of manure bedded with straw at 22 %TS with different organic loading rates using this plug flow reactor (Paper II). The reactor was monitored continuously to avoid failure of the process and to identify the critical organic loading rate, above which instability can occur in the reactor. Thereafter, we carried out a study on dry fermentation of solid wastes in a textile bioreactor (Paper IV). The plug flow bioreactor studied earlier is suitable for dry-AD processes, but it requires expert skills and constant monitoring, so there is a need for simple technologies that can be accessed easily by developing countries where the required expertise may not be available. Therefore, we worked on the dry digestion of solid wastes in the textile bioreactor and discovered that it is very simple to operate; the reactor is a robust and cost effective solution for rural and developing countries. We also proposed an industrial-scale concept for dry digestion of solid wastes using the textile bioreactor for farmers. Additionally, we carried out a general study on biogas bioreactors and the challenges associated with them while introducing a novel textile bioreactor (Paper V).

In view of the fact that easily degraded feedstocks are not highly available and some problems associated with pretreatments, we focused on dry anaerobic co-digestion of solid wastes in batch and continuous processes using plug flow reactors during my fourth year (Paper VI).

Working on biogas production from solid wastes under various conditions using textile and plug flow bioreactors has been very interesting. I am looking forward to setting up a biogas laboratory in a prominent university in Nigeria and subsequently a biogas plant.
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AD</td>
<td>Anaerobic digestion</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BMP</td>
<td>Biomethane potential</td>
</tr>
<tr>
<td>C/N</td>
<td>Carbon to nitrogen ratio</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>CSTR</td>
<td>Continuous stirred tank reactor</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
</tr>
<tr>
<td>HMF</td>
<td>5-hydroxymethylfurfural</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>LCFA</td>
<td>Long chain fatty acids</td>
</tr>
<tr>
<td>MSW</td>
<td>Municipal solid waste</td>
</tr>
<tr>
<td>MP-AES</td>
<td>Microwave plasma-atomic emission spectrometer</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>PF</td>
<td>Plug flow</td>
</tr>
<tr>
<td>STR</td>
<td>Solid retention time</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>UASB</td>
<td>Upflow anaerobic sludge blanket</td>
</tr>
<tr>
<td>VFA</td>
<td>Volatile fatty acids</td>
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<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS

ABSTRACT ...................................................................................................................................................................... iii

LIST OF PUBLICATIONS ................................................................................................................................................... vii

RESEARCH JOURNEY ...................................................................................................................................................... ix

NOMENCLATURE ................................................................................................................................................................ xi

1. Introduction............................................................................................................................................................................. 1

1.1 Aims of studies ................................................................................................................................................................... 2

1.2 Thesis structure ................................................................................................................................................................. 2

1.3 Research ethics and social aspects .................................................................................................................................... 3

2. Solid wastes as potential biomass resources .................................................................................................................... 5

2.1 Emissions from solid wastes ............................................................................................................................................. 5

2.2 Potential of solid wastes ....................................................................................................................................................... 6

2.2.1 Agricultural residues ...................................................................................................................................................... 6

2.2.1.1 Chicken feathers ..................................................................................................................................................... 7

2.2.1.2 Animal manure ..................................................................................................................................................... 8

2.2.1.3 Forest and crop residues ........................................................................................................................................... 8

2.2.2 Municipal solid wastes ................................................................................................................................................... 9

2.2.3 Industrial residues ........................................................................................................................................................... 10

3. An overview of Biogas production .................................................................................................................................. 13

3.1 Basic reactions involved in the anaerobic digestion process .......................................................................................... 14

3.2 Challenges of biogas production and possible solutions .................................................................................................. 15

3.2.1 Temperature fluctuations ................................................................................................................................................. 15

3.2.2 Feedstock composition .................................................................................................................................................... 16

3.2.2.1 Organic content and nutrients ............................................................................................................................... 16

3.2.2.2 Impurities in feedstock .............................................................................................................................................. 17

3.2.2.3 Long chain fatty acid content ............................................................................................................................. 17

3.2.2.4 Total solid content ................................................................................................................................................... 18

3.2.3 Foam formation ............................................................................................................................................................... 19

3.2.4 Process instability ........................................................................................................................................................... 20

3.2.5 Reactor design ............................................................................................................................................................... 21

3.3 Biogas in Nigeria .............................................................................................................................................................. 25

3.3.1 Biogas challenges and pioneered projects in Nigeria ................................................................................................ 26
# TABLE OF CONTENTS

ABSTRACT ......................................................................................................................................................... iii

LIST OF PUBLICATIONS ................................................................................................................................. vii

RESEARCH JOURNEY ........................................................................................................................................ ix

NOMENCLATURE ............................................................................................................................................... xi

1. Introduction .................................................................................................................................................. 1
   1.1 Aims of studies ........................................................................................................................................ 2
   1.2 Thesis structure ....................................................................................................................................... 2
   1.3 Research ethics and social aspects ........................................................................................................ 3

2. Solid wastes as potential biomass resources ............................................................................................... 5
   2.1 Emissions from solid wastes .................................................................................................................. 5
   2.2 Potential of solid wastes ......................................................................................................................... 6
      2.2.1 Agricultural residues ......................................................................................................................... 6
         2.2.1.1 Chicken feathers .......................................................................................................................... 7
         2.2.1.2 Animal manure ............................................................................................................................. 8
         2.2.1.3 Forest and crop residues ................................................................................................................ 8
      2.2.2 Municipal solid wastes ..................................................................................................................... 9
      2.2.3 Industrial residues ............................................................................................................................. 10

3. An overview of Biogas production ............................................................................................................... 13
   3.1 Basic reactions involved in the anaerobic digestion process .................................................................... 14
   3.2 Challenges of biogas production and possible solutions ......................................................................... 15
      3.2.1 Temperature fluctuations ................................................................................................................ 15
      3.2.2 Feedstock composition ..................................................................................................................... 16
         3.2.2.1 Organic content and nutrients ....................................................................................................... 16
         3.2.2.2 Impurities in feedstock ................................................................................................................ 17
         3.2.2.3 Long chain fatty acid content ..................................................................................................... 17
         3.2.2.4 Total solid content ....................................................................................................................... 18
      3.2.3 Foam formation .................................................................................................................................. 19
      3.2.4 Process instability ............................................................................................................................ 20
      3.2.5 Reactor design .................................................................................................................................... 21
   3.3 Biogas in Nigeria ....................................................................................................................................... 25
      3.3.1 Biogas challenges and pioneered projects in Nigeria ........................................................................ 26
3.3.2 Strategies for improved biogas technology ................................................................. 28

4. Dry anaerobic digestion (dry-AD) ..................................................................................... 31
4.1 Comparison of wet and dry anaerobic digestion ............................................................. 31
4.2 Start-up phase in dry anaerobic digestion ..................................................................... 32
4.3 Dry anaerobic digestion – Batch process ....................................................................... 33
   4.3.1 Dry digestion in textile bioreactor ............................................................................ 34
   4.3.2 BEKON dry digestion technology ......................................................................... 36
4.4 Dry anaerobic digestion – Continuous process ............................................................... 36
   4.4.1 Plug flow reactor for continuous dry digestion process ........................................... 37
   4.4.2 Continuous dry digestion of manure bedded with straw ........................................ 37
   4.4.3 Continuous dry co-digestion of solid wastes ........................................................... 39
   4.4.4 Continuous dry-AD technologies .......................................................................... 40

5. Improving biogas production from solid wastes .............................................................. 43
5.1 Pretreatment to overcome biomass recalcitrance ......................................................... 44
   5.1.1 Mechanical pretreatment .................................................................................... 43
   5.1.2 Thermal pretreatment ....................................................................................... 44
   5.1.3 Chemical pretreatment ..................................................................................... 44
   5.1.4 Biological pretreatment .................................................................................... 44
   5.1.4.1 Hydrolysis of chicken feathers for biogas production ....................................... 45
5.2 Co-digestion to achieve balance nutrient .................................................................... 46
5.3 Acclimatisation of microorganisms to substrates ......................................................... 47

6. Digestate as biofertiliser .................................................................................................... 49
6.1 Composition and quality of the digestate ..................................................................... 49
6.2 Benefits of digestate .................................................................................................... 50
6.3 Dewatering process ..................................................................................................... 51
6.4 Digestate from dry anaerobic digestion in textile reactor ................................................ 52

7. Concluding remarks and future directions .................................................................... 55
7.1 Conclusions .................................................................................................................... 55
7.2 Future directions .......................................................................................................... 56

ACKNOWLEDGEMENT ....................................................................................................... 57
REFERENCES ....................................................................................................................... 61
World population is growing rapidly, and this explosion has led to rapid consumption of oil resources and a tremendous increase in the volume of wastes generated. Globally, about 17 billion tonnes of total solid wastes are generated per year [1], and the amount is estimated to reach 27 billion tonnes in 2050 [2]. Continuous emissions of carbon dioxide, methane, and other greenhouse gases from these waste streams and the burning of fossil fuels has led to a global environmental crisis. The intensive agriculture practised to produce food also damages the environment through the use of chemical fertilisers. Additionally, about 16 % of the global population does not have access to electricity and about 38 % of the population [3, 4] uses solid wastes (forest residue, animal manure, crop and other wastes residues) for residential heating and cooking in poorly ventilated areas, which results in environmental and health hazards. Concerns about these environmental pressures and energy insecurity have increased the need for research on energy generation from renewable sources. The undervalued and abundant solid wastes that are generated have great potential as sources of biomass for energy production if properly harnessed and could lead to reduced environmental pollution and increased renewable energy production.

Biogas production through anaerobic digestion is a resource effective way of managing the large volume of organic waste generated. It is a well-established and sustainable process for energy generation from organic wastes. In addition to the biogas produced for energy generation, the digestate residue can be utilised as bio-fertilizer. Anaerobic digestion of organic wastes for biogas production is generally done through both wet and dry digestion processes. In industry, biogas production from wastes with high water content is most common process; however the utilization of solid wastes from agricultural, municipal, and industrial activities, including forest and crop residues, is becoming more and more important as well. Solid wastes usually have a solid content of between 15 % and 50 %; hence conventional wet anaerobic digestion treatment of these kinds of wastes requires a lot of water. Therefore, processing these waste streams for biogas production using dry-AD technology is a better option making it possible to manage waste streams with low moisture content.
A significant drawback regarding some of these solid wastes fractions is their recalcitrant nature, which limits their biological degradation and results in slow processes and low biogas production. Therefore, it is necessary to overcome the recalcitrant structure and make them accessible to microorganisms for improved biogas production [5], so there is a need for a suitable pretreatment prior to biogas production. Additionally, dry anaerobic digestion of solid wastes for biogas production has proven to be very challenging for industries and developing nations due to problems with feeding, mixing, and obtaining the appropriate technology for operation. Hence, there is great need for reactors that are simple to operate, robust in nature, and cost effective. Agricultural sectors, communities, and industries can install on-site anaerobic digesters to treat solid wastes and produce energy. Anaerobic digestion of these wastes is sustainable and can play an important role in reducing greenhouse gas emissions and the continuing dependence on fossil fuels.

1.1 Aims of studies

The main goals of this study were to improve biogas yield from recalcitrant and inhibitory solid wastes and to assess dry anaerobic digestion of solid wastes through the development and use of novel bioreactors. To attain this goal, this study was divided into four parts:

- Biological pretreatment of solid wastes (chicken feathers) prior to biogas production (Paper I)
- Study of pretreatment and non-pretreatment strategies for improved biogas production from recalcitrant and inhibitory solid wastes (Paper III)
- Investigation of the potential of dry anaerobic digestion (dry-AD) using novel biogas bioreactors: plug flow and textile bioreactors (Papers II, IV, and VI)
- Study of conventional biogas bioreactors and the challenges associated with them while introducing a novel textile bioreactor (Paper V)

1.2 Thesis structure

This thesis is divided into seven main chapters as follows:

- Chapter 1 introduces the thesis, motivation, and the main objectives of this research.
- Chapter 2 provides information on emissions from solid wastes, the potentials of solid wastes, and the challenges associated with the processing of these wastes fractions for biogas production.
• **Chapter 3** presents a general overview of biogas production, the reactions involved, biogas production challenges and possible solutions. It also presents an overview of the potential of biogas in Nigeria and strategies for advancement of biogas technology in Nigeria.

• **Chapter 4** describes dry anaerobic digestion (dry-AD) for biogas production and its advantages over wet fermentation. It also presents the start-up phase for dry digestion processes, the batch dry digestion process in a textile bioreactor, dry digestion in continuous plug flow reactors, and available continuous dry digestion technologies in the industries (**Papers II, IV, V and VI**).

• **Chapter 5** describes pretreatment and non-pretreatment strategies to improve biogas production from recalcitrant and inhibitory solid wastes (**Papers I and III**).

• **Chapter 6** presents a short review on the digestate residue after biogas production and discusses the composition and quality. Suitability of digestate residue obtained after dry digestion of manure with straw in a textile bioreactor was also presented (**Paper IV**).

• **Chapter 7** provides the main conclusions of research and discusses potential future work.

### 1.3 Research ethics and social aspects

Access to a safe ecosystem and undamaged natural resources is a public right because human health depends on the quality of the surrounding environment. Many people die prematurely, especially in developing countries, as a result of improper management of solid wastes, which leads to air pollution, flooding, health and environmental challenges. It is estimated that 38% of the global population [3, 4] uses solid biomass (wood, forest debris, manure, crop, and other waste residues) for home heating and cooking in poorly ventilated areas. My research on biogas production from wastes will help improve quality of life by reducing environmental pollution and increasing renewable energy production.

Biogas production sometimes requires a lot of water, and this use of water should be reduced if possible because access to water is an essential human right. Biogas production for the generation of energy should not be at the expense of this essential right. Therefore, this research was focused on dry anaerobic digestion, in which a reduced amount of water is used (**Papers II, IV, and VI**).
Biogas production also becomes a human rights issue when it destroys ecosystems and natural resources that are critical to the health and subsistence of people [6], hence, the residue remaining after biogas production must be free of pathogens, viruses, and toxic compounds that could contaminate soil, air, or water.

Methane, which is the major product of biogas production, is a greenhouse gas. It is the second most important greenhouse gas after CO$_2$, causing roughly 25 % of contribution to greenhouse warming [7]. Emissions of methane during biogas production contribute to global warming, so precautions should be taken to reduce methane emissions to the barest minimum. Methane can also reduce the amount of life-sustaining oxygen in the air, especially in confined spaces.

Additionally, genetically modified bacteria can have an effect on natural ecosystems [8] when the digestate residue from biogas production is released into the environment. In this research, a wild strain of bacteria (Bacillus pumilus), which is sustainable and ecologically safe, was used for the biological pretreatment prior to biogas production (Paper I).

Biogas production should be environmentally sustainable to preserve life, protect our ecosystem, improve air quality, and ameliorate current climate problems.
Solid wastes as potential biomass resources

A tremendous amount of solid wastes is generated on a daily basis, and this amount will increase due to population growth and increasing rates of consumption. A large amount of these wastes is still being disposed with limited recycling, combustion without energy recovery, and landfilling without gas control [9], resulting in global environmental issues. These wastes are potential sources for new material and renewable energy generation if harnessed properly.

2.1 Emissions from solid wastes

As solid wastes from municipal communities and agricultural and industrial activities are disposed, the organic fraction of these wastes is degraded over a period of time releasing CH$_4$, CO$_2$, and other gases to the atmosphere and thereby contributing to global warming. The estimated global emissions from solid waste sites are in the range of 20 to 40 million tonnes of CH$_4$ per year [10] and livestock sector also contributes significantly to anthropogenic greenhouse gas emissions.

Solid wastes are often disposed in landfills, which results in emissions of methane and nitrous oxides and thus contribute to the greenhouse effect. Another common methods for the treatment of these waste streams are composting and incineration; composting results in emissions of volatile compounds (ketones, aldehydes, ammonia, and methane) [11] and incineration often leads to significant releases of dioxins to the environment if the exhaust gas is not treated properly. The environmental impacts of these conventional management methods are listed in Table 2.1.

Anaerobic digestion is a promising technology for reducing emissions from solid wastes: it is a cost effective, resourceful, and viable method for solving waste problems. Studies have shown that anaerobic digestion of these wastes is sustainable and has a great advantage over aerobic treatment because of its improved energy balance [11].
Table 2.1. Environmental impacts of conventional solid waste management methods [12]

<table>
<thead>
<tr>
<th></th>
<th>Landfill</th>
<th>Composting</th>
<th>Incineration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Air</strong></td>
<td>Emissions of methane (CH$_4$) and carbon monoxide (CO)</td>
<td>Emissions of methane (CH$_4$) and carbon monoxide (CO)</td>
<td>Emissions of SO$_2$, NO$_x$, HCl, HF, CO, CO$_2$, N$_2$O, dioxins, furans, heavy metals (Zn, Pb, Cu, As)</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td>Leaching of salts, heavy metals, and biodegradable and persistent organics to groundwater</td>
<td>N/A</td>
<td>Deposition of hazardous substances on surface water</td>
</tr>
<tr>
<td><strong>Soil</strong></td>
<td>Accumulation of hazardous substances in soil</td>
<td>N/A</td>
<td>Landfilling of ashes and scrap</td>
</tr>
<tr>
<td><strong>Landscape</strong></td>
<td>Soil occupancy; restriction on other land uses</td>
<td>Soil occupancy; restriction on other land uses</td>
<td>Visual intrusion; restriction on other land uses</td>
</tr>
<tr>
<td><strong>Ecosystem</strong></td>
<td>Contamination and accumulation of toxic substances in the food chain</td>
<td>Contamination and accumulation of toxic substances in the food chain</td>
<td>Contamination and accumulation of toxic substances in the food chain</td>
</tr>
<tr>
<td><strong>Urban areas</strong></td>
<td>Exposure to hazardous substances</td>
<td>N/A</td>
<td>Exposure to hazardous substances</td>
</tr>
</tbody>
</table>

2.2 Potential of solid wastes

Solid wastes are potential biomass sources because they are readily available and their conversion to energy through biological processes is feasible with low capital investment. Biomass comes from a range of sources, as shown in Fig. 2.1, which can be classified according to the activities generating these wastes or the locations where these wastes are generated [13]. Solid wastes generated by agricultural sectors, communities, and industries along with their potential as renewable energy sources are discussed in this section. Table 2.2 shows the major characteristics of solid wastes used during this study.

2.2.1 Agricultural residues

Agricultural wastes account for the largest potential feedstock, and wide varieties of these wastes can be used as sources of biomass energy. The most common sources (chicken feathers, animal manure, forest and crop residues) are discussed in this section.
2.2.1.1 Chicken feathers (CF)

Currently, the world’s average stock of chicken is about of 22 billion live chickens [14], and about 5–7% of the total weight of a normal chicken is feathers [15]. Therefore, chicken feathers are available in tremendous amount around the world, causing environmental challenges. The major component of feather is β-keratin [16], which is an insoluble structural protein. The polypeptides chain, as shown in Fig. 2.1, is tightly packed and highly cross-linked with disulphide bonds, hydrogen bonds, and hydrophobic interactions [17]. This structure makes the protein insoluble and resistant to enzymatic attack, which is a major limitation to the biological processing of these wastes [5]. Chicken feather is a potential source of biomass for energy generation if an appropriate pretreatment method is applied prior to anaerobic digestion. The methane potential of protein wastes is as high as 0.496 Nm$^3$/kgVS [18] if all insoluble proteins are converted to protein. In this research, chicken feathers were used as feedstock for methane production at various concentrations, and the recalcitrant nature of feathers were altered prior to anaerobic digestion using biological pretreatment (Paper I).
2.2.1.2 Animal manure

Farm animals produce billions of tons of manure annually, and the amount of manure bedded with straw increases as the number of housed dairy animals increases. Storing these immense quantities of manure can lead to significant anthropogenic emissions of methane and N\textsubscript{2}O [20]. Additionally, untreated manure can also contain pathogens and viruses that can lower the productivity of livestock farms [21] and pose a threat to human health when applied to agricultural land. However, the large volume of animal manure generated by agricultural sectors is a great source for energy production; methane from the manure can be captured and used as a source of energy, thereby preventing the environmental harm caused by these waste streams. Freshness of manure is required for effective conversion to energy through anaerobic digestion process: the older the manure, the higher the degree of previous decomposition and the lower the amount of undecomposed matter available for conversion to methane [22]. Cattle manure bedded with straw at 22 %TS, 27 %TS, and 30 %TS was used as feedstock for biogas production through the dry anaerobic digestion process in this study (Papers II, IV, and VI).

2.2.1.3 Forest and crop residues

Forest and crop residues are lignocellulosic biomass with a yearly production of approximately 200 billion tons [23]. They are the most abundant renewable source for energy production. Forest residues include dead trees, litter fall, and wastes generated in the process of culling and logging. Crop residues include straw, husk, leaf, stem, stalk, shell, cob, and bagasse, which come from cereals, cotton, groundnut, fruits, legumes, palm oil, and sugar.
cane. These waste streams are composed of polysaccharide-rich walls, as shown in Fig. 2.3. They are difficult to break down as a result of the matrix polymers (hemicellulose, pectins, and lignin) surrounding the cellulosic microfibrils in the plant cell wall [24]. The degree of resistance of these wastes to microbial attack varies depending on the type of lignocellulose, various compartments of the cell wall, cell age, and processing phenomena such as drying and heating [25].

Effective biological processing of this biomass for energy production requires an appropriate pretreatment to open the structure and increase the accessibility for the cellulose degrading microorganisms. Wheat straw was used in co-digestion of cattle manure to increase the C/N of the substrate and thereby enhance the performance of the anaerobic digestion process (Paper IV). It was also used in dry co-digestion of different solid waste fractions i.e. chicken feathers, citrus wastes, wheat straw, and manure with straw to reduce the inhibitory effect of citrus waste (Paper VI).

![Fig. 2.3. Different layers in cell wall of a wood fiber or tracheid including middle lamella, primary wall, and secondary wall (S1, S2, S3). Cellulosic microfibrils in Plant Cell wall surrounded by matrix polymers (reprinted with permission) [26].](image)

2.2.2 Municipal solid wastes (MSW)

Municipal solid wastes usually include all the wastes generated in a community, such as residential, commercial, and institutional wastes. They are the most concentrated of all waste fractions, containing food wastes, paper, cardboard, textiles, plastics, glass, metals, yard
trimmings, and others. Globally, the volume of MSW generated is about 1.3 billion tonnes per year, and this volume is expected to increase to about 2.2 billion tonnes per year in 2025 [27]. MSW usually contains an easily biodegradable organic fraction of up to 40%, and about 60% of the dry weight of the average municipal solid waste is composed of lignocelluloses [28]. A major challenge in the biological processing of these wastes is the composition; there are varieties of different fractions involved, depending on the source of the wastes, which can have adverse effects on the digestion process. Therefore, MSW has to be mechanically, magnetically, or hand-sorted to remove large stones, plastics, glass, metals, and other debris and then shredded to reduce its particle size to be able to access and process the organic fraction.

### 2.2.3 Industrial residues

Major organic industrial residues are those generated from food and fruit processing industries. These industries produce large volumes of solid wastes, such as peels, pulp, molasses, seeds, skins, pomace, spoiled food, and fruits. The composition and quantity of the solid wastes generated varies from plant to plant and also depends on the technology employed by the industry. Solid wastes generated from these industries contain large amounts of organic matter such as carbohydrates, proteins, and lipids, which makes them potential sources of biomass for energy production through biological processes. The major limitation is that some of these wastes contain chemicals or substances that inhibit the activity of microorganisms and thereby have an adverse effect on energy generation through anaerobic digestion processes [29, 30]. Therefore, the wastes often need to be pre-treated or co-digested with other substrates to reduce the inhibitory effect on biogas yield. Installation of anaerobic digesters can be an integral part of industry services to treat solid wastes while also meeting the industry’s energy demands. In this research, citrus waste was co-digested with chicken feather, wheat straw, and cattle manure to reduce the inhibitory effect of D-limonene on methane yield and enhance the buffering capacity of the digestion process (Paper VI).

### Table 2.2: Major characteristics of solid wastes used during this study (mean values and standard deviations based on triplicate measurements) (modified from Paper VI)

<table>
<thead>
<tr>
<th>Solid wastes</th>
<th>pH</th>
<th>Bulk density (g/L) ±</th>
<th>TS (%) ±</th>
<th>VS (%) ±</th>
<th>Ash (%) ±</th>
<th>Total carbon (%) ±</th>
<th>TKN (%) ±</th>
<th>C/N</th>
<th>COD gCOD/gVS substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken feather</td>
<td>ND</td>
<td>ND</td>
<td>93.47 ± 0.06</td>
<td>99.26 ± 0.01</td>
<td>0.74 ± 0.01</td>
<td>55.14 ± 0.01</td>
<td>15.50 ± 0.30</td>
<td>3.6</td>
<td>ND</td>
</tr>
<tr>
<td>Citrus wastes</td>
<td>3.24</td>
<td>753.60  ± 24.32</td>
<td>23.42 ± 2.23</td>
<td>96.07 ± 0.74</td>
<td>3.93 ± 0.74</td>
<td>53.37 ± 0.41</td>
<td>0.99 ± 0.08</td>
<td>1.03</td>
<td>55.5</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>ND</td>
<td>190.47 ± 6.93</td>
<td>89.07 ± 0.08</td>
<td>94.96 ± 0.14</td>
<td>5.04 ± 0.14</td>
<td>52.76 ± 0.08</td>
<td>0.83 ± 0.08</td>
<td>69</td>
<td>0.82</td>
</tr>
<tr>
<td>Manure with straw</td>
<td>8.01</td>
<td>542.00  ± 26.87</td>
<td>25.84 ± 0.92</td>
<td>77.21 ± 2.74</td>
<td>22.79 ± 2.74</td>
<td>42.89 ± 1.52</td>
<td>2.26 ± 0.04</td>
<td>19</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Extractives 8.27% , Total lignin 16.52% , Cellulose 42.74% , Hemicellulose 27.99% , dry basis. ND – not determined. C/N – carbon to nitrogen ratio. COD – chemical oxygen demand.
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<th>C/N</th>
<th>COD gCOD/gVS_substrate</th>
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*dry basis. ND – not determined. C/N – carbon to nitrogen ratio. COD – chemical oxygen demand
An overview of biogas production

Biogas is produced from organic wastes through anaerobic digestion processes. This reduces the effect of feedstock costs on biogas production and makes biogas production and utilisation a good solution for addressing both waste and energy challenges [31]. Waste and residues are also considered the most abundant renewable resources, and their use as feedstock for biogas production reduces the competition between fuel and food, which is important for the long-term success of biofuel [32]. The process of biogas production includes preparation of the feedstock for digestion and the anaerobic digestion process, which consists of four basic steps, hydrolysis, fermentation, acetogenesis, and methanogenesis, as explained in Fig. 3.1. In this process, the first stage is very essential because microorganisms cannot use large molecules directly unless they are disintegrated into smaller molecules; the rate of disintegration during this stage depends on the nature of the substrate. During fermentation, the products from the previous stage are used as substrates except fatty acids released during the decomposition of fats and aromatic structures [33]. The third stage is very complex because it requires interaction between the acetogens and the methanogens; the process is closely linked to the concentration of hydrogen gas [33] and will stop if the hydrogen produced is not continuously consumed. The most important substrates for methane formation are H₂, CO₂ and acetate but some methanogens can also use methanol, formate and methylamines [7]. Additionally, optimal conditions such as neutral pH, constant temperature, balanced nutrient composition, and consistent feed rate are required for effective conversion of organic wastes to biogas. Biogas produced through this process is a mixture of gases, consisting mainly of methane (usually between 50 and 80 % [34]), carbon dioxide (between 20 and 45 %), and some quantities of water vapour, hydrogen sulphide, ammonia, and traces of other gases. The composition of the biogas depends on types of substrates, process condition, activities of the microorganism during the digestion process [35], and various technical designs of the plant [36]. Biogas can be used for heating, generation of electricity, and as a fuel for transportation, and the nutrient rich residue remaining after biogas production can be used as a bio-fertiliser. The sub-sections of this chapter outline the basic reactions, challenges of biogas production, and assess biogas in Nigeria.
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3.1 Basic reactions involved in the anaerobic digestion process

Themelis and Kim [37] showed that the mixture of organic wastes can be approximated by the chemical formula $C_6H_{10}O_4$, excluding nitrogen and sulphur because they are relatively minor and occur principally in mixed food wastes. The hydrolysis reaction can then be written as shown in equation 1.

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + H_2$$  \hspace{1cm} (1)

The hydrolysed organic compounds (sugars and amino acids) are converted to alcohols and organic acids by fermentative bacteria, as written in equations 2 and 3 [38]

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$ \hspace{1cm} (2)

$$C_6H_{12}O_6 + 2H_2 \rightarrow 2CH_3CH_2COOH + 2H_2O$$ \hspace{1cm} (3)

All of the organic acids, except the acetic acids [39], are consumed by the acetogenic bacteria and thereafter converted to acetic acid and hydrogen, as written in equation 4 [38].

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$ \hspace{1cm} (4)

The last step is the formation of methane, in which methanogens consume acetic acid, hydrogen, and carbon dioxide to produce methane. Some methanogens can feed on methanol and several other substrates, including methylated compounds [7]. The basic reactions involved in methane formation are written in equations 5, 6, 7, and 8 [38].

$$...$$

Fig. 3.1. Stages of the anaerobic digestion process for active biogas production and required different microbial communities
\[ CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \] (5)

\[ CH_3COOH + CO_2 \rightarrow CH_4 + 2CO_2 \] (6)

\[ CH_3OH + H_2 \rightarrow CH_4 + H_2O \] (7)

\[ 2CH_3CH_2OH + CO_2 \rightarrow 2CH_3COOH + CH_4 \] (8)

3.2 Challenges of biogas production and possible solutions

Despite the benefits of biogas production, a negative effect on any of the processes explained in Fig. 3.1 has a direct effect on the other processes and the biogas process can become unsteady or fail completely [40]. The major challenges discussed in this subsection are those related to temperature fluctuations, feedstock composition, foam formation, process instability, and reactor design.

3.2.1 Temperature fluctuations

Microorganisms require optimum temperature for growth and active performance during biogas process and can be divided into psychrophilic (optimum temperature around 10 °C), mesophilic (optimum temperature around 37 °C), thermophilic (optimum temperature around 55 °C) and hyper thermophilic (optimum temperature around 85 °C) [33]. Temperature fluctuations affect the performance of a biogas process adversely because the activity of the microorganism is reduced if temperature is above or below their optimum range. A decrease in temperature may result in a reduced volatile fatty acid production rate, substrate decomposition rate, and metabolic rate of the microorganism [41, 42]. Navickas, Venslauskas [43] investigated the influence of temperature variations on the performance of anaerobic digestion of industrial wastes and observed that a change in temperature from 52 °C to 57 °C (at constant total solids concentration, organic load, and pH) resulted in a 24 % reduction in biogas yield. The degradation rate is higher thermophilic processes, however they are more sensitive to changes in temperature [44] and moreover, they require increased energy input to maintain a stable temperature. In this study, all experiments were operated at mesophilic temperature, at 37 °C (Papers I, II, and VI) and at 25 °C for dry digestion of cattle manure with straw in a textile bioreactor (Paper IV), to check the suitability of the process in tropical regions, but the reactor can be heated from below to attain the desired temperature in cold conditions.
regions. This is a good strategy because mesophilic processes are less sensitive to temperature changes, although they may require longer degradation time.

One of the ways of overcoming the major challenge of temperature fluctuations is using a two-phase anaerobic digestion process in which the hydrolysis/acidogenesis stage is operated under thermophilic conditions and the methanogenic stage is operated under mesophilic conditions [44, 45]. Another way is avoiding heat loss by insulating the reactor with low cost materials such as Styrofoam [46]; this material was used to insulate the plug flow reactors used in this study (Paper II and VI). Previous research has also shown that between 12 % and 50 % of the biogas produced, depending on system design characteristics, is used in heating the reactor and maintaining constant temperature within the system [47, 48]. Therefore, the use of solar thermal energy to heat the reactor is a better option with low operating cost and better optimisation of biogas yield [49]. Additionally, dry-AD is a good solution as it requires less energy for heating due to low water content. The textile biogas bioreactor is also an appropriate technology for tropical countries because it does not require heating and the reactor is UV treated and weather resistant.

3.2.2 Feedstock composition

The biogas yield from anaerobic digestion of organic solid wastes depends on factors such as, the organic and nutrient contents, impurities in the feedstock, the presence of possible inhibitors (e.g. antibiotics, disinfectants, solvents, herbicides, salts, and heavy metals) [50], the total solids content as well as the long chain fatty acids content.

3.2.2.1. Organic content and nutrients

The organic content of a feedstock determines the theoretical yield of biogas that can be produced from it. It is necessary to determine the methane potential of the feedstock in order to estimate the extent to which the specific feedstock can be degraded [51, 52]. The organic content and the nutrients present in the feedstock affect the rate of growth and the activity of microorganisms. Microorganisms are dependent on macro and micro nutrients, trace elements, and vitamins for their growth, and these are vital for effective conversion of organic matter to methane [7, 53]. The most important nutrients are carbon and nitrogen, and the C/N ratio of feedstock is a vital factor for the choice of feedstock. Excess availability of nitrogen during the degradation leads to the formation of NH₃, which inhibits microbial growth at higher concentrations [36, 54], and its deficiency causes the biogas process to fail.
Feedstocks used in this study were prepared according to Zupančič and Roš [55] by blending 1g of the sample with water (dilution factor of 50) to allow homogenization [56]. The theoretical methane potential of the feedstocks were then calculated from the amount of feedstock used and the chemical oxygen demand (COD) concentration using equation (9) [57], assuming that the equation is valid for any substance or product [58].

\[
\text{BMP}_{\text{thCOD}} = \frac{n_{\text{CH}_4}RT}{pV_{\text{S}_\text{added}}},
\]

where:

- \(\text{BMP}_{\text{thCOD}}\) (theoretical yield under laboratory condition);
- \(R\) (gas constant, 0.082 atm L/mol K);
- \(T\) (working temperature, 310 K);
- \(P\) (atmospheric pressure, 1 atm);
- \(V_{\text{S}_\text{added}}\) (volatile solids of the substrate added, g);
- \(n_{\text{CH}_4}\) (methane produced, mol) determined according to equation (10)

\[
n_{\text{CH}_4} = \frac{\text{COD}}{64(\frac{R}{mol})}
\]

### 3.2.2.2 Impurities in the feedstock

Clean feedstock is important among others for the quality of the digestate residue and for the overall efficiency of the anaerobic digestion process. Inadequate preparation of feedstock before feeding can lead to blockage of gas pipes and the formation of foam in the reactors, thereby causing a significant reduction in biogas yield and a great effect on the overall digestion process [59]. Additionally, inhibitors (e.g. antibiotics, disinfectants, solvents, herbicides, salts, and heavy metals) that enter the reactor through the feedstock can slow the process, or the accumulation of inhibitors in the reactor can lead to the death of the microorganisms at high concentrations.

### 3.2.2.3. Long chain fatty acid content

Lipid-rich wastes such as wastes from slaughterhouses, oil processing industry, dairy product industry, and wool scouring contain high methane content compared to carbohydrates and proteins-rich wastes [60]. These waste streams are easily degraded to glycerol and LCFAs; excessive amount of LCFAs could inhibit the activity of the microorganisms thereby resulting into low biogas yield or failure of the process. Angelidaki et al. [61] investigated the effect of long chain fatty acids in cattle manure under a thermophilic biogas process and observed that low concentrations of oleate and stearate acid inhibited all steps of the anaerobic digestion.
Dasa, Westman [62] also reported that palmitic and oleic acids with concentrations of 3.0 and 4.0 g/l, respectively, resulted in > 50% inhibition of biogas production and that stearate acids had an even greater inhibitory effect.

3.2.2.4. Total solids content

The total solids content of the feedstock can affect gas yield because there is limited mass transfer if the total solids content is high and, as a result, the microorganisms are only able to decompose the substrate in their immediate environment. At very high contents ≥ 40%, digestion can come to a complete halt as there is insufficient water available for the growth of the microorganisms [36, 63]. Additionally, a high content of total solids can cause problems if inhibitors are present in the feedstocks as these are present in concentrated forms because of the low water content. In this study, feedstocks with total solid contents between 20% and 32% were investigated in batch and continuous dry digestion processes (Paper II, IV and VI).

To overcome the challenges with feedstock composition, the feedstock can be sorted mechanically, magnetically, or by hand to remove debris and then shredded to reduce its particle size. Pretreatment of the feedstock is also vital, especially for lignocellulosic and keratin-rich wastes, because it helps to increase the availability of the feedstock to the microorganisms and thereby increase the biogas yield [64-66]. Ammonia inhibition from protein-rich wastes can be resolved by low loading or by dilution [67]. Membrane applications have also been reported to be effective in protecting the microorganisms from the toxic effects of substrate and from product inhibition [62, 68]. Dry digestion processes allow flexibility in the choice of feedstocks, less pre-treatment, and are better options for treating fibre-rich feedstocks compared to wet digestion processes. Additionally, nutrient medium should be used during anaerobic digestion unless these nutrients are present in the feedstock or the inoculum used [53]. The nutrient medium is necessary to supply the macronutrients, trace elements, and vitamins needed for growth and microbial activity. In this study, the nutrient medium described by Angelidaki, Alves [53] was used (Papers I, II, and IV), except in the dry co-digestion of solid wastes (Paper VI), in which four different substrates (citrus wastes, chicken feather, cattle manure, and wheat straw) were used and it was presumed that all nutrients needed would be supplied by the individual substrates.
3.2.3. Foam formation

Foam is a dispersion of gas in liquid consisting of a large proportion of gas, and its formation in anaerobic digestion processes is a major challenge for plant operators [69]. The presence of surface active substances such as volatile fatty acids, oil, grease, detergents, proteins, particulate matter (grit, metals, sand, etc.) [69-71] is the major cause of foam formation. Reactors operated with a high organic loading rate are more prone to foaming because at higher loading rate the organic compounds are not fully degraded [72]. This incomplete degradation results in accumulation of these surface-active agents and by-products that promote foaming. The organic loading rate for mesophilic anaerobic digestion of municipal sludge varies from 0.7 kgVS/m$^3$/d to 7.2 kgVS/m$^3$/d [70], but it has been reported that operating reactors at organic loading rates higher than 4.5 kgVS/m$^3$/d can result in foaming even though these rates are still within the recommended range [71]. Excessive mechanical mixing also increases the amount of bubbles in the bulk phase, enhancing the attachment of surface active and hydrophobic compounds and thereby causing foaming [73]. On the other hand, insufficient mixing during digestion results in stratification, which causes the surface active agents and other non-degraded hydrophobic materials to rise to the surface of the bulk phase and thus cause foaming [70].

