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Integration of the first and second generation bioethanol processes and the importance of by-products

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Abstract

Lignocellulosic ethanol has obstacles in the investment costs and uncertainties in the process. One solution is to integrate it with the running dry mills of ethanol from grains. However, the economy of these mills, which dominate the world market, are dependent on their by-products DDGS (Distiller’s Dried Grains and Solubles), sold as animal feed. The quality of DDGS therefore must not be negatively influenced by the integration. This puts restraints on the choice of pretreatment of lignocelluloses and utilizing the pentose sugars by food-grade microorganisms. The proposed solution is to use food related filamentous Zygomycetes and Ascomycetes fungi, and to produce fungal biomass as a high-grade animal feed from the residues after the distillation (stillage). This also has the potential to improve the first generation process by increasing the amount of the thin stillage directly sent back into the process, and by decreasing the evaporator based problems.

Keywords: Bioethanol; DDGS; Filamentous fungi; Integration; Lignocellulose
1. Introduction

From a human perspective, the world is dependent on fossil fuels for its primary energy supply. In 2010, we consumed 12.7 billion tons of oil equivalents (IEA, 2012) globally, including 32.4% oil, 27.3% coal and peat, and 21.4% natural gas, while biofuels and waste contributed with 10.0%. Amongst the oil consumers, the transport sector completely dominated with 61.5% of the total consumption. Consequently, renewable alternatives for the transportation fuel should be seriously considered, if the fossil fuels are to be replaced.

During the last decade(s), concerns regarding global warming, fossil fuel depletion, and energy security resulted in a wide interest in renewable and environmentally friendly fuels. The dominating biofuel for transportation is ethanol with the annual world production rising from 17.0 to $86.1 \times 10^6$ m$^3$ from 2000 to 2011 (REN21, 2012). It is followed by biodiesel with an annual world production of $21.4 \times 10^6$ m$^3$ in 2011. The largest ethanol producing countries are U.S.A. and Brazil, responsible for the production of $54 \times 10^6$ and $21 \times 10^6$ m$^3$ in 2011, respectively (REN21, 2012). Currently, all industrial scale production of ethanol belongs to the first generation of biofuels. However, the technology to produce second generation ethanol does exist. One of the main obstacles for its implementation is the combination of high risk investments (including technological risks and political/policy risks) with low potential returns.

The aim of the present paper is to present an alternative to the direct implementation of an industrial scale second generation bioethanol process with the integration of the second generation into the existing first generation bioethanol processes, which aims to reduce the current barriers to process change/investments. The challenge of a pentose-rich substrate is also taken into account.
2. Bioethanol production

2.1. First generation bioethanol

The first generation ethanol plants utilize either sugars or starch. The sugar-based ethanol plants are predominantly produced in Brazil from sugarcanes. The starch-based ethanol is generally from corn but also from grains, and is dominated by the U.S. followed by other major ethanol producing countries such as China, Canada, France, Germany, and Sweden. In the global market, ca. 21 million m³ ethanol is produced from sugarcane, while ca. 60 million m³ ethanol is produced from corn and grains (REN21, 2012). The starch-based process will be in focus here. There are more than 200 such plants in the U.S. with an average capacity of about 260,000 m³