Foaming can be overcome by identifying a critical organic loading threshold above which foaming can occur in each reactor [74]. Overloading of reactors should be avoided by daily monitoring of organic loading and process performance. Ensuring sufficient but not excessive mixing by monitoring reactors regularly and preventing grit accumulation is a good preventive measure against foaming. Foam formation is a common challenge in wet digestion processes, so dry-AD is a better technology to avert this problem. In this study, continuous plug flow reactors were used (Papers II and VI). The impeller in this reactor allows minimal mixing by moving the reactor contents manually to the inlet and back to the outlet for better performance of the process. Increasing the C/N ratio of feedstock is also an option for reducing foaming in reactors. Tanimu, Mohd Ghazi [75] investigated the effect of feedstock C/N ratio on foam formation in anaerobic digestion and found that increasing the C/N ratio of food waste from 17 to 26 and 30 by adding other wastes reduced foaming in the reactors by up to 60%.
3.2.4 Process instability

Process instability in biogas reactors occurs due to temperature fluctuations, nutrient unavailability, feeding problems, high organic loading rate, and the presence of inhibitors in feedstocks, as explained above. Possible good operation practises to overcome instability in biogas reactors are explained in this section.

The initial start-up phase is essential for continuous processes. This phase enables the microbial community to adapt to the feedstock and the conditions in the reactor. A moderate amount of substrate should be fed at the initial stage and then the loading can be increased gradually to reach the optimum value for the digestion process. If the organic loading rate (OLR) is too high, there can be volatile fatty acid (VFA) accumulation, which causes instability by a decrease in pH which can lead to inhibition of methanogens resulting into process failure. Early indicators of process instability are VFA concentration, alkalinity ratio (VFA/alkalinity ratio), hydrogen concentration (should be typically less than 100 ppm) and redox potential (should be lower than -300 mV) [40]. In this study (Papers II and VI), VFA and VFA/alkalinity ratio were used for monitoring the biogas process. The VFA/alkalinity ratio is a two way titration measurement; titration is carried out until a pH of 5.0 is reached (bicarbonate alkalinity) and then until pH of 4.4 (alkalinity caused by VFA) and was calculated based on the Nordmann method [76]. The VFA/alkalinity ratio lower than 0.3 is generally considered to indicate stable processes [40]. A continuous feed rate is vital for the stability of the process [77] because variations in feed rate often result in variations in biogas production rates, which can affect the biogas yield. Alterations in the energy content of two different batches of the feedstock can also result in biogas production instability, even though the feed rate remains the same [40].

The process performance of a continuous plug flow reactor treating manure with straw at 22 %TS was monitored by regular measurement of the VFA/alkalinity ratio, as shown in Fig. 3.2 (Paper II). The figure shows the variation of pH and VFA/alkalinity ratio at different OLRs. At 2.8 gVS/L/d (OLR 1), the VFA/alkalinity ratio was lower than the reported limit of 0.3, indicating stable process conditions. When the OLR was increased to 4.2 gVS/L/d (OLR 2), the VFA/alkalinity ratio was still below 0.3. However, increasing the organic loading rate to 6 gVS/L/d (OLR 3) led to a gradual increase in the VFA/alkalinity ratio. The process started to show signs of instability during the first retention time of 28 days with fluctuations in the daily biogas production (Paper II). Continuing the loading at the same conditions (6
gVS/L/d) into the second retention time caused considerable disturbances in the system, with a sharp increase in VFA/alkalinity ratio, which is a typical sign of overloading. However, the process could be restored back to stability by reducing the organic loading rate back to 4.2 gVS/L/d (OLR 2).

Fig. 3.2. pH and VFA/Alkalinity ratio variation at different organic loading rate (OLR) during experiments. The symbols represent pH (◊), VFA/Alkalinity ratio (□). Presented values are mean values of duplicate measurements with error bars as standard deviation between the two values. (Paper II)

3.2.5 Reactor design

Anaerobic reactors for biogas production also pose some challenges to the effectiveness of the biogas process depending on the process configurations and operating conditions of the reactors. A reactor may be suitable and economical for a particular type of feedstock or co-substrate but may not be suitable for another. Therefore, for overall effectiveness of the biogas production process, reactors must be selected with consideration of the feedstock composition, amount of feedstock to be treated, desired product, and process economy [78]. For an anaerobic reactor to accommodate high loading rates, the following basic conditions must be met [79]:

- High retention of viable microorganisms in the reactor under operational conditions
- Viable microorganisms that are sufficiently adapted and/or acclimatised
- Sufficient contact between viable microorganisms and the feedstock
• High reaction rates and absence of mass transfer limitations
• Favourable environmental conditions for the microbial communities in the reactor under the operating conditions

Plug flow reactors were used for continuous dry digestion processes in this study (Papers II and VI), and these were effective for dry digestion of manure bedded with straw and dry co-digestion of different solid waste fractions. The textile bioreactor newly developed by FOV Fabrics AB (Borås, Sweden) was also used in this study (Paper IV) for dry batch digestion of manure bedded with straw, and the reactor was suitable and efficient for this process. The basic reactors used in biogas production processes and the major challenges associated with these reactors are presented in Table 3.1.
Table 3.1 Overview of biogas reactors and possible challenges

<table>
<thead>
<tr>
<th>Basis of Classification</th>
<th>Reactor</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding mode</td>
<td>Batch</td>
<td>Larger volume required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insufficient mixing can induce dead zones, which make the digestion process inefficient</td>
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<tr>
<td></td>
<td></td>
<td>Limited opportunity to control the process</td>
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<tr>
<td></td>
<td></td>
<td>Lower biogas yield</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possibility of explosions during methane-to-air transition phase when unloading the reactor</td>
</tr>
<tr>
<td></td>
<td>Continuous</td>
<td>Longer start-up times required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased VFA accumulation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Require high skill in operation</td>
</tr>
<tr>
<td>Solids content</td>
<td>Wet</td>
<td>Larger amounts of water and energy required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dilute digestate residue requiring costly dewatering processes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frequent clogging of nozzles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sedimentation at the bottom creating extensive operational problems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Foam formation</td>
</tr>
<tr>
<td></td>
<td>Dry</td>
<td>Feedstock handling and mixing are difficult</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Longer retention time required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Might be prone to substrate/product inhibition due to low water content</td>
</tr>
<tr>
<td>Operating temperature</td>
<td>Mesophilic</td>
<td>Lower reaction rates, low enzyme activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Digestate may contain higher pathogen concentration</td>
</tr>
<tr>
<td></td>
<td>Thermophilic</td>
<td>Higher heat energy demand</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prone to temperature fluctuations, so the process is less stable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher risk of ammonia nitrogen toxicity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Higher odour formation due to higher VFA concentration</td>
</tr>
<tr>
<td>Process stage</td>
<td>Single-stage</td>
<td>Higher retention time</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Formation of foam</td>
</tr>
<tr>
<td></td>
<td>Multi-stage</td>
<td>Higher energy consumption; high investment cost. More complex process, required high skill</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in operation</td>
</tr>
<tr>
<td>Reactor design/material of construction</td>
<td>Plug flow</td>
<td>Reactor has to be emptied if impeller requires servicing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Feedstock can lead to blockage of gas pipes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Start-up may take a longer time</td>
</tr>
<tr>
<td></td>
<td>CSTR</td>
<td>Prone to foam formation. Intermixing and process control is more complex as size increases.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High capital and operational cost</td>
</tr>
</tbody>
</table>
Table 3.1 Overview of biogas reactors and possible challenges (Cont’d.)

<table>
<thead>
<tr>
<th>Basis of Classification</th>
<th>Reactor</th>
<th>Challenges</th>
</tr>
</thead>
</table>
| Reactor design/material of construction | UASB                   | Requires constant monitoring to avoid washout of cells  
Sensible to sudden temperature changes  
resulting in granule disintegration  
Start-up may take a longer time, especially  
when reactor is inoculated with sludge instead of dense granules  
Prone to shock loading and risk of odour problems. Effluent requires further treatment |
| Fixed dome              |                        | Requires high technical skill for construction  
Prone to leakage  
Utilisation of gas produced is less effective because the gas pressure fluctuates substantially |
| Anaerobic filter        |                        | Requires expert design and construction  
Requires constant monitoring to ensure good functioning and a water tight system  
Effluent require further treatment  
Risk of clogging, depending on pre- and primary treatment  
Cleaning the clogged filter is cumbersome  
Long start-up and risk of foul odour |
| Floating drum           |                        | Steel drum is expensive and requires rigorous maintenance to avoid leakage  
Not suitable for fibrous substrates  
Gas holder can get stuck in the resulting floating scum |
| Covered lagoon          |                        | Longer retention time and lower degradation rate  
Poor interaction between substrate and microorganisms. Seasonal variation in biogas production |
| Polyethylene tubular reactors |                        | Susceptible to mechanical damage  
Short lifespan  
Sensitive to sunlight and deteriorate rapidly due to seasonal changes |
| Plastic tanks           |                        | Expensive  
Not suitable for large-scale biogas plants |
| Reinforced plastic reactors |                      | Expensive  
Floating where underground water level persists |

References: [36, 39, 44, 80-91]
3.3 Biogas in Nigeria

Nigeria, with a growing population of about 186 million [92], has the largest economy among the nations in Africa with a gross domestic product (GDP) of about $405 billion, according to World bank 2016 [93], yet Nigeria continues to face energy and environmental challenges. About 96% of the population of Nigeria is connected to the national grid, but only 18% of the functioning connections have a reliable supply of electricity [94], which is a major challenge that depletes the energy and enterprises necessary for the country’s development. Also, continuous burning of fossil fuels and solid wastes has resulted in 94% of the population of Nigeria being exposed to air pollution levels (measured in PM$_{2.5}$) that exceed the WHO guidelines and air pollution damage costs of about 1% post of gross national income [95]. Water and soil pollution is also a challenge due to improper management of human sewage and the tremendous amounts of solid wastes generated. As water, soil, and air pollution as well as energy poverty pose great challenges to human health and the environmental and economic development of the country, the need for renewable energy cannot be overemphasised.

Renewables accounted for about 62% of the net additions to global power generating capacity in 2016, and the vast majority of renewable energy for heating was supplied by biomass, with smaller contributions from solar thermal and geothermal energy [4]. Biogas production is an appropriate technology needed in Nigeria to ease the nation’s energy and environmental challenges. According to Ngumah, Ogbulie [96] Nigeria generates about 542.5 million tonnes of total wastes per annum (livestock wastes, human excreta, crop residues, and municipal solid wastes). This tremendous amount of wastes has the potential to produce an estimated 25.53 billion m$^3$ of biogas and 88.19 million tonnes of bio-fertilisers annually, as shown in Table 3.2. The biogas produced can be used for heating, cooking, transportation, and generation of electricity if properly harnessed, and the residue remaining after biogas production is suitable for improving agricultural development in the country. Biogas can augment the conventional energy sources in the country, thereby improving the quantity and quality of the energy supply while also reducing environmental pollution.
Table 3.2 Estimated potential of waste biomass in Nigeria (modified from [96])

<table>
<thead>
<tr>
<th>Biomass resource</th>
<th>Total biomass generated (million tons/year)</th>
<th>Estimated biogas potential (billion m³/year)</th>
<th>Biomethane potential (BMP) (billion m³/year)</th>
<th>Energy potential (Terajoules/year)</th>
<th>Estimated biofertiliser (dry) potential (million tons/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle excreta</td>
<td>197.60</td>
<td>6.52</td>
<td>3.65</td>
<td>142350</td>
<td>25.69</td>
</tr>
<tr>
<td>Sheep &amp; goat excreta</td>
<td>39.60</td>
<td>2.30</td>
<td>1.61</td>
<td>62790</td>
<td>3.71</td>
</tr>
<tr>
<td>Pig excreta</td>
<td>15.30</td>
<td>0.92</td>
<td>0.55</td>
<td>21450</td>
<td>1.68</td>
</tr>
<tr>
<td>Poultry excreta</td>
<td>32.60</td>
<td>2.50</td>
<td>1.65</td>
<td>64350</td>
<td>1.89</td>
</tr>
<tr>
<td>Abattoir waste</td>
<td>83.30</td>
<td>4.42</td>
<td>2.65</td>
<td>103350</td>
<td>6.12</td>
</tr>
<tr>
<td>Human excreta</td>
<td>52.00</td>
<td>2.60</td>
<td>1.69</td>
<td>65910</td>
<td>6.45</td>
</tr>
<tr>
<td>Crop residue</td>
<td>83.00</td>
<td>4.98</td>
<td>3.00</td>
<td>117000</td>
<td>36.20</td>
</tr>
<tr>
<td>Municipal solid wastes</td>
<td>39.10</td>
<td>1.29</td>
<td>0.85</td>
<td>33150</td>
<td>6.45</td>
</tr>
<tr>
<td>Total</td>
<td>542.50</td>
<td>25.53</td>
<td>15.65</td>
<td>610350</td>
<td>88.19</td>
</tr>
</tbody>
</table>

3.3.1 Biogas challenges and pioneered projects in Nigeria

The fixed-dome reactor is one of the commonly used biogas reactors in Nigeria because of its long lifespan, but the technology is expensive, labour intensive, and requires skilled supervision [86]. The lack of government commitment and poor continuity of previous biogas programme initiatives through successive governments is a major factor limiting the advancement of this technology [97]. An additional challenge is corruption, which increases the investment costs for biogas implementation and thereby reduces the rate of return for the investment [98]. Many biogas plants have failed due to poor planning, non-suitable biogas site selections, poor reactor construction technology, poor maintenance, non-skilful operators of the digestion process, and unavailability of spare parts.

In spite of these challenges, there are some existing biogas plants in Nigeria: less than 20 pilot projects, including the United Nations Development Programme (UNDP) project in Kano state, have been established [99]. The Cows-to-Kilowatts Project, located in Ibadan, the
capital of Oyo State, was begun in May 2008 [100] with collaboration among the Global Network for Environment and Economic Development Research, Nigeria (NGO), the Biogas Technology Research Centre, KMUTT, Thonburi, Thailand (research institute), the Centre for Youth, Family and the Law, Nigeria (community-based organisation), and the Sustainable Ibadan Project, Nigeria (UN-HABITAT Programme) [101]. The biogas plant, shown in Fig. 3.3, employs an anaerobic fixed-film reactor with a volume of 3000 m$^3$ for treatment of abattoir waste to produce biogas and organic fertiliser. The Bodija market in Ibadan slaughters about 1000 cows per day, and the plant is capable of producing about 1500 m$^3$ of biogas (900 m$^3$ of methane) [102], which is sufficient to fill 5400 cylinders of cooking gas per month. The plant is also capable of producing about 1500 litres of bio-fertiliser per day for farmers.

NASRUN Nig. Ltd. was established in 2002 as a crop and livestock enterprise. It has a biogas plant (Kutunku Farms Biogas Plant) of about 140 m$^3$ located in Minna, Niger State, Nigeria [103], where a fixed-dome reactor was designed to use farm waste feedstock for biogas production.

![Fig. 3.3. Biogas plant in Nigeria for the treatment of abattoir wastes from Bodija market, Ibadan (reprinted with permission) [101]](image)

Another fixed-dome bio-digester of 20 m$^3$ which was built by the Energy Commission of Nigeria ECN-SERC/UDU in 1998 is fed with cow dung. The reactor is located at the Mayflower Secondary School, Ikenne Ogun state [100], and produces gas for cooking and bio-fertiliser for farmers. Additionally, the National Centre for Energy Research and Development, University of Nigeria Nsukka (NCERD/UNN) built a biogas plant of 10 m$^3$ for
Women at Achara, Nsukka, Enugu state. The biogas plant is fed with domestic animal wastes, cassava peels and wastes from the milling of cowpea, and bambara nuts from a food processing plant [104]. The Sokoto Energy Research Centre (SERC) also constructed a 30 m$^3$ biogas reactor at the National Animal Production Research Institute (NAPRI) in Zaria. The reactor is fed with human excreta, and the biogas produced is used for cooking at the Zaria prison.

3.3.3 Strategies for improved biogas technology in Nigeria

Successful implementation of biogas technology in Nigeria requires innovative strategies to motivate investment in biogas production, which will lead to economic growth, environmental safety, and national security. Strategies that have been used by developed countries for successful implementation are discussed in this section. Nigeria needs to adopt some of these strategies to stimulate the growth of biogas in the country.

Technology: The new textile bioreactor used in this study for biogas production (Paper IV) is a promising technology for developing countries such as Nigeria, where the biogas process should be cost-effective. The laboratory- and pilot-scale results demonstrated the suitability of this technology for advancement of global energy production (Paper V). Farmers and individual households can easily install such reactors and produce biogas for heating and cooking. A number of reactors can be run in parallel for continuous gas production, depending on the amount of wastes generated. In this way, farmers can become self-sufficient in generating renewable energy while also producing bio-fertiliser for enhancement of agricultural development in the country.

Funding: One major problem for successful large-scale implementation of biogas technology in Nigeria is funding. Financial support from the government and from non-governmental organisations is needed for significant improvements in the technology. In Sweden, there have been significant advances in biogas production for energy generation and after upgrading it’s used as fuel for vehicles, and this development has been made possible by many years of central government funding through local investment programs (LIP) and climate investment programs (KLIMP) [105]. In Denmark, the first plant built in Vester Hjermitslev, with the goal of making the town energy self-supporting [106], was implemented with almost complete governmental funding: a 4 million DKK grant from the government and an 8.4 million DKK loan from the North Jutland County Council [107]. There have also been
investment grants for centralised biogas plants (up to 40 % of costs) and loan schemes with long-term low interest rates.

Policies: There is need for policies that will support the development of biogas production for energy generation in the country. The government should develop state-of-the-art policies at all levels (federal, state, and local government) that will attract investment and encourage flexibility in energy generation. Policies such as feed-in tariffs, net metering, virtual net metering, and tax incentives have provided great support for renewable energy in developed countries [4]. Policies in Sweden supporting renewable transport fuel include tax exemptions or reductions of taxes applied on fossil fuels and laws mandating refilling stations to provide at least one renewable fuel [108].

Awareness campaigns and education: People should be educated on the importance of proper management of the wastes generated daily and the benefits of biogas produced from these waste fractions. There is need for continuous counselling and training in communities, schools, and market places about how to manage their wastes effectively, reduce the amount of wastes generated as much as possible, reuse for a longer time, recycle to new products, and recover energy from wastes.

Research development: The government should support research development by ensuring strong collaboration between universities and industries in Nigeria. Pilot projects can be conducted in universities to evaluate the feasibility of the technology and obtain optimum conditions for successful implementation and thereafter the technology can be adopted by industries.
Chapter 4

Dry anaerobic digestion (dry-AD)

The anaerobic digestion process can be defined according to the total solid content of the feedstock: wet digestion for total solid contents between 0.5 % and 15 % [109] and dry digestion for total solid contents greater than 20 % [110, 111]. In industries, wet anaerobic digestion processes are most common, but recently there has been great concern about the large amount of water used while treating organic solid wastes for biogas production and the huge water content of the digestate residue. In addition, the use of solid wastes (total solid content between 15 % and 50 %) from agricultural, municipal, and industrial activities, including forest and crop residue, is becoming more attractive. Therefore, the impetus for developing dry anaerobic digestion processes is increasing in both research and industry.

4.1 Comparison of wet and dry anaerobic digestion

Wet anaerobic digestion of solid wastes requires a lot of water, which is a challenge for countries with water shortage. Presently, there is global water scarcity; about 1.4 billion people live in river basins in which water use rates exceed recharge rates [112]. It is obvious that there will be competition for water as population increases and industrial development progresses. Also, the digestate residue remaining after biogas production through the wet process contains a lot of water, and dewatering of the digestate requires high energy consumption and results in loss of nutrients. Therefore, dry anaerobic digestion is a promising technology to avert these problems. Compared with the wet anaerobic digestion processes, dry-AD provides better economic feasibility because reactor volume is minimised due to the reduced volume of water [113, 114]. It is also beneficial because it is more robust, flexible in its acceptance of feedstocks which requires less pre-treatment [114].

The total solid content of feedstocks affects the performance of anaerobic digestion processes, and the microbial morphology in the reactor changes as the total solid content changes [63, 111]. The microbial communities that are dominant in dry digestion processes may differ from those in wet digestion processes as the TS content increases [115, 116]. Yi, Dong [111] investigated microbial communities using pyrosequencing technology while treating food waste with TS contents from 5 % to 20 % under the mesophilic condition. The result showed
Dry anaerobic digestion (dry-AD)

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better VS reduction and methane yield in reactors with higher TS content and that Methanosarcina was dominant in three reactors showing an increasing trend with increasing TS content. Li, Chu [117] reported that Anaerococcus species are abundant in solid-state anaerobic digestion (dry-AD) and that these species are responsible for improved degradation efficiency and methane yield.

However, dry-AD requires pumps specifically designed for handling high-solid slurries, which may be more expensive than the centrifugal pumps used in wet digestion systems [114, 118]. It also requires a longer start-up period and is sometimes prone to inhibition because inhibitors that enter the reactor through feedstocks are in higher concentration due to the reduced water content.

4.2 Start-up phase in dry anaerobic digestion

In dry-AD, there is minimal or no mixing during the digestion process due to the high solid content in the reactor. This necessitates the need for proper conditioning of the feedstock. The start-up phase is important for the generation of microbial cultures adapted to the feedstock and can be very long due to the low growth rates of the anaerobic microorganisms. If inoculum adapted to feedstock similar to the one of interest is available then start-up can be shorter, just a few days otherwise it can take longer period up to 1 or 2 months or longer if the right microorganisms are not acclimatised. In this study, the start-up period took 40 days (Paper II) for dry digestion of manure with straw and 28 days (Paper VI) for initial start-up in dry co-digestion of chicken feathers, citrus wastes, wheat straw, and manure with straw in plug flow reactors, as shown in Fig. 4.1.

The inoculum (anaerobic sludge) should be collected from an active anaerobic digester, preferably from a dry digestion plant because inoculum with high solid content is needed. Obtaining inoculum with high solid content is vital because centrifuging the inoculum at high speed to obtain as many bacteria as possible (solid inoculum) may result in significant reductions in bacteria viability [119], which can have a potential effect on the outcome of the experiment. Furthermore, appropriate inoculum to substrate ratio is needed; the amount of solid inoculum needed depends on the substrate and the feedstock of the previous digester from which the inoculum is taken. In this study, an inoculum-to-substrate ratio (VS_{inoculum} to VS_{substrate}) of 1 was used in a textile batch reactor treating manure with straw, but this ratio was reduced to 0.5 after an acclimatisation period of 232 days (Paper IV). This study also
used, an inoculum-to-substrate ratio of 2 in the co-digestion of chicken feathers, wheat straw, and citrus wastes in continuous plug flow reactors, but after a start-up period of about 1 month (adaptation period), this ratio was reduced to 0.4 to increase the solid content in the reactor (Paper VI). The inoculum must be mixed with the substrate prior to placing them into the reactor, but the mixing must not be too rigorous. Rigorous mixing can results rapidly overwhelmed methanogenic areas by acid inhibition [120].

Fig. 4.1. Daily biogas production and accumulated methane yield obtained during the initial startup phase carried out in batch mode for the digestion of the best mixing ratio at 20 %TS (1:1:6 referring to chicken feather: citrus wastes: wheat straw) in plug flow reactors; reactor 1 (R1) and reactor 2 (R2). Colours represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: ◊ and □ for daily biogas production and △ and x for cumulative methane yield. (Paper VI).

4.3 Dry anaerobic digestion – Batch process

In the batch digestion processes, an appropriate amount of inoculum is mixed with the substrate, the mixture is loaded into the reactor, the reactor is closed, and the substrate is digested under anaerobic conditions. The reactor remains closed until degradation is completed. Usually little or no mixing is possible inside the reactor. Biogas production starts slowly, increases gradually to a peak, and then declines until there is no more gas production.
The reactor is opened and the digestate is replaced with a new batch. The tank is then closed again and is ready for operation [121]. Batch processes are best operated in parallel, so at least one reactor is always producing biogas. The principle of the batch digestion process is technically simple, but supplying the substrates to the microbial communities at once can repress the digestion process. Additionally, insufficient mixing can induce settlement layers making the digestion process inefficient. Thus, the conditions in the process change continually due to cell metabolism and lack of control; hence steady biogas production is not attainable. The following subsections describe the experimental batch digestion processes carried out in this study (textile bioreactor) as well as the BEKON dry digestion technology.

4.3.1 Dry anaerobic digestion in textile bioreactor

Textile biogas reactor technology is designed to make biogas technology accessible and attractive to developing countries. This new bioreactor is made of textiles, which makes it less combustible, resistant to tearing, light-weight, and environmentally benign. This reactor is UV treated to prevent easy degradation due to exposure to sunlight and weather resistant, which increases its lifespan. The reactor is also composed of coated polymers as a protection against corrosive gases such as H₂S. Textile biogas reactors range in size from 1 m³ to 1000 m³ and are suitable for both small- and large-scale biogas processes. Conventional biogas reactors, such as the fixed-dome, floating-drum, and plastic biogas reactor, along with the challenges associated with these reactors were studied while introducing the textile biogas bioreactor (Paper V). Textile bioreactors are a promising technology for dissemination, and this will enhance global advancement of biogas technology, especially in developing countries.

Dry digestion of manure bedded with straw was investigated using the newly developed textile bioreactor (Paper IV). Figure 4.2 shows a schematic sketch of the experimental set-up. Repeated batches were carried out. During the first digestion process, the total feedstock with 22 %TS was inoculated with the initial inoculum (unacclimatised inoculum) maintaining a volatile solids (VS) ratio of inoculum to substrate at 1:1 and thereby having a total TS of 10 % in the reactor. In order to increase the TS in the textile reactor and investigate the response of microorganisms after acclimatisation, a second batch digestion (acclimatised low TS) was performed; the TS of the substrate was increased to 27 %, thereby increasing the TS in the reactor to 12 %. To increase the TS in the reactor further, a third batch digestion process (acclimatised high TS) was also performed. The VS ratio was reduced to 0.5 and the TS of the substrate was increased to 30 %, thereby increasing the TS in the reactor to 17 %.

Fig. 4.2. Schematic sketch of the experimental set-up (Paper IV).

It was found that the methane yield increased from 183 to 290 NmlCH₄/gVS with decreasing degradation time from 136 to 92 days after a long-term acclimatisation period of 232 days, as shown in Fig. 4.3. The bioreactor worked successfully for more than 324 days without any gas leakage or major maintenance.

Fig. 4.3. Cumulative methane yield and production rate obtained during the digestion process with gradual increase of total solid (TS) content of the feedstock and reactor mixture. 22 %, 27 %, and 30 % (TS of feedstock); 10 %, 12 %, and 17 % (TS in reactor) (Paper IV).
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4.3.2 BEKON dry digestion technology

The BEKON process allows digestion of solid wastes with high solid content. It is suitable for processing wastes with total solid contents of up to 50 % [122]. The solid waste is inoculated with fermented substrate, loaded into the reactor, closed in the reactor, and digested under the anaerobic condition. The process is a single-step batch process; all digestion steps, as explained in Fig. 3.1, take place in the same reactor. There is no addition of feedstock or mixing inside the reactor applied during the digestion. The fermentation process runs at the mesophilic condition (34 – 37 °C), and the temperature is regulated through heated floor and walls. To allow continuous inoculation and to provide moisture for the microorganisms during the digestion, percolation liquid is collected and recirculated back continuously into the reactor. This is a good practice because recirculation of the liquid can facilitate the growth of the microorganisms [123] and thereby improve methane yield while also reducing degradation time. However, liquid recirculation sometimes can result in a high risk of acidification, thereby leading to instability in the process. BEKON technology allows several reactors to run simultaneously, and the biogas produced is used for electricity generation. A detailed illustration of the process is shown in Fig. 4.4.

![BEKON dry batch digestion technology](image)

**Fig. 4.4.** BEKON dry batch digestion technology (reprinted with permission [122])

4.4 Dry anaerobic digestion – Continuous process

In the continuous digestion process, the substrate is supplied to the microorganisms continuously in small amount, which minimises toxic effects of the substrate or product on the cells. The substrate is fed continuously into the reactor, and an equal amount is removed; this is done to maintain a stable working volume and steady state conditions. A moderate amount
of the substrate is fed at the initial stage and then the loading rate is increased gradually to reach the target. This continuous supply of substrate without alteration results in steady biogas production, and the process can be controlled easily. Plug flow bioreactors are commonly used in the industry for continuous digestion processes.

4.4.1 Plug flow reactors for continuous dry digestion processes

Horizontal plug flow reactors, as shown in Fig. 4.5, for continuous dry anaerobic digestion processes were used in this study. The reactor was operated under mesophilic conditions (37 °C), and the temperature was sustained by circulating water from a heater, a thermostatic water bath through a water jacket surrounding the reactor, and 10-mm-thick styrofoam was used to insulate the reactor against heat loss. The plug flow reactor has three main sections, namely the inlet zone, main zone, and outlet zone as shown in Fig. 4.5.

![Plug flow reactors](image)

**Fig. 4.5.** Plug flow reactors used in this study for continuous dry anaerobic digestion processes

4.4.1.1 Continuous dry digestion of manure bedded with straw

Cattle manure bedded with straw was used as feedstock and digested at 22 %TS using the continuous plug flow reactor (Fig. 4.5). Figure 4.6 shows the details of the experimental set-up and other accessories.
The feedstock was inoculated and the experiment started in batch mode until no further gas production was monitored (40-day digestion start-up period). The continuous feeding operation began after the start-up period and was investigated at organic loading rates of 2.8 gVS/L/d (OLR 1), 4.2 gVS/L/d (OLR 2), and 6 gVS/L/d (OLR 3) with corresponding retention times of 60, 40, and 28 days. Figure 4.7 shows the biogas production at the various stages. Overloading was observed when the OLR of 6 gVS/L/d was applied, so the procedure of this OLR was repeated to check the stability of the process under this condition. However, the daily gas production remained unstable leading to increased VFA/alkalinity ratio which is an indication of instability (explained in section 3.3.4). However the process could be restored back to stability by reducing the OLR to 4.2 gVS/L/d with the corresponding retention time of 40 days.
Fig. 4.7. Biogas production at different organic loading rates (OLRs) during continuous experiments. The symbols represent daily biogas production (◊) and cumulative biogas production (□). OLR 1 (2.8 gVS/L/d), OLR 2 (4.2 gVS/L/d) and OLR 3 (6 gVS/L/d). (Paper II).

4.4.1.2 Continuous dry co-digestion of citrus wastes with chicken feathers and wheat straw

Dry co-digestion of citrus wastes with chicken feathers, wheat straw and manure bedded with straw was investigated in batch digestion assay (Paper VI). The long term effects in process performance were investigated for the best mixing ratio indicating synergy, determined through the batch assays, in continuous plug flow reactors at different organic loading rates. Reactor 1 (R_{TS21}) was fed with a feedstock having 21 %TS and reactor 2 (R_{TS32}) was fed with a feedstock having 32 %TS. The OLR was kept first at 2.0 gVS/L/d (OLR 1) and then it was increased to 3.8 gVS/L/d (OLR 2) with corresponding retention times of 50 and 30 days, respectively. During the feeding appropriate amounts of the digestate residue withdrawn every day from the reactors was mixed with the fresh feedstock in a ratio of 1:2 (feedstock:digestate; wet basis). Process parameters such as volume biogas produced, methane content of the biogas, pH, TS, VS, VFA/Alkalinity ratio, total VFA and total ammonia concentration were monitored regularly during the digestion process. At OLR of 2 gVS/L/d, the methane yield of 362 NmlCH₄/gVS_{added} was obtained from R_{TS21} which is 13.5 % higher than the yield obtained from R_{TS32} (319 NmlCH₄/gVS_{added}) (Paper VI). However, the
stability of the process declined for both R\textsubscript{TS21} and R\textsubscript{TS32} as OLR was increased to 3.8 gVS/l/d resulting in high total VFA and VFA/alkalinity ratio as shown in Fig. 4.8.

Fig. 4.8. Variation in pH, VFA/alkalinity ratio, total VFA and total ammonia concentration in reactor 1 (R\textsubscript{TS21}) and reactor 2 (R\textsubscript{TS32}) during continuous operation; startup (35 days), OLR1 (2 gVS/L/d for 50 days) and OLR2 (3.8 gVS/L/d for 30 days). Colours represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: (a) ◊ and □ pH, ∆ and x VFA/alkalinity ratio (b) ◊ and □ total VFAs concentration, ∆ and x total ammonia concentration. (Paper VI).

4.4.2 Continuous dry-AD technologies

Väst Blekinge Miljö AB (VMAB) in Sweden uses a technology designed for dry digestion of household wastes to produce biogas fuels for transportation. The process uses plug flow reactors for continuous dry digestion of solid wastes with a total solid content of about 35 %. The reactor is capable of treating 15,000 tons of wastes per year, and the wastes typically
consist of 75 % sorted household wastes and 25 % garden wastes [124]. The household wastes are pre-treated by crushing and separation of non-digestive wastes. The feedstock is stored for 1 – 3 days in a buffer tank and thereafter pumped continuously into the plug flow reactors for anaerobic digestion. This process runs at thermophilic conditions (55 °C), and horizontal impellers inside the reactor, shown in Fig. 4.9, allow minimal mixing in the reactor. Presently, the continuous dry fermentation process works properly without liquid recirculation, which is similar to the process used in this research study (Paper II).

![Fig. 4.9. Plug flow reactor with impeller inside the reactor for flow of material and mixing](image)

DRANCO technology which was developed in Belgium, is a single-stage system for treatment of solid wastes, mostly municipal solid wastes, with total solid content of 15 % to 40 % in the reactor [125]. It is a vertical plug flow reactor, as shown in Fig. 4.10a. Feedstock is pumped to the top of the reactor through feeding tubes and extracted through a conical outlet at the bottom. There is no mixer inside the reactor, but mixing is enhanced by recycling the digestate (one part of fresh wastes mixed with six parts of the digestate) [118]. Manual recycling of the digestate at feedstock to digestate ratio of 1 to 2 was also investigated in this research for dry co-digestion of solid wastes (Paper VI).

STRABAG technology is designed for the treatment of solid waste with TS content between 15 % and 45 % using horizontal plug flow reactors, as shown in Fig. 4.10b. The reactors are equipped with agitators arranged transversely to the flow direction to prevent formation of floating scum and settlement of materials [126].
KOMPOGAS technology is similar to STRABAG technology. It is aided by slow rotation of impellers inside the reactors for homogenisation and degassing [118]. The process requires careful adjustment of the solid content in the reactor to around 23 %TS; higher TS values may cause excessive resistance to flow [118].

**Fig 4.10.** Reactor designs for adequate mixing of solid wastes (a) DRANCO technology [125] (b) STRABAG technology
Improving biogas production from solid wastes

Anaerobic digestion of agricultural, municipal, and industrial solid wastes often results in low biogas yields and slow degradation rates because some of these wastes have recalcitrant molecular structures that make them difficult to degrade by microorganisms and some contain chemicals/substances that can inhibit microbial growth. Strategies to overcome the challenges associated with these wastes in order to ascribe value to them are discussed in this section.

5.1 Pretreatment to overcome biomass recalcitrance

Pretreatment of the substrate is needed either for making it easier to handle at the biogas plant or for altering its structure for easy degradation, hence enhancing its methane potential. There are different pretreatment methods that can be used depending on the types of substrates and the goals of the pretreatment. The most suitable pretreatment methods for agricultural, municipal, and industrial solid wastes are discussed in this section.

5.1.1 Mechanical pretreatment

Mechanical pretreatment is required for biomass with high total solids content (e.g. forest residue, crop residue, chicken feather wastes) to reduce the particle size and crystallinity of the feedstock. It can also reduce viscosity in biogas reactors making mixing easier [127]. Milling is commonly used, and optimising the size reduction process is important for improving biogas yield. Lindner, Zielonka [128] pretreated non-degraded digestate by ball milling in order to feed it back into the fermentation process, and they observed that ball milling pretreatment enhanced the methane yield of two-stage maize silage digestate by 9 % and 17 % using two-stage hay/straw digestate, respectively. However, the electrical demand of this pretreatment method is very high, so it is not viable as a stand-alone pretreatment for industrial applications but can be combined with other pretreatments that are cost effective [5]. In Paper I, the chicken feather used was chopped to particle sizes between 1 and 10 mm and then biologically pretreated prior to biogas production. The untreated wheat straw used in Papers IV and VI was milled to particle sizes between 0.5 mm and 2 mm prior to use as a co-substrate for biogas production.
5.1.2 Thermal pretreatment

The feedstock is heated to a temperature between 125 and 190 °C under pressure and maintained at that temperature for one hour [127, 129]. Autoclaves and microwaves are commonly used in the laboratory for this method. The method’s effectiveness depends on the feedstock, pretreatment temperature, and amount of time. Thermal pretreatment of solid wastes requires additional water. The presence of heat and water causes swelling of the biomass by disrupting the hydrogen bonds between cellulose and matrix polymers (hemicellulose and lignin) [129]. Higher pretreatment temperature can enhance the solubility of organic matter [130], although this might not result in increased biogas production [131]. Liquid hot-water pretreatment at lower temperature is effective for hydrolysis of hemicellulose [65], and moderate pretreatment of biomass is required for improved anaerobic digestion. This process requires a lower amount of chemicals for neutralisation of the hydrolysate and produces a lower amount of neutralisation residue compared to dilute-acid pretreatment [64].

5.1.3 Chemical pretreatment

The chemical pretreatment method involves the use of acids and bases at different concentrations and conditions for hydrolysis of recalcitrant biomass. It is most suitable for forest and crop residues (lignocellulosic biomass) but can be used for hydrolysis of chicken feathers as well [132]; it enhances the degradation rate of this biomass and improves biogas production. Dilute acid pretreatment under controlled conditions is generally used for solubilising hemicellulose from this lignocellulose biomass and thereby increasing the accessibility of cellulose to the microorganisms [5]. High temperature and high acid concentration, can however cause the release of inhibitory products [133]. Acid pretreatment of wheat straw and sugarcane bagasse was studied by Bolado-Rodriguez, Toquero [134], and the result showed acid solubilisation of high concentrations of sugars generating furfural and HMF with strong inhibition effects. However, Xiao and Clarkson [135] reported 80 % lignin solubilisation when newsprint was pretreated with 35 % acetic acid and 2 % nitric acid, resulting in a three times higher methane yield than the yield obtained from the untreated material, although there was no effective recovery of the acids used.
5.1.4 Biological pretreatment

Biological pretreatment makes use of microorganisms (mostly bacteria and fungi) to degrade recalcitrant biomass for improved biogas production. Studies have shown that white-rot fungi produce enzymes that are capable of extensive degradation of lignin and that brown-rot fungi break down cellulose and hemicellulose resulting in increased biomass digestibility [136], although there is a loss of cellulose and hemicellulose during this process [137]. Based on the enzyme production patterns of white-rot fungi, Hatakka [138] suggested three categories of fungi for lignin degradation, namely a lignin-manganese peroxidase group (e.g. *P. chryso sporium* and *Phlebia radiata*), manganese peroxidase-laccase group (e.g. *Dichomitus squalens* and *Rigidoporus lignosus*), and lignin peroxidase-laccase group (e.g. *Phlebia ochraceofulva* and *Junghuhnia separabilima*). Additionally, the soft rot ascomycetal fungus (*Trichoderma viride*) has been applied to MSW (kitchen wastes, garden wastes, grass clippings, foliage, and wood pellets) in an aerobic upstream process prior to anaerobic digestion [139]. The results showed a threefold increase in methane yield compared to the case of untreated MSW.

Bacteria and fungi have also been reported capable of producing enzymes that degrade keratin-rich wastes. Some species of *Bacillus* [66, 140-144] and some species of saprophytic and parasitic fungi [145, 146] have been reported effective for hydrolysing keratin-rich wastes. Commercial enzymes (cellulase, hemicellulase, proteases, amylases, and lipases) are also suitable for effective hydrolysis of complex biomass. Multiple commercial enzymes (consortium) are more effective than single commercial enzymes because they act synergistically to enable efficient conversion of the substrate by the concerted action of several species [65]. Commercial enzymes are very expensive, but the enzymes can be produced on-site from cheap feedstocks, which makes the process cost effective [147]. The biological pretreatment method is usually carried out at low temperatures and pressures without expensive equipment or chemical reagents. This method is also sustainable, ecological, and cost effective; however, the enzymatic reactions are slow and as such require longer pretreatment times compared to other pretreatments method [148].

5.1.4.1 Hydrolysis of chicken feathers for biogas production

The keratin-degrading bacterium used in this study was a naturally occurring strain of *Bacillus* isolated from compost and identified as *Bacillus sp. C4* (2008) [141]. Chicken
feathers at total solid concentrations of 5, 10, and 20 % (dry weight of feathers) were biologically pretreated. The increase in feather concentration from 5 to 20 %TS caused a suppression of keratinase activity, which resulted in a decrease in the percentage of feathers degraded, as shown in Fig. 5.1a.

**Fig. 5.1** (a) Substrate degradation at different feather concentrations (b) Soluble crude protein from TKN value (modified from Paper 1)

After 8 days of degradation, the amount of soluble crude protein increased from an average of 4.13 g/l to 35.97 g/l, 36.97 g/l, and 45.94 g/l at initial feather concentrations of 5, 10, and 20 %TS, respectively, as shown in Fig. 5.1b.

Both the hydrolysate and the whole broth of the pretreated feathers were investigated as substrates for biogas production using anaerobic sludge and bacteria granules as inocula (Paper 1). Pretreatment improved methane yield by 292 % and 105 % when anaerobic sludge and granules were used on the feather hydrolysate, respectively. Bacteria granules worked effectively on the total broth, yielding 445 NmLCH₄/gVS methane, which is 124 % more than that obtained with the same type of inoculum from untreated feather.

### 5.2 Co-digestion to achieve balance nutrient

Co-digestion of different feedstocks with an appropriate mixing ratio is a possible solution to overcome the challenges associated with mono-digestion of potential feedstocks and obtain improved biogas yield. Mono-digestion of protein-rich wastes (animal manure and chicken feathers) results in low biogas yield due to high nitrogen content, which may inhibit methanogens. Lignocellulosic wastes are seasonal biomass with high carbon content but very
low nitrogen content, but digestion of these feedstocks alone also results in a slow process leading to low biogas yield. Additionally, some industrial wastes such as fruit and food wastes may contain chemicals and substances that inhibit methanogens and thereby result in low biogas yield. Choosing an appropriate mixing ratio is vital for effective performance of the co-digestion process, and it is important to consider the C/N ratio, biodegradability, possible inhibitors, and the TS of the individual feedstocks [5]. Considering the fact that easily degraded feedstocks are not highly available and the problems associated with pretreatments, co-digestion is a good strategy for improving biogas production because it favours synergisms, dilutes harmful compounds, optimises biogas production, and increases digestate quality [149]. Co-digestion of different solid wastes has been reported by various researchers to have substantial impacts on anaerobic digestion processes, as shown in Table 51.

In this study, a methane yield of 163 NmlCH₄/gVS was obtained from manure bedded with straw at 22 %TS and C/N ratio of 16.8 using the plug flow reactor (Paper II). However, digestion of manure bedded with straw at 22 %TS and C/N ratio of 25 due to the addition of untreated wheat straw in the textile reactor resulted in a 12 % increase in methane yield due to the increased C/N produced by the wheat straw (Paper IV). Additionally, co-digestion of citrus wastes with chicken feathers, wheat straw and manure bedded with straw was investigated at 20 %TS content to reduce the inhibitory effect of the citrus waste (Paper VI). The best mixing ratio (1:1:6; referring to chicken feathers: citrus wastes: wheat straw) enhanced the methane yield by 14 % compared to the expected yield calculated from the methane potentials obtained for the individual fractions.

### 5.3 Acclimatisation of microorganisms to substrates

One strategy for overcoming the problem of low methane yield due to substrate or product inhibition and preventing a lag phase is long-term acclimatisation of microbial communities to the feedstock and conditions in the reactor. Studies have shown changes in microbial communities compared with the initial inoculum due to adaptation [150-152]. Carucci, Carrasco [153] investigated the effect of acclimatised inoculum on co-digestion of fresh vegetables and sludge mixture and observed that inhibition of methanogenesis was overcome by a long acclimatisation period of 120 days. Adaptation of the microbial community to supplied feedstock during a long-term (337 days) anaerobic digestion of maize and sugar beet silage has also been reported [152]. This shows that acclimatisation of microorganisms to feedstock and conditions in the reactor is vital for improved biogas production. In this study, a
long-term acclimatisation period of 232 days resulted in a 58 % increase in methane yield when manure bedded with straw was digested in the textile bioreactor (Paper IV).

Table 5.1 Co-digestion of solid wastes for improved biogas production (modified from [147])

<table>
<thead>
<tr>
<th>Feedstocks</th>
<th>Effect of co-digestion</th>
<th>Influencing factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food, vegetable, fruit, leaf and paper wastes</td>
<td>Reduced ammonia-nitrogen inhibition</td>
<td>C/N ratio</td>
<td>[154]</td>
</tr>
<tr>
<td>Solid energy crops and pig manure</td>
<td>Enhanced process performance</td>
<td>High buffering capacity</td>
<td>[155]</td>
</tr>
<tr>
<td>Crops (grass silage, oat straw, and sugar beet tops) and cow manure</td>
<td>Increased methane yield, Increased VS removal</td>
<td>Mixing ratio</td>
<td>[156]</td>
</tr>
<tr>
<td>Cattle manure with wheat straw</td>
<td>Increased methane yield</td>
<td>C/N ratio</td>
<td>[157]</td>
</tr>
<tr>
<td>Fresh vegetable and precooked food wastes</td>
<td>Increased methane production yield and rate</td>
<td>Dilution to non-inhibiting initial concentration Synergetic effect</td>
<td>[153]</td>
</tr>
<tr>
<td>Yard and food wastes</td>
<td>Increased methane yield, and volumetric productivity Increased VS reduction</td>
<td>Mixing ratio (C/N ratio)</td>
<td>[158]</td>
</tr>
<tr>
<td>MSW, manure, crop residues, and slaughterhouse wastes</td>
<td>Increased methane yield</td>
<td>High buffering capacity (synergetic effect)</td>
<td>[159]</td>
</tr>
<tr>
<td>Paper tube residues and nitrogen-rich substrate mixture</td>
<td>Stabilized the process Reduced HRT Reduced VFA accumulation</td>
<td>High buffering capacity</td>
<td>[160]</td>
</tr>
<tr>
<td>Crop silage and cow manure</td>
<td>Increased methane yield, and allowed higher organic loading rate</td>
<td>Mixing ratio</td>
<td>[161]</td>
</tr>
<tr>
<td>Citrus wastes, chicken feathers and wheat straw</td>
<td>Improved methane yield, Buffering capacity and C/N ratio</td>
<td></td>
<td>Paper VI</td>
</tr>
<tr>
<td>Cassava silage and excess sludge</td>
<td>Reduced propionate concentration, Increased methane yield</td>
<td>C/N ratio</td>
<td>[162]</td>
</tr>
</tbody>
</table>

C/N = carbon to nitrogen ratio, VS = volatile solids, HRT = hydraulic retention time, VFA = volatile fatty acids
Digestate as biofertiliser

Digestate is the residue remaining after biogas production by anaerobic digestion of organic wastes. The organic wastes used as feedstocks for biogas production contain some macronutrients (nitrogen, phosphorus, potassium, calcium, sulphur, and magnesium) and micronutrients (boron, chlorine, manganese, iron, zinc, copper, molybdenum, and nickel), and additional nutrients/trace elements are sometimes added to enhance the digestion process. As these feedstocks degrade during the digestion process, the nutrients are released and concentrated in the residue [33]; therefore, the digestate residue is a valuable fertiliser which contains most of the macronutrients and minor nutrients in plant-available form. This residue after biogas production can result in similar or even better crop yields than obtained by using commercial fertilisers [163].

6.1 Composition and quality of the digestate

The composition of the digestate determines its quality, and this varies from one biogas reactor to another. The nutrients content of the digestate i.e. its composition depends on the composition of the feedstock, TS content of the feedstock, process conditions, and pre-treatment methods.

Feedstock composition: Most of the nutrients in the feedstock end up in the digestate [163]. The nutrients content of the digestate can be controlled by regulating the composition of the feedstock and as such the feedstock should be free of heavy metals (Cadmium, Lead and Mercury) which in higher concentration are toxic to plant growth [164, 165]. The most suitable feedstocks for biogas production with digestate used as fertiliser are animal manure, crops, the organic fraction of MSW, vegetable by-products and residues, and wastes from agriculture, horticulture, and forestry. Furthermore, during co-digestion of different feedstocks, there may be variations in the digestate nutrients compared to those of mono-digestion [166].

TS content of the feedstock: The higher the TS of the feedstock, the higher the TS of the digestate residue. Digestate with higher TS contains larger amounts of carbon and nitrogen that can be broken down further in the soil, which in the long-run results in the release of more nutrients [33, 166].
Process conditions: The retention time for the feedstock inside the reactor, at constant process temperature, influences the digestate quality [167]. If the organic loading rate of the anaerobic digestion process is high and the retention time is short, the digestate might contain a large amount of undigested organic matter, which is not economical [168]. In continuous processes, it is possible that fractions of feedstock (and the impurities contained in them) may escape through the digestate. Appropriate process temperatures and retention times must be selected during the digestion process to ensure a quality digestate. A thermophilic process increases the rate of pathogen reduction in the digestate, but a mesophilic digestion process alone may not be adequate [168]. Pasteurisation (70 °C, 60 min) is required before or after anaerobic digestion, especially when animal by-products are used as substrates [169]. The waste may sometimes be heated at a lower temperature but for a longer time prior to mesophilic or thermophilic digestion aiming to achieve similar effects [170].

Pre-treatment methods: Recalcitrant feedstocks are often pretreated to enhance digestibility and thereby improve AD performance. However, the pretreatment method should be mild and must not generate inhibitors or retain chemicals that can have a negative effect on the quality of the digestate.

6.2 Benefits of digestate

Improved quality of soil: Digestate has some active microorganisms which increase the biological activity of the soil and enhance the formation of microscopic biofilms within the soil. The microorganisms in soil are mostly heterotrophic (use organic carbon as a source of carbon and energy), and the use of digestate stimulates the growth of these microorganisms in soil, thereby facilitating nutrient mineralisation for plant uptake and protection of plants against disease [33, 171]. Digestate increases the buffering capacity of soil and enhances retention of water and air in the soil profile [33].

During anaerobic digestion of organic wastes, organic nitrogen is converted to ammonium nitrogen, so the digestate residue contains a high proportion of mineralised nitrogen, especially in the form of ammonium, which is available for plants. It also contains other macro- and micro-elements essential for plant growth [172, 173]. In addition, the digestate contains a certain amount of carbon and nitrogen, which is broken down further in soil resulting in the release of more nutrients in the long run. In this way, digestate improves crop yield.
During anaerobic digestion of organic wastes, the volatile solid content and viscosity of the waste is reduced, which makes the digestate easier to spread on agricultural land than untreated manure. The use of digestate as bio-fertiliser decreases the cost of crop production, and the income of farmers can be increased through the selling of their bio-fertiliser when they have a surplus of nutrients on their farm.

6.3 Dewatering process

The digestate is usually separated into solid and liquid fractions to reduce the volume, making the solid fraction easier to handle and reducing the cost of transportation. The liquid fraction usually contains higher amounts of ammonium and potassium [174], but phosphorus is retained in the solid fraction. The liquid fraction of the digestate can be used directly as organic fertiliser. The solid fraction can also be used on soil to improve soil structure, retain nutrients, and prevent leaching, or the solid fraction can be post-treated using an aerobic composting process thereby closing the production cycle [175-177]. The digestate is usually separated into different fractions using the following techniques.

Screw press: The screw press separates the digestate into liquid and solid fractions. The press can be set to the desired amount of total solid content needed in the solid fraction [178]. This method can achieve between 30 % to 38 % total solid contents in the solid fraction. There is also a belt press that functions as a screw press but has a higher separation efficiency [179].

Centrifuge: This method uses centrifugal force to separate the digestate. In this research, the digestate was separated into solid and liquid fractions by centrifuging at 5,000g for 10 min prior to analysis (Paper IV).

Evaporation: This method utilises thermal energy and is applied to concentrate the digestate and increase the total solid content. Concentrations ranging up to 20 % total solids can be achieved, but at high temperature ammonia can be released. This can be avoided by decreasing the pH of the digestate prior to evaporation [180].

Chemical separation: This method involves chemical precipitation, adsorption, and removal of major nutrients in the liquid fraction. Concentrated nitrogen, potassium, and phosphorus can be obtained in solid form from the liquid fraction [179]. Another separation technique is membrane filtration and reverse osmosis. This technique is still new, but it can allow total separation of the solid fraction to obtain a clean liquid.
6.4 Digestate from dry digestion of manure with straw in textile bioreactor

In this study, the digestate residue remaining after dry anaerobic digestion of manure bedded with straw was analysed for its suitability as a bio-fertiliser (Paper IV). The digestate residue was separated into liquid and solid fractions, and the available macro and minor nutrients, heavy metals were quantified using MP-AES (Agilent technologies), as shown in Table 6.1.

Table 6.1 Composition of digestate from dry anaerobic digestion of manure bedded with straw (Paper IV)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Digestate residue</th>
<th>Unit</th>
<th>Liquid fraction</th>
<th>Solid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.01 – 8.06</td>
<td>g/L</td>
<td>2.45 – 2.8</td>
<td>6.77 – 9.22 (mg/g)</td>
</tr>
<tr>
<td>Total carbon (%)(\ast)</td>
<td>34.36 – 36.23</td>
<td>g/L</td>
<td>1.25 – 1.55</td>
<td>ND</td>
</tr>
<tr>
<td>TS (%)(\ast)</td>
<td>7.97 – 13.39</td>
<td>g/L</td>
<td>1.19 – 4.29</td>
<td>0.025 – 0.051</td>
</tr>
<tr>
<td>VS (%)(\ast)</td>
<td>61.84 – 65.15</td>
<td>g/L</td>
<td>10.03 – 30.54</td>
<td>14.71 – 36.52</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td></td>
<td>mg/L</td>
<td>15 – 50.50</td>
<td>48.33 – 64.33</td>
</tr>
<tr>
<td>Ammonium nitrogen</td>
<td></td>
<td>mg/L</td>
<td>3.35 – 60</td>
<td>13.4 – 16.73</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td>mg/L</td>
<td>0.13 – 0.19</td>
<td>0.14 – 0.35</td>
</tr>
<tr>
<td>Phosphorus</td>
<td></td>
<td>mg/L</td>
<td>0.11 – 0.19</td>
<td>0.33 – 0.69</td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td>mg/L</td>
<td>0.28 – 0.37</td>
<td>0.01</td>
</tr>
<tr>
<td>Magnesium</td>
<td></td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>0.02 – 0.05</td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td>mg/L</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td></td>
<td>mg/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\ast\)Fresh matter; ND = not determined; \(\ast\) dry basis

The pH of the digestate was slightly alkaline, which should increase the buffering capacity of soil because most agricultural lands are somewhat acidic. The liquid fraction had higher nitrogen and potassium contents compared to the solid fraction, but the phosphorus content was low in both fractions. Studies have shown a loss of some amount of phosphorus during anaerobic digestion processes [173, 181]. Phosphorus can be added as a supplement to avoid...
phosphorus deficiency in the soil; however, nutrient requirements vary from one soil to another, so the digestate can be supplied to soil with major deficiencies of nitrogen and potassium. Some amounts of Ca, Mg, Cu, Fe, Zn, and Ni were found in the digestate, but heavy metals that are toxic to plants, such Cd and Cr had insignificant concentrations, as shown in Table 6.1.
Chapter 7

Concluding remarks and future directions

Dry anaerobic digestion (dry-AD) is an appropriate technology for processing solid organic waste; potentials of textile and plug flow bioreactors for biogas production through dry-AD were assessed in this study. The major solid wastes treated for biogas production in this study were chicken feather, manure bedded with straw, wheat straw, and citrus wastes.

7.1 Conclusions

The major conclusions of this research are presented below.

- Anaerobic digestion of chicken feather wastes with bacteria granules is possible even without any pretreatment. Bacteria granules used as inoculum resulted in almost two times more methane production from the untreated feather than when anaerobic sludge was used.

- Pretreatment improved methane yield by 124% and 237% compared to the untreated when bacteria granules and anaerobic sludge were used on the total broth of pretreated feathers, respectively. Anaerobic sludge as inoculum performed better on the feather hydrolysate, whereas bacteria granules worked better on the total broth of the pretreated feathers.

- The plug flow reactor was efficient for dry-AD of manure bedded with straw at 22%TS with increasing organic loading rates of 2.8, 4.2, and 6 gVS/L/d and corresponding retention times of 60, 40, and 28 days. Organic loading rates of up to 4.2 gVS/L/d with a corresponding retention time of 40 days produced better process stability with methane yields up to 0.163 LCH$_4$/gVS added/d, which is 56% of the theoretical yield.

- The textile bioreactor is robust and simple to operate. The reactor worked successfully for the dry digestion of manure with straw at 22–30% TS (solid content of the feedstock). A long-term acclimatisation period of 232 days resulted in a 58% increase in methane yield.

- The digestate residue after biogas production from manure with straw in the textile bioreactor was determined to be suitable as a bio-fertiliser.
Dry anaerobic digestion (dry-AD) is an appropriate technology for processing solid organic waste; potentials of textile and plug flow bioreactors for biogas production through dry-AD were assessed in this study. The major solid wastes treated for biogas production in this study were chicken feather, manure bedded with straw, wheat straw, and citrus wastes.

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- Anaerobic digestion of chicken feather wastes with bacteria granules is possible even without any pretreatment. Bacteria granules used as inoculum resulted in almost two time more methane production from the untreated feather than when anaerobic sludge was used.

- Pretreatment improved methane yield by 124 % and 237 % compared to the untreated when bacteria granules and anaerobic sludge were used on the total broth of pretreated feathers, respectively. Anaerobic sludge as inoculum performed better on the feather hydrolysate, whereas bacteria granules worked better on the total broth of the pretreated feathers.

- The plug flow reactor was efficient for dry-AD of manure bedded with straw at 22 %TS with increasing organic loading rates of 2.8, 4.2, and 6 gVS/L/d and corresponding retention times of 60, 40, and 28 days. Organic loading rates of up to 4.2 gVS/L/d with a corresponding retention time of 40 days produced better process stability with methane yields up to 0.163 LCH_4/gVS_{added}/d, which is 56 % of the theoretical yield.

- The textile bioreactor is robust and simple to operate. The reactor worked successfully for the dry digestion of manure with straw at 22 – 30 % TS (solid content of the feedstock). A long-term acclimatisation period of 232 days resulted in a 58 % increase in methane yield.

- The digestate residue after biogas production from manure with straw in the textile bioreactor was determined to be suitable as a bio-fertiliser.
Co-digestion of citrus wastes with chicken feather and wheat straw with mixing ratio of 1:1:6 gave the best performance in batch assays.

Best mixture investigated in continuous dry digestion experiments using plug flow reactors. At OLR of 2 gVS/l/d and retention time of 50 days, the methane yield of 362 NmlCH$_4$/gVS$_{added}$ was obtained from $R_{TS21}$, which is 13.5 % higher than the yield obtained from $R_{TS32}$ (319 NmlCH$_4$/gVS$_{added}$). Higher OLR of 3.8 gVS/l/d and retention time of 30 days shows process instability in both reactors.

7.2 Future directions

Dry-AD is gaining interest due to reduced amount of water needed, less pretreatment, more flexibility in acceptance of waste and easy handling of digestate residue. However, there are still some challenges associated with this process for biogas production, such as longer start up and retention time, inhibition problems due to reduced amounts of water, problems of mixing, process instability and suitability of the digestate as bio-fertiliser. The following future research will be necessary to overcome these challenges.

- Dry-AD in this study was carried out under mesophilic condition. It will be necessary to investigate dry-AD under the thermophilic condition to shorten the start-up period as well as the retention time. This will probably also improve the methane yield.
- The methane yield obtained from dry-AD using acclimatised inoculum under this condition was higher than that obtained from the original inoculum (unacclimatised inoculum). It will be interesting to investigate the dominant microbial communities in dry-AD and compared with wet-AD.
- Considering the problem of mixing in the dry-AD process, it is necessary to investigate the significance of liquid, digestate, and biogas recirculation for optimising the performance of dry-AD.
- Acceptability of the digestate residue after biogas production is important for the agricultural sector. Therefore, investigating the influence of single substrates, co-substrates, process design, and pre-treatment methods on composition and quality of the digestate is essential.
- Further investigations on the synergistic and antagonistic effects of co-digestion of different solid wastes with an appropriate mixing ratio, considering the C/N ratio, inhibitors, feedstock biodegradability, and total solid content will be of great value.
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Regina Jijoho Patinvoh
December 2017
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Biological Pretreatment of Chicken Feather and Biogas Production from Total Broth

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Abstract  Chicken feathers are available in large quantities around the world causing environmental challenges. The feathers are composed of keratin that is a recalcitrant protein and is hard to degrade. In this work, chicken feathers were aerobically pretreated for 2–8 days at total solid concentrations of 5, 10, and 20 % by Bacillus sp. C4, a bacterium that produces both α- and β-keratinases. Then, the liquid fraction (feather hydrolysate) as well as the total broth (liquid and solid fraction of pretreated feathers) was used as substrates for biogas production using anaerobic sludge or bacteria granules as inoculum. The biological pretreatment of feather waste was productive; about 75 % of feather was converted to soluble crude protein after 8 days of degradation at initial feather concentration of 5 %. Bacteria granules performed better during anaerobic digestion of untreated feathers, resulting in approximately two times more methane yield (i.e., 199 mlCH4/gVS compared to 105 mlCH4/gVS when sludge was used). Pretreatment improved methane yield by 292 and 105 % when sludge and granules were used on the hydrolysate. Bacteria granules worked effectively on the total broth, yielded 445 mlCH4/gVS methane, which is 124 % more than that obtained with the same type of inoculum from untreated feather.

Keywords  Chicken feather · Pretreatment · Bacillus substilis strain · Keratinase · Biogas production · Mesophilic · Hydrolysate · Total broth · Bacteria granules

Introduction

The world’s average stock of chicken is about 22 billion live chickens [1], and about 5–7 % of the total weight of a normal chicken are feathers [2]. The poultry industries produce about six billion tons of chicken feathers annually. Landfilling is a major practice of disposing feather wastes in the...
world today, resulting in landfill gas including methane and nitrous oxides. The second largest treatment of feather waste is incineration [3]; however, a minor portion of the feathers are converted to feather meals used in animal feed preparation and in organic fertilizer through hydrothermal or chemical pretreatment methods. Nevertheless, this product has a low nutritional value due to the denaturing of the amino acid content [4, 5] and, moreover, the process is capital intensive.

The organic fraction of solid organic wastes and materials (agricultural wastes, sludge, animal byproducts, energy crops, and other substrates) is used for biogas production, but the use of keratin wastes such as chicken feathers, wools, hair, or nails is not yet fully explored. These wastes, if properly pretreated, can serve as a valuable feedstock for biogas production. It is important to alter the recalcitrant structure of the keratin to enhance bio-digestibility of the wastes for biogas production resulting in improved biogas yields [6]. The high degree of crosslinking of the polypeptide chain caused by extensive formation of disulfide bonds is a major challenge in processing these wastes. This strong covalent bond makes them insoluble in polar solvents [7] and resistant to proteases [8, 9].

Current processing of these wastes is based on strong acid or alkaline hydrolysis and other physicochemical methods such as hydrothermal and superheated water treatments. These treatment methods result in severe degradation and destruction of feather keratins [10]; however, the energy and/or chemicals demand related to these methods leads to economical or sustainability challenges. Enzymatic hydrolysis using keratinases is also an option, but the high cost of enzymes makes this method limited for industrial applications. Hence, the recent research focuses on biological pretreatment methods, which are sustainable, ecological, and cost effective for extracting soluble keratins. Several microorganisms (bacteria and fungi), such as Bacillus [4, 11, 12] and Aspergillus sp. [13–15] were reported to degrade keratins (chicken feathers). Hence, introducing these kinds of pretreatment can be promising and therefore there is a need to explore these effectively for their use prior to biogas production. In a previous study [16], a recombinant Bacillus megaterium strain was used for pretreatment of chicken feathers. An initial feather concentration of 4 % was pretreated prior to biogas production and then only the hydrolysate of the pretreated feather was further investigated as a biogas substrate.

In this study, the goal was to use a wild strain of bacteria for the pretreatment, and then the whole broth of the pretreated feathers including the bacteria was investigated as a substrate to produce biogas, a concept that is closer to be applied in future industrial applications. Chicken feathers at total solid concentrations of 5, 10, and 20 % (dry weight of feathers) were biologically pretreated with a naturally occurring Bacillus strain and thereafter the total broth of pretreated feathers, as well as only the feather hydrolysate for a comparison, was used for biogas production. This protein-degrading bacteria was isolated from compost and identified as Bacillus sp. C4 (2008) [4]. Moreover, this paper assesses investigations at higher initial total solid concentrations in order to minimize reactor volume and utilize larger amount of wastes. Additionally, the effects of incubation time, as well as the type of the inoculum on the biogas yield were also evaluated.

**Experimental**

**Biological Treatment of Chicken Feather**

White chicken feathers were collected from a slaughterhouse (Håkantorp Slaughterhouse AB, Genomfattarsvägen, Sweden). The feathers were washed in dilute soap solution immediately after
collection and rinsed with tap water and then distilled water. The washed feathers were air-dried for 3 days and then chopped into particle sizes between 1 and 10 mm, thereafter stored at room temperature before use.

The keratin-degrading bacteria used in this study was a Bacillus sp., isolated from compost and identified as Bacillus sp. C₄ (2008) [4], stored at −80 °C in 50 % glycerol. The bacteria were incubated at 37 °C for 18 h on agar plates containing 1.5 % bacteriological agar in Luria-Bertani broth. It was then inoculated in 100 ml of Luria-Bertani broth (tryptone 1 % (w/v), yeast extract 0.5 % (w/v), and NaCl 0.5 % (w/v)) and incubated in a water bath at 37 °C and shaking with 160 rpm overnight (18 h).

The feathers with different total solid concentrations of 5, 10, or 20 % (dry weight of feather) were mixed with 90, 84, or 73 ml, respectively, of a modified basal medium (NH₄Cl 0.5 g/l, NaCl 0.5 g/l, K₂HPO₄ 0.5 g/l, KH₂PO₄ 0.4 g/l, MgCl₂.6H₂O 0.1 g/l, yeast extract 1.5 g/l, peptone 1 g/l), and the pH was adjusted to 7.0 using K₂HPO₄ or KH₂PO₄ (1 M) in 500-ml Erlenmeyer flasks, sterilized and inoculated with 5 ml of the overnight culture, i.e., 5 % (v/v) of Bacillus sp. C₄ (2008), making a total volume of 100 ml. The flasks were then placed in a water bath, and all of the cultivations were running under aerobic conditions at 37 °C, and 160 rpm for 2, 4, 6, or 8 days. All experimental setups were carried out in duplicates.

The remaining feathers in each culture was removed by vacuum filtration using 10-μm paper filters, and the percentage of feather degradation was determined according to the method presented by Park and Son [17]. After removing the un-degraded feathers, 15 ml of the liquid fraction (called hydrolysate) was used to measure the total crude protein and keratinase activity. The hydrolysate was then stored at −20 °C prior to use for biogas production.

Another setup of the experiments was carried out with identical conditions as the abovementioned one, except that the un-degraded feathers were not separated after the biological pretreatment, so that the total broth of pretreated feathers was stored at −20 °C and then used for biogas production.

**Soluble Keratin Preparation and Keratinase Activity Assay**

Soluble keratin was prepared from chicken feathers according to Wawrzkiewicz et al. [18] with slight modifications and used as substrate for the keratinase activity measurements. The chicken feathers (2 g) were treated at 100 °C for 2 h with 100 ml of dimethyl sulfoxide (DMSO) using a reflux condenser. Then, 300 ml of acetone cooled at −20 °C for 2 h was added to 100 ml of cooled keratin solution in DMSO and left in a refrigerator for 1 h for better precipitation. The solution was then centrifuged at 4000g for 10 min. The precipitate (cake) was washed with distilled water, and then the acetone was allowed to evaporate from the uncapped tube at room temperature for 30 min and finally the cake was suspended in 50 ml distilled water.

This prepared soluble keratin was used as substrate for the enzyme activity determination, and the assay was performed according to the method of Wawrzkiewicz et al. [18], with slight modifications. The prepared soluble keratin suspension (0.5 ml) was mixed with 3.5 ml of 0.1 M phosphate buffer pH 7.5 and 1 ml of sample solution containing the enzyme. Thereafter, the reaction mixture was incubated for 5 h at 45 °C, followed by stopping the enzymatic reaction using 3 ml of 10 % (w/v) trichloroacetic acid (TCA) and left for 30 min at 4 °C. In the control test, the reaction was stopped first with 3 ml of 10 % w/v TCA before adding 0.5 ml of prepared soluble keratin suspension. The test and control suspensions were centrifuged at
4000g for 10 min at 4 °C, and the absorbance of supernatant was measured at 280 nm using a spectrophotometer. One unit (U) keratinase activity was defined as the amount of enzyme causing 0.01 absorbance increase between the sample and the control at 280 nm under the conditions above [17].

**Anaerobic Batch Digestion Assays**

Anaerobic batch digestion assays were carried out using either feather hydrolysate or total broth of biologically pretreated chicken feathers as substrates, performed in accordance to the method described by Angelidaki et al. [19]. The assays were carried out under mesophilic conditions (37 ± 1 °C) using 118-ml serum glass bottles as reactors with active volume of 56 ml and headspace of 62 ml. The inoculum was either granulated bacteria obtained from a UASB reactor treating municipal wastewater (Hammarby Sjöstad, Stockholm, Sweden) or digested sludge from a digester treating activated sludge and operating at mesophilic conditions at a municipal wastewater treatment plant (Getteröverket, Varberg, Sweden). The inoculants were filtered through a 2-mm porosity sieve to remove large and undigested particles, and then acclimated for 5 days in an incubator at 37 °C prior to use. Substrates (feather hydrolysate or total broth) with loading of 0.25 gVS (amount of solubilized volatile solids for feather hydrolysate and volatile solids of both solubilized and undegraded feathers for total broth) was used, and then inoculum corresponding to 0.5 gVS was added, keeping a VS ratio (VS\text{substrate} to VS\text{inoculum}) at 1:2 in all setups. The pH in each reactor was adjusted to 7.0 using hydrochloric acid solution (2 M), and the nutrient composition was kept according to Angelidaki et al. [19]. Inoculum and water instead of substrate were used as blank to disclose any methane production by the inoculum itself. The reactors were then sealed with rubber septa and aluminum caps, and the headspace was flushed with a gas mixture of 80 % N\textsubscript{2} and 20 % CO\textsubscript{2} for 2 min to create anaerobic environment in each setup [19]. The reactors were then placed in an incubator at 37 ± 1 °C, and they were shaken manually once a day during the incubation period of 55 days. All experimental setups were performed in duplicates. Gas samples were taken twice a week at the beginning and once a week towards the end of the digestion period from the headspace of each reactor using a pressure-tight syringe (VICI, precious sampling Inc., USA) and then they were analyzed using a gas chromatograph (GC). Gas measurement and analysis were carried out as described previously [20]. All methane volumes are presented at standard conditions (0 °C and 1 atm).

**Analytical Methods**

Moisture content, pH, total nitrogen, total solids, and volatile solids were determined according to biomass analytical procedures [21]. Total nitrogen contents were measured using the Kjeldahl method [22], and then the total crude protein content was calculated by multiplying the Kjeldahl nitrogen content by 6.25. The total organic carbon was obtained by correcting the total dry weight carbon value for the ash content [23]. The fat content was determined using the Soxhlet extraction procedure [24].

The methane produced was determined by a GC (Perkin-Elmer, USA) equipped with a packed column (6’ × 1.8” OD, 80/100, Mesh, Perkin Elmer, USA) and a thermal conductivity detector (Perkin-Elmer, USA), with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. A 250-μl pressure-lock gas syringe (VICI, precious sampling Inc., USA) was used for taking
samples for the gas analysis, and the accumulated methane production was calculated accordingly [20].

**Statistical Analysis**

The experiments were randomized during biogas production analysis using statistical software, MINITAB® (version 17.1.0). Randomization was used in order to ensure that equal treatment was given to all reactors during gas sampling and analysis.

Factor effects, pretreatment impacts, confidence intervals, and standard deviations were analyzed for the anaerobic digestion experiments. The set of experimental runs was analyzed using general linear model analysis of variance (ANOVA) with accumulated methane yield as response variable, with three different factors (pretreatment time, initial percentage of feathers, and type of inoculum used with either hydrolysate or total broth of pretreated feathers) and respectively four, three, and four factor levels. The interaction effects between pretreatment time and type of inoculum used, as well as initial percentage of feathers were also evaluated. ANOVA was used to analyze data, which generate confidence intervals and the significance difference between the different factors considered in the anaerobic digestion experiments. The factors were considered significant when the probability (p value) was less than or equal to 0.05.

**Results and Discussion**

**Untreated Chicken Feathers and Inoculants Characterization**

The feathers used in this study contained 92.05 % dry matter of which 97.59 % was organic matter (Table 1). Crude protein constituted 92.65 % of the feathers, and due to high nitrogen content in the feathers, the C/N ratio was low (3.66). The fat content was also very low (0.87 % TS). The characteristics of the two different inoculants, i.e., anaerobic sludge and granular sludge, used for the anaerobic batch digestion assays are as shown in Table 1. The anaerobic sludge used in this work contains 62.18 % of total solid as organic matter, which is slightly

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Chicken feather</th>
<th>Anaerobic sludge</th>
<th>Bacteria granules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (%)</td>
<td>92.05 ± 0.17</td>
<td>3.53 ± 0.08</td>
<td>10.20 ± 0.40</td>
</tr>
<tr>
<td>Volatile solids (%TS)</td>
<td>97.59 ± 0.95</td>
<td>62.18 ± 0.55</td>
<td>68.00 ± 0.19</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>7.95 ± 0.17</td>
<td>96.47 ± 0.08</td>
<td>89.80 ± 0.40</td>
</tr>
<tr>
<td>Total organic carbon (%TS)</td>
<td>54.21 ± 0.53</td>
<td>34.54 ± 0.31</td>
<td>37.78 ± 0.11</td>
</tr>
<tr>
<td>Total nitrogen (%TS)</td>
<td>14.82 ± 0.13</td>
<td>4.16 ± 0.15</td>
<td>4.83 ± 0.11</td>
</tr>
<tr>
<td>Crude protein (%TS)</td>
<td>92.65 ± 0.13</td>
<td>26.00 ± 0.15</td>
<td>30.18 ± 0.11</td>
</tr>
<tr>
<td>C/N</td>
<td>3.66 ± 0.04</td>
<td>8.30 ± 0.07</td>
<td>7.82 ± 0.02</td>
</tr>
<tr>
<td>Fat content (% TS)</td>
<td>0.87 ± 0.19</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Bulk density (kg/m³)</td>
<td>ND</td>
<td>965.3 ± 17.38</td>
<td>1012.7 ± 14.18</td>
</tr>
<tr>
<td>pH</td>
<td>ND</td>
<td>8.21 ± 0.01</td>
<td>6.96 ± 0.03</td>
</tr>
</tbody>
</table>

*ND not determined

*Standard deviation based on at least duplicate measurements
lower than that measured in the bacteria granules (68 %). C/N ratio was almost the same in both inoculants used, and the total solids in granules were substantially higher than that in the sludge.

**Bacteria Growth and Keratinase Activity**

The feather broth was sterilized prior to aerobic pretreatment with *Bacillus* sp. in order to deactivate all forms of biological agents. *Bacillus* sp. prefers yeast extract and peptone as nutrients for keratinase enzyme production [4] and therefore the medium supplemented with yeast extract and peptone. These materials will be degraded by the bacteria during the biological pretreatment. However, the excess amount of the yeast extract and peptone can be further assimilated in the subsequent digestion leading to more biogas production.

The *Bacillus* sp. grew well in the medium and produced keratinase resulting in degradation of feathers to soluble crude protein. The pH of the medium increased from 7.0 to 8.9, 8.6 and 8.7 for 5, 10, and 20 % concentrations, respectively, during the 8 days of degradation. This increase in pH was likely due to the production of ammonia and other alkaline compounds during the degradation of feathers as reported by Cai et al. [11]. Bacteria growth reached a maximum of $9.0 \times 10^9$, $14.3 \times 10^9$, and $1.6 \times 10^9$ CFU/ml on day 4 for 5, 10, and 20 % feather concentrations, respectively. This shows an increase in bacteria population as the feather concentration increased from 5 to 10 %. However, the population of the bacteria was reduced at higher feather concentration of 20 %, probably because at this concentration, the contact between the bacteria and its substrate was limited, since at this high initial feather concentration, larger aggregated feather particles were observed reducing the accessibility of feathers. An increase in pH from 7.5 to 8.5 was reported by Suntornsuk and Suntornsuk [25]. They found maximum bacteria growth of $8.3 \times 10^8$ CFU/ml after 3 days of incubation, and these values also increased when concentration of feathers increased using feather concentrations between 1 and 5 %.

The keratinase activity of the enzyme produced by the *Bacillus* sp. is presented in Fig. 1. There was a mass loss of 77, 38, and 24 %, respectively for 5, 10, and 20 % of initial feather concentrations, determined after 8 days of degradation, which is an indication of the ability of the produced keratinase to degrade feathers [26]. The activity of the enzyme produced increased from day 2 to day 6 when 5 % initial concentration of feathers was applied and then decreased slightly after 6 days of incubation. However, at higher initial feather concentrations of 10 and 20 %, a sharp decrease in the enzyme activity was
observed already after 4 days of degradation (Fig. 1). Ghasemi et al. [27] also reported a decrease in keratinase activity after 3 days of incubation, when 1 % of feather was used, and this was linked to feedback inhibition of the enzyme by its product.

Increase in feather concentrations from 5 to 20 % caused a suppression of keratinase activity, which resulted in a decrease in the percentage of feathers degraded (Fig. 2). At 5 % initial concentration, a maximum activity of 31.9 U was observed at day 6, corresponding to 72.3 % of substrate degradation. Furthermore, a maximum activity of 28.4 U was observed at day 4 when 10 % initial concentration of feathers was applied of which 25.0 % was degraded. Finally, a maximum enzyme activity of 26.2 U was determined for 20 % initial feather concentration at day 4 resulting in 24.9 % degradation of the substrate. Similarly, Cai et al. [11] reported reduction in keratinase activity as feather concentration increased from 1 to 20 g/l.

**Degradation of Feather to Soluble Crude Protein**

Feather degradation during the bacterial pretreatment was observed by visualizing the disappearance of feathers into the culture medium and measuring the amount of feathers that disappeared. At different initial concentrations of 5, 10, and 20 %, 77, 38, and 24 %, respectively, (Fig. 2) of the total initial amount of feathers were dissolved after 8 days of degradation. The percentage of feather degraded increased as the duration of the degradation were prolonged; however, there was no significant difference in the amount of degraded feathers after 6- and 8-day-long degradation times, and this was observed at all the different initial feather concentrations. Park and Son [17] reported 100 % feather degradation when the initial feather concentration was 0.1 % after 10 days of treatment with *B. megaterium* F7–1. On the other hand, Deivasigamani and Alagappan [28] reported 85 % degradation for initial concentration of 1 % feathers and after 5 days of treatment with *Bacillus* sp. FK 46, while Bálint et al. [29] obtained 75 % degradation when 4 % TS feather concentration was applied after 138 h (approximately 6 days) of treatment with *Bacillus licheniformis* KKL. Moreover, similar results were reported by Suntornsuk and Suntornsuk [25], where 1 to 5 % of feather concentrations were investigated. These previously reported results are all in accordance with our observations, where the percentage of feathers degraded decreased as the initial feather concentration increased, which is possibly an indication of product inhibition for the enzyme. Moreover, as it was mentioned earlier at the highest initial feather concentration, i.e., at 20 %, the bacterial growth itself was also limited leading to less overall biomass concentration compared to those achieved at lower concentration feathers.

![Fig. 2 Substrate degradation at feather concentrations of 5 %TS (blue diamond suit), 10 %TS (orange square), and 20 % TS (grey up-pointing triangle)](image-url)
On day zero, the amount of soluble crude protein was very low (an average of 4.13 g/l), but this amount increased as days of degradation increased, which is an indication that the feathers most probably converted to soluble crude protein by the keratinase enzyme produced (Fig. 3). Beside soluble proteins, other intermediate degradation products, such as amino acids, ammonia, and sulfate can also be formed during feather degradation [26].

After 8 days of degradation, the amount of soluble crude protein increased from an average of 4.13 to 35.97 g/l, 36.97 and 45.94 g/l at initial feather concentration of 5, 10, and 20 %, respectively (Fig. 3). If we compare these values with the feather degradation shown on Fig. 2, it is clear that the main products formed during feather degradation are soluble proteins. The soluble crude protein increased slightly up to day 8, when total solid concentrations of 5 and 10 % were applied. However, this increased only up to day 4 in the case of 20 % initial concentration of feathers and thereafter it was approximately the same, as shown in Fig. 3. Our results are in line with the results obtained by Deivasigamani and Alagappan [28], where 75 % of the feather keratin was converted to soluble crude protein after 5 days of incubation when 1 % of feather concentration was used.

**Anaerobic Digestion of Hydrolysate of Pretreated Feathers**

Accumulated methane profiles from digesters using hydrolysates as substrate are shown in Fig. 4. Bacteria granules performed better during anaerobic digestion of untreated feathers, resulting in approximately two times more methane yield (i.e., 199 mlCH\textsubscript{4}/gVS compared to 105 mlCH\textsubscript{4}/gVS when anaerobic sludge was used). However, the highest methane yield of around 430 mlCH\textsubscript{4}/gVS was achieved from the hydrolysate of the pretreated feathers independently of which kind of inoculum was applied during the anaerobic batch digestion assays (Fig. 4). Hence, the methane yield was improved by 292 % after 55 days of anaerobic digestion when sludge was used as inoculum and, respectively, the yield was improved by 105 % when granules were used as inoculum, showing that pretreatment with *Bacillus* sp. C4 significantly improved the digestibility in both cases.

At 5 % initial concentration of feathers, increasing the pretreatment time did not give any significant effect on the methane production from the hydrolysate irrespective of the inoculum used. An average of 433 ± 12 mlCH\textsubscript{4}/gVS yield was observed, reaching the maximum yield after 19 days of digestion in the case of using sludge as inoculum (Fig 4a). However, when the granules were used, the maximum average methane production of 426 ± 22 mlCH\textsubscript{4}/gVS was achieved only after 45 days (Fig. 4d).
At higher initial feather concentrations of 10 and 20 %, the accumulated methane yield from hydrolyzates obtained after 2 days of pretreatment was lower and it reached its maximum of $331 \pm 3 \text{ mlCH}_4/\text{gVS}$ after 19 days when sludge was used (Fig. 4b, c) and a maximum yield of $324 \pm 20 \text{ mlCH}_4/\text{gVS}$ after 45 days when granules were used (Figs. 4e, f) as inoculum.

Fig. 4 Effect of inoculum (sludge (a, b, c) and granules (d, e, f)) and initial feather concentrations (5, 10, or 20 %) on accumulated methane yield of hydrolysate of pretreated feathers at different days of degradation compared with the untreated. Day 2 (blue diamond suit), day 4 (red square), day 6 (green up-pointing triangle), day 8 (X), and untreated (x).
However, as pretreatment time increased from day 4 to day 8, the accumulated methane yield was not significantly different in cases of when either sludge or granules were used as inoculum. The reason behind this is probably the fact that at higher concentrations of 10 and 20 %, the percentage of soluble crude protein (Fig 3) in the total feather degraded (Fig. 2) at day 2 was slightly lower compared to longer pretreatment times, but at lower concentration of 5 %, the percentage of soluble crude protein in total feather degraded was almost the same independently on the length of the pretreatment time. These results are in accordance with what was found by Forgács et al. [16] who used 4 % of feather concentration in the pretreatment prior to biogas production.

Regarding the degradation rate achieved during the first 10 days of the digestion period, it was higher when anaerobic sludge was used as inoculum compared to that when granules were used (Fig. 4). The maximum methane yield at all conditions could be reached within 19 days when sludge was used while it was reached only after 45 days in the case of granules (Fig. 4). Though the free cells had a faster rate than the bacteria granules, the same overall yield was obtained in the end of the digestion time (Fig. 4).

**Anaerobic Digestion of Total Broth of Pretreated Feathers**

Accumulated methane profiles from digesters when the total broth of pretreated feathers was used as substrate are shown in Fig. 5. Overall, lower methane yields and slower degradation rates were obtained using anaerobic sludge as inoculum, compared to those determined in cases of granules as inoculum. For example, the accumulated methane yield of total broth of feathers pretreated for 2 days was 49 % higher when bacteria granules were used than that when sludge was used as inoculum. These results show that the granular sludge was probably able to adapt to possible inhibitors such as ammonia and sulfate [26, 30] in the total broth of pretreated feathers better than the sludge. When granules were used, the remaining un-degraded feathers were broken down better; and thereafter the intermediate products could be converted to methane on a higher rate than those obtained in the case of the free cells present in the anaerobic sludge (Fig. 5). The same trend is shown during anaerobic digestion of the untreated feathers. The methane yield from the untreated feather was 89 % higher when granules were used as inoculum compared to that when sludge was applied (Figs. 4 and 5). This suggests that granules perform better both on the untreated feather and on total broth containing a larger amount of un-degraded feathers than the sludge.

Furthermore, in the cases of anaerobic sludge as inoculum, the methane yield was slightly lower after 2-day-long pretreatment times at all initial concentration of feathers, compared to those obtained after the longer treatments (Fig. 5a, b, c). However, there was no significant difference in the methane yields obtained (an average of 445 ± 12 mlCH₄/gVS) as pretreatment time increases in cases when granules were used as inoculum (Fig. 5d, e, f).

The result from the statistical analysis (Table 2) supports our results, showing that pretreatment duration of 2 days has significant effect on the accumulated methane yield but this significant effect could be observed only at higher initial concentrations of 10 and 20 % of feathers (Fig. 6). This also shows that in the case of hydrolysate as substrate, the shortest pretreatment time of 2 days was not enough to obtain higher methane yields at higher initial concentrations irrespective of which kind of inoculum was used. The statistical analysis also shows that using total broth of pretreated feather instead of hydrolysate as substrate, gave a significant effect ($p = 0.000$) on the accumulated methane yields (Table 2). It is also shown on Fig. 6 that the pretreatment time had no significant effect on the accumulated methane yield.
Fig. 5  Effect of inoculum (sludge (a, b, c) and granules (d, e, f)) and initial feather concentrations (5, 10, or 20 %) on accumulated methane yield of total broth of pretreated feathers at different days of degradation compared with the untreated. Day 2 (blue diamond suit), day 4 (red square), day 6 (green up-pointing triangle), day 8 (X), and untreated (x)
Table 2 General linear model ANOVA on anaerobic digestion of hydrolysate and total broth of pretreated feathers with accumulated methane yield as response variable

<table>
<thead>
<tr>
<th>Factors</th>
<th>Coef</th>
<th>SE Coef</th>
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<th>p value</th>
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<tbody>
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<td>Constant</td>
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<td>3.75</td>
<td>107.99</td>
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<td>Pretreatment time</td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
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<td>6.50</td>
<td>-5.10</td>
<td>0.000</td>
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<tr>
<td>4</td>
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<td>Initial % of feather</td>
<td></td>
<td></td>
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<tr>
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<td>6.09</td>
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<tr>
<td>TBS</td>
<td>-49.44</td>
<td>6.50</td>
<td>-7.61</td>
<td>0.000</td>
</tr>
</tbody>
</table>

HG hydrolysate with granules, HS hydrolysate with sludge, TBG total broth with granules, TBS total broth with sludge

$p ≤ 0.05$ (95%CI) denoted significance. $S = 36.7744$, R-sq. = 53.50 %; R-sq.(adj) = 49.23 %

Fig. 6 General linear model ANOVA interaction plot of pretreatment time with type and initial %, accumulated methane yield as response variable. HG (hydrolysate with granules), HS (hydrolysate with sludge), TBG (total broth with granules), and TBS (total broth with sludge)
when total broth was used as substrate together with bacteria granules as inoculum, but the accumulated methane yield was highly affected by the length of the pretreatment time when total broth was digested with anaerobic sludge.

The results of the statistical analysis also support our experimental results determined from total broth of pretreated feather using bacteria granules as inoculum, which show that the accumulated methane yield was basically constant after different pretreatment times and irrespective of the initial feather concentrations used.

Conclusions

1. Increase in feather concentration from 5 to 20 % did not result in significant increase in the amount of feathers degraded. Pretreatment of feather wastes with Bacillus sp. C 4 (2008) was successful, as an average of 75.5 % of the feather keratin was converted to soluble crude protein by the enzyme produced after 8 days of degradation.

2. Chicken feather wastes can effectively be used for biogas production. Anaerobic digestion of these wastes is possible even without any pretreatment; the granules used as inoculum resulted in almost two times more methane production from the untreated feather (an average yield of 199 mlCH 4 /gVS) than when anaerobic sludge was used as inoculum (an average yield of 105 mlCH 4 /gVS). However, the final methane yield observed from samples treated biologically by using naturally occurring bacteria that can produce both α- and β-keratinases reached approximately the same level. Accordingly, the methane yield could be increased by 292 % when sludge was used as inoculum on the hydrolysate of the pretreated feather (initial concentration of 5 %) after 55 days of anaerobic digestion and respectively by 105 % when granules were used as inoculum on the hydrolysate compared to those obtained from untreated feathers. The same trend was observed in the case of anaerobic digestion of total broth of treated feathers, an increase by 237 % was obtained when sludge was used as inoculum and, respectively, an increase by 124 % was achieved when bacteria granules were applied as inoculum.

3. Sludge used as inoculum performed better on the feather hydrolysate, while granules worked better on total broth of the pretreated feathers. A pretreatment duration of 2 days is recommended when 5 % initial feather concentration is applied while 4 days are recommended for higher, i.e., 10 % and 20 %, initial concentrations, when hydrolysate of pretreated feathers is used for methane production. Total broth of pretreated feathers can be used effectively with granules for methane production at shorter pretreatment duration of 2 days irrespective of the initial feather concentrations in between the investigated range of 5–20 %.

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References


Dry fermentation of manure with straw in continuous plug flow reactor: Reactor development and process stability at different loading rates

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Swedish Centre for Resource Recovery, University of Borås, Sweden

**Highlights**
- New plug flow reactor developed for continuous dry digestion processes.
- Reactor worked successfully for 230 days using untreated manure with straw at 22\% TS.
- Methane yield of 56\% of the theoretical value was obtained at OLR of 4.2 gVS/L/d.
- OLR of 6 gVS/L/d caused process instability with 41\% VS removal efficiency.

**Graphical Abstract**

**Abstract**

In this work, a plug flow reactor was developed for continuous dry digestion processes and its efficiency was investigated using untreated manure bedded with straw at 22\% total solids content. This newly developed reactor worked successfully for 230 days at increasing organic loading rates of 2.8, 4.2 and 6 gVS/L/d and retention times of 60, 40 and 28 days, respectively. Organic loading rates up to 4.2 gVS/L/d gave a better process stability, with methane yields up to 0.163 LCH\(_4\)/gVS\(_\text{added}\)/d which is 56\% of the theoretical yield. Further increase of organic loading rate to 6 gVS/L/d caused process instability with lower volatile solid removal efficiency and cellulose degradation.

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**1. Introduction**

Anaerobic digestion of organic wastes for biogas production has been successfully applied but there is a need for improved processes and design of less expensive reactors. In this vein the drive for dry anaerobic digestion processes is increasing both in research and the industry because of technical simplicity in design, together with low construction and operational costs (Karthikeyan & Visvanathan, 2013; Kothari et al., 2014). Dry anaerobic digestion technology is designed to process more organic wastes per reactor volume with total solids (TS) content greater than 20\% (Demirer & Chen, 2008; Fernández et al., 2008) treating food wastes, manure bedded with straw, garden wastes and other high solid waste fractions. In comparison with the wet anaerobic digestion process, this process allows higher organic loading rates (OLR), less pretreatment and gives better economic feasibility (Karthikeyan & Visvanathan, 2013) since the reactor volume is minimized and it is easier to handle the digestate residue.

Animal wastes and crop residues are one of the most abundant waste fractions generated worldwide, the amount of manure

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bunded with straw produced increases daily as the number of housed dairy herd increases. Cattle manure bedded with straw usually have a TS greater than 20%, farmers can use low cost anaerobic digesters to convert these enormous waste streams to biogas, thereby improve water quality, reduce methane and nitrous oxide emissions and improve soil fertility. Dry anaerobic digestion is therefore a better option for processing these wastes. Since biogas production involves a complex biological process, monitoring of the process is essential to avoid process instability and failure of the digester (Drosog, 2013). For enhanced performance of dry anaerobic digestion processes, a suitable reactor is required; considering the reactor composite composition, amount of substrate to be treated, and process economy of the reactor.

Plug flow reactors have been reported to be efficient for dry anaerobic digestion processes. These reactors are inexpensive and easy to build which make them a suitable technology to improve the livelihoods of farmers (Lansing et al., 2010, 2008). Plug flow reactors have also been reported to have the highest success rate in the United States, where 42% out of the 242 anaerobic digesters operating at livestock farms in 2015 were plug flow designs (USEPA, 2016). Nevertheless, some shortcomings, such as lower mass transfer due to lack of mixing, thermal stratification and solid sedimentation problems have been reported (Lansing et al., 2010). These problems can be minimized by the use of impellers in plug flow reactors. The impellers allow minimal mixing for better performance in the reactors. In high solid digestion processes, however, continuous mixing have been reported to indicate unstable performance at high OLR and it was observed that the continuously mixed unstable reactor became stable when the mixing level was reduced (Stroo et al., 2001). Researchers have also investigated the effectiveness of plug flow reactors on manure and other substrates with solid content in the range of 11–14% TS (Adl et al., 2012; Cantrell et al., 2008). There have been studies on dry digestion of different substrates in batch reactors but little information is available on dry digestion in continuous plug flow reactors. The innovation of this paper in meeting this gap was the development of a novel type solid-state plug-flow laboratory reactor to treat substrates at higher TS levels, i.e. greater than 20%.

The efficiency of this reactor was then investigated in a continuous dry anaerobic digestion process treating manure bedded with straw at 22% TS content. The main objective was to identify the critical OLR, above which instability can occur in the reactor. The process was monitored by measuring VFA/Alkalinity ratio, pH, volatile fatty acid (VFA) and total ammonia nitrogen concentrations regularly. This is vital because the cost of starting the entire process over again far outweighs monitoring of the process.

2. Materials and methods

2.1. Reactor design

A horizontal plug flow reactor (Fig. 1) for continuous dry anaerobic digestion process was designed and made-up at the University of Borås, Sweden. This reactor has 9.2 L total volume, 1565 mm length and maximum inside pressure of 10 kPa. It was mounted on a base surface with which a clamp to the two edges of the reactor was associated for suspending the reactor. Mesophilic (37 °C) conditions were maintained by circulating water from a heater, a thermostatic water bath (GD 100, Grant Instruments Ltd., Cambridge, UK), through a water jacket (150 mm outer diameter with 4 mm wall thickness and capacity of 5 L) surrounding the reactor. The reactor was then shielded with a 10 mm thick Styrofoam to avoid heat loss.

The reactor was sub-divided into 3 zones as follows. Inlet zone – this part was made of polyethylene material with sealed buffer system to avoid the release of gas and air entering the system. This part can be considered as an inactive zone in the inlet system. A shutoff valve was connected at the inlet with a piston rod (made gas tight by O-rings). The inlet storage volume is about 0.256 L with 0.0053 L of feedstock per rotation.

Main Zone – the main zone of the reactor was made of a Poly-methylmethacrylate (PMMA) material with 110 mm outer diameter and 5 mm thickness for easy handling and to make the reactor transparent. An impeller, installed on a hexagonal shaft that runs through the reactor connected the inlet to the outlet. The hexagonal shaft had two oil seals that prevented leakage of materials and several O-rings making the different parts gas tight. The impeller allowed mixing of the feedstock at the bottom part of the inlet, and transported the materials very slowly towards the outlet taking several rotations: 100 cm² per rotation but it can also depend on the viscosity of feedstock and working volume of reactor.

Outlet zone – this part was also made of polyethylene material with an outlet pipe for the collection of the digestate residue. The material was transferred from inlet towards outlet at the end of the reactor by rotating of the impeller shaft; as a result the digested residue was discharged through the outlet pipe while new materials were added. The impeller handle was connected to the outlet for manual rotation. In this zone, the gas outlet was also connected where the daily volume of biogas produced was measured by the tipping device in the automated methane potential test system (AMPTS); meanwhile with an inserted thermocouple the temperature of the reactor was controlled.

Safety measures – a construction was made with a polyethylene material at the inlet and outlet with specific rubber connectors for sealing. If pressure inside the reactor would increase beyond 10 kPa due to blockage in the gas outlet or for any reason, this part of the reactor could open up avoiding an explosion. All interior parts were carefully selected in order to avoid any chemical reaction with the experimental materials as well as any corrosion during the test.

Fig. 1 shows a schematic diagram of the reactor and the experimental set up together with other accessories, such as heater and water bath for maintaining the temperature, sampling point for biogas composition analysis and the AMPTS system for measuring the biogas produced.

2.2. Substrates and inoculum

The substrate, cattle manure bedded with straw, was collected from a cattle farm outside Borås (Sweden) and used as feedstock for the continuous anaerobic digestion process. During the experimental period two different batches, with similar content of total solids (TS) and volatile solids (VS) were obtained from the same farm. The manure was shredded manually to reduce the particle size of straw; then it was characterized, weighted and stored in plastic containers at ~20 °C to prevent biodegradation until further use. During experiment, weighted frozen substrate was defrosted at room temperature and thoroughly mixed to gain a homogenized feed before use. Sludge used as inoculum was obtained from a digester treating waste water sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). The inoculum was filtered through a 2 mm porosity sieve to remove sand, plastic and other unwanted particles after which it was acclimated for five days in an incubator at 37 °C prior to use. The inoculum was centrifuged at 10,000g for 10 min to obtain a TS content of 7.8 ± 0.24%. Table 1 shows the most important characteristics of the substrate and the inoculum used during the investigations.

2.3. Experimental procedure

The substrate was inoculated with inoculum to start-up the reactor, keeping a volatile solids (VS) ratio (VSsubstrate to VSinoculum)
at 1:2 in the reactor. The pH of the mixture was adjusted to 7.47 by 2 M HCl solution. Furthermore, a nutrient solution with composition according to Angelidaki et al. (2009) was also added into the reactor. The experiment was started at batch mode until no further gas production was monitored (40 days of digestion period). After that the continuous feeding operation was started with an OLR of 2.8 gVS/L/d. The experiments continued with gradually increasing OLRs, thereby decreasing retention times with the aim of maintaining a fixed working volume. The OLR values investigated were 2.8 gVS/L/d (OLR 1), 4.2 gVS/L/d (OLR 2) and 6 gVS/L/d (OLR 3) with corresponding retention times of 60, 40 and 28 days. For the experimental period (start-up and OLR1) the first batch of manure was utilized, while another batch of the feedstock was used during the experimental period of OLR 2 and OLR 3. Each OLR condition was kept until a period of at least one corresponding retention time. The reactor was fed regularly in every second day and the digestate residue was withdrawn before feeding, and kept for analysis, while biogas composition was monitored daily. Process parameters, such as pH, VFA/Alkalinity ratio, VFA and total ammonia nitrogen concentration, TS, VS, as well as lignin, cellulose and hemicellulose contents in the digestate residue were also measured to monitor the digestion process. The reactor was mixed manually (once daily) with the impeller by moving its content to the inlet and back to the outlet to minimize stratification in the reactor. Overall, the experiment lasted for 230 days.

2.4. Theoretical BMP of experimental feedstock

The feedstock being solid was prepared according to Zupančič and Roš (2012) by blending 1 g of the sample with water (dilution factor of 50) to reduce the particle size and allow homogenization (Raposo et al., 2012). Thereafter, theoretical methane potential of the substrate used during digestion process was calculated from the amount and chemical oxygen demand (COD) concentration of the actual feeding using Eq. (1) (Nielfa et al., 2015) assuming that the equation is valid for any substances or products (Tarrin & Buswell, 1934).

\[
\text{BMP}_{\text{theoretical}} = \frac{n_{\text{CH}_4}RT}{\text{pVS}_{\text{added}}}
\]

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Manure with Straw</th>
<th>Anaerobic sludge</th>
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<tr>
<td>Total solids (%)</td>
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<td>BMP_{theoretical} (LCH₄/gVS_{added})</td>
<td>0.290 ± 0.01</td>
<td>ND</td>
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</tbody>
</table>

ND = Not determined; COD = chemical oxygen demand; C/N = carbon nitrogen ratio.

a Dry basis.
where:

\[
\text{BMP}_\text{thCOD} = \text{theoretical yield under laboratory condition}
\]

\[
R = \text{gas constant (R = 0.082 atm L/mol K)}
\]

\[
T = \text{working temperature (310 K)}
\]

\[
P = \text{atmospheric pressure (1 atm)}
\]

\[
\text{VS}_\text{added} = \text{volatile solids of the substrate added (g)}
\]

\[
\text{nCH}_4 = \text{methane produced (mol) determined according to Eq. (2)}
\]

\[
\text{nCH}_4 = \frac{\text{COD}}{64\ (\text{gC/mmol})}
\]

2.5. Analytical procedure

Moisture content, pH, total nitrogen, TS and VS were determined according to biomass analytical procedures (APHA-AWWA-WEF, 2005). Total nitrogen contents were measured using the Kjeldahl method, and the protein content was estimated by multiplying the Kjeldahl nitrogen content with a factor of 6.25 according to Gunaseelan (2009). The total carbon was obtained by correcting the total dry weight carbon value for the ash content (Haug, 1993; Zhou et al., 2015). The fat content was determined using the Soxhlet extraction procedure (Carpenter, 2010). Alkalinity measured as the total inorganic carbonate and was determined by the Nordmann titration method according to Lossie and Pütz (2008). Samples were centrifuged at 4000 g for 15 min and then 5 ml of the supernatant was titrated with 0.1 N sulfuric acid to pH 5, the titration was then continued until pH 4.4 was reached in order to determine the VFA concentration measured as acetic acid equivalent. Digestate samples for total ammonia nitrogen concentration were centrifuged (15 min at 4000g); supernatant diluted 50 times in deionized water to a final volume of 5 ml. Samples were then analyzed using Ammonium 100 test kit (Norcolour, MACHEREY-NAGEL GmbH & Co. KG, Germany) and concentration measured using Nanocolor 5000 Photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany). The free ammonia concentration was calculated from the total ammonia nitrogen concentration and pH values of samples according to Kayhanian (1999). Total cellulose, hemicellulose and lignin content of the solid fraction of the digestates were determined according to NREL protocols (Sluiter et al., 2011). The digestate was centrifuged at 4000g for 15 min and the solid fraction was washed with 100 ml distilled water and then air dried until moisture content was less than 10%. The samples were then hydrolyzed using 72% H2SO4 in a water bath at 30 °C for 60 min, samples were stirred every 5 min to ensure uniform hydrolysis, and then a second hydrolysis was performed using 4% H2SO4 in an autoclave at 121 °C for 60 min. Monomeric sugars contained in the hydrolysis liquid were determined by HPLC (Waters 2695, Waters corporation, Milford, MA, USA). Mannose, glucose, galactose, xylose and arabinose were analyzed using Aminex HPX-87P column (Bio-Rad) at 85 °C and 0.6 ml/min ultrapure water as eluent. Acid soluble lignin (ASL) was determined using a UV spectrophotometer (Libra S60, Biochrom, England) at 320 nm. Acid insoluble lignin (AIL) was gravimetrically determined as residual solid after hydrolysis corrected with ash content. The ash content was determined as the remaining residue after keeping the samples in the muffle furnace at 575 °C for 24 h. The percentage of cellulose degraded was calculated according to Zhou et al. (2015).

The daily volume of biogas produced was measured by the tipping device in the Automatic Methane Potential Testing System (AMPTS, Bioprocess control AB, Lund, Sweden), which is based on the principle of water displacement buoyancy. The composition (methane and carbon dioxide) of the produced gas was determined using a GC (Perkin-Elmer, USA) equipped with a packed column (6' × 1.8” OD, 80/100, Mesh, Perkin Elmer, USA), and a thermal conductivity detector (Perkin-Elmer, USA), with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. A 250-μl pressure-lock gas syringe (VICI, precious sampling Inc., USA) was used for taking samples for the gas composition analysis.

VFAs in the digestate filtrates were measured using a high-performance liquid chromatograph (HPLC, Waters 2695, Waters Corporation, Milford, MA, USA) equipped with an RI detector (Waters 2414, Waters Corporation Milford, MA, USA) and a biohydrogen-ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) operating at 60 °C. A UV absorbance detector (Walters 2487), operating at 210 nm wavelength was used in series with a refractive index (RI) detector (Walters 2414) operating at 60 °C.

3. Results and discussion

3.1. Reactor development

During preliminary studies a syphon mechanism was applied initially at the inlet of the reactor to make it gas tight and avoid entering of air during feeding. This mechanism worked well at lower TS rates up to 11%. However, it was very troublesome when working with higher TS concentrations; i.e. when feedstock with 13% TS was applied, this completely blocked the syphon system for the feed. After that, the inlet part of the reactor was modified to a sealed buffer system described in Section 2.1 and shown in Fig. 1. The shutoff valve connected at the inlet was always closed at the start of feeding to avoid gas leakage. When materials were fed in, the piston rod was inserted and tightened, to avoid the release of any gas. After this, the valve was opened, and the material was pressed down into the reactor via the piston rod and then the valve was closed again. The impeller in this reactor allows minimal mixing by moving the reactor content manually to the inlet and back to the outlet for better performance of the process. This new modification worked well even with an inlet feed of 22% TS without any blockage at the inlet. The developed plug flow reactor worked successfully treating cattle manure bedded with straw at 22% TS for 230 days using organic loading rates of 2.8, 4.2, and 6 gVS/L/d at retention times of 60, 40 and 28 days respectively.

3.2. Substrate characterization

The most important characteristics of the substrate as collected from the cattle farm are shown in Table 1. The substrate contains 22.29% TS of which 70.44% are organic matter. The carbon nitrogen (C/N) ratio was 16.8:1 which is within the required range for stable anaerobic digestion process. Previously, optimal C/N ratios between 20:1 and 35:1 have been stated (Habiba et al., 2009; Kayhanian, 1999), also, other researchers, i.e. Friehe et al. (2010), Pagès Díaz et al. (2011) reported a wider range of between 10:1 and 30:1 as optimal C/N ratios. However, the availability of carbon and nitrogen for the microorganisms is more significant than the actual C/N ratio calculated on the basis of elementary composition of the feedstock. Hence the C/N ratio of straw might be overestimated due to the limited availability of carbon fraction in the lignocellulosic structure of straw (Herrmann et al., 2016). The theoretical methane potential of the feedstock used in this experiment was calculated to be 0.290 LCH4/gVSadded.

3.3. Biogas production

The startup batch digestion period ran for 40 days, the daily gas production was high at the beginning; hence the digestion process started up properly and the feedstock were degraded until the end
of this batch digestion period. The methane content in the produced gas reached 63% ± 3.86 at the end of the startup (batch) period as shown in Fig. 2b. This start-up period was allowed until gas production nearly stopped (40 days) after which the continuous digestion mode with feeding and withdrawing in every second day was started.

Fig. 2a illustrates the daily biogas production at different OLR. Experiments were carried out using progressive organic loading rates; the loading rate of 2.8 gVS/L/d (OLR 1) produced only about 1 L/d in the initial days. Then it gradually increased to around 3 L/d after 36 days and remained at the same level until the end of the OLR1 (2.8 gVS/L/d) period. Similar pattern was observed for the period when OLR 2, (4.2 gVS/L/d) was applied, it started from 3.5 L/d daily methane production initially which increased gradually to around 5 L/d after 18 days, and thereafter it remained stable. The process performed differently when OLR 3, i.e. 6 gVS/L/d was introduced. There were larger fluctuations observed in the daily gas production and only a slightly stable period could be achieved after 20 days maintaining a daily biogas production of around 7 L/d. The reactor was then fed keeping the OLR 3 (6 gVS/L/d) for an additional corresponding retention time of 28 days to check the stability of the process under this condition. However, the daily gas production could not be kept stable with decreasing values towards the end of this period. In parallel, the VFA concentration continued to increase (Fig. 4a) leading to increased VFA/alkalinity ratios with a maximum of around 0.9 at the end of the period (Fig. 3a) showing instability in the process. To examine if VFA could be reduced and restore stability in the process, the

![Fig. 2. Biogas production (a) and biogas composition (b) at different organic loading rate (OLR) during experiments. The symbols represent daily biogas production (△), cumulative biogas production (○), methane composition (●) and carbon dioxide composition (■).](image)
OLR was then reduced to 4.2 gVS/L/d (OLR 2) again. As a consequence of the reduced load the biogas production reduced gradually and remained stable after 16 days maintaining a daily biogas production around 4.2 L/d which is slightly lower than that obtained when the same loading rate (OLR 2) was used previously.

The average methane content in the biogas was 64.9%, 65.1% and 63.3% for OLR of 2.8, 4.2 and 6 gVS/L/d respectively. Overall, OLR up to 4.2 gVS/L/d gave a better process stability with methane yield of 0.163 LCH4/gVS added accounting around 56% of the theoretical methane yield of 0.290 LCH4/gVS added as shown in Table 2. The methane yield at OLR 2.8 gVS/L/d was slightly lower than the yield at OLR 4.2 gVS/L/d probably due to slight differences in the feedstock composition, since these two experimental periods were performed with two different batches of the substrate. This lower methane yield could be associated with the presence of untreated straw in the cattle manure. Hydrolysis of the cellulose in untreated straw has been reported to be the rate limiting step (Noike et al., 1985) especially when it is mixed with cattle manure (Myint & Nirmalakhandan, 2006). However, our result is similar to the methane yield of 0.170 LCH4/gVS added obtained by Kusch et al. (2008) when the digestion of horse dung with straw was investigated in batch-operated solid phase digestion.

### 3.4. Process performance

![Fig. 3. pH and VFA/Alkalinity ratio variation (a) and composition of cellulose, hemicellulose and lignin in digestate (b) at different organic loading rate (OLR) during experiments. The symbols represent pH (○), VFA/Alkalinity ratio (△), total lignin (●), cellulose (■) and hemicellulose (▲). Presented values are mean values of duplicate measurements with error bars as standard deviation between the two values.](image-url)

Fig. 3a shows the variation of pH and VFA/Alkalinity ratio at different OLR while Fig. 4a illustrates the variation of total and individual VFA for all the experiments carried out. At 2.8 gVS/L/d (OLR 1), the pH was increased slightly to around 8 initially but dropped to around 7.5 after 4 days of digestion and it remains...
steady afterwards which is within the favourable pH range of 7–8 for anaerobic digestion (Drosg, 2013). The VFA/Alkalinity ratio was lower (Fig. 3a) than the reported failure limit value of 0.3 which shows that the process was stable during these conditions. Anaerobic digestion process has been reported to be working favourably without acidification possibility when this ratio is less than 0.3 (Drosg, 2013). When the OLR was increased to 4.2 gVS/L/d, the pH remained still within the favourable range around 7.6 throughout this period and the VFA/Alkalinity ratio was below 0.3. So, an appropriate buffering capacity and high stability of the process was observed for OLR of 2.8 and 4.2 gVS/L/d and at corresponding retention times of 60 and 40 days.

No VFA accumulation was observed during the process applying 2.8 gVS/L/d (OLR 1) and 4.2 gVS/L/d (OLR 2) as shown in Fig. 4a. This signifies the complete degradation of the intermediary produced volatile fatty acids. Fast consumption of VFAs was also reported by Liu et al. (2007) as a proof of stable and properly functioning process without danger of failure. However, when the OLR was increased to 6 gVS/L/d with corresponding decreased retention time of 28 days the stability of the system deteriorated. At this 6 gVS/L/d loading (OLR 3), the process started to show signs for instability already during the first retention time of 28 days.

![Fig. 4. Total and individual volatile fatty acids (VFA) variation (a) and Total ammonia nitrogen with free ammonia concentration (b) at different organic loading rate (OLR) during experiments. The symbols represent Total VFA (○), Acetic (C), Butyric (△), isobutyric (•), isovaleric (●), propionic (□), valeric (I), Total ammonia nitrogen (●) and free ammonia (■).](image)

### Table 2
Experimental Results at different loading rate (values are averages with standard deviation).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OLR 1</th>
<th>OLR 2</th>
<th>OLR 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading rate (gVS/d)</td>
<td>13.80</td>
<td>20.70</td>
<td>29.90</td>
</tr>
<tr>
<td>Organic Loading rate (OLR) (gVS/L/d)</td>
<td>2.80</td>
<td>4.20</td>
<td>6.00</td>
</tr>
<tr>
<td>Retention time</td>
<td>60</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Biogas produced (L/gVSadded)</td>
<td>0.23 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Average methane content (%)</td>
<td>64.90 ± 1.75</td>
<td>65.10 ± 2.81</td>
<td>63.30 ± 2.06</td>
</tr>
<tr>
<td>Methane yield (LOU/gVSadded)</td>
<td>0.15 ± 0.01</td>
<td>0.163 ± 0.01</td>
<td>0.146 ± 0.01</td>
</tr>
<tr>
<td>Initial VS concentration (%)</td>
<td>17.17</td>
<td>17.17</td>
<td>17.17</td>
</tr>
<tr>
<td>Final VS concentration (%)</td>
<td>4.31 ± 0.64</td>
<td>7.31 ± 1.19</td>
<td>10.10 ± 0.82</td>
</tr>
<tr>
<td>VS removal efficiency (%)</td>
<td>74.87 ± 3.75</td>
<td>57.42 ± 6.96</td>
<td>41.17 ± 4.80</td>
</tr>
</tbody>
</table>
though the pH was still within the favourable range, i.e. an average of 7.7. However, the VFA/Alkalinity ratio increased to 0.4 and the acetic acid concentration increased to 2100 mg/L which signifies a slightly unstable process (Drosg, 2013). There was fluctuations in the daily biogas production (Fig. 2a) as well as at this loading rate (OLR3). Continuing the loading at the same conditions, i.e. 6 gVS/L/d, into the second retention time period caused considerably disturbances in the system. As shown in Fig. 3a, the VFA/Alkalinity ratio increased up to 0.9 and the total VFA content also increased gradually up to around 7000 mg/L (Fig. 4a) which is a typical sign for overloading. The acetic acids increased up to around 3800 mg/L and the propionic acid concentration increased slightly to around 1000 mg/L during the process. Propionic acid accumulation within the range of 250–1000 mg/L has been reported as an indication of instability in the process (Drosg, 2013) and concentrations greater than 1000 mg/L has been reported as a first sign for overloading in the system (Björnsson et al., 1997). Running the process at a higher OLR (6 gVS/L/d) led to a shorter retention time of 28 days, since the reactor was kept at a fixed working volume during the whole experimental period. This shorter retention time could cause the removal of the slow-growing methanogenic community, which led to process instability as well as overloading of the reactor.

Despite the increase in VFA the pH still remained within the range of 7.6 and 8 during the second retention period of 6 gVS/L/d (OLR3). Weiland (2010) has also reported that manure can have a surplus of alkalinity which stabilizes the pH value even at higher VFA accumulation and as such will not always result in pH drop. To examine if the VFA could be reduced and restore the process back to stability, the loading rate was reduced again to 4.2 gVS/L/d (OLR 2). It was observed that the VFA/Alkalinity ratio reduced gradually to 0.2 (Fig. 3a) and the VFA accumulation reduced drastically (Fig. 4a) and so the process was restored back to stability.

During digestion, at OLR of 2.8 and 4.2 gVS/L/d the total ammonia nitrogen concentration was between 1350 and 2000 mg/L (Fig. 4b) and the VFA/Alkalinity ratio was below 0.3 which shows good buffering capacity. However, at OLR of 6 gVS/L/d, the total ammonia nitrogen concentration was slightly reduced to around 1000 mg/L probably due to reduced time for feedstock degradation and as such the buffering capacity was slightly lower. This together with the VFA accumulation resulted to higher VFA/Alkalinity ratio showing instability in the process. The free ammonia concentration throughout the process at different OLR was between 47 and 145 mg/L which shows that the reported tolerable free ammonia concentration of 150 mg/l (Kayhanian, 1999) was not exceeded, hence the process was not inhibited by free ammonia.

3.5. Volatile solids removal efficiency

An important factor for assessing the efficiency of an anaerobic digestion process is following up the reduction in VS. As shown in Table 2, 2.8 gVS/L/d (OLR 1) has the highest VS removal efficiency, an average of 74.87 ± 3.75%, while 6 gVS/L/d (OLR 3) has the lowest with an average of 41.17 ± 4.80%. It was observed that as VFAs getting accumulated the removal efficiency reduces. The removal efficiency decreases as the OLR increases, since with increasing OLR the retention time in the digester decreases, so that the organic matter loaded into the digester has not enough time for the degradation, especially not if difficult to digest fractions, as untreated straw, is present in the feedstock.

3.6. Degradation of lignocellulosics

As shown in Fig. 3b, the amount of cellulose and hemicellulose decreases gradually compared to those of the initial composition in the feed during the digestion process, while the lignin content increases. This shows that the carbohydrates were consumed during the process at all loading rates examined. However, the highest cellulose degradation was obtained at organic loading rate of 2.8 gVS/L/d (OLR 1); an average of 60.90% ± 6.37, while at 4.2 gVS/L/d (OLR 2) an average of 48.8% ± 6.73 cellulose degradation was observed, and finally 6 gVS/L/d (OLR 3) having the lowest cellulose degradation of an average of 30.1 % ± 7.05. For the same reason as explained above the amount of cellulose degraded decreases as the loading rate increases and with reducing retention time.

4. Conclusions

The new plug flow reactor developed can operate successfully for continuous dry digestion of manure bedded with straw at 22% TS when operated at OLR of 2.8 and 4.2 gVS/L/d with retention time of 60 and 40 days respectively, OLR of 6 gVS/L/d and retention of 28 days favoured process instability decreasing the VS removal efficiency and cellulose degradation. Digestion of manure bedded with straw without pretreatment at 22% TS was successful, methane yield 0.163 LCH4/gVSadded counting around 56% of the theoretical yield with 57% VS removal efficiency was obtained on organic loading rate of 2.42 gVS/L/d.

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References


Innovative pretreatment strategies for biogas production. 
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Innovative pretreatment strategies for biogas production

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highlights

Substrates for biogas might be indigestible, hard to digest, toxic materials. Indigestible materials can be gasified to syngas and then fermented to biogas. Recalcitrant biomass can go through a variety of pretreatments. Toxic substrate can be digested with or without a pretreatment. Right choice of digesting reactor and criteria can resolve some of these challenges.

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abstract

Biogas or biomethane is traditionally produced via anaerobic digestion, or recently by thermochemical or a combination of thermochemical and biological processes via syngas (CO and H₂) fermentation. However, many of the feedstocks have recalcitrant structure and are difficult to digest (e.g., lignocellulosic or keratin), or they have toxic compounds (such as fruit flavors or high ammonia content), or not digestible at all (e.g., plastics). To overcome these challenges, innovative strategies for enhanced and economically favorable biogas production were proposed in this review. The strategies considered are commonly known physical pretreatment, rapid decompression, autohydrolysis, acid- or alkali pretreatments, solvents (e.g. for lignin or cellulose) pretreatments or leaching, supercritical, oxidative or biological pretreatments, as well as combined gasification and fermentation, integrated biogas production and pretreatment, innovative biogas digester design, co-digestion, and bio-augmentation.

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Contents

1. Introduction ........................................................................................................... 1
2. Digestion of potential feedstocks for biogas production and their challenges .............................................................................. 1
3. Pretreatment methods ......................................................................................... 1

1.1. Biochemistry of biogas production. ................................................................ 1
1.1.1. Biological route. .......................................................................................... 1
1.1.2. Combined thermochemical and biological route ................................................ 1
1.1.3. Theoretical yield ......................................................................................... 1

2.1. Indigestible feedstocks ..................................................................................... 1
2.2. Hard-to-digest feedstocks ............................................................................... 1
2.2.1. Lignocellulosic wastes .............................................................................. 1
2.2.2. Keratin-rich wastes .................................................................................... 1
2.3. Feedstock with inhibitors ............................................................................... 1
2.3.1. Inhibitors as part of the feedstock—fruit wastes ............................................ 1
2.3.2. Inhibitors released during digestion—wastes from food processing .................... 1
2.3.3. Inhibitors released during digestion—ammonia inhibition from protein wastes .......................................................... 1
2.3.4. Inhibitors released during gasification .......................................................... 1

3. Pretreatment methods ......................................................................................... 1

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HIGHLIGHTS
• Substrates for biogas might be indigestible, hard to digest, toxic materials.
• Indigestible materials can be gasified to syngas and then fermented to biogas.
• Recalcitrant biomass can go through a variety of pretreatments.
• Toxic substrate can be digested with or without a pretreatment.
• Right choice of digesting reactor and criteria can resolve some of these challenges.

ABSTRACT
Biogas or biomethane is traditionally produced via anaerobic digestion, or recently by thermochemical or a combination of thermochemical and biological processes via syngas (CO and H₂) fermentation. However, many of the feedstocks have recalcitrant structure and are difficult to digest (e.g., lignocellulosic or keratins), or they have toxic compounds (such as fruit flavors or high ammonia content), or not digestible at all (e.g., plastics). To overcome these challenges, innovative strategies for enhanced and economically favorable biogas production were proposed in this review. The strategies considered are commonly known physical pretreatment, rapid decompression, autohydrolysis, acid- or alkali pretreatments, solvents (e.g. for lignin or cellulose) pretreatments or leaching, supercritical, oxidative or biological pretreatments, as well as combined gasification and fermentation, integrated biogas production and pretreatment, innovative biogas digester design, co-digestion, and bio-augmentation.

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Contents
1. Introduction .......................................................... 14
  1.1. Biochemistry of biogas production ......................... 14
    1.1.1. Biological route ........................................ 14
    1.1.2. Combined thermochemical and biological route ........ 15
    1.1.3. Theoretical yield ....................................... 15
  2. Digestion of potential feedstocks for biogas production and their challenges ........................................ 16
    2.1. Indigestible feedstocks ..................................... 16
    2.2. Hard-to-digest feedstocks .................................. 16
      2.2.1. Lignocellulosic wastes ............................... 17
      2.2.2. Keratin-rich wastes ................................. 17
    2.3. Feedstock with inhibitors ................................ 17
      2.3.1. Inhibitors as part of the feedstock-fruit wastes ......... 17
      2.3.2. Inhibitors released during digestion – wastes from food processing .... 17
      2.3.3. Inhibitors released during digestion – ammonia inhibition from protein wastes ..... 18
      2.3.4. Inhibitors released during gasification ............... 18
  3. Pretreatment methods ......................................... 18

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3.1. Gasification of indigestible feedstock ................................................................. 18
3.2. Hard-to-digest feedstocks and feedstocks with inhibitors ................................... 19
3.2.1. Accessible surface area and dehydratization ....................................................... 20
3.2.2. Hydrolysis of hemicellulose ............................................................................. 20
3.2.3. Lignin removal ................................................................................................ 20
3.2.4. Hydrolysis of keratin ....................................................................................... 20
3.2.5. Removal of inhibitors present in the feedstocks-fruit flavors ................................. 20
4. Innovative non-pretreatment strategies ................................................................. 20
4.1. Integrated biogas production strategy .................................................................. 21
4.2. Innovative digester design strategy ..................................................................... 21
4.3. Co-digestion strategy ........................................................................................ 22
4.4. Bio-augmentation strategy ................................................................................ 22
5. Conclusion ............................................................................................................ 22
References ............................................................................................................... 22

1. Introduction

Biogas production through anaerobic digestion technology has advanced tremendously over the years. Presently, due to high energy demand and environmental concerns as the world’s population increases, the drive for anaerobic digestion processes is gaining momentum within research and industry for sustainable energy generation. In this vein, there is an increasing focus on better feedstock utilization for improved biogas production. Nevertheless, the challenges of low biogas yield, high retention time, and high investment cost impede the maximum performance of biogas production in anaerobic digestion systems. These bottlenecks are highly dependent upon the availability, composition, and degradability of the feedstock used for biogas production. Great potential lies in biogas production from various feedstocks such as crop residues, livestock residues, municipal waste, landfill waste, food waste, aquatic biomass, keratin waste, and lignocellulosic feedstocks because of their availability and abundance. However, most of these feedstocks have slow degradation rates and as such require longer retention times. In addition, some of these feedstocks form toxic intermediates or contain toxic compounds, which inhibit the biogas production process. Nevertheless, the abundance and thus low cost of these feedstocks confirm that there is a need for new strategies for a better utilization of such kinds of waste streams.

Theoretically, biogas can be produced from the organic fraction of any material, such as wood, crop residue, textile wool, chicken feathers, lignocellulosic waste, industrial food waste, fruit waste, etc. However, today, biogas is typically produced only from feedstocks that are easily utilisable by the microbial community responsible for transforming these feedstocks into biogas. However, these easily digestible feedstocks, i.e., crop and livestock residues, source sorted municipal waste, food waste, waste water with high organic content, etc., are not as abundant or readily available for biogas production, thereby, limiting the amount of biogas that can be produced. Nevertheless, the development of innovative technologies aiming for the utilization of feedstocks that are readily available but not easily degradable would result in an increase in biogas production.

The major reasons why some feedstocks are not ideal for biogas production are: (a) they cannot be digested by microorganisms, (b) digestion by microorganisms is very difficult to achieve, (c) digestion could be achieved but in a very slow way, and (d) the presence of inhibitors in the feedstock or the production of inhibitory compounds during microbial degradation. The goal of the “pretreatment” is to facilitate the digestion process by removing these barriers and to make the organic content of the substrate easily accessible and utilisable by the microbial community. There have been several approaches toward pretreatment, which can be classified as physical, chemical, physicochemical, and biological (Taherzadeh and Karimi, 2008). This review considers some innovative strategies, helping the utilization of indigestible, slow, hard to digest, and inhibitory feedstocks for biogas production. The ideal pretreatment to be employed for processing these feedstocks should be cost-effective, increase feedstock accessibility to microorganisms, and not use or produce substances that inhibit biogas production, should not demand high energy, and should not generate by-products that are harmful to the environment. A short review on the biochemistry of biogas production, theoretical yield, available potential feedstocks, and the challenges associated with these feedstocks will help in understanding how innovative strategies toward pretreatment can be implemented.

1.1. Biochemistry of biogas production

1.1.1. Biological route

Feedstock for biogas production is prepared prior to digestion by removing contaminants such as grits, metals, and other debris depending on the source of feedstock. Moreover, the size of the feedstock may be reduced (for feedstock whose available surface area is not accessible by hydrolyzing bacteria), and inhibitors from feedstocks such as fruit flavors and the oil from palm oil mill effluent (POME) can be removed.

Organic feedstocks undergo different degradation steps during the anaerobic digestion (AD) process (Fig. 1), and they are briefly discussed as follows. Step one is hydrolysis, here the feedstock is disintegrated by the action of a diverse community of hydrolytic bacteria producing exoenzymes. The products of this first step are simple sugars, amino acids, and fatty acids. This step has been reported as being the rate limiting step for hard-to-digest biomass (Fernandes et al., 2009) such as lignocellulose and keratin-rich wastes. Moreover, some toxic by-products can be formed during this step (Neves et al., 2006). Step two is acidogenesis, in this step monomers from the hydrolysis are converted into short chain organic acids, alcohols, a few organic-nitrogen and organic-sulfur compounds, together with hydrogen and carbon dioxide. This step has been reported to be the fastest step in the AD process (Vavilin et al., 1996). If the feedstock has a low buffering capacity and the organic loading rate is high, the accumulation of volatile fatty acids can result in a pH drop, which would inhibit the methanogens that produce methane in the final step. Step three is the acetogenesis, here the homoacetogenic microorganisms reduce hydrogen and carbon dioxide to acetic acid (Deublein and Steinhauser, 2011). In this step, the aceticogenic bacteria can only survive at a very low hydrogen concentration, so excessive production of hydrogen from the acidogenesis step can inhibit these bacteria (Deublein and Steinhauser, 2011). Step four is methanogenesis, where methane production takes place under strict anaerobic conditions.
This step has been reported as the rate limiting step for easily degraded and those with low buffering capacity feedstocks (Rozzi and Remigi, 2004). There are mainly two groups of methanogenic bacteria that can be distinguished, i.e., hydrogenotrophic methanogens and acetotrophic methanogens converting hydrogen and carbon dioxide, and acetic acid, respectively, to methane. The balance between the hydrogen-forming and hydrogen-consuming microorganisms is very important, since anaerobic oxidation, i.e., acetate formation can only take place at a low partial pressure of hydrogen because of thermodynamic reasons.

1.1.2. Combined thermochemical and biological route

The increasing generation of indigestible organic waste increases the interest in the combination of thermochemical and biochemical processes such as gasification and fermentation. Both gasification and fermentation consist of several process steps. Initially, the feedstock is fed to the gasifier in which temperature increases to approx. 1200 °C. As the temperature rises, the feedstock’s water evaporates (at 100 °C), tar and char are produced, and pyrolysis gases are generated. The main reaction steps at this point are the water gas and the Boudouard reaction (Eqs. (1) and (2)). Moreover, gases react with each other (gas-phase reactions) and with the carbon (gas-solid reactions). Methane can also be produced by the reaction of carbon and hydrogen.

Water gas reaction: \( \text{H}_2\text{O} + \text{C} \rightarrow \text{H}_2 + \text{CO} \). \( \Delta H_{\text{BGR}} = 131.3 \text{ kJ/mol} \) (1)

Boudouard reaction: \( \text{CO}_2 + \text{C} \rightarrow 2\text{CO} \). \( \Delta H_{\text{BGR}} = 172.5 \text{ kJ/mol} \) (2)

The gas produced from the gasification contains fermentable hydrogen, carbon monoxide, and carbon dioxide. This gas mixture is fed into a fermenter where it is converted into methane. More specifically, carbon dioxide and hydrogen are converted into methane and water by hydrogenotrophic methanogens, while acetotrophic methanogens convert carbon monoxide and water into methane and carbon dioxide.

1.1.3. Theoretical yield

The organic content of a feedstock determines the theoretical yield of biogas production. When the elemental composition is known, the theoretical methane production can be calculated using Eq. (3) (Symons and Buswell, 1933):

\[ \text{C}_n\text{H}_m\text{O}_x\text{NS} + y\text{H}_2\text{O} \rightarrow x\text{CH}_4 + n\text{NH}_3 + s\text{H}_2\text{S} + (c - x)\text{CO}_2 \] (3a)

Moles of methane produced \((x)\)  
\[ = 1/8(4c + h - 2o - 3n - 2s) \] (3b)

If carbohydrates \((\text{C}_6\text{H}_{10}\text{O}_5)\), protein \((\text{C}_5\text{H}_7\text{O}_2\text{N})\), or fat \((\text{C}_3\text{H}_{12}\text{O}_4\text{N})\) are used as substrates, the theoretical yield will be 0.42, 0.50, and 1.01 N m\(^{-3}\)-CH\(_4\)/kg VS respectively (Schnürer and Jarvis, 2010). When both the elemental composition and the proportion of carbohydrates, proteins, and fats are not known, the theoretical methane yield can also be calculated from the chemical oxygen demand of the feedstock using Eq. (4):

\[ \text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} \] (4)

From this equation, 2-kmols of \(\text{O}_2\) (or 64 kg COD) are needed for the complete oxidation of 1-kmol of methane, so 1 kg COD is equivalent to 1/64-kmol of methane or 0.35 m\(^3\) CH\(_4\) at standard
Wastes from food processing industries (Russ and Meyer-Pittroff, 2004) and theoretical methane yield from the different substrates (Schnürer and Jarvis, 2010).


challenges are discussed below in order to ascribe value to them.

As discussed earlier, biomass that is easily processed is mainly used as feedstock for anaerobic digestion process. Common, easily processed, feedstocks include livestock manure, food-processing wastes, and sewage sludge. On the other hand, biomass that is difficult to process is in high abundance and accumulates tremendously. This biomass, when properly pretreated, can be a valuable feedstock for biogas production, thereby reducing environmental pollution and enhancing the recovery of renewable energy. For instance, lignocellulosic wastes have about 40–60% cellulose and 20–40% hemicellulose (Kang et al., 2014), which is a potential solution to increase the digestibility and ascribe value to the lignocellulosic wastes.

Furthermore, the digestion of cotton/polyester textiles was investigated in another study (Jeihanipour et al., 2013). However, these processes are premature, and more research is required for scaling up. One way to convert indigestible waste into fermentable feedstock is to apply gasification step before AD. During gasification, the feedstock is converted into a gas mix called syngas, which is thereafter converted into methane by anaerobic microbes. The combination of two already industrial processes gathers many advantages such as efficient know-how and existing infrastructures. However, the main bottleneck of syngas AD or fermentation is the low gas-liquid mass transfer, especially of hydrogen and carbon monoxide. However, the use of a gas dispenser such as an innovative hollow fiber membrane can drastically increase the gas diffusion rate (Shen et al., 2014). In addition, the diffusion of syngas in a more economical way would be a very positive step for scaling up the process.

### 2.2. Hard-to-digest feedstocks

The recalcitrant nature of some feedstocks limits their biological degradation for biogas production. This recalcitrant nature is a result of several properties such as the crystalline structure of the feedstock, different layers of the plant cell wall, and their interactions with other polymers (Taherzadeh and Jeihanipour, 2012).

### 2.3.2. Inhibitors released during digestion – wastes from food processing industries

As discussed earlier, feedstocks that are indigestible, hard to digest, slow to digest, or contain inhibitors are discussed as potential feedstocks for anaerobic digestion. Challenges associated with these feedstocks are discussed as well.

#### 2.1. Indigestible feedstocks

A large fraction of the globally produced waste is composed of organic indigestible materials, which are not biologically degradable or their degradation is extremely slow and cost-inefficient. Landfills contain many indigestible compounds such as processed paper and impregnated wood. Because of the indigestible and heterogeneous nature of these wastes, their volumes increase exponentially posing an environmental threat. According to the world bank, these materials make up the highest proportion of municipal solid waste in high income countries, while they accounted for 27% of the global solid waste generated in 2009 (TheWorldBank, 2010).

Indigestible wastes contain components that can be used by microbial catalysts during the biogas production. Recently, some studies were conducted on the biological degradation of traditionally indigestible feedstock for biogas production. For example, a novel bacterium, Ideonella sakkastensis 201-P6, was proven to degrade polyethylene terephthalate (PET) (Yoshida et al., 2016). Furthermore, the digestion of cotton/polyester textiles was investigated in another study (Jeihanipour et al., 2013). However, these processes are premature, and more research is required for scaling up. One way to convert indigestible waste into fermentable feedstock is to apply gasification step before AD. During gasification, the feedstock is converted into a gas mix called syngas, which is thereafter converted into methane by anaerobic microbes. The combination of two already industrial processes gathers many advantages such as efficient know-how and existing infrastructures. However, the main bottleneck of syngas AD or fermentation is the low gas-liquid mass transfer, especially of hydrogen and carbon monoxide. However, the use of a gas dispenser such as an innovative hollow fiber membrane can drastically increase the gas diffusion rate (Shen et al., 2014). In addition, the diffusion of syngas in a more economical way would be a very positive step for scaling up the process.

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Table 1

<table>
<thead>
<tr>
<th>Industry</th>
<th>Application</th>
<th>Waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td>Dairy processing</td>
<td>Diluted whole milk, whey, separated milk, cleaning water</td>
</tr>
<tr>
<td></td>
<td>Yogurt processing</td>
<td>Diluted milk, cleaning water, whey</td>
</tr>
<tr>
<td></td>
<td>Cheese processing</td>
<td>Dry milk particle, whey, cleaning water</td>
</tr>
<tr>
<td>Oil</td>
<td>Palm oil processing</td>
<td>Palm kernel shell, empty fruit bunches, palm oil mill effluent</td>
</tr>
<tr>
<td></td>
<td>Olive oil processing</td>
<td>Vegetable water, olive husk and skin</td>
</tr>
<tr>
<td></td>
<td>Vegetable oil processing</td>
<td>Oil seed cake</td>
</tr>
<tr>
<td></td>
<td>Coconut oil processing</td>
<td>Outer coat fiber (coir), desiccated coconut meat (copra)</td>
</tr>
<tr>
<td>Meat</td>
<td>Slaughterhouse</td>
<td>Slaughterhouse waste water, slaughterhouse blood</td>
</tr>
<tr>
<td>Brewery</td>
<td>Beer making</td>
<td>Malt dust, spent grain, kieselguhr sludge, yeast</td>
</tr>
<tr>
<td>Grain</td>
<td>Cereals 300–400</td>
<td>Bran, middlings, shells, husk, broken grain, ergot</td>
</tr>
<tr>
<td></td>
<td>Oat husking</td>
<td>Bran, husk</td>
</tr>
<tr>
<td></td>
<td>Rice mill</td>
<td>Rice bran, brown rice waste</td>
</tr>
<tr>
<td></td>
<td>Malt processing</td>
<td>Malt dust, grain separator waste</td>
</tr>
<tr>
<td>Sugar</td>
<td>Sugar cane processing</td>
<td>Bagasse, molasses</td>
</tr>
<tr>
<td></td>
<td>Sugar beet processing</td>
<td>Molasses, exhausted beet pulp, carbonation sludge</td>
</tr>
<tr>
<td>Substrate</td>
<td>Methane yield (m^3/ton-vs)</td>
<td></td>
</tr>
<tr>
<td>Food waste</td>
<td>400–600</td>
<td></td>
</tr>
<tr>
<td>Fruit waste</td>
<td>200–500</td>
<td></td>
</tr>
<tr>
<td>Grass</td>
<td>200–400</td>
<td></td>
</tr>
<tr>
<td>Straw</td>
<td>100–320</td>
<td></td>
</tr>
<tr>
<td>Municipal sludge</td>
<td>160–350</td>
<td></td>
</tr>
<tr>
<td>Protein wastes</td>
<td>496</td>
<td></td>
</tr>
<tr>
<td>Manure (pigs, cattle, chickens)</td>
<td>100–300</td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse waste</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>Cereals</td>
<td>300–400</td>
<td></td>
</tr>
</tbody>
</table>
These complex polymers are tightly packed and highly crosslinked with hydrogen bonds, disulfide bonds (for keratin wastes), and hydrophobic interactions (Daroit et al., 2009). Hydrolysis is the rate-limiting step for these feedstocks; therefore, it is necessary to overcome the recalcitrant nature and make them accessible to the microorganisms for effective biogas production. Some of these feedstocks and their challenges as well as how to overcome these challenges are discussed below in order to ascribe value to them.

2.2.1. Lignocellulosic wastes
Lignocellulosic wastes are one of the most abundant renewable organic resources on earth with a yearly production of approximately 200 billion tons (Zhang, 2008). The increasing expansion of the agricultural industry and its activities has led to an increase in the production and the consequent accumulation of a large volume of these wastes. Forestry by-products, woody crops, and municipal solid waste also contribute to an enormous amount of these waste streams. About 60% of the dry weight of the average municipal solid waste is composed of lignocellulosic (Holtzapple et al., 1992). The abundance of lignocellulosic wastes makes them a potential feedstock for biogas production, and it has been reported that these kinds of wastes can add up to a significant energy value of approximately 1500 MJ/year (Kang et al., 2014) depending on the yield from the biomass, total cultivable area, and the technology used. Although these wastes are rich in fibers, readily available, and have a significant energy value, they have a low biogas yield due to their resistance to microbial attack.

Lignocellulosic wastes are difficult to digest as a result of matrix polymers (hemicellulose, pectins, and lignin) surrounding the cellulose microfibrils in the plant cell wall (Himmel and Picataggio, 2009). The degree of resistance of these wastes to microbial attack varies depending on the type of lignocellulose, various compartments of the cell wall, and cell age, while it is also affected by processing phenomena such as drying and heating (Taherzadeh and Jeihanipour, 2012). However, effective use of lignocellulosic biomass for biogas production requires a hydrolysis step in order to open up the structure and increase the accessibility for cellulose degrading microorganisms. It is only after this step that the material will be ready for conversion. The rate and extent to which cellulose can be hydrolyzed depends on the connection between the lignin, hemicellulose, and cellulose and also the degree of crystallinity in the cellulose itself. A potential solution is an innovative pretreatment method to increase the digestibility and ascribe value to the lignocellulosic wastes.

2.2.2. Keratin-rich wastes
Keratin-rich wastes such as chicken feather, wool, hair, nails, horns, hooves, and claws are produced worldwide by the poultry, wool, meat, and fish industries. For instance, the world’s average stock of chicken is about 22 billion (FAOSTAT, 2013); thus, the poultry industries produce a tremendous amount of feathers yearly. Moreover, the global production of wool amounts to 2.1 million tons per year (IWTO, 2015). Assuming that all the insoluble protein (keratin) are converted into soluble protein, the methane potential of keratin wastes is as high as 0.496 Nm3/kgVS (Angelidaki and Sanders, 2004). However, the methane yield obtained is usually low due to the recalcitrant structure of keratin. Keratin is an insoluble structural protein where the polypeptides chain is tightly packed and highly crosslinked with disulfide bonds, hydrogen bonds, and hydrophobic interactions (Daroit et al., 2009). This structure makes the protein insoluble and resistant to enzymatic attack, which is a major hindrance in the biological processing of these wastes. The keratin in hair, wool, and horn is tightly packed in an α-helix, and it is different from the keratin in the chicken feathers (β-helix) because it contains a higher amount of cysteine (Moreira et al., 2007), which makes α-keratins even more difficult to hydrolyze than β-keratins. An appropriate pre-treatment is required, therefore, prior to biogas production for keratin-rich wastes to be utilized for biogas production.

2.3. Feedstock with inhibitors
Inhibitors are compounds or substances that slow down or stop the ability of enzymes, catalysts, or microorganisms to transform reactants to desired products. From the biogas pretreatment perspective, inhibitors could be classified based on their biodegradability or the point at which they enter the biogas production process. Based on the biodegradability, some inhibitors such as furfural, long chain fatty acids, and organic pollutants are biodegradable, while others such as heavy metals are non-biodegradable (Chen et al., 2008). Under optimal conditions, microbes in the digesters can digest feedstocks containing low concentrations of biodegradable inhibitors, while the accumulation of non-biodegradable inhibitors can lead to the death of microbes in the digester (Chen et al., 2008). The other categories of inhibitors either come into the digester as part of the feed (such as fruit flavors, aromatic compounds) or are released during digestion or pre-treatment (such as NH3, long chain fatty acids). Feedstocks with inhibition based on point of entry or release are discussed in the following subsections.

2.3.1. Inhibitors as part of the feedstock-fruit wastes
The Food and Agricultural Organization (FAO) of the United Nations 2013 statistics showed that the global fruit production has increased by 3% annually in the past decade, with 804.4 million tons produced in 2012 (FAO, 2013). The FAO also reported that 36–56% of the produced fruits end up as waste (FAO, 2011). Fruit waste could be generated as a result of poor management or storage, diseases or infestation as well as mechanical damage during harvesting or fruit processing. Fruit processing generates two types of waste: solid waste that consists of peels, skin, seed, stones, etc., and liquid waste from juice and wash-waters (Gustavsson et al., 2011). The average VS of fruit waste consists of 78.3% carbohydrates, 8.5% protein, and 6% fat (Schürner and Jarvis, 2010), so the theoretical yield of methane from fruit waste is 0.43 Nm3·CH4/kg·VS or 0.04 Nm3·CH4/kg fruit waste. Using the estimated global fruit production for the past decade, the estimated fruit production in 2016 would be 905.4 million tons, and if 46% ends up as waste, 16.7 × 109 Nm3 of methane could be produced from the fruit waste. However, the actual methane production from fruit waste is much less than this theoretical yield because of the presence of inhibitory flavor compounds affecting the AD process negatively (Wikandari et al., 2013).

These flavor compounds can be classified into esters, alcohols, aldehydes, ketones, lactones, and terpenoids. Compounds like geraniol, thymol, carvacrol, etc. belonging to the terpenes family have been reported as cytotoxic (Rosato et al., 2007). Wikandari et al. (2013) found that 0.5% of terpenoids, aldehydes, and alcohols in an AD feedstock can reduce the methane production by 99%. Limonene, a terpenoid and a major component of peel oil from citrus fruits, has been reported to cause failure in a continuous mesophilic AD process at a concentration of 400 μL/L (Mizuki et al., 1990), while at a concentration of 450 μL/L it can lead to the failure of a thermophilic AD process (Forgács, 2012). Due to these flavor compounds, it is essential for fruit waste to be pre-treated before it is used for biogas production.

2.3.2. Inhibitors released during digestion – wastes from food processing
Wastes from the food processing industries are a good choice for biogas production because the waste produced consists mostly of the organic fraction of the raw materials used by the industry.
These wastes usually have a high biological oxygen demand (BOD) and a high chemical oxygen demand (COD), with varying contents of suspended solids depending on their source (Russ and Meyer-Pittroff, 2004). The wastes from food processing are either solid or liquid, and most of the solid part can be classified as a lignocellulosic residue (Table 1). As lignocellulosic wastes have been discussed in Section 2.2.1, in this section only the liquid fraction of the waste from food processing which is associated with a high volume of production and negative environmental effects, will be discussed. More specifically, the liquid waste from the oil industry has attracted a lot of attention because of its potential for biogas production and its negative effect on the environment.

Global vegetable oil production increased from 90.5 × 10^6 MT in 2000 to 160.59 × 10^6 MT in 2012 (Statista, 2016) with oil-palm, soybean, rapeseed, and sunflower accounting for 75% of the global oil production (FAO, 2013). Over this period, the palm oil production increased from 21.75 × 10^6 MT to 51.74 × 10^6 MT making palm oil to be the most produced vegetable oil in the world. The production of vegetable oil is usually associated with the production of wastewater. Taking palm oil as an example, for every ton of crude palm oil produced, 2.5–3.75 tons of palm oil wastewater, called palm oil mill effluent (POME) is produced (Chin et al., 2013). POME is a thick brownish liquid generated from the clarification and sterilization of palm oil during the oil extraction process in the palm oil mills. It is considered to be one of the most polluting effluents from the agricultural industry because of its properties such as high COD (15–100 g/L), high BOD (10.25–43.75 g/L), and acidic pH (3.4–5.2) (Ahmad et al., 2011). Biogas production from POME could solve the problem of treating POME for the environment and also generating an additional valuable product (biogas) for the oil-palm industries. Using 50 g/L as an average COD of POME, every 1 m³ of POME can produce 18.78 Nm³ of CH₄ or 37.56 Nm³ of biogas. Taking into account the production of 3.125 tons of POME per ton of the palm oil, theoretically 2.9 × 10¹⁵ Nm³ of CH₄ or 5.8 × 10¹⁵ Nm³ of biogas could have been produced from the palm oil produced globally in 2012. With this tremendous potential, it is important both from energy and environmental perspectives that POME is properly utilized.

Despite the tremendous potential of producing biogas from POME or other waste water from oil production, in reality most AD digesters fail when fed continuously with daily COD load of between 35 and 65 g/L (Zinatizadeh et al., 2007), which is challenging as the COD of these feedstocks could be as high as 100 g/L (Ahmad et al., 2011). One of the reasons for this failure is that POME or other waste water from the oil production contains polymeric fat, which are broken down during hydrolysis to long chain fatty acids such as stearic acid, oleic acid, and arachidic acid, and long chain fatty acid accumulation has been shown to inhibit the AD process (Angelidaki and Ahring, 1992). Another reason for the failure when using POME is the accumulation of volatile fatty acids (VFAs) in the digester. Thus, to fully harness the potential of waste water from the oil production, such as POME, for biogas production, it is important to come up with innovative strategies.

### Inhibitors released during digestion – ammonia inhibition from protein wastes
Presently, about 1 million tons of protein-rich wastes are produced annually (Kovács et al., 2013), and about 30% of the weight of animals processed for food ends up as slaughterhouse waste (Kovács et al., 2015). These wastes mainly contain manure and blood; they are highly rich in protein, readily available, and can easily digested, which makes them a potential feedstock for biogas production. The theoretical methane potential of protein wastes is 0.496 Nm³/kgVS (Angelidaki and Sanders, 2004), and slaughterhouse wastes have a theoretical potential of about 0.70 Nm³ CH₄/kgVS (Schnürer and Jarvis, 2010); however, since they have a low C/N ratio, digestion of these feedstocks as a single substrate is usually unsuccessful due to the accumulation of ammonia during digestion. During the breakdown of protein-rich wastes, depending on the pH and temperature, free ammonia (NH₃) or ammonium ion (NH₄⁺) are produced, which at higher concentrations inhibit the anaerobic digestion process (Yenigün and Demirel, 2013). Studies have shown that inhibition is due to the presence of free ammonia rather than ammonium ion (Kayhanian, 1999), with a free ammonia nitrogen (FAN) concentration of 150 mg/L causing complete inhibition (Yenigün and Demirel, 2013). Therefore, proper treatment of these feedstocks before digestion and control of ammonia concentration during the digestion process is necessary to maximize the potential of these wastes for biogas production.

### 2.3.4. Inhibitors released during gasification
During feedstock gasification, several inhibitors can be generated mainly because of the feedstock composition and the operating conditions. Some of these inhibitors are char, H₂S, NH₃, NOₓ, ethane, ethylene, and acetylene as well as heavy metals and particles. A high concentration of these components is known to cause cell dormancy, enzyme inhibition, low cell growth, and limited hydrogen uptake. The amounts of these components can be limited by cleaning the syngas with cyclones, reforming, and by controlling the operating conditions and the feedstock composition.

### 3. Pretreatment methods
#### 3.1. Gasification of indigestible feedstock
During gasification, the feedstock is gasified by exposure to high temperatures (1000–1200 °C) and an oxidizing agent. Steam, oxygen, and air are mainly used as oxidizing streams. The produced gas (syngas) is mainly composed of H₂, CO, and CO₂, which can thereafter be fermented for biogas production. In addition, at the end of the gasification, a residual ash, in the form of slags, is left in the gasifier. The composition of the syngas and the ash is affected greatly by the feedstock composition and the process temperature. For example, feedstocks with high amount of carbon generate syngas with high CO content. Furthermore, at lower operating temperatures below 1000 °C, the gas produced contains higher amounts of impurities. The remaining ashes, from thermochemical processes, have been mainly used as building material in road construction. However, ashes consist of toxic components such as heavy metals, which pose an environmental threat. Therefore, alternative uses have been considered. For example, it has been reported that the addition of ash enhanced the biogas production, benefitted the alkalinity, and pH of anaerobic digesters (Banks and Lo, 2003).

One of the main advantages of gasification as a pretreatment process is that it can treat a wide variety of heterogeneous feedstocks. Furthermore, gasification has a high conversion rate, and both syngas and the remaining ash can be used for biogas production (Banks and Lo, 2003). In addition, syngas production can be economically efficient if parameters such as pressure, gasification agent, syngas and feedstock composition are taken into consideration. More specifically, an economic assessment showed that it is possible to produce syngas with an approximate cost of $0.25/Nm³ based only on biomass feedstock. Moreover, the production costs can almost be reduced by half when considering co-gasification of biomass and coal (Trippe et al., 2011). On the other hand, another study argues that challenges such as high operational costs should be addressed in order to upgrade the combined gasification and fermentation process to a commercial scale (Richter et al., 2013).
Table 2

Conventional pretreatment processes for biogas production.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Processes</th>
<th>Function</th>
<th>Economic challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical pretreatment1,2</td>
<td>Milling, irradiation, pyrolysis, ultrasonic</td>
<td>Increase of surface area and pore size</td>
<td>Consumes high electric energy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disruption of biomass structure and decrease of crystallinity</td>
<td>Equipment repairs can be very expensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of degree of polymerization</td>
<td>Effective recovery of ammonia can be expensive</td>
</tr>
<tr>
<td>Rapid decompression</td>
<td>High pressure steaming, steam explosion</td>
<td>Partial hydrolyzation of hemicellulose</td>
<td></td>
</tr>
<tr>
<td>pretreatment3</td>
<td>ammonia fibre explosion (AFEX) CO2 explosion</td>
<td>Partial decrystallization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SO2 explosion</td>
<td>Partial lignin solubilization</td>
<td></td>
</tr>
<tr>
<td>Autohydrolysis pretreatment</td>
<td>Liquid hot water bath, liquid hot water percolation</td>
<td>Hydrolization of cellulose at very high and lower temperature</td>
<td></td>
</tr>
<tr>
<td>Acid pretreatment5,6</td>
<td>Sulfuric, hydrochloric, phosphoric, nitric and carbonic acid</td>
<td>High removal of hemicellulose and lignin</td>
<td>Recovery of acid is expensive</td>
</tr>
<tr>
<td>Alkaline pretreatment6,9</td>
<td>Sodium hydroxide, ammonia, lime, potassium hydroxide</td>
<td>Increase of internal surface area</td>
<td>High cost of bases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduction of degree of polymerization</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrystallization</td>
<td></td>
</tr>
<tr>
<td>Solvent pretreatment6</td>
<td>Organic solvents e.g. methanol, ethanol, acetone, ethylene glycol and hydrodurfuryl alcohol</td>
<td>Solubilization of lignin</td>
<td>High cost of solvent</td>
</tr>
<tr>
<td></td>
<td>Cellulose-dissolving solvents e.g. cadoxen, concentrated mineral acids, DMSO, NMPO and zinc chloride</td>
<td>Disruption of lignin structure</td>
<td>Effective recovery of solvent is costly</td>
</tr>
<tr>
<td>Leaching5</td>
<td>Organic solvents e.g. hexane, diethyl ether, dichloromethane or ethyl acetate</td>
<td>Removal of inhibitors in citrus waste</td>
<td></td>
</tr>
<tr>
<td>Supercritical fluid</td>
<td>Utilizing water, CO2 or ammonia</td>
<td>Removal of lignin</td>
<td>Highly expensive</td>
</tr>
<tr>
<td>Oxidative pretreatment4</td>
<td>Oxidizing compounds e.g. hydrogen peroxide or peracetic acid</td>
<td>Increase of biomass digestibility</td>
<td></td>
</tr>
<tr>
<td>Biological pretreatment4,5,6</td>
<td>White rot fungi, brown rot fungi, Enzyme products (cellulase, hemicellulase and β-glucosidase) Bacillus sp., Aspergillus sp. and alkaline endopeptidase</td>
<td>Degradation of lignin, degradation of cellulose and hemicellulose</td>
<td>High cost of chemical</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hydrolysis of keratin</td>
<td></td>
</tr>
</tbody>
</table>

1 Dimethyl sulfoxide.  
2 N-Methylmorpholine N-oxide.  
3 Bochmann et al. (2013).  
4 Onyech et al. (2002).  
6 Hendricks and Zeman (2009).  
7 Johnson and Elander (2009).  
8 Singh et al. (2014).  
10 Sun and Cheng (2002).  
11 Wikandi et al. (2015).  
12 Li and Kiran (1988).  
13 Fellihi et al. (2014).  
14 Forgacs et al. (2011a).  
15 Mazotto et al. (2013).  
16 Patinvoh et al. (2016).  
17 Zaghloul et al. (2011).  
18 Wang et al. (2016).  
19 Xiao and Clarkson (1997).  
20 Kumar and Murthy (2011).  
21 Kabir et al. (2015).  
22 Li et al. (2013).

3.2 Hard-to-digest feedstocks and feedstocks with inhibitors

Due to the recalcitrant nature and strong elasticity, recalcitrant feedstock can only result in a low biogas yield. Furthermore, feedstocks such as fruit wastes are easily degraded but also result in low yield due to the presence of inhibitors. Therefore, these wastes are pretreated prior to the biogas production. The main purpose of pretreatment for lignocellulosic wastes is to break the lignin layer that protects the cellulose and hemicellulose, in order to make the feedstock more accessible for digestion. Pretreatment also helps to decrease the crystallinity of cellulose and to increase the porosity. For other feedstocks like keratin-rich wastes, if the crosslinking between the polypeptides chain breaks, the keratin is more accessible and easier to digest. Another purpose of pretreatment is to remove the inhibitors, which are present in potential feedstocks. Several pretreatment methods have been reported in detail aiming to make these feedstocks viable to digestion by microorganisms, thereby increasing the biogas yield (Table 2). Some pretreatments lead to changes in the feedstock composition by dissolving the hemicellulose or lignin or both, while some other pretreatments result in changes in the cellulose crystallinity, molecular weight, biomass porosity, and particle size (Johnson and Elander, 2009). Specific pretreatment methods suitable for (i) increasing accessible surface area, (ii) decrystallization of cellulose, (iii) hydrolysis of...
hemicellulose, (iv) removal of lignin, (v) hydrolysis of keratin, and (vi) removal of inhibitory compounds are discussed in the following subsections.

3.2.1. Accessible surface area and decrystallization

Commination, such as ball milling, is a physical pretreatment method that increases the feedstock surface area and decrystallizes cellulose. Silva et al. (Silva et al., 2012) investigated the effect of grinding on wheat straw. The results showed that ball milling samples yield 46% total carbohydrates and 72% glucose as a result of the reduction in the cellulose crystallinity from 22% to 13%. Ball milling was also applied to non-degraded digestate in order to feed it back into the digestion process (Lindner et al., 2015). Enhanced methane production by 9% was reported in the case of two-stage maize sillage digestate, and an increase of 17% was detected when using two-stage hay/straw digestate. Irradiation is also an effective pretreatment method that disrupts the structure of the biomass cell wall and decreases the crystallinity of the cellulose (Johnson and Elander, 2009). The effect of microwave pretreatment was evaluated on the solubilization of microalgae as a substrate for biogas production, and it was observed that at a specific energy of 21.8, 43.6, and 65.4 MJ/kgTS, the pretreatment increased the biogas production rate by 27–75% and the biogas yield by 12–78%, according to the results applied from the BMP assays. These pretreatments are suitable methods for increasing the surface area and decrystallizing recalcitrant feedstocks; however, they are not economically feasible due to the high energy consumption. Therefore, they are not viable as stand-alone pretreatments for industrial applications but can be combined with other pretreatments that are cost effective.

3.2.2. Hydrolysis of hemicellulose

Dilute acid pretreatment at controlled conditions can solubilize almost all the hemicellulose from a biomass and thereby increase the accessibility of microorganisms to cellulose. This method breaks the intramolecular bonds between the lignin, hemicellulose, and cellulose in the cell wall and hydrolyzes the hemicellulose (Taherzadeh and Jeihanpour, 2012). However, at a high temperature and acid concentration, undesirable dehydration occurs resulting in the hemicellulose and cellulose being degraded into different types of inhibitory compounds, such as furfural (Taherzadeh et al., 1997). Hence, it is necessary to carry out the pretreatment at mild conditions to prevent excessive sugar degradation. Un-catalyzed steam explosion is also an alternative pretreatment method for the hydrolysis of hemicellulose. However, the xylose yield from the hemicellulose is usually not higher than 65% of the theoretical yield due to the excessive sugar degradation (Brownell and Saddler, 1987). Liquid hot water pretreatment at a lower temperature (200–230 °C) has also been reported as an effective method for the hydrolysis of hemicellulose (Johnson and Elander, 2009). A higher yield of soluble sugars is possible with this method compared to the un-catalyzed steam explosion method. However, the liquid hot water pretreatment method liberates the sugars in the oligomeric form (Johnson and Elander, 2009).

3.2.3. Lignin removal

Alkaline pretreatment is a suitable method for solubilizing the lignin; it can be carried out at different concentrations of lime, sodium hydroxide, or ammonia. This method causes swelling of the fibers, which results in an increased accessible surface area, reduced degree of polymerization, and decrystallization of the cellulose. Alkaline treatment breaks the intramolecular bonds between the lignin, hemicellulose, and cellulose and disrupts the lignin structure (Hsu, 1996). Lime (Ca(OH)$_2$) pretreatment in the presence of water (9 ml/g dry biomass) was investigated for 2 h on switchgrass at 100–120 °C with 0.1 g Ca(OH)$_2$/g dry biomass loading (Chang et al., 1997). The results showed that 29% lignin was solubilized. Moreover, Sambusiti et al. (2013) investigated the effect of alkaline (NaOH) pretreatment on ensiled sorghum forage in semi-continuous digesters. It was observed that pretreatment with 10 g NaOH/100gTS increased the methane yield by 25% compared to untreated sorghum and did not cause any inhibition of the process. Organic or organic-aqueous solvent mixtures utilizing ethanol, benzene, ethylene glycol, or butanol have also been reported as being effective for lignin removal (Taherzadeh and Karimi, 2008). Studies have also shown that white-rot fungi produce enzymes that are capable of extensive degradation of lignin. As a result, the biomass digestibility is increased (Adney et al., 2009), although there is a loss of cellulose and hemicellulose during this process (Hatakka, 1983).

3.2.4. Hydrolysis of keratin

Pretreatment of keratin-rich waste using a strong acid, alkaline hydrolysis, and other physicochemical methods results in severe degradation and destruction of the keratin (Barone et al., 2006). Hence, recent research focuses on the biological pretreatment methods, which have been reported as successful for keratin degradation. Microorganisms (bacteria and fungi) produce enzymes that can break down the keratin and make it soluble. The keratin-rich feedstock is inoculated with appropriate bacteria or fungi cultures and incubated for days or weeks depending on the activity of the microorganism used and the properties of the substrate. Several microorganisms such as Bacillus sp. (Cai et al., 2008; Fellahi et al., 2014; Forgács et al., 2011a; Patinvoh et al., 2016; Zaghloul et al., 2011) and Aspergillus sp. (Mazotto et al., 2013) have been investigated and reported as being effective for hydrolyzing keratin.

3.2.5. Removal of inhibitors present in the feedstocks-fruit flavors

Inhibitory compounds that are part of the feedstocks used for the biogas production such as flavor compounds present in fruits have been shown to be adversely affecting the anaerobic digestion process even at low concentrations. Steam explosion has been reported to successfully remove these types of inhibitors from citrus waste, while also breaking up the lignocellulose structure in the feedstock (Forgács et al., 2011b). Steam explosion is a thermal pretreatment method that requires a very high pressure of up to 60 bar and a temperature of up to 150 °C. Combined chemical and steam explosion pretreatment methods have been found to reduce the required temperature and residence time of the pretreatment, while also increasing the degradation rate in contrast to when chemical or steam explosion methods are used separately (Forgács et al., 2011b).

4. Innovative non-pretreatment strategies

Although several pretreatment methods have emerged over the past few years, certain challenges such as high cost, harmful environmental by-products, low biogas yield, and high energy requirement still persist. To address these challenges, some innovative non-pretreatment strategies aiming to improve the use of the readily available and low cost feedstocks for biogas production are discussed in this section. The strategies discussed include integrated biogas production, innovative digester design, co-digestion, and bio-augmentation. An integrated biogas production strategy addresses the cost challenge, the innovative digester design strategy addresses the inhibition challenge, and the co-digestion strategy addresses both the challenge of low yield and inhibitors, while the bio-augmentation strategy addresses the challenge of inhibitors.
4.1. Integrated biogas production strategy

Process integration refers to combining production systems either at the level of individual processes or whole factories with the goal of efficient utilization of resources, while reducing the cost and environmental emissions (Hallale, 2001). Although researchers agree that having biofuel production facilities close to each other can reduce the costs associated with the waste treatment, energy utilization, and shared logistics, the economics of integrating different biofuel production facilities have not been fully explored. Despite this, there have been successful instances where integrating an additional process route into an already established biofuel production facility resulted in better economics for the biofuel production company. As an example, Derer et al. (2011) used thermochromically pretreated oat straw for ethanol production and after the removal of ethanol, the residue was used for biogas production. The results showed that sequential ethanol and biogas production resulted in 28–34% higher energy yield than direct biogas gasification. Biogas production was also faster with fermentation residues compared to direct digestion, showing that enzymatic saccharification and ethanol fermentation make the feedstock more digestible.

Detailed techno-economic analysis of this kind of integration has not been investigated, however a brief understanding of how integration affects the economics can be investigated by comparing the production cost with the revenue. The cost of methane production from wheat straw using steam pretreatment was estimated as $0.85/m³, with the cost from pretreatment as $0.11/m³ CH₄ (13% of the total cost) (Shaheef et al., 2013). The cost of ethanol production from lignocellulose residue in California was estimated as $0.43/L ethanol (Williams et al., 2007), excluding pretreatment (as the pretreatment cost has been factored into the biogas production cost), the cost could drop to $0.33/L ethanol. Using the current selling price of ethanol ($0.4/L) (USEIA, 2016) and the experimental results from Derer-Rut et al. (2011), integrating ethanol and biogas production facility could increase the profitability of the biogas facility by $0.014/kg-dry matter of wheat straw than when pretreated wheat straw was used to produce only biogas. This shows that the ethanol production process can be used for pretreating biogas feedstocks, while also bringing the benefits of process integration such as increased productivity.

Considering citrus waste as an example of fruit waste, the high costs associated with the pretreatment could be a deterrent from using it for biogas production. However, integrating pretreated citrus waste into already established ethanol and biogas production facilities could make the process more economically attractive, because in addition to ethanol and biogas, limonene and pectin would be valuable products that could generate additional revenue to offset the cost of pretreatment. Pourbafrani et al. (2010) found that from a ton of citrus waste at 20% dry weight, 39.64 L of ethanol, 45 m³ of methane, 8.9 L of limonene, and 38.8 kg of pectin can be produced. Furthermore, oranges, a type of citrus waste, accounted for 8.5% of the total global fruit waste in 2012 (FAO, 2013). Putting this amount of orange waste into the results found by Pourbafrani et al. (2010) would result in the production of 2.7 x 10³ m³ of ethanol, 3.1 x 10⁹ Nm³ of biogas, 6.1 x 10³ m³ of limonene, and 2.7 x 10³ tons of pectin.

Apart from the fruit waste, the integration concept can be applied to the other readily available feedstocks. From the biorefinery principles, diverse valuable products or energy can be generated from these feedstocks. For example, lignocellulosic waste conversion into ethanol and biogas has gained a lot of attention from researchers, but in addition to this, the US Department of Energy (DOE) identified fifteen high value building block chemicals that can be attained from lignocellulosic sugar conversion (Werpy et al., 2004). Integrating any of the identified high value chemicals production with biogas production could enhance the economics of biogas production from lignocellulosic waste. A similar analogy applies to other easily available feedstocks. Thus, process integration could be vital in making the economics of biogas production more favorable.

4.2. Innovative digester design strategy

Conventionally, a biogas digester design could be simple or detailed depending on the scale of application, the nature of the feedstock as well as the microorganisms involved. The design usually aims for the digester to provide a good environment that enables efficient contact between the microorganisms and the substrate. However, in case of some pretreatment methods or the use of certain chemicals, the AD process can be inhibited, even though these methods can successfully release dissolved intermediates for the AD process (Bolado-Rodriguez et al., 2016). To make these methods beneficial for methane production, it is important to develop a new digester concept that shields the microorganisms from the inhibitors, and in this way allow for a higher biogas yield to be achieved.

One way for this is to allow for a good contact between the microorganisms and the substrate, while selectively removing the inhibitors. In a recent study (Ezeji et al., 2013), a bioreactor design was applied, called continuous bioreactors with simultaneous product recovery and bleeding, to overcome the inhibition challenges in the bio-butanol production. This design allowed for a good contact between the microorganisms and the substrate, while bleeding away the inhibitory materials and recovering the bio-butanol to prevent an inhibitory concentration. With this design, 25 times higher production of butanol was achieved than in the traditional bioreactor used as a control (Ezeji et al., 2013). Apart from the inhibitory by-products or chemicals, feedstock such as POME could be inhibitory to the AD process. POME is produced in large volumes, so to minimize the costs associated with a large digester volume, a digester design that can allow for a short hydraulic retention time (HRT) is necessary. Najtallpur et al. (2006) used a modified form of an up-flow anaerobic sludge blanket (UASB) reactor called an up-flow anaerobic sludge fixed film (UASFF) digester and in this way they were able to achieve 90% reduction in the COD of POME at an organic loading rate (OLR) of 23.15 g-COD/d and HRT of 1.5 days. This modification of the UASB concept was essential since POME contains suspended and colloidal components. Furthermore, these components consist of fat, protein, or cellulose, which can lead to bed failure and washout of microorganisms from the traditional UASB system (Zinatizehad et al., 2007). Hence, an innovative digester design can be helpful, both in overcoming inhibition challenge and for reducing the retention time needed for the biogas production.

Another innovative design is the use of two- or multi-stage digesters. Separating the methanogenesis step from the acidogenesis step during the AD process has been found to give optimal conditions to the different microorganisms involved in the AD processes. Moreover, it has achieved a better process control and ability to handle higher OLRS than that in the single digesters (Demirel and Yenigün, 2002). This is due to the difference in the nutritional needs, environmental sensitivity, and the growth rate of the acidogenesis microorganism from those of the methanogens. Theoretically, using the two-stage AD system means that optimal conditions can be provided to the rate limiting steps within the AD process, leading to a better methane yield or shorter retention time, especially for the difficult-to-digest substrates. Using 20 g/L cellulose loading, Jeihanpour et al. (2013) found that a two-stage digester system resulted in 80% increase in the maximum methane production and a decrease in the lag phase from 15 to 4 days. However, the cost and the added complexities of the two-stage AD sys-
tems have been hindering the wide scale establishment of commercial two-stage AD facilities (Blumensaat and Keller, 2005).

The use of membranes for biological processes, referred to as membrane bioreactor, is another interesting application of the innovative digester strategy for biogas production. Membrane bioreactor applications have been reported to be effective in protecting the microorganisms from the toxic effect of substrate and product inhibition (Youngsukkasem et al., 2013). Youngsukkasem et al. (2013) reported that using a membrane bioreactor enabled biogas production from a substrate with 3% D-limonene at a loading rate of 15 g-COD/L, while the control digester with free cells failed at 7.5 g-COD/L loading. The use of a membrane is also a suitable option for handling inhibition caused by ammonia accumulation. The use of a hollow fiber membrane was reported to successfully reduce the free ammonia nitrogen concentration by about 70% (Lauterbück et al., 2012). The ability of the membrane bioreactors to shield the microorganisms from inhibitors means that the pretreatment of inhibitory feedstock and the costs associated with it could be avoided.

4.3. Co-digestion strategy

Mono-digestion of the recalcitrant feedstocks, highly protein-rich substrates, or feedstocks with harmful compounds often results in a slow process and a low biogas yield. These restrictions can be overcome by the co-digestion of different feedstocks with an appropriate mixing ratio, considering the C/N ratio, inhibitors, feedstock biodegradability, and total solid content. This is an effective strategy for reducing the ammonia inhibition during the digestion process since it aims at favoring synergisms, dilutes harmful compounds, optimizes the biogas production, and increases the digestate quality (Mata-Alvarez et al., 2014). Comino et al. (2010) investigated the co-digestion of chopped crop silage with cow manure at different feeding rates. It was observed that feeding with 70% VS of cow in the feedstock increased the methane yield by 109% compared to manure mono-culture. Co-digestion of various substrates (manure, slaughterhouse wastes, municipal solid wastes, and crop residues) at different mixing ratios was investigated by Pagés Díaz et al. (2011). Synergic effects were reported, giving rise to up to 43% more methane yield, compared to that calculated from the methane yields of individual feedstocks. Paper tube residuals added to a nitrogen-rich substrate mixture used in an industrial digestion plant was also reported as having stabilizing effects on the digestion process (Teghammar et al., 2013). Paper addition prevented the accumulation of VFAs, increased the methane yield by 15–34%, and decreased the hydraulic retention time by five days. Considering the fact that easily degraded feedstocks are not highly available and the problems associated with pretreatments, co-digestion is a good strategy to increase the amount of produced biogas, while avoiding the costs and challenges associated with the pretreatments. Nevertheless, more research is necessary in this area for a better understanding of the appropriate mixing ratios, interaction effects, and the impact of co-digestion.

Another benefit of co-digestion is the reduction of ammonia accumulation when feedstocks with high protein content are being digested. Zeshan et al. (2012) reported that adjustment of the C/N ratio to 32 resulted in 30% less ammonia concentration when food wastes, fruit and vegetable wastes, garden and paper wastes were co-digested in a dry digestion process.

4.4. Bio-augmentation strategy

Biological pretreatment of lignocellulose biomass and keratin-rich wastes has been reported to be successful thereby, improving the biogas production (Forgács et al., 2011a; Patinvoh et al., 2016; Rouches et al., in press). Nevertheless, the need for two-stage processes or for pretreatment makes these options unfeasible for industrial applications. A promising option is bio-augmentation with anaerobic cellulolytic bacteria and with keratinase enzymes to enhance hydrolysis and improve the biogas yield, which has previously been demonstrated by several researchers. Peng et al. (2014) applied the bio-augmentation strategy to improve the hydrolysis of wheat straw using Clostridium cellulolyticum. According to the results, the biochemical methane potential of wheat straw was improved. Bio-augmentation with 10% inoculation of Acetobacteroides hydrogenigenes on the anaerobic digestion of corn straw has also been reported (Zhang et al., 2015) leading to an increase of 19–23% of the methane yield. Forgács et al. (2013) investigated the co-digestion of the organic fraction of municipal solid waste (OFMSW) with chicken feathers bio-augmented with savinase, an alkaline endopeptidase (0.53 ml/gVSfeathers). The addition of this anaerobic enzyme resulted in a methane yield of 0.485 Nm³/kgVS/d with stable reactor performance, while the process without bio-augmentation resulted in a low yield and accumulation of un-degraded feathers.

Another bioaugmentation strategy that could be helpful in handling the inhibitory feedstocks and some inhibitory byproducts formed during the pretreatment is the use of flocculating bacteria (flocs). Flocculation has been shown as an effective way for yeast cells to overcome the inhibitory effect of some by-products (e.g., furfural), which are formed during the pretreatment of lignocellulosic biomass (Westman et al., 2014). The formation of the local high cell density by flocs allows the outer cells to shield the cells inside the flocs from the inhibitory surrounding, thus, allowing the flocs to utilize the biodegradable content of the feedstock. In another similar work, flocculating cells were used by Najafpour et al. (2006) to treat POME with a pH of between 3.8–4.4, which could otherwise be lethal to methanogens. Apart from pH, ammonia toxicity can also be handled by using floccs. Koster and Lettinga (1988) studied the anaerobic digestion of potato juice at extreme ammonia concentrations using flocs, showing that methanogenensis can occur even at 11.8 g ammonia-nitrogen/L concentration. The flocs were adapted to a high concentration of ammonia nitrogen, and after the adaptation processes the maximum tolerable ammonia concentration was 6.2 times higher than the initial toxicity threshold level. Bioaugmentation, either in the form of addition of new microbial community or the formation of high local cell density (flocs), is a promising strategy for possible industrial applications utilizing recalcitrant and inhibitory feedstocks for biogas production.

5. Conclusion

The feedstock for biogas production may contain indigestible, hard-to-digest, and inhibitory compounds. Therefore, there is a need for pretreatment or other innovative methods in order to facilitate the biological digestion. The indigestible materials can be treated by a combined gasification-fermentation process. Moreover, hard-to-digest compounds such as recalcitrant lignocelluloses or keratin can be pretreated by thermal, chemical, physicochemical, or biological methods. In addition, feedstock with inhibitors can be pretreated by several methods such as steam explosion, or non-pretreatment methods developed to facilitate their digestion, such as innovative digesters and/or bio-augmentation.

References

Cost effective dry anaerobic digestion in textile bioreactors: Experimental and economic evaluation. *Bioresource Technology* 245(Part A): 549-559
Cost effective dry anaerobic digestion in textile bioreactors: Experimental and economic evaluation

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1. Introduction

Solid wastes from agricultural, municipal and industrial activities are major sources of environmental pollution. Large volumes of these wastes are being generated and are increasing immensely due to population growth and high consumption rate. Therefore, processing of solid wastes for biogas production is essential that other waste management options which are both environmentally friendly and economical are explored. Biogas production through anaerobic digestion is an interesting waste management option for handling the organic fraction of solid waste, as biogas production usually leads to reduced pollution and increased energy production. Studies have shown that anaerobic digestion of these wastes is sustainable and has a great advantage over aerobic treatment because of its improved energy balance (Mata-Alvarez et al., 2000). In addition to biogas production, digestate residue from anaerobic digestion usually contains high content of phosphate and nitrogen, especially in the form of ammonium which is available for plants; it also contains other macro-nutrients and trace elements essential for plant growth (Makádi et al., 2012).

Anaerobic digestion of organic wastes is carried out generally through wet (feedstock with solid concentration between 0.5% and 15%) (Li et al., 2011) and dry (feedstock with solid concentration greater than 20%) (Bolzonella et al., 2003) anaerobic digestion processes. When treating solid wastes in wet anaerobic digestion processes, fresh and recycled water must be added while large reactor volumes, high energy for heating and costly dewatering process of the digestate is required. Therefore, processing of solid wastes for biogas production through dry-AD is a better option since they usually have low moisture content. However, for enhanced performance of dry-AD process, a suitable reactor is required; considering the substrate composition, amount of substrate to be treated, and process economy of the reactor (Patinvoh et al., 2017). It also requires an appropriate technology for operation. In this vein, several continuous and batch reactors for dry-AD processes have been employed such as plug flow reactors (Deng et al., 2016; Patinvoh et al., 2017) for continuous dry-AD processes, and completely mixed reactor (Guendouz et al., 2010) for batch dry-AD processes. However, some of these reactors required expert design and constructions, constant monitoring, high capital and operational cost.

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The most commonly used reactors in developing countries are fixed-dome reactors, floating-drum reactors and polyethylene tubular reactors (usually modelled as plug flow reactors) (Rowse, 2011). The cost of a fixed-dome reactor is relatively low and the reactor is usually constructed underground which makes it less sensitive to seasonal temperature changes but this reactor requires high technical skill for construction (Kossmann et al., 1999). It is also prone to leakages (Kossmann et al., 1999; Rajendran et al., 2013) (porosity and cracks) and utilization of gas produced is less effective as the gas pressure fluctuates substantially. Floating-drum reactor is easy to operate, it provides gas at constant pressure but the steel drum is relatively expensive and requires rigorous maintenance (Kossmann et al., 1999). It requires constant removal of rust and regular painting to avoid gas leakage, this reactor is not also suitable for fibrous substrates because the gas-holder can get stuck in the resulting floating scum (Kossmann et al., 1999). Hence, there is a need for reactors that are robust in nature, easy to maintain, suitable for dry digestion processes and cost effective. An innovative textile-based bioreactor which is durable, cost effective and easy-to-operate was developed for biogas production, and it was found to be effective and economical for wet anaerobic digestion of organic fraction of municipal solid wastes (Rajendran et al., 2013).

In this work, the potential of dry anaerobic digestion in the textile-based bioreactor was studied in batch process, and its technical and economic aspects were evaluated. Manure bedded with straw was used as an example of the substrate abundantly available at farms. Repeated batch digestion processes were investigated, the TS in the reactor was gradually increased in order to acclimatize the inoculum to the substrates under solid state condition and thereafter increase the amount of wastes treated. The residue after biogas production was analysed to check its suitability as bio-fertilizer. Additionally, the economic analysis was carried out to evaluate this technology.

2. Materials and methods

2.1. Bioreactor

A new design of innovative textile reactor at laboratory scale was developed and supplied by FOV Fabrics AB (Borås, Sweden). It was made of advanced textiles and sophisticated coated polymers which makes the bioreactor durable, resistant to digesting bacteria and chemicals, easily transportable and highly resistant to UV light and high temperature (up to 80 °C) (Rajendran et al., 2013). The reactor is made of high quality polyester PVC coated fabrics; it is a horizontal bioreactor of 184 cm length, 54 cm breadth and had a total volume of about 90 L. The bioreactor was maintained at 25 °C by circulating water through water jacket. This bioreactor had an air tight zip which was opened for feeding and closed after feeding; it also had an opener for gas outlet. The reactor was examined for leakages by filling with water and air before starting the experiment. Fig. 1 shows the schematic sketch of the experimental set up together with other accessories, such as gas collection bag, sampling point for biogas compositional analysis and the gas flow meter (Drum-type gas meter, TG 05 Model 5, Ritter, Germany) for measuring the produced biogas volume.

2.2. Substrate and inoculum preparation

Cattle manure bedded with straw and untreated wheat straw was obtained from a farm outside Borås (Rådde Gård, Sweden). During the experimental period, two different batches of the substrates were obtained; the amount of straw in manure was higher in the second batch collected compared to the first batch. The first batch was used for first experiment while second batch was used for the second and third experiments. The manure was shredded manually to reduce the particle size of straw in the manure. The untreated wheat straw was milled to particle size of 0.5–2 mm, after which the feedstocks were characterized.

Inoculum was obtained from a digester treating wastewater sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). The inoculum was filtered through a 2-mm porosity sieve to remove sand, plastic and other unwanted particles after which it was acclimatized for five days prior to use. The inoculum was centrifuged at 7,600 × g using continuous centrifuge to obtain a TS content of 7.0 ± 0.24%. The inoculum from wet fermentation with TS content of 3.53 ± 0.01% was centrifuged in order to reduce its water content since the textile reactor is studied under dry anaerobic digestion. The inoculum after centrifuge having TS and VS content of 7% and 4% respectively was then used for the first batch experiment.

2.3. Experimental procedure

Manure bedded with straw was mixed with required amount of untreated wheat straw to increase the C/N ratio; the C/N ratio was kept between 20:1 and 25:1 throughout the experiment. The amount of untreated straw needed to increase the C/N was calculated according to Eq. (1) (AAFCRD, 2005) and the required amount added is shown in Table 3. During the first digestion process (unacclimatized inoculum), the total feedstock with 22% TS was inoculated with the initial inoculum (7%TS and 4%VS) keeping a volatile solids (VS) ratio (VSinoculum to VSsubstrate) at 1:1 thereby having total TS of 10% in the reactor. A nutrient solution with composition according to Angeldaki...
et al. (2009) was added only at this start-up period and the pH of the mixture was adjusted to 7.45 using 2 M HCl solution. The reactor was maintained at 25 °C by circulating water through water jacket arranged under the reactor as shown in Fig. 1 and there was no mixing inside the reactor during the digestion process. The biogas produced was collected in a gas bag and volume was measured once the gas collection bag was filled. Biogas samples were taken through the gas sampling point for compositional analysis. The first digestion process lasted for 136 days. In order to increase the TS in the textile reactor and investigate the response of bacteria after acclimatization, the second batch digestion (acclimatized low TS) was performed. Second digestion process was performed the same way as in the first one, except that the digestate residue from the first digestion was used as inoculum (7.97%TS and 5.19%VS) and no nutrient was added. The volatile solids (VS) ratio (VS_{inoculum} to VS_{substrate}) was kept at 1:1 and TS of the substrate was increased to 27% thereby increasing the TS in the reactor to 12%. To further increase the TS in the reactor thereby treating more wastes, a third batch digestion process (acclimatized high TS) was also performed. The digestate residue from the second digestion was used as inoculum (9.6%TS and 6.2%VS). The volatile solids (VS) ratio (VS_{inoculum} to VS_{substrate}) was reduced to 0.5 and TS of the substrate was increased to 30% thereby increasing the TS in the reactor to 17%. The compositions in different batch digestion setup are summarized in Table 2.

\[
a = \text{total weight of straw} \\
b = \text{total weight of manure with straw} \\
M_0 = \text{moisture content of straw} \\
M_w = \text{moisture content of manure with straw} \\
N_0 = \text{% nitrogen of straw on dry basis} \\
N_m = \text{% nitrogen of manure with straw on dry basis} \\
R_n = C/N \text{ ratio of straw} \\
R_m = C/N \text{ ratio of manure with straw} \\
R = \text{desired C/N of mixture}
\]

\[
a = \frac{N_0}{N_m} \times \frac{R_n - R_m}{R_m - 1 - M_w} \times \frac{1 - M_w}{1 - M_0}
\]

2.4. Analytical methods

Moisture content, pH, total nitrogen, TS and VS were determined according to biomass analytical procedures (APHA-AWWA-WEF, 2005). Total nitrogen contents were measured using the Kjeldahl method, and the protein content was estimated by multiplying the total nitrogen contents by a factor of 6.25 according to Gunaseelan (2009). The total carbon was obtained by correcting the total dry weight carbon value for the ash content (Haug, 1993; Zhou et al., 2015). The bulk density was determined according to Zhang et al. (2012).

Extractives in untreated straw samples were determined according to NREL protocols (Sluiter et al., 2008) using Soxhlet method with water and ethanol extraction for 24 h. Total carbohydrate and lignin content of extractive free straw samples were determined according to NREL protocols (Sluiter et al., 2011). The extractive free straw samples were air-dried until moisture content was less than 10%. Samples were then hydrolyzed using 72% H_2SO_4 in a water bath at 30 °C for 60 min while samples were stirred every 5 min to ensure uniform hydrolysis, and then a second hydrolysis was performed using 4% H_2SO_4 in an autoclave at 121 °C for 60 min. Monomeric sugars obtained during the hydrolysis were determined by HPLC. Mannose, glucose, galactose, xylose and arabinose were analyzed using Aminex HPX-87 P column (Bio-Rad) at 85 °C and 0.6 mL/min ultrapure water as eluent. Acid soluble lignin (ASL) was determined using a UV spectrophotometer (Libra S60, Biochrom, England) at 320 nm. Acid insoluble lignin (AIL) was gravimetrically determined as residual solid after hydrolysis corrected with ash content. The ash content was determined as the remaining residue after keeping the samples in the muffle furnace at 575 °C for 24 h.

The biogas volume was measured with Drum-type gas meter (TG 05 Model 5, Ritter, Germany), and then it was corrected for reporting at standard temperature and pressure (0 °C, and 1 bar) using ideal gas law. The methane composition of the produced biogas was determined using a GC (Perkin-Elmer, USA) equipped with a packed column (6′ × 1.8 OD, 80/100, Mesh, Perkin Elmer, USA), and a thermal conductivity detector (Perkin-Elmer, USA), with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. A 250-μl pressure-lock gas syringe (VICI, precious sampling Inc., USA) was used for taking samples for the gas composition analysis. The degree of digestion was calculated using Eq. (2) according to Schnürer and Jarvis (2010).

\[
\text{Degree of Digestion} = \frac{((TS_{in} + VS_{in}) - (TS_{out} + VS_{out}))/((TS_{in} + VS_{in}))}{100}
\]

2.5. Digestate analysis

The digestate residue was centrifuged at 5,000 × g for 10 min and the liquid fraction was passed through 0.2 μm filter prior to analysis. 0.2g of the solid fraction was dissolved in 2 ml concentrated HNO_3 (65%), 0.5 ml concentrated HCl (37%), 0.1 ml concentrated HF (48%) and 1 ml H_2O_2 (30%). Thereafter, it was digested at 200 °C (800 W) for 20 min in the microwave oven. Dissolved samples were then diluted with milli-Q water to 25 ml. The available heavy metals, trace elements and macro-nutrients in both the liquid and solid fraction of the diges-tate were quantified using microwave plasma-atomic emission spectrometer (Agilent Technologies 4200 MP-AES). The liquid fraction of the digestate was analyzed for ammonium nitrogen concentration using Ammonium 100 test kit (Nanolor, MACHEREY-NAGEL GmbH & Co. KG, 10 Germany) and concentration measured using Nanolor 500D Photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany).

2.6. Economic analysis

The annual cost of producing biogas or any of the fermentative biofuels in US$/Volume can be estimated using Eq. (3) (Bergeron, 1996). A 15-year biogas production lifetime was assumed for the textile bioreactor (Rajendran et al., 2013). The interest rate (i) used for the analysis was 7.8% which is the average deposit interest rate in Nigeria (a tropical country) from 2010 to 2016 (World Bank, 2017a).

\[
\text{Annual biogas production cost} = \frac{FC \times Y + (ACC + OC) \times Ye \times EC}{1 + Ye \times EC}
\]

where FC = feedstock cost ($/tonne), Y = yield (Nm^3/tonne), ACC = annual capital cost ($/Nm^3), OC = capital cost, i = interest rate, OC = operating cost ($/Nm^3), Ye = electricity yield (kWh/L), EC = electricity credit ($/kWh), n = 15 (years).

Economic analysis was performed on the cost of replacing agricultural solid waste generation by collection through waste management providers and by composting with dry-AD at three different dry-AD scenarios which are: no acclimatization of the inoculum using solid waste with a density of 542 kg/m^3 (scenario A), short acclimatization (136 days) of the inoculum using solid waste with a density of 542 kg/m^3 (scenario B) and longer acclimatization (232 days) of the inoculum using solid waste with a density of 542 kg/m^3 (scenario C). The calculations were performed at three different waste generation levels: 3 tons/year for a typical small scale farm with animal husbandry and vegetable cultivation or 2 mature cows (Ho et al., 2013), 5200 tons/year for a medium mechanized farm or over 100 mature cows and 51,000 tons/year for a large scale mechanised farm or over 1000 mature cows (Chen, 2016; Hoornweg et al., 1999). The unit cost of composting from a study in Taiwan (which is similar to many tropical or...
subtropical country) was $67/ton for highly mechanized privatized large scale compost production facility, $97/ton for mid scale and $404/ton for small scale manual government affiliated composting facility or farms (using a conversion factor of 1 NTS = 0.033 and 53% conversion of waste to compost) (Chen, 2016; Hoomweg et al., 1999). For the waste collection by waste managers option, the estimated cost of collecting waste for lower mid income countries in 2010 was between $30–$75 per ton of solid waste (World Bank, 2016). Additionally farms that employ waste disposal by waste managers also have to purchase compost or organic fertilizer (Ruggieri et al., 2009) and this cost an additional $211–$356 per ton (Chen, 2016), making the total cost handling solid waste by waste managers to be from $241 to $431 per ton.

The annual profit (AP) was calculated by subtracting the annual cost of biogas production (Eq. (3)) from the annual cost of handling waste through other means plus the revenue generated from biogas sales as shown in Eq. (4);

$$ AP = \text{Annual cost of waste handling} - \text{Annual revenue from biogas} $$

The revenue (R) from biogas was gotten by multiplying the current commercial selling price of natural gas of $0.222/1000-Nm³ (Selling price in February 2017) by the annual methane production from the dry anaerobic digestion divided by the methane fraction in natural gas (95%) (EIA, 2017). The revenue calculations (Eq. (5)) were done using the methane production values for the experiments performed.

$$ R = \text{Vol of methane produced} \times \text{Volume of methane produced} \times \text{Methane fraction} \times \text{Price of methane} \times \text{Number of years} $$

The payback period (PBP) was calculated using Eq. (6), where the initial project delay time was the time needed for completing the first anaerobic digestion batch. The net present value (NPV) was calculated using Eq. (7), with the profit from the first year different from those of the other years, and the internal rate of return which makes the NPV zero was also calculated.

$$ PBP = \frac{CC}{AP + \text{Initial project delay time}} $$

$$ NPV = C_0 + \sum_{n=1}^{\infty} \frac{AP}{(1 + i)^n} $$

where C0 = the initial capital investment.

3. Results and discussion

The batch dry anaerobic digestion (dry-AD) of manure bedded with straw was carried out in a textile-based bioreactor. The reactor is built from textile (Rajendran et al., 2013) and has undergone drag, tears and pressure testing prior to the experimental study and was found very durable. It was used successfully for over 324 days without any gas leakage or major maintenance. The first batch anaerobic digestion of the feedstock took longer degradation time and the methane yield was slightly lower. Subsequent batch digestion processes resulted to improved methane yield and reduced degradation time. The digestate after biogas production was analyzed as supplement for soil deficient of major nutrients. Then economic analysis of replacing waste collection by waste managers or composting with dry-AD in a textile bioreactor was carried out using payback period, net present value and internal rate of return as profitability criteria. A sensitivity analysis of what effect changes in key cost and waste generation factors could have on the profitability of dry-AD in the textile bioreactor was also investigated.

3.1. An industrial concept for dry-AD in rural areas

The results obtained in this work would be beneficial for rural areas, farmers, some industrial applications and developing countries where a cost-effective and simple solution is needed. The solid waste with higher TS is treated while generating energy and bio-fertilizer. Dry-AD of solid wastes in the textile-based bioreactors function on a simple principle (Fig. 2a) as a few reactors should run in parallel. Fresh solid wastes are mixed with inoculum (needed only at the initial stage), fed to the bioreactor (with shovel for small scale or tractor/pumps for large scale) and then the bioreactor is closed. As the materials inside the bioreactor have higher TS, and it is not mixed, it has low heat transfer so it can perform better than wet digestion in colder climates. However, it can be heated underneath to attain desired temperature in cold
regions, but this is not needed for tropical regions. The bioreactor is closed until degradation time is completed while gas produced is collected continuously in a gas holder. When there is no more gas production, the bioreactor is opened and a portion of the digestate will be pumped or moved for storage in a separate bioreactor, while a portion of the digestates remains inside the bioreactor, mixed with fresh wastes and the bioreactors is closed and sealed for the next run of gas production. The number of the bioreactors needed in parallel depends on the feeding time and the gas production duration in each batch. For example, if 3 months would be the gas production period in each batch and 15 days would be enough for emptying, and refilling the bioreactors, 6 bioreactors can work in parallel to fulfill continuous receiving of the fresh wastes. The volume of the textile bioreactor needed to handle different amount of waste is shown in Table 2, and they can be arranged as shown in Fig. 2a.

3.2. Feedstock characterization

The characteristics of the feedstocks used in this work are shown in Table 1. The result showed that the substrates have different moisture content at different batches, probably because they were collected under different storage conditions. The C/N of the manure bedded with straw was the same but the C/N ratio of wheat straw was considerably different from one batch to another. The variation in the C/N ratio of wheat straw could be due to the age of the straw; the younger the tissues of the straw, the lower the C/N ratio (Hicks, 1928). Cellulose and hemicellulose content of wheat straw were similar for the two batches. The cellulose was between the range of 39–43% and hemicellulose was between the ranges of 28–32% but not all these carbon will be accessible during the anaerobic digestion process. The manure bedded with straw collected has a total solid content between 18% and 26% which makes it suitable for dry digestion compared to other means of handling. This waste also has a C/N of 19:1 which means it can be used directly by farmers for the digestion process; the C/N can also be adjusted slightly using untreated wheat straw to enhance the buffer capacity of the process and also reduce the possibility of substrate or product inhibition (Wang et al., 2011).
Fig. 2b shows the cumulative methane production for the three (3) batch digestion processes during the whole experiment and the methane production rates were estimated from the cumulative methane production as shown. For the first digestion process (22% TS of feedstock; 10% TS in reactor), there was a lag phase of 10 days and after wards the methane production increased steadily indicating the beginning of the exponential growth. The digestion process lasted for 136 days to 92 days. Furthermore, the methane production rates increased with gradual increase in the TS which shows gradual acclimatization of microbial communities to the feedstock resulting in an increase in methane yield by about 13% compared to that during the first digestion, while the degradation time was reduced from 136 days to 96 days. After a long-term acclimatization of 232 days (first and second digestion), the VS ratio for inoculum to feedstock was reduced from 1 to 0.5 thereby increasing the TS in the reactor to 17%. The biogas production started almost immediately even here, although there was a slight reduction in the methane yield at the beginning compared to that during the second digestion. Then, there was steady increase in the methane yield resulting in about 58% increase compared to that obtained in the first digestion. Accordingly, the degradation time was reduced from 136 days to 92 days. Furthermore, the methane production rates increased with gradual increase in the TS which shows gradual acclimatization of microbial communities to the conditions in the reactor and probably new predominant microbial community for high solid-state digestion were formed (Li et al., 2013). However, the degree of digestion was low; only 29% of the organic matter was broken down and converted to biogas during the digestion period. This could be due to lack of internal mixing and slow degradation rate of the feedstocks (manure with straw and untreated wheat straw). An approximate degree of digestion of 35% has been reported for cattle manure (Schriener & Jarvis, 2010) which is low compared to readily degradable substrates. The residue can undergo post storage stage where biogas production can continue over a long period of time.

The results showed that farmers can only treat small volume of wastes at the beginning which will also result to low biogas yield and longer degradation time. Then, the volume of wastes can be increased and subsequent digestion processes will results to higher methane yield and shorter degradation time.

Table 2
Composition and experimental results at different batch digestion setup Volume and cost of textile reactor needed for the different scenarios and scales.

<table>
<thead>
<tr>
<th>Setup</th>
<th>Feedstock (g)</th>
<th>TS (%)</th>
<th>Reactor Mixture TS (%)</th>
<th>Reactor Mixture VS (%)</th>
<th>Cumulative Methane yield (Nml/gVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Manure with straw</td>
<td></td>
<td>Wheat straw</td>
<td>C/N</td>
<td>In</td>
</tr>
<tr>
<td>Unacclimatized inoculum (inoculum)</td>
<td>3478</td>
<td>235</td>
<td>17425</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>Acclimatized (Low TS)</td>
<td>5000</td>
<td>86</td>
<td>20617</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>Acclimatized (High TS)</td>
<td>7021</td>
<td>473</td>
<td>14516</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Volume of textile reactor (m³)</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost of textile reactor ($)</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Setup</th>
<th>Feedstock (g)</th>
<th>TS (%)</th>
<th>Reactor Mixture TS (%)</th>
<th>Reactor Mixture VS (%)</th>
<th>Cumulative Methane yield (Nml/gVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 tons/year</td>
<td>Unacclimatized inoculum</td>
<td>5200 tons/year</td>
<td>51000 tons/year</td>
<td>2</td>
<td>915</td>
<td>3 tons/year</td>
</tr>
<tr>
<td>3 tons/year</td>
<td>Acclimatized (Low TS)</td>
<td>237 367</td>
<td>2 094</td>
<td>2 420 208</td>
<td>16 615 659</td>
<td></td>
</tr>
<tr>
<td>3 tons/year</td>
<td>Acclimatized (High TS)</td>
<td>1 871</td>
<td>1 487 124</td>
<td>10 209 675</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Dry basis.

Standard deviation based on at least duplicate measurements.

* Dry basis; ND = Not determined; C/N = carbon nitrogen ratio.
AD has not yet been reported in literature. In this work, economic replacing composting or waste collection by waste managers with dry-generation (Trendewicz & Braun, 2013). However, economic analysis of lands are mostly acidic. The liquid fraction of the digestate has higher phosphorus content of the digestate is low probably part of the phosphorus has been lost during the digestion process (Möller & Müller, 2012) and as such phosphorus or phosphate can be added as supplement to avoid the internal rate of return. The economics associated with replacing composting and waste collection by waste managers with dry anaerobic digestion was investigated for three different dry digestion scenarios; no acclimatization of the inoculum and solid waste with a density of 907 kg/m³ (scenario A), short acclimatization of the inoculum and solid waste with a density of 542 kg/m³ (scenario B) and longer acclimatization of the inoculum and solid waste with a density of 542 kg/m³ (scenario C). The analyses were performed at three different scales corresponding to solid waste generation from typical small, mid and large scale farms.

The volume of the textile bioreactors needed for the different scales and scenarios were calculated as discussed in Section 2.6 and are shown in Table 2. How the reactor volumes can be used for gathering waste, dry-AD, digestate storage and biogas storage is shown in Fig. 2a and was discussed in Section 3.1. Building on that, this is how the reactor volumes will be allocated for different purposes: For the first anaerobic digestion batch; 6% (scenario A), 16% (scenario B), 32% (scenario C) of the total reactor volume is needed for storing the daily produced farm waste prior to dry anaerobic digestion at all scales, while 6% (scenario A), 10% (scenario B), 16% (scenario C) of the total reactor volume is needed for storing the digestate after the completion of the dry-AD. For the subsequent batches 6% (scenario A), 10% (scenario B), 16% (scenario C) of the total reactor volume would be needed for storing the daily produced waste, while 6% (scenario A), 10% (scenario B), 16% (scenario C) of the total reactor volume would be needed for storing the digestate after the dry-AD. Details on how to perform this kind of calculations and optimal reactor sizing can be found in literature (Mährert & Linke, 2009). The investment cost of the textile bioreactor (Table 2) were calculated using; $150/volume for small scale (in volumes of 2 m³) (Rajendran et al., 2013), $100/volume for mid-scale (in volume in 100s of m³) and $70/volume for large-scale (volume in 1000s of m³) (Osadolor et al., 2014). The total capital investment needed for dry-AD for the different scenarios and scales with the annual cost associated with managing the waste either through composting or through waste managers is shown in Table 4.

### 3.4. Digestate quality

The pH of the digestate was slightly alkaline as shown in Table 3; this will increase the buffering capacity of the soil as many agricultural lands are mostly acidic. The liquid fraction of the digestate has higher nitrogen and potassium content compared to the solid fraction as shown in Table 3. Möller et al. (2010), also reported high nitrogen and potassium content in liquid fraction after separation of the biogas effluent. The digestate (liquid fraction) contains an average of 2.63 g/L of total potassium (an average of 2.74 g/L) which is also a major macronutrient needed for plant growth. However, the phosphorus content of the digestate is low; the concentration did not exceed the quality standards for phosphorus or phosphate can be added as supplement to avoid phosphorus deficiency in the soil or the digestate supplied to soil lacking majorly nitrogen and potassium. Additionally, concentration of heavy metals (Cadmium, Chromium, Nickel, Zinc and Copper) in the digestate is low; the concentration did not exceed the quality standards; retain nutrients and avoid leaching. Therefore, the whole digestate can be used as bio-fertilizer or the solid fraction post treated using aerobic composting process thereby closing the production cycle (Fouda, 2011; Meissl & Smidt, 2007; Poggi-Varaldo et al., 1999).

### 3.5. Economic evaluation

For biogas to be used as a means for handling manure and straw waste, it has to be more profitable than other possible methods of handling the waste. Several techno-economic analyses have been done in comparing the profitability of biogas production against other methods such as; replacing kerosene or LPG utilization with biogas at household level (Rajendran et al., 2013), biogas for electricity generation (Gebrezgabher et al., 2010), biogas for combined heat and power generation (Trendewicz & Braun, 2013). However, economic analysis of replacing composting or waste collection by waste managers with dry-AD has not yet been reported in literature. In this work, economic profitability was measured by the payback period, net present value and the internal rate of return. The economics associated with replacing composting and waste collection by waste managers with dry anaerobic digestion was investigated for three different dry digestion scenarios; no acclimatization of the inoculum and solid waste with a density of 907 kg/m³ (scenario A), short acclimatization of the inoculum and solid waste with a density of 542 kg/m³ (scenario B) and longer acclimatization of the inoculum and solid waste with a density of 542 kg/m³ (scenario C). The analyses were performed at three different scales corresponding to solid waste generation from typical small, mid and large scale farms.

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Digestate residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.01-8.06</td>
</tr>
<tr>
<td>Total carbon (%)</td>
<td>34.36-36.23</td>
</tr>
<tr>
<td>TS (%)</td>
<td>7.97-13.39</td>
</tr>
<tr>
<td>VS (%)</td>
<td>61.84-65.15</td>
</tr>
<tr>
<td>Unit (Liquid fraction)</td>
<td>2.45-2.8</td>
</tr>
<tr>
<td>Solid fraction*</td>
<td>0.25-0.55</td>
</tr>
<tr>
<td>Total Nitrogen g/L</td>
<td>1.25-1.55</td>
</tr>
<tr>
<td>Ammonium nitrogen g/L</td>
<td>0.19-4.29</td>
</tr>
<tr>
<td>Potassium mg/L</td>
<td>0.03-0.35</td>
</tr>
<tr>
<td>Phosphorus mg/L</td>
<td>10.30-30.54</td>
</tr>
<tr>
<td>Calcium mg/L</td>
<td>15.50-50.50</td>
</tr>
<tr>
<td>Magnesium mg/L</td>
<td>3.35-60</td>
</tr>
<tr>
<td>Copper mg/L</td>
<td>0.13-0.19</td>
</tr>
<tr>
<td>Iron mg/L</td>
<td>5.45</td>
</tr>
<tr>
<td>Zinc mg/L</td>
<td>0.11-0.19</td>
</tr>
<tr>
<td>Nickel mg/L</td>
<td>0.28-0.37</td>
</tr>
<tr>
<td>Chromium mg/L</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cadmium mg/L</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Dry basis.

### Table 4

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Small scale</th>
<th>Mid-scale</th>
<th>Large-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2980</td>
<td>3 518 040</td>
<td>24 257 700</td>
</tr>
<tr>
<td>B</td>
<td>3032</td>
<td>3 518 040</td>
<td>24 957 700</td>
</tr>
<tr>
<td>C</td>
<td>1967</td>
<td>2 318 040</td>
<td>16 557 700</td>
</tr>
</tbody>
</table>

Total annual operation cost needed for biogas production ($)

### Table 5

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Small scale</th>
<th>Mid-scale</th>
<th>Large-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>152</td>
<td>170 007</td>
<td>1 155 007</td>
</tr>
<tr>
<td>B</td>
<td>155</td>
<td>170 007</td>
<td>1 190 007</td>
</tr>
<tr>
<td>C</td>
<td>102</td>
<td>110 007</td>
<td>770 007</td>
</tr>
</tbody>
</table>

Revenue from biogas sales ($)

### Table 6

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Small scale</th>
<th>Mid-scale</th>
<th>Large-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>42</td>
<td>409</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>53</td>
<td>519</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>83</td>
<td>817</td>
</tr>
</tbody>
</table>

Annual composting cost

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Small scale</th>
<th>Mid-scale</th>
<th>Large-scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1200</td>
<td>504000</td>
<td>3417000</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>1250000</td>
<td>12300000</td>
</tr>
</tbody>
</table>

A dense substrate without inoculum acclimatization; B less dense substrate with short inoculum acclimatization; C less dense substrate with long inoculum acclimatization.
3.5.1. Payback period

The payback period for replacing composting and using waste managers are shown in Fig. 3 for the different scenarios considered. The initial project delay time for the first anaerobic digestion batch completion for scenario A was 0.37 years (calculated by dividing the number of needed for batch completion by 365), 0.64 for scenario B and 0.89 for scenario C. Replacing composting or the use of waste managers by dry-AD in the textile reactor always resulted in a payback period less than the proposed project life of 15 years. In addition, it can be observed that the longer the acclimatizing time of the inoculum, the shorter the payback period. Another possible explanation for the reduction in the payback period is that more amount of waste per year is processed in scenario B compared to A, and scenario C processes the most amount of waste per year. Considering the result for small scale,
the payback period for all dry anaerobic digestion scenarios considered were all less than 3.5 years, indicating that using the textile bioreactor for dry digestion is more profitable than composting or waste collection by waste managers for small scale farmers. As the farm scale increased, dry anaerobic digestion in the textile bioreactor would still be the most profitable method of handling solid waste, particularly dry anaerobic digestion after long inoculum acclimatization which had a payback period of 5 years or less for all cases considered.

### 3.5.2. Net present value and internal rate of return

The result for the net present value (NPV) and internal rate of return (IRR) calculated for the different scenarios using an interest rate of 7.8% are shown in Fig. 4. For the first year, the annual profit was multiplied by 0.627 for scenario A (1 – delay fraction used for PBP

### Table 5

Sensitivity analysis on replacing composting with dry anaerobic digestion.

<table>
<thead>
<tr>
<th>Replacing composting with dry anaerobic digestion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Net present value (NPV) ($)</strong></td>
<td><strong>Internal rate of return (IRR) (%)</strong></td>
</tr>
<tr>
<td>Small scale Mid-scale Large scale</td>
<td>Small scale Mid-scale Large scale</td>
</tr>
<tr>
<td>Scenario A 5320 1,733,125 5,948,401</td>
<td>31.4 4.00 3.32</td>
</tr>
<tr>
<td>Scenario B 6152 428,723 1,874,533</td>
<td>10.47 9.48</td>
</tr>
<tr>
<td>Scenario C 6959 1,553,297 9,783,711</td>
<td>20.53 19.31</td>
</tr>
</tbody>
</table>

### Additional Scenarios

| Scenario A 6086 151,842 127,233 39.86 8.7 7.91 |
| Scenario B 6898 1,059,041 6,373,146 50.09 15.38 14.37 |
| Scenario C 7291 1,937,377 12,420,569 67.1 26.02 24.48 |

| Scenario A 4555 1,618,091 12,024,036 25.47 0.26 −0.37 |
| Scenario B 5607 201,595 2,281,603 34.2 6.68 5.95 |
| Scenario C 6627 1,937,377 12,420,569 67.1 26.02 24.48 |

### 20% less textile reactor cost

| Scenario A 5343 1,693,487 5,559,649 31.61 4.20 3.60 |
| Scenario B 6174 467,841 2,429,422 40.57 10.21 9.29 |
| Scenario C 6981 1,591,915 10,162,472 56.15 20.11 18.73 |

### 20% more auxiliary equipment cost

| Scenario A 5298 1,772,762 6,337,153 31.26 3.81 3.05 |
| Scenario B 6130 389,606 1,662,121 40.57 10.21 9.29 |
| Scenario C 6937 1,514,678 9,494,590 56.15 20.11 18.73 |

### 20% less waste generation

| Scenario A 4331 584,030 4,758,721 31.8 4.02 3.22 |
| Scenario B 4994 345,376 1,636,617 41.24 10.49 9.64 |
| Scenario C 5637 1,244,965 7,826,969 57.1 20.55 19.31 |

### 20% more waste generation

A dense substrate without inoculum acclimatization; B less dense substrate with short inoculum acclimatization; C less dense substrate with long inoculum acclimatization.
calculation, 0.36 for scenario B and 0.11 for scenario C. Considering waste disposal through waste managers, irrespective of inoculum acclimatization, dry anaerobic digestion is more profitable as evident by the positive NPVs. In addition, the IRR for all scales and scenarios considered were from 30.1 to 69.6%, implying that dry anaerobic digestion using the textile reactor would remain the most profitable option for all farm scale with or without inoculum acclimatization until the interest rate in that country exceeds the IRR. Currently there are only three countries in the world where the interest rate exceeds 30%, and there is no country with an interest rate greater than 50% (World Bank, 2017b) so the odds of solid waste management by using waste managers becoming more profitable than dry anaerobic digestion in the textile reactor is rear.

Comparing dry-AD to composting (Fig. 4), both the scale of waste generation and the duration inoculum acclimatization influences what option is more profitable. For small scale, irrespective of inoculum acclimatization or the nature of the feed, dry-AD is more profitable than composting as evident by the positive NPV’s and IRR greater than 33%. As the scale of waste generation increases, the profitability of dry-AD in the textile reactor over composting increases with the duration of the acclimatization of the inoculum used, as evident by the increasing positive NPV’s and the increasing IRR from short to long inoculum acclimatization time at all scales. The minimum IRR for replacing composting with dry anaerobic digestion when long acclimatization is used was 19.3%, this indicates that the NPV would remain positive over a high interest rate, with it only becoming negative when the interest rate in that country exceeds the IRR. In the cases where composting was found to be a better option than anaerobic digestion because of negative NPV, the IRR were 4% or less indicating that the NPV at those cases is sensitive to the interest rate assumed for the calculations, if an interest rate of 3% was used for the calculations all the NPV’s will be positive. A 3% interest rate is feasible because there are many countries whose current interest rate are 3% or less (World Bank, 2017b), using the interest rate of any of those countries would make NPV of replacing composting with dry anaerobic digestion positive for all scale with or without inoculum acclimatization.

3.6. Sensitivity analysis

The result from the sensitivity analysis calculations for replacing composting with dry-AD is shown in Table 5. At small scale, the NPVs were insensitive to a ± 20% change in reactor cost, equipment cost and the amount of waste generated. For cases that had a positive or negative NPV in the base scenario, a ± 20 % change in the cost of equipment and amount of waste generated was not sufficient in changing the sign notation of the NPV of any of the base cases. So, the profitability criteria were not sensitive to a ± 20% change in the cost of equipment and amount of waste generated.

The profitability criteria for replacing composting with dry anaerobic digestion at mid and large scale was sensitive to changes in the cost of the reactor when short or no inoculum acclimatization time was used. For the base case, the NPV of using scenario A for replacing composting with dry anaerobic digestion were negative at mid (IRR = 4 %) and large scale (IRR = 3.32%), however, a 20% reduction in the cost of the textile reactor made the NPV positive for both mid (IRR = 8.7%) and large scale (IRR = 7.91%) scenarios. A 20% increase in the cost of the textile reactor resulted in the positive NPV at the base case of scenario B at mid (IRR = 10.47) and large scale (IRR = 9.48) becoming negative for both mid (IRR = 6.68%) and large scale (IRR = 5.95%). The sensitivity of the textile reactor cost for mid- and large-scale scenario A and B cases would mean that using other reactors more expensive than the textile reactor for dry-AD would not be economical when compared with composting. However, replacing composting with dry anaerobic digestion after long acclimatization (scenario C) was not sensitive to a 20% change in the cost of the textile reactor at all scales, as seen from the positive NPV in all cases and high

IRR values ranging from 15.35% to 67.1%. This indicates that dry anaerobic digestion of solid farm waste in the textile reactor after a long period of inoculum acclimatization is a more economical way of handling the waste than composting.

4. Conclusion

Textile-based bioreactor is a cost-effective solution and the technology is simple to operate. It can be accessed easily by developing countries where required expertise may not be available. For small scale farm, irrespective of inoculum acclimatization or the nature of the feed, dry-AD is more profitable than composting as evident by the positive NPV’s and IRR greater than 33%. A long acclimatization period makes dry-AD in the textile reactor more profitable than composting for small, mid and large scale farms when handling solid waste as evident by the positive NPV’s and IRR greater than 19%.

Acknowledgements

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Biogas digesters: from plastics and bricks to textile bioreactor —
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Biogas digesters: from plastics and bricks to textile bioreactor – A review

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Biogas digesters: from plastics and bricks to textile bioreactor – A review

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Abstract

Biogas technology and anaerobic digestion is a well-established process for energy generation but there are still technical and economic barriers to advancement of this technology especially in developing countries. Considering the relatively long retention time of the materials inside the bioreactors, the material of construction of the bioreactor is important for commercial applications and should be selected considering cost, lifespan, effectiveness, design and operation of the bioreactor. The design and operation of particularly household and smaller bioreactors with their challenges are reviewed in this study. A particular attention is made to the new textile bioreactor that are relatively new in the market. The laboratory and pilot scale results shows that the innovative textile bioreactor is a promising technology for global energy generation advancement.

Keywords:

Biogas digesters; materials of construction; plastic bioreactors, textile bioreactors;
1. Introduction

Renewable energy is gaining widespread acceptance; renewables accounted for about 62% of net additions to global power generating capacity in 2016 and majority of renewable heat were supplied by biomass with smaller contributions from solar thermal and geothermal energy (REN21, 2017). Biogas technology has been used quite extensively in developed countries and some developing countries for renewable energy generation thereby reducing environmental impacts of wastes disposal. The technology has improved significantly through support from government and non-governmental organizations (Dahlquist, 2013; Geels & Raven, 2007; Nilsson et al., 2012). However, many countries are still under environmental pressures and energy insecurity which has led to increased interest in biogas technology around the world. This technology is currently the most sustainable way to utilize the energy content of organic wastes while also recycling the nutrients and reducing harmful emissions (Luostarinen et al., 2011). Acceptance of the digestate residue as bio-fertilizer, ban of land-filling and the limitation of incineration of organic waste are possible factors favoring the development of this technology around the globe (Torrijos, 2016).

During the last decades, a number of reactors have been developed and examined for small- and large-scale biogas production, but there are still technical barriers for successful operation of biogas reactors which impede the development of this technology. In addition, the cost of many biogas reactors (capital, operational and maintenance costs) is high; most farmers and many developing countries are not financially buoyant to install such reactors despite increased interest in biogas technology. Therefore, improving energy efficiency worldwide through biogas technology requires innovative strategies and initiatives to motivate investment in biogas production; this will lead to increased economic growth, environmental safety and national security.
Biogas reactor is at the heart of any process plant; its demand and performance are usually the most important factors in the design of a whole plant; and the performance of the reactor is important for economic assessment of the technology as a whole (Peacock & Richardson, 2012). A suitable bioreactor for biogas production should be easy to operate, effective in producing biogas, easy to install and cost effective in order to promote development of biogas technology. Additionally, for an anaerobic reactor to accommodate high loading rates, basic conditions must be met such as high retention of viable microorganisms in the reactor under operational conditions, sufficient contact between viable microorganisms and the feedstock, high reaction rates, and favourable environmental conditions for the microbial communities in the reactor under the operating conditions (Lettinga, 1995). Therefore, for overall effectiveness of the biogas production process, reactors must be selected putting into consideration the design of the reactor, its mode of operation (Peacock & Richardson, 2012) as well as feedstock composition, amount of feedstock to be treated, desired product and process economy of the reactor (Patinvoh et al., 2017a). A biogas reactor must be well designed since the technical conception of a typical biogas plant is towards achieving an optimum biogas yield considering the overall economy of the process (Werner et al., 1989).

This work aims at reviewing conventional biogas reactors and challenges associated with them while introducing an advanced textile-based reactor for biogas production, which is relatively new in the market. Detailed knowledge of the textile bioreactor technology for small- and large-scale biogas plants will contribute to development of biogas technology and aid optimizing its economy.

2. Conventional Biogas reactors

Anaerobic reactors are devices designed for microbial degradation of organic wastes in oxygen-free condition for biogas production; such reactors must be water tight and gas tight. Additionally, biogas reactors must be protected against UV light, chemicals, corrosive gases,
and insulated against extreme weather conditions. There are different types of anaerobic reactors; what is common to them all is that they produce biogas for energy generation and biofertilizer for soil amendment in agriculture. They are, however, differentiated either by mode of operation, solid content, operating temperature, process stages, reactor design or material of construction. Commonly used materials for construction of biogas reactors include steel, stainless steel, bricks, concrete and plastics (Cheng et al., 2013; Cheng et al., 2014a; ISSF, 2012). Major factors for the choice of material are technical suitability, availability, maintenance and cost-effectiveness (Kuria & Maringa, 2008); reactors are designed for small and large scale biogas production.

The size of the biogas reactor depends on the amount of organic wastes to be treated, site location, demand of natural gas and consumption pattern, and technical skill of staff regarding operation of the biogas plant (Samer, 2012). Small scale reactors convert organic wastes such as kitchen wastes, animal manure, human excreta and garden wastes to biogas for household and community use; commonly used reactors are fixed-dome, floating-drum and rubber-balloon (Rowse, 2011). Large scale reactors are typically obtainable in developed countries but also gaining interest in developing countries (DeBruyn et al., 2006) due to environmental pollution and energy poverty. These reactors are used for conversion of large volume of wastes such as municipal solid wastes, agricultural wastes, industrial wastes and sludge from wastewater treatment plant to biogas (Werner et al., 1989). The biogas produced is usually fed into public grid (De Mes et al., 2003) for heating and electricity generation; it can also be upgraded and used as transportation fuel. The most commonly used large scale reactors are plug flow, continuous stirred tank, upflow anaerobic sludge blanket (UASB), and complete mix reactors; these types of reactors are complex in design and operation (usually require skillful personnel).

Search for new technologies to enhance global advancement of biogas technology has led to invention of plastics and textile-based biogas reactors. A short review on conventional biogas
reactors and challenges associated with them will advance the implementation of textile-based bioreactors for small and large scale biogas production.

2.1 Fixed-dome and floating-drum reactors

A typical fixed-dome reactor consists of a cylindrical or hemispherical chamber constructed underground; it is made of bricks and cement (An et al., 1997; Samer, 2012; Sasse, 1988). It has both mixing and overflow tanks connected to the inlet and outlet valves of the reactor respectively. The biogas produced is collected in upper chamber (fixed gas holder) and as pressure increases due to continuous gas production the digestate is discharged through the outlet to the overflow tank. The use of fixed-dome reactors for biogas production is an established technology in many parts of the world especially in China, India, Nepal, Vietnam, Bangladesh, Cambodia, Pakistan and Tanzania (Cheng et al., 2013; Ghimire, 2013).

Fixed dome reactors are commonly used for rural households because of their long lifespan (Ghimire, 2013). However, the technology is expensive (Pérez et al., 2014), the construction is labor intensive and required skilled supervision (Kossmann et al., 1999). It is also prone to porosity and cracks as a result of atmospheric temperature fluctuations or earthquake. Cheng et al. (2014b) reported only 53 % of fixed dome reactors in good operation during a survey of 94 household plants in Nepal; most plants are not functioning well due to plumbing problems, damages at the slurry chamber and cracks in the reactor (Tomar, 1994). Utilization of the gas produced is less effective as the gas pressure fluctuates and possibility of explosion of the fixed gas holder due to excessive biogas pressure (Kuria & Maringa, 2008). Additionally, large fixed-dome plants always require a separate gas holder and an agitator; floating gas holders are sometimes used in cold region which is more expensive (Sasse, 1988).

Floating-drum reactors are similar to the fixed-dome reactors in operation; the major difference with floating drum reactor is the floating drum on top of the reactor that separates gas
production and collection (Shi, 2014). They are made of mild steel and the reactor walls and bottom are made of bricks; the reactor has a moveable gas holder, cylindrical digestion chamber with the inlet and outlet pipes connected (Kumar et al., 2015). These types of reactors are easy to operate and the drum can provide gas at constant pressure. However, the steel drum is relatively expensive and requires constant removal of rust and regular painting to avoid gas leakage (Kossmann et al., 1999). Recently the steel drum is replaced with fiber reinforced plastics gas holders to solve the problem of corrosion but they are more expensive; sometimes high density polyethylene (HDPE) is used (Mostajir et al., 2013).

2.2 Plastic biogas reactors

Construction of biogas reactors from plastics is an alternative option to solve challenges associated with fixed-dome and floating drum reactors; the technology evolved from the need for new materials that are non-corrosive, good insulator, cheaper and easier to construct (Kumar & Bai, 2005).

Flexible and rigid plastics materials have been used for construction of biogas reactors, gas holders, digestate storage and membrane covers; polymeric materials used include polyethylene (PE), polyvinylchloride (PVC), polypropylene (PP), polystyrene (PS), polymethyl methacrylate (PMMA), acrylonitrile butadiene styrene (ABS) and fiber reinforced plastics (Cheng et al., 2013). Several plastic biogas reactors have been developed and tested in countries like China, India Vietnam, Kenya, Bolivia, Bangladesh, Rwanda, Taiwan, Tanzania, Ethiopia, Columbia and Honduras (An et al., 1997; Cheng et al., 2013; Cheng et al., 2014a; GTZ/EnDev, 2010; Nzila et al., 2012; Rakotojaona, 2013). These reactors and challenges associated with them are discussed in subsequent subsections.

2.2.1 Polyethylene tubular reactors
The first plastic reactor was a tubular reactor; an inexpensive red mud PVC reactor of 15 m³ total volume introduced in Taiwan and evaluated by Pound et al. (1981) using plug flow principles; the content of the reactor was unmixed and unheated. This model was later simplified in Ethiopia and then Colombia (An et al., 1997). Polyethylene tubular reactors also known as balloon tubular reactors were introduced within the project supported by FAO and SIDA (college of agriculture and forestry in Ho Chi Minh City) (An et al., 1994). Since 1990s (Rakotojaona, 2013), several of these reactors have been implemented and commercialized for small scale biogas plants due to low cost and simplicity of installation. Biogas produced is either stored in the upper part of the digester (Nzila et al., 2012) or in a similar rubber tubes made from polyethylene material (Nazir, 1991) and used through the gas vent when required. The first-generation plastic reactors are susceptible to mechanical damage, have short lifespan (2 to 5 years), sensitive to sunlight and deteriorate rapidly due to seasonal changes (Kossmann et al., 1999; Nazir, 1991; Nzila et al., 2012).

2.2.2 Plastic tanks

Rigid plastic tanks are developed for biogas production and they are made of hard polymeric materials such as hard polyvinylchloride (PVC), high density polyethylene (HDPE) and modified plastics except reinforced plastics (Cheng et al., 2013). They are composed of two pre-built rigid plastic tanks (Rakotojaona, 2013); the first tank for anaerobic digestion and the second tank for gas storage. They are easy to install, longer lifespan compared to polyethylene tubular reactors and quick biogas production start-up (3 to 4 days) (Rakotojaona, 2013) but they are expensive and not suitable for large scale biogas plants. Currently there are very few plastic tanks installed.

2.2.3 Latest generation plastic reactors

The problem of low reliability (40 %) (Nzila et al., 2012) with the low-cost polyethylene tubular reactors led to modified plastic biogas reactors. Several PE tubular reactors installed in Kenya
were damaged (burst) and as such reactors were modified to PE/PVC; inner layer PE and outer layer UV treated PVC and further strengthen by synthetic mesh to avoid failure due to stress and high pressure (GTZ/EnDev, 2010). The new model of reinforced plastics in China is made of unsaturated polyester resin, gel-coated resin, chopped strand mat and high-quality glass fiber cloth (Cheng et al., 2013). About 200,000 of these modified reactors have been installed in China with volume ranges from 2.5 m³ to 10 m³. Reinforced plastic reactors are developed for biogas production in order to make them weather or UV resistant (Kumar et al., 2015) thereby increasing their lifespan. Research has shown that methane content of biogas produced using reinforced plastics is 15.3% higher than that obtained when concrete reactors are used (Cheng et al., 2013). However, these modified reactors are expensive and floating where underground water lever persist (Cheng et al., 2013).

Reactor covers are used to store gas produced, insulate the reactor and prevent emission of odor and gases such H₂S, CH₄, NH₃, N₂O, CO₂, and volatile organic compound; reactors covers must be able to withstand exposure to moisture, corrosive gases and digestion feedstock (Stenglein et al., 2011). There are new reactor covers made from plastics which are different from conventional fixed and floating covers. Plastic covers are made of high density polyethylene (HDPE), linear low density polyethylene (LLDPE), polyvinylchloride (PVC). Recently plastic covers are made of composites polymeric materials to reduce permeability, reinforced and strengthen the membrane and also to provide protection against UV light and chemicals (Stenglein et al., 2011).

3. Textile biogas reactor technology

3.1 History and development

Plastic biogas reactor technology was introduced to solve some of the problems associated with conventional biogas reactors as discussed in section 2. However, the lifespan of the material is short due to easy degradation and plastic biogas reactors are not robust for large scale
application (Chen et al., 2012; Cheng et al., 2013). GTZ/EnDev (2010) also reported several of biogas plants installed failing due to low quality of plastic reactor bags. These shortcomings led to the development of a textile biogas reactor for reliable biogas production considering efficiency, economy and large-scale application of the reactor. This invention aims at making the biogas technology accessible and attractive to several countries especially developing countries that may not have substantial subsidy support for biogas implementation.

This new biogas reactor is made of textiles which makes it less combustible, resistant to tearing, light weight and environmentally friendly. The textile reactor is UV treated to prevent easy disintegration due to exposure to sunlight; they are also insulated against extreme weather conditions thereby increasing lifespan. Additionally, the reactor is composed of sophisticated coated polymers as a protection against corrosive gases such as H2S. Textile biogas reactors range in size from 1 m³ to 1000 m³ and they can be used for small and large scale biogas plants treating sludge from wastewater treatment plants, industrial wastes, agricultural and organic fraction of municipal solid wastes.

3.2 Laboratory studies

The first textile biogas reactor developed was of a pyramid shape as shown in Fig.1; it was a continuous reactor with a total volume of about 112 L. The inlet and outlet pipes were fixed on opposite sides of the reactor, the gas outlet was fitted on the upper part and opener fixed to the reactor for discharging the digestate residue when desired. The efficiency of this reactor was investigated by Rajendran et al. (2013) for biogas production using synthetic medium and treating organic fraction of municipal solid waste; the reactor was operated at room temperature. The goal of this study was to check the suitability and efficiency of this new reactor since textile material has never been used for constructing biogas reactor. The results showed a stable biogas operation with OLR of 1.0 gVS/L/d yielding 570 L/kgVS/d when the synthetic medium was used. The same yield was obtained when organic fraction of municipal solid waste was treated
with an increasing OLR from 0.1gVS/L/d to 1.0 gVS/L/d; this shows that the reactor was appropriate for effective degradation of the treated waste. Rajendran et al. (2013) also carried out an economic evaluation of the process; the result shows the sum of investment and 15 years operational cost of the reactor was 656 USD which is a positive investment when compared to 1455 USD for subsidized LPG and 975 USD for kerosene.

A horizontal textile bioreactor was thereafter produced for batch anaerobic digestion process as shown in Fig. 2. The reactor has a volume of about 90 L with a gas outlet and a zip for opening and closing the reactor; it was operated at a temperature of 25°C. The potential of this reactor for dry anaerobic digestion was evaluated by Patinvoh et al. (2017b) treating manure bedded with straw at 22 % to 30 %TS of feedstock. Additional goal of this study was to evaluate the technical and economic aspect of this new reactor technology. The reactor was very easy to operate and worked successfully for over 324 days without leakage or major maintenance. Patinvoh et al. (2017b) reported methane yield of 290 NmlCH4/gVS at 30 %TS after long acclimatization of the inoculum and the digestate residue analyzed showed suitability as biofertilizer; this shows there was no reaction between the material of construction and the digestate. The economic evaluation on the experimental results also shows dry-AD with this reactor a profitable method of handling the waste with maximum payback period of 5 years, net present value from $7,000 to $9,800,000 (small to large bioreactors) with internal rate of return from 56.6 to 19.3 %.

3.3 Stress analysis and test for large scale application
A biogas reactor is liable to failure if the gas pressure exceeds a limit that the reactor can withstand; it is therefore important to understand and quantify stress in the reactor (Kaminski, 2005) as a safety measure. GTZ/EnDev (2010) reported some of the plastic biogas reactors installed in Kenya were damaged (burst) due to stress or high pressure in the reactor.
The developed textile biogas reactor is made of unique composite materials to increase the tensile strength of the reactor for large scale application. However, for safety measures the stress associated with this reactor was determined using curvature and numerical analysis by Osadolor et al. (2016); the analysis was performed for large scale application of bioreactors with volume from 100 m$^3$ to 1000 m$^3$. The calculated tensile stress in a 1000 m$^3$ reactor was reported to be 14.2 MPa. The result showed that using textile material can increase the tensile strength more than 14.2 MPa thereby preventing failure while also reducing cost. The strength of the reactor was also tested for large scale application; this reactor was filled up to half of its capacity (Rajendran, 2015) as shown in Fig. 2 and there was no damage to the reactor after the test.

4 Biogas plant in India

India has a long history in development of small scale biogas plants; there were several biogas plants installed in India during the past 30 years. Most commonly used biogas reactors are fixed dome reactors and floating drum reactors of which there has not been much innovation in the design during the past several years. Additionally, these reactors require high technical skill for construction and also prone to leakages. The steel drum is relatively expensive and requires rigorous maintenance; it requires constant removal of rust and regular painting to avoid gas leakage. After studying operations of existing reactors and challenges involved, FOV fabrics developed plug flow textile-based reactors as shown in Fig. 4 which are easy to install and operate, cost effective, portable, modular in nature and easy to maintain. FOV fabrics have successfully installed more than 20 textile reactors for biogas production in India treating food wastes, cow dung, human excreta and mixed feedstocks.

4.1 Process Description

Total volume of the textile reactor is about 100 m$^3$ and the biogas plant operates on continuous process; the complete system of the plant is shown in Fig. 5. Initially the reactor is loaded with inoculum for a start-up and thereafter fed with the substrates. One MT of cow dung (sometimes
food wastes) was mixed with 1 m$^3$ of water to make fluent slurry; 2 m$^3$ of slurry was fed into the reactor everyday through the inlet and the same amount withdrawn. Part of the slurry is recycled back along with fresh cow dung as replacement for water; this is done in order to minimize the volume of water used. The retention time of the process is about 30 days and under optimized condition generates about 40 m$^3$ of biogas per day. Biogas produced accumulates at the upper part of the reactor and flows through the gas vent to a separate gas storage tank and used afterwards for cooking or for power generation using biogas generator. Digestate residue after biogas production is projected to be used as organic fertilizer to improve the quality of agricultural soil thereby generating additional revenue. The projected economic remunerations from the biogas plant are shown in Table 1.

5. Conclusions

Bioreactor design for biogas production are getting better in order to reduce cost and construction time of biogas installations; advanced textile bioreactors are promising technology for dissemination. This will enhance global advancement of biogas technology especially in developing countries.

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Figure Captions

Fig. 1. Schematic sketch of the first generation textile bioreactor (Rajendran et al., 2013)

Fig. 2. Schematic sketch of textile bioreactor for dry-AD (Patinvoh et al., 2017b)

Fig. 3 Strength testing for a pilot textile reactor (Rajendran, 2015)

Fig. 4. Plug flow textile-based reactors installed in India (a) 100 m³ (b) 10 m³

Fig. 5. Scheme of complete textile biogas reactor plant in India
Table 1: Economic estimate of products from the biogas plant in India

### LPG Savings by setting up a 1 Mt/day of cow dung based textile biogas reactor

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual savings on LPG Cylinder</td>
<td></td>
</tr>
<tr>
<td>Number of kg cow dung generated per day</td>
<td>1000</td>
</tr>
<tr>
<td>Gas generated (m³/day)</td>
<td>40</td>
</tr>
<tr>
<td>Number of kg of LPG saved per day</td>
<td>20</td>
</tr>
<tr>
<td>Savings on LPG per day in Rs</td>
<td>Rs 1,600</td>
</tr>
<tr>
<td>Savings in no. of kg of LPG per year</td>
<td>7,300</td>
</tr>
<tr>
<td>Annual Savings on LPG in Rs</td>
<td>Rs 5,84,000</td>
</tr>
</tbody>
</table>

### Electricity Savings by setting up a textile biogas reactor

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual savings on Electricity</td>
<td></td>
</tr>
<tr>
<td>Number of kgs’ cow dung waste generated per day</td>
<td>1000</td>
</tr>
<tr>
<td>Power generated (Kw/day)</td>
<td>40</td>
</tr>
<tr>
<td>Savings on electricity per day in Rs</td>
<td>Rs 400</td>
</tr>
<tr>
<td>Power generated in Kw annually</td>
<td>14,600</td>
</tr>
<tr>
<td>Annual savings on Electricity in Rs</td>
<td>Rs 1,46,000</td>
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</tbody>
</table>

### Savings from organic fertilizer obtained from the reactor

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
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<tbody>
<tr>
<td>Annual savings on Organic Manure</td>
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</tr>
<tr>
<td>Number of liters of diluted waste fed in per day</td>
<td>2000</td>
</tr>
<tr>
<td>Organic manure obtained from bio-slurry per day in kg</td>
<td>160</td>
</tr>
<tr>
<td>Savings on organic manure per day in Rs</td>
<td>Rs 800</td>
</tr>
<tr>
<td>Organic manure obtained from bio-slurry per year in kg</td>
<td>58,400</td>
</tr>
<tr>
<td>Annual savings on Organic Manure in Rs</td>
<td>Rs 2,92,000</td>
</tr>
</tbody>
</table>

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*average cost of commercial LPG is considered at Rs. 80 per kg.

*b average cost of electricity is considered at Rs. 10 per KW.
Fig. 1.
Fig. 2.
Fig. 4.
Storage and mixing

Waste

Water for dilution

Storage and mixing

Biogas

FOV Biogas

Disposal Fertilizer

Cleaning, if required

Storage close to kitchen

Stove and kitchen

Fig. 5.
Dry anaerobic co-digestion of citrus wastes with keratin-rich and lignocellulosic solid organic wastes: Batch vs. Continuous Processes

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Abstract

Dry anaerobic co-digestion of citrus wastes (CW) with chicken feather (CF), wheat straw (WS) and manure bedded with straw (MS) was investigated in batch and continuous processes. Experiments were designed with different mixing ratios considering the inhibitory effect of citrus wastes, C/N ratio, and total solid content of individual feedstocks. With the best mixing ratio (CF:CW:WS:MS) of 1:1:6:0, enhanced methane yield by 14 % was obtained compared to the expected yield calculated according to the methane yields obtained from the individual fractions. The process performance of this mixture was then investigated in continuous plug flow reactors at different organic loading rates (OLR) using feedstock total solid contents of 21 %TS (RTS21) and 32 % TS (RTS32). At OLR of 2 gVS/L/d, methane yield of 362 NmlCH\(_4\)/gVS\(_{added}\) was obtained from RTS21, which is 13.5 % higher than the yield obtained from RTS32 (319 NmlCH\(_4\)/gVS\(_{added}\)). However, it was not possible to achieve a stable process when the OLR was further increased to 3.8 gVS/L/d, which resulted in high total VFAs concentrations, VFA/alkalinity ratios and a decline in the biogas production.

Keywords:
Solid wastes; Dry co-digestion; Batch process; Continuous process; Process stability
1. Introduction

Organic fraction of solid wastes from agricultural and industrial activities degrades over a period of time. This process releases CH₄, CO₂ and other gases into the atmosphere; leading to global warming. Global emissions from solid wastes are estimated between 20 to 40 million tonnes of CH₄ per year (Jensen and Pipatti 2002). Waste streams like chicken feathers, solid manure and wheat straw account for the largest potential feedstock as sources of biomass energy and are readily available. Converting them into energy through anaerobic digestion is a feasible option with low capital investment. Chicken feather is a good potential source of nitrogen with over 90 % of crude protein content (Patinvoh et al. 2016; Salminen et al. 2003) and wheat straw is a potential source of carbon with a composition of about 35 - 43 % of cellulose and 21 – 32 % of hemicellulose (Kabir et al. 2015; Karthikeyan and Visvanathan 2013; Patinvoh et al. 2017c). Additionally, fruit wastes contain large amount of organic matter that are easily degraded; about 78.3 % carbohydrates, 8.5 % protein, and 6 % fat (Schnürer and Jarvis 2010), which make them potential biomass source for energy production. Over 40 million tons of industrial citrus wastes are generated globally (Sharma et al. 2017).

Notably, because of the recalcitrant structure of most agricultural solid wastes such as chicken feathers and wheat straw, they degrade slowly: resulting in low biogas. Several research works have been conducted to improve methane yield from keratin-rich and lignocellulosic solid wastes by chemical pretreatment (Aslanzadeh et al. 2011; Bolado-Rodríguez et al. 2016; Chandra et al. 2012; Forgács et al. 2014; Lohrasbi et al. 2010), steam explosion pretreatment (Theuretzbacher et al. 2015), and biological pretreatment (Forgács et al. 2011; Patinvoh et al. 2016). Additionally, the presence of peel oil with D-limonene as main ingredient in citrus wastes inhibit microbial growth (Mizuki et al. 1990), which leads to low biogas yield. This inhibition challenges in citrus wastes for biogas production have been addressed by leaching (Wikandari et al. 2015), steam explosion pretreatment (Forgács et al. 2012) and membrane technology (Wikandari et al. 2014). Although these methods have been reported effective in solving problems with low biogas yield, there are still problems of high energy consumption, expensive technology, harmful environmental by-products, and release of inhibitors during some of the pretreatment methods (Patinvoh et al. 2017b). Other problem associated with the digestion of these waste streams is the high nitrogen (i.e. chicken feather) or carbon (i.e. straw and citrus waste) content leading to nutrient imbalance when they are digested as sole substrate (mono-digestion).
In view of the fact that easily degraded feedstocks are not readily available and the problems associated with some of the pre-treatments methods, co-digestion can be an alternative strategy for improving the biogas yield from these types of solid wastes. This concept favours synergisms, dilutes harmful compounds, optimizes the biogas production, and increases the digestate quality (Mata-Alvarez et al. 2014). Co-digestion of these solid wastes with an appropriate mixing ratio will lead to a balanced nutrient supply and will enhance the buffering capacity of the digestion system. In choosing appropriate mixing ratio, it is important to consider the C/N ratio, biodegradability, possible inhibitors and total solid content of individual feedstocks (Patinvoh et al. 2017b). Although nitrogen is an essential nutrient for the microorganisms, its excess leads to the formation of ammonia during the degradation; which inhibits microbial growth at higher concentrations (Bachmann 2015; Frieh et al. 2010b). Additionally, when the amounts of easily degradable carbon is too high, the process tends to be susceptible to acids accumulation (Liu et al. 2008). Co-digestion of crop residues (i.e. carbon-rich) with livestock residues (i.e. nitrogen-rich) could balance the C/N ratio, thereby improve the biogas yield (Comino et al. 2010a). A considerable wider C/N ratio, e.g. between 10:1 and 30:1, has been reported in the literature for a stable digestion process (Frieh et al. 2010a; Pagés Díaz et al. 2011). Nevertheless, the composition of feedstocks (Gunaseelan 2007), the availability of carbon and nitrogen to the microbial community (Herrmann et al. 2016) and different operation parameters during the digestion process are also significant for an effective anaerobic digestion.

Furthermore, with a total solid (TS) greater than 20 %, there has been great concern about the large amount of water usually used while treating these solid wastes and which consequently leads to huge amounts of digestate residue with high water content to handle. To combat this problem, recent studies have been focused on dry anaerobic digestion of these waste streams both in pilot and large-scale applications (Baere 2012; Patinvoh et al. 2017a; Patinvoh et al. 2017c; Tyrberg 2013; Zeshan et al. 2012).

The main purpose of this work was to investigate co-digestion of citrus wastes with chicken feathers, wheat straw and manure bedded with straw in dry anaerobic digestion process. Experiments were designed with different mixing ratios; the synergetic and antagonistic interactions, which might arise in different mixtures, were evaluated through anaerobic batch digestion assays. In addition, the long term effects in process performance were investigated for the best mixture indicating synergy in continuous plug flow reactors at different organic loading rates with feedstock total solid contents of 21 %TS and 32 %TS.
2. Materials and Methods

2.1 Substrates and inoculum

Different substrates used during this study were chicken feathers (CF), citrus wastes (CW), wheat straw (WS) and cattle manure bedded with straw (MS). The chicken feather waste was collected from a slaughterhouse (Håkantorps Slakteri AB, Håkantorps, Sweden) and prepared according to Patinvoh et al. (2016) prior to use. Citrus wastes were obtained from Brämhults juice AB (Borås, Sweden) and chopped manually. Wheat straw and cattle manure bedded with straw were obtained from a farm outside Borås (Rådde Gård, Sweden). The manure was shredded manually to reduce the particle size of straw in the manure and the wheat straw was milled to particle size of 0.5 to 2 mm. Chicken feather wastes and wheat straw were stored at room temperature, while citrus wastes and manure bedded with straw were stored in plastic containers at -20 °C to prevent biodegradation until further use. During the experiment, weighted frozen substrates were thawed at room temperature and thoroughly mixed to gain a homogenized feed before use.

Prior to analyses, the feedstocks being solids were prepared according to Zupančič and Roš (2012) by blending 1g of the sample with water (dilution factor of 50) to reduce the particle size and allowing homogenization (Raposo et al. 2012). Thereafter, the theoretical methane potential (BMP_{th,COD}) of the feedstocks used were calculated related to their chemical oxygen demand (COD) content as described previously (Patinvoh et al. 2017a), after determining the COD content for the individual feedstocks. However, the COD for chicken feathers was not detectable; therefore, the theoretical methane yield of 496 Nml methane produced per g volatile solids (VS) for proteins (Angelidaki and Sanders 2004) was used for the calculations, which is applicable if all insoluble protein (keratin) are converted to soluble protein.
Anaerobic sludge used as inoculum was obtained from a digester treating wastewater sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). The inoculum was incubated at 37 °C for 2 weeks prior to use. The inoculum with 3.8 %TS and 2.7 %VS was then centrifuged at 10,000g for 10 min to increase its TS content to 9.43 % and VS content to 6.44 %. Table 1 shows the characteristics of the substrates and the anaerobic sludge (inoculum) used during the experiments.

2.2 Statistical design

The experiment was designed with statistical software MINITAB® (version 17.1.0), using 3-factor simplex lattice design, consisting of pure substrates and mixtures of two, three and four substrates at wet weight ratios. The chicken feather (CF) fraction was kept constant to maintain the C/N ratio of the mixtures between 12 and 21. The experiments were replicated according to the same setups two times and methane yield was used as response variable. The linear mixing model without synergetic or antagonistic interaction was used to obtain the expected methane yield from all mixtures which correspond to VS fraction from individual substrates while the quadratic model was used to obtain the predicted methane yield from all mixtures with synergetic and antagonistic interaction between VS fractions of individual substrates. Table 2 shows the mixture compositions following the simplex lattice design.

2.3 Batch anaerobic dry digestion of individual substrates and mixtures

Anaerobic batch digestion tests on individual substrates and the co-digestion mixtures were performed according to the method described by Angelidaki et al. (2009). The assays were carried out under mesophilic conditions (37 ± 1°C) using 118 ml serum glass bottles as reactors; each reactor contains 39 ml of inoculum and 1.25 gVS of individual substrates and mixtures keeping a VS ratio (VSsubstrate to VSinoculum) at 1:2 in all setups. The total solid content of individual substrates and mixtures were adjusted to 20 %TS thereby having a final TS of 11 % in all reactors; the mixture compositions used in the various set ups are shown in Table
2. Inoculum and water was used as blank to disclose any methane production by the inoculum itself. The pH in all reactors were adjusted to 7.0 ± 1 using 2M NaOH and HCl solution, then the reactors were sealed with rubber septa and aluminum caps, and the headspace was flushed with a gas mixture of 80 %N₂ and 20 %CO₂ for 2 minutes to create anaerobic environment in each setup. The reactors were placed in an incubator at 37 ± 1°C and were shaken manually once a day during the incubation period of 82 days. All experimental setups were performed in duplicates, and gas samples taken twice in a week during the process and once a week towards the end of the digestion period.

2.4 Co-digestion of the best mixture in plug flow reactors

The best mixing ratio, i.e. the mixture with highest methane yield, as determined during the batch assays, was applied in the continuous co-digestion process using plug flow reactors. The reactors worked as described previously (Patinvoh et al. 2017a) with a working volume of 5 L. The first part of the experiment was to carry out batch digestion in plug flow reactors with the same condition used in serum bottles (118 ml); this was done to adapt the inoculum to the feedstock (best mixing ratio) and examine the performance in plug flow reactors. The plug flow reactors were loaded with the feedstock at 20 %TS and inoculated with anaerobic sludge. VS ratio (VSsubstrate to VSinoculum) was kept at 1:2 thereby having a TS of 11 % in the reactors. The process was operated in batch mode for 28 days while the TS in the bioreactors were reduced to around 3 to 4 %TS. Since, the co-digestion process was studied under dry digestion; the TS in the reactors were increased to 15 % by adding fresh feedstock to the acclimatized inoculum achieving a VS ratio (VSsubstrate to VSinoculum) of 2.5:1. Thereafter, the continuous feeding operation began after a startup phase of 32 days. Reactors were fed every day; reactor 1 (RTS21) was fed with a feedstock having 21 %TS with bulk density of 511.7g/L and reactor 2 (RTS32) was fed with a feedstock having 32 %TS with bulk density of 322.9 g/L. The OLR was kept first at 2.0 gVS/L/d (OLR 1) and then it was increased to 3.8 gVS/L/d
(OLR 2) with corresponding retention times of 50 and 30 days, respectively. During the feeding equivalent amounts of the digestate residue was withdrawn every day from the reactors; digestate was mixed with fresh feedstock in a ratio of 1:2 (feedstock: digestate; wet basis) prior to feeding. The reactors were then mixed manually (once daily) with the impeller by moving its content to the inlet and back to the outlet to minimize stratification in the reactor. Process parameters such as volume of biogas produced, methane content of produced biogas, pH, TS, VS, VFA/Alkalinity ratio, total VFA and total ammonia concentration were monitored regularly during the digestion process.

2.5 Analytical methods

Total solids (TS), volatile solids (VS), pH, and ash content were determined according to biomass analytical procedures (APHA-AWWA-WEF 2005). The total nitrogen (TKN) was determined using the Kjeldahl method. The total carbon was obtained by correcting the total dry weight carbon value for the ash content (Haug 1993; Zhou et al. 2015) and the bulk density was determined according to Zhang et al. (2012). COD concentrations were analyzed using a COD test kit (Nanocolor, MACHEREY-NAGEL GmbH & Co. KG. Germany) and the concentrations were then measured using Nanocolor 500D Photometer (MACHEREY-NAGEL GmbH & Co. KG. Germany). Extractives in wheat straw samples were determined according to the NREL protocol (Sluiter et al. 2008) using Soehllet method with water and ethanol extraction for 24 h. Total carbohydrate and total lignin content of extractive free straw samples were then determined according to NREL protocols (Sluiter et al. 2011). Monomeric sugars obtained during the hydrolysis were determined by HPLC. Mannose, glucose, galactose, xylose and arabinose were analyzed using Aminex HPX-87P column (Bio-Rad) at 85 °C and 0.6 mL/min ultrapure water as eluent. Acid soluble lignin (ASL) was determined using an UV spectrophotometer (Biochrom Ltd, Cambridge, England) at 320 nm. Acid insoluble lignin (AIL) was gravimetrically determined as residual solid after hydrolysis
corrected with the ash content. The ash content was determined as the remaining residue after keeping the samples in the muffle furnace at 575 °C for 24 h.

Samples of digestates obtained from reactors in the continuous experiment were centrifuged (5,000 g for 10 min), and then the supernatant diluted 50 times were analyzed for total ammonia concentration using Ammonia rapid test kits (Megazyme, Megazyme International Ireland, Ireland) and the concentrations were measured at 340 nm wavelength using a spectrophotometer (Biochrom Ltd, Cambridge, England). Alkalinity was measured as the total inorganic carbonate and was determined by the Nordmann titration method according to Lossie and Pütz (2008). Digestate samples were centrifuged at 5,000 g for 10 min and then 5 ml of the supernatant was titrated with 0.1 N sulfuric acid to pH 5, the titration was then continued until pH 4.4 was reached in order to determine the VFA concentration measured as acetic acid equivalent. Total VFAs in the digestate filtrates were measured using a high-performance liquid chromatograph (HPLC, water 2695, Waters Corporation, Milford, MA, USA) equipped with an RI detector (Waters 2414, Waters Corporation Milford, MA, USA) and a biohydrogen-ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) operating at 60 °C. A UV-absorbance detector (Walters 2487), operating at 210 nm wavelength was used in series with a refractive index (RI) detector (Walters 2414) operating at 60 °C.

Gas samples, taken from the headspace of each batch reactor using a 250-µl pressure-lock gas syringe (VICI, precious sampling Inc., USA), were analyzed with gas chromatograph (Perkin-Elmer, USA) equipped with a packed column (6’×1.8” OD, 80/100, Mesh, Perkin Elmer, USA), and a thermal conductivity detector (Perkin-Elmer, USA) with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. Gas measurement and analysis were carried out as described previously (Teghammar et al. 2010). The biogas volume from the continuous plug flow reactors was measured with Drum-type gas
meter (TG 05 Model 5, Ritter, Germany), and was corrected for reporting at standard
temperature and pressure (0 °C, and 1 atm) using the ideal gas law. The methane content of
biogas produced was determined with gas chromatograph as described above.

3. Results and discussion

3.1 Substrates and Inoculum composition

Characteristics of the feedstocks examined in this study are presented in Table 1; all the
substrates had a solid content of between 23 % and 93 %, which make them suitable for dry
anaerobic digestion. Chicken feather had the lowest C/N ratio (3.6) due its high nitrogen
content while wheat straw had the highest C/N ratio (69) as a result of its high carbon content.
Citrus wastes also contained high carbon, but very low nitrogen, while manure bedded with
straw had higher nitrogen content compared to that in citrus waste and wheat straw.
Additionally, citrus wastes was the only acidic waste of all the substrates investigated with its
pH of 3.24 and moreover, it had previously been reported to contain 3.78 % of limonene
(Pourbafrani et al. 2010) inhibiting methanogens (Mizuki et al. 1990). Several studies
reported that mono digestion of these solid wastes had resulted in low biogas yields or total
failure due to acid accumulation (Liu et al. 2008) or ammonia inhibition (Resch et al. 2011) or
inhibition by D-limonene (Forgács et al. 2012; Wikandari et al. 2014).

All the different mixing ratios examined in this study had a C/N ratio between 12 and 21 as
shown in Table 2; this is within the optimal C/N range required for a stable anaerobic
digestion process (Friehe et al. 2010a; Pagés Díaz et al. 2011).

3.2 Methane yield from individual substrates

The theoretical maximum methane potential of the individual feedstocks calculated based on
the COD content, together with the experimental results of methane yields obtained during the
batch anaerobic digestion assays are presented in Fig. 1. Mono-digestion of citrus wastes
resulted in methane yield of 137 NmlCH₄/gVS, which is 33 % of the theoretical maximum yield. This is in accordance with methane yields of 102 NmlCH₄/gVS (Forgács et al. 2012) and 131 NmlCH₄/gVS (homogenized) (Wikandari et al. 2015) for similar substrate obtained earlier under thermophilic wet digestion conditions. Citrus wastes degrade easily but the methane yield is low due to the presence of D-limonene which is inhibitory to the microbial community as reported previously by several researchers (Mizuki et al. 1990; Wikandari et al. 2014). Furthermore, the methane yield from chicken feathers was 132 NmlCH₄/gVS corresponding to 27 % of the theoretical yield. Previously, Patinvoh et al. (2016) and Forgács et al. (2011) found methane yield of 105 (mesophilic condition) and 180 (thermophilic condition), respectively. These yields are low due to the slow degradation of chicken feathers as a result of its recalcitrant structure. The major component of chicken feather is β-keratin (Suh and Lee 2001), which is an insoluble structural protein. Experimental methane yield of 191 NmlCH₄/gVS was obtained from the cattle manure bedded with straw, which is 54 % of the theoretical maximum yield, while 65 % of the theoretical methane yield was obtained from wheat straw, as shown in Fig. 1. Other studies reported methane yields between 100 to 300 NmlCH₄/gVS for wheat straw and manure bedded with straw obtained under different conditions (Aslanzadeh et al. 2011; Kusch et al. 2008; Patinvoh et al. 2017a; Patinvoh et al. 2017c; Schnürer and Jarvis 2010).

3.3 Methane yield from mixtures – synergetic and antagonistic interactions

Fig. 2 shows the cumulative methane production curves obtained for anaerobic dry co-digestion of chicken feather (CF), citrus waste (CW), wheat straw (WS) and manure bedded with straw (MS) during 82 days of digestion period. The compositions of the different mixtures are presented in Table 2. The co-digestion of the various substrate mixtures resulted in methane yields ranging from 165 to 238 NmlCH₄/gVS, with the mixing ratio (CF:CW:WS:MS) of 1:1:6:0 (M4) giving the highest methane yield. Table 3 shows the
comparison between the experimental values and the response variables (methane yield) obtained from the linear and quadratic mixing models.

In this work, the expected and predicted methane yield from all mixtures were modeled using the linear mixing model (without synergetic or antagonistic interaction between individual substrates) and the quadratic mixing model (including synergetic and antagonistic interactions between individual substrates), respectively. Corresponding methane yields are presented in Table 3 and Fig. 3. Of the mixing models, mixing ratios (CF:CW:WS:MS) of 1:1:5:1 (M2) and 1:1:6:0 (M4) corresponding to C/N ratios of 19 and 21, respectively, gave the best performance, i.e. the methane yield was increase by 12 % and 14 %, respectively, compared to values calculated with the linear model. On the other hand, mixing ratios of 1:0:5.8:1.2 (M16) and 1:0:7:0 (M18) having the same C/N ratios of 19 and 21 showed antagonistic effects, since the measured methane yields, and calculated methane yields using the quadratic model were 9 % and 11 % lower compared to values calculated according to the linear model, as shown in Table 3 and Fig.3.

Co-digestion of different substrates often results in synergetic effects due to the balance of necessary nutrients achieved in the mixture (Mata-Alvarez et al. 2000). The experimental and statistical results from the mixing ratios show that the higher the amount of citrus wastes and wheat straw in the mixture, the better the methane yield. The results obtained did show synergistic and antagonistic effects but these were not statistically significant (p-value > 0.05). Chicken feather and manure bedded with straw are the once contributing least to the methane yield, however, individual fractions in the mixing ratios may have provided macro nutrients and trace elements needed by the microorganisms for improved methane yield (Mata-Alvarez et al. 2014; Pagés-Díaz et al. 2014). Furthermore, addition of chicken feather may have contributed to buffering the digestion system by releasing ammonium cations (Lal 2005). Co-digestion of citrus wastes with chicken feather and wheat straw with mixing ratio
of 1:1:6:0 (M4) gave the highest methane yield, it was therefore chosen for further investigations in continuous processes.

3.4 Best mixing ratio in continuous process

3.4.1 Initial phase (adaptation period)

This phase was carried out in batch mode using the best mixing ratio; this allows microbial adaptation and conditioning feedstock prior to the continuous process. This phase lasted for 28 days and the daily as well as the accumulated biogas production is presented in Fig. 4. The anaerobic digestion of the best mixing ratio 1:1:6:0 (M4) in the plug flow reactors; reactor 1 (R1) and reactor 2 (R2) was stable. The cumulative methane yield was 270 and 282 NmlCH4/gVS in R1 and R2, respectively. During the 28 days long digesting period, 57 l and 60 l of biogas was produced in R1 and R2 resulting in a TS reduction of 64 % and 73 %, respectively. The methane content was 63 % ± 2.5 in R1 and 62 % ± 2.9 in R2. The methane yield obtained during this adaptation phase in the plug flow reactors was between 13 % and 18 % higher than that obtained from this mixing ratio (M4) during the batch digestion test. Minimal mixing in the plug flow reactor by moving its content with impeller to the inlet and back to the outlet provides good contact between the microorganisms and the feedstock, thereby improving degradation rate and methane yield. After this adaptation period and since the co-digestion process was aimed to be studied at dry digestion conditions, the TS in the reactors was increased to 15 % by adding fresh feedstock to the acclimatized inoculum keeping a VS ratio (VSsubstrate to VSinoculum) at 2.5:1.

3.4.2 Process performance at different loading rates

The continuous digestion process started after 32 days of a startup at 15 %TS. By the end of this startup phase, the TS content decreased to 7.6 % in R_{TS21} and to 8 % in R_{TS32}. Then, continuous operations were carried out at organic loading rates of 2.0 gVS/L/d (OLR 1) and thereafter at 3.8 gVS/L/d (OLR 2) in both reactors. During this operation the TS in the
digestate increased with the increase in OLR both in RTS21 and RTS32. At OLR1 (2.0 gVS/L/d) the TS content of digestate was 9.8 % ± 0.34 in RTS21 and 11.12 ± 0.69 in RTS32. As the OLR increased to 3.8 gVS/L/d, the TS content of the digestate increased to 10.02 ± 0.55 % in RTS21 and to 12.1 ± 0.45 % in RTS32. Additionally, reactor 2 (RTS32) which was fed with a feedstock with 32 %TS was blocked towards the end of this period (OLR 2); there were problems both with the feeding and with the discharge of the digestate residue.

The daily biogas production and methane content in the produced biogas during continuous operation at different organic loading rates is presented in Fig. 5. During OLR 1, a biogas production of about 4 L/d was obtained from both reactors at the beginning, which increased slightly and became stable towards the end of the digestion period at OLR1 showing an average of 6.1 L/d in RTS21 and 5.7 L/d in RTS32. The methane proportion in the biogas was 60 % in RTS21 and 57 % in RTS32. The methane yield in RTS21 was 362 NmlCH4/gVSadded, which is 13.5 % higher than the yield obtained from RTS32 (319 NmlCH4/gVSadded). Chen et al. (2015) also reported a decrease in the biogas yield during continuous dry digestion of swine manure as the TS in the feedstock was increased from 20 % to 35 %. However, when the organic loading rate was increased to 3.8 gVS/L/d (OLR 2), the daily biogas production increased slightly at the beginning but this could not be sustained and the biogas production dropped as feeding continued. The methane content also dropped slightly to an average of 58 % in RTS21 and 56 % in RTS32.

The pH was stable, between 7.4 and 7.8 for both reactors during the digestion period at OLR1 (2.0 gVS/L/d), as shown in Fig. 6, in both reactors. Nevertheless, the pH decreased slightly as OLR was increased to 3.8 gVS/L/d but did not drop below 7.0, hence it was within the favorable pH range of 6.8 to 8 for the anaerobic digestion processes (Drosg 2013; Lahav and Morgan 2004), although according to Jabeen et al. (2015) this can vary with substrates and digestion techniques. At OLR of 2.0 gVS/L/d (OLR 1), the VFA/alkalinity ratio was below
0.3 for both reactors which is also within the optimum range required for a stable operation (Drosg 2013; Liu et al. 2012). Furthermore, the total VFA was also below 1 g/l during this organic loading rate signifying a stable process. However, the stability of the process declined as the OLR increased to 3.8 gVS/L/d with corresponding retention time of 30 days. The total VFA concentration increased to 8 g/l in R1 and to 4.4 g/l in R2 by the end of the digestion period together with the VFA/alkalinity ratio, which increased sharply, indicating problems in both reactors (Fig. 6). This resulted in a decline in biogas production as shown in Fig. 5. In this study, process instability was observed at lower organic loading rate of 3.8 gVS/L/d compared to OLR of 6 gVS/L/d reported previously for instability while treating manure bedded with straw at 22 % TS using the continuous plug flow reactor (Patinvoh et al. 2017a). This can be attributed to the presence of citrus waste in the co-digestion mixture in this study. For rapidly biodegradable substrates, such as citrus wastes, the acidogenic reactions can occur quickly resulting in VFA accumulation (Comino et al. 2010b). Co-digestion of fruit, vegetable and food wastes have been reported less stable at OLR > 2.0 gVS/L/d (Shen et al. 2013) because of the accumulation of propionate. In addition, total ammonia concentration was slightly higher in RTS32 than that in RTS21 as shown in Fig. 6; this could be the result of the moisture content reduction in RTS32. Chen et al. (2015) also reported high ammonia nitrogen concentration when TS concentration of feedstock was increased from 20 % to 35 % in continuous dry fermentation of swine manure.

4. Conclusion

Co-digestion of citrus wastes with chicken feather and wheat straw with mixing ratio of 1:1:6 gave the best performance in batch assays, with a methane yield of 238 Nml/gVS. This mixture was therefore chosen for further investigations in plug flow reactors at different TS contents in the feed. The methane yields obtained during the adaptation phase in the plug flow reactors (batch mode using the same condition as in serum glass bottles) were between 13 %
Anaerobic sludge used as inoculum was obtained from a digester treating wastewater sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). The inoculum was incubated at 37 °C for 2 weeks prior to use. The inoculum with 3.8 %TS and 2.7 %VS was then centrifuged at 10,000g for 10 min to increase its TS content to 9.43 % and VS content to 6.44 %. Table 1 shows the characteristics of the substrates and the anaerobic sludge (inoculum) used during the experiments.

2.2 Statistical design

The experiment was designed with statistical software MINITAB® (version 17.1.0), using 3-factor simplex lattice design, consisting of pure substrates and mixtures of two, three and four substrates at wet weight ratios. The chicken feather (CF) fraction was kept constant to maintain the C/N ratio of the mixtures between 12 and 21. The experiments were replicated according to the same setups two times and methane yield was used as response variable. The linear mixing model without synergetic or antagonistic interaction was used to obtain the expected methane yield from all mixtures which correspond to VS fraction from individual substrates while the quadratic model was used to obtain the predicted methane yield from all mixtures with synergetic and antagonistic interaction between VS fractions of individual substrates. Table 2 shows the mixture compositions following the simplex lattice design.

2.3 Batch anaerobic dry digestion of individual substrates and mixtures

Anaerobic batch digestion tests on individual substrates and the co-digestion mixtures were performed according to the method described by Angelidaki et al. (2009). The assays were carried out under mesophilic conditions (37 ± 1°C) using 118 ml serum glass bottles as reactors; each reactor contains 39 ml of inoculum and 1.25 gVS of individual substrates and mixtures keeping a VS ratio (VS_substrate to VS_inoculum) at 1:2 in all setups. The total solid content of individual substrates and mixtures were adjusted to 20 %TS thereby having a final TS of 11 % in all reactors; the mixture compositions used in the various set ups are shown in Table


Figure Captions

Figure 1. Cumulative methane yield during batch anaerobic digestion and theoretical maximum methane potential obtained from COD compositions of individual substrates. Symbols represent: grey (■) experimental yield and black (■) theoretical maximum potential.

Figure 2. Cumulative methane yield obtained during dry co-digestion of the various mixing ratios in batch experiments. The composition of the different mixtures (i.e., M1…M18 can be found in Table 2.

Figure 3. Synergetic and antagonistic effect of the various mixtures investigated. Symbols represent: grey (■) obtained methane yield from quadratic mixing model (with synergetic and antagonistic effect) and black (■) obtained methane yield from linear mixing model (without synergetic and antagonistic interactions). The composition of the different mixtures (i.e., M1…M18 can be found in Table 2.

Figure 4. Daily biogas production and accumulated methane yield obtained during the initial stage (adaptation period) carried out in batch mode for digestion of the best mixing ratio (M4) in plug flow reactors. The digestion period lasted for 28 days and the initial load was 20 %TS of feedstock in both reactors; reactor 1 (R1) and reactor 2 (R2). Colours represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: ◊ and □ for daily biogas production and ∆ and × for cumulative methane yield.

Figure 5. Daily biogas production and the methane content of the produced biogas in reactor 1 (R_{TS21}) and reactor 2 (R_{TS32}) during continuous operation; startup (35 days) OLR1 (2 gVS/L/d for 50 days) and OLR2 (3.8 gVS/L/d for 30 days). Colours represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: ◊ and □ for daily biogas production and ∆ and × for methane content of the biogas.
Figure 6. Variation in pH, VFA/alkalinity ratio, total VFA and total ammonia concentration in reactor 1 (R_TS21) and reactor 2 (R_TS32) during continuous operation; startup (35 days), OLR1 (2 gVS/L/d for 50 days) and OLR2 (3.8 gVS/L/d for 30 days). Colours represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: (a) ◊ and □ pH, ∆ and x VFA/alkalinity ratio (b) ◊ and □ total VFAs concentration, ∆ and x total ammonia concentration.
Table 1 Characteristics of substrates and inoculum (mean values and standard deviations based on triplicate measurements)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>pH</th>
<th>Bulk density (g/L)</th>
<th>TS (%)</th>
<th>VS (%)*</th>
<th>Ash (%)*</th>
<th>Total carbon (%)*</th>
<th>TKN (%)*</th>
<th>C/N</th>
<th>COD gCOD/gVS&lt;sub&gt;substrate&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken feather</td>
<td>ND</td>
<td>ND</td>
<td>93.47</td>
<td>± 0.06</td>
<td>99.26</td>
<td>0.74</td>
<td>± 0.01</td>
<td>± 0.01</td>
<td>15.50</td>
</tr>
<tr>
<td>Citrus wastes</td>
<td>3.24</td>
<td>753.60</td>
<td>23.42</td>
<td>± 2.23</td>
<td>96.07</td>
<td>3.93</td>
<td>± 0.74</td>
<td>± 0.74</td>
<td>0.99</td>
</tr>
<tr>
<td>Wheat&lt;sup&gt;a&lt;/sup&gt; straw</td>
<td>ND</td>
<td>190.47</td>
<td>89.07</td>
<td>± 6.93</td>
<td>94.96</td>
<td>5.04</td>
<td>± 0.14</td>
<td>± 0.14</td>
<td>0.83</td>
</tr>
<tr>
<td>Manure with straw</td>
<td>8.01</td>
<td>542.00</td>
<td>25.84</td>
<td>± 26.87</td>
<td>77.21</td>
<td>22.79</td>
<td>± 2.74</td>
<td>± 1.52</td>
<td>2.26</td>
</tr>
<tr>
<td>Anaerobic sludge</td>
<td>8.19</td>
<td>1006.7</td>
<td>9.43</td>
<td>± 5.52</td>
<td>68.29</td>
<td>39.43</td>
<td>± 0.09</td>
<td>± 0.35</td>
<td>4.73</td>
</tr>
</tbody>
</table>

<sup>a</sup>Extractives 8.27 %*, Total lignin 16.52 %*, Cellulose 42.74 %*, Hemicellulose 27.99 %*

*dry basis. ND – not determined. C/N – carbon to nitrogen ratio. COD – chemical oxygen demand
Table 2 Mixture compositions used in batch anaerobic co-digestion assays.

<table>
<thead>
<tr>
<th>Mixing ratios&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mixtures</th>
<th>C/N</th>
<th>Mixing compositions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chicken feather (CF)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1:1:1:5</td>
<td>M1</td>
<td>14</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1:0:6</td>
<td>M3</td>
<td>13</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1:6:0</td>
<td>M4</td>
<td>21</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:2.2:4.3</td>
<td>M5</td>
<td>15</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:3:2:2</td>
<td>M6</td>
<td>17</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:1.1:5.4</td>
<td>M7</td>
<td>14</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:5.4:1.1</td>
<td>M8</td>
<td>19</td>
<td>7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:6:5:0</td>
<td>M9</td>
<td>13</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:6:5.0</td>
<td>M10</td>
<td>21</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.25:2.25:4.5</td>
<td>M11</td>
<td>14</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.25:4.5:2.25</td>
<td>M12</td>
<td>17</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.25:0:6.75</td>
<td>M13</td>
<td>12</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1:0.25:6.75:0</td>
<td>M14</td>
<td>21</td>
<td>10.6</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1:0.1:2:5.8</td>
<td>M15</td>
<td>13</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0.5:8:1:2</td>
<td>M16</td>
<td>19</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0:0:7</td>
<td>M17</td>
<td>12</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:0:7:0</td>
<td>M18</td>
<td>21</td>
<td>11.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratios of CF:CW:WS:MS respectively; the mixing ratios are based on VS<sub>added</sub>.

<sup>b</sup> Wet basis.
Table 3  Experimental values and response variable ($Y_{CH4}$) obtained from all mixtures investigated (mean values and standard deviations based on duplicate samples)

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Experimental $Y_{CH4}$ (Nml/gVS$_{added}$)</th>
<th>Quadratic model$^b$ $Y_{CH4}$ (Nml/gVS$_{added}$)</th>
<th>Linear model$^b$ $Y_{CH4}$ (Nml/gVS$_{added}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>170.26 (± 13.71)</td>
<td>186.19 (± 6.88)</td>
<td>181.82 (± 22.69)</td>
</tr>
<tr>
<td>M2</td>
<td>232.52 (± 5.75)</td>
<td>226.60 (± 6.88)</td>
<td>201.61 (± 22.69)</td>
</tr>
<tr>
<td>M3</td>
<td>188.18 (± 5.71)</td>
<td>174.36 (± 9.12)</td>
<td>176.91 (± 26.43)</td>
</tr>
<tr>
<td>M4</td>
<td>238.05 (± 11.36)</td>
<td>234.98 (± 9.12)</td>
<td>206.55 (± 26.43)</td>
</tr>
<tr>
<td>M5</td>
<td>164.65 (± 37.01)</td>
<td>192.07 (± 4.92)</td>
<td>190.98 (± 21.32)</td>
</tr>
<tr>
<td>M6</td>
<td>226.51 (± 9.78)</td>
<td>206.41 (± 4.92)</td>
<td>201.69 (± 21.32)</td>
</tr>
<tr>
<td>M7</td>
<td>175.98 (± 12.82)</td>
<td>183.69 (± 4.98)</td>
<td>185.63 (± 24.16)</td>
</tr>
<tr>
<td>M8</td>
<td>221.64 (± 4.21)</td>
<td>212.36 (± 4.98)</td>
<td>207.04 (± 24.16)</td>
</tr>
<tr>
<td>M9</td>
<td>191.22 (± 3.87)</td>
<td>174.49 (± 6.82)</td>
<td>180.27 (± 28.28)</td>
</tr>
<tr>
<td>M10</td>
<td>200.24 (± 38.51)</td>
<td>217.50 (± 6.82)</td>
<td>212.39 (± 28.28)</td>
</tr>
<tr>
<td>M11</td>
<td>198.34 (± 5.89)</td>
<td>188.40 (± 4.31)</td>
<td>193.08 (± 22.02)</td>
</tr>
<tr>
<td>M12</td>
<td>192.02 (± 30.79)</td>
<td>199.37 (± 4.31)</td>
<td>204.20 (± 22.02)</td>
</tr>
<tr>
<td>M13</td>
<td>168.53 (± 5.57)</td>
<td>173.93 (± 6.67)</td>
<td>181.96 (± 29.27)</td>
</tr>
<tr>
<td>M14</td>
<td>217.70 (± 13.21)</td>
<td>206.83 (± 6.67)</td>
<td>215.32 (± 29.27)</td>
</tr>
<tr>
<td>M15</td>
<td>180.04 (± 13.76)</td>
<td>178.97 (± 6.40)</td>
<td>189.41 (± 25.86)</td>
</tr>
<tr>
<td>M16</td>
<td>177.84 (± 18.69)</td>
<td>193.58 (± 6.40)</td>
<td>212.47 (± 25.86)</td>
</tr>
<tr>
<td>M17</td>
<td>166.51 (± 8.85)</td>
<td>172.97 (± 8.90)</td>
<td>183.64 (± 30.31)</td>
</tr>
<tr>
<td>M18</td>
<td>189.76 (± 38.02)</td>
<td>194.88 (± 8.90)</td>
<td>218.24 (± 30.31)</td>
</tr>
</tbody>
</table>

$^b$ Response variable ($Y_{CH4}$) from linear and quadratic mixing models. $Y_{CH4}$ = methane yield.
Fig. 1

The chart illustrates the methane yield (Nm³CH₄/g VS) for individual substrates: Chicken feather, Citrus wastes, Wheat straw, and Manure with straw. The methane yield for Chicken feather is the highest among the substrates shown. The x-axis represents the individual substrates, while the y-axis represents the methane yield in Nm³CH₄/g VS.
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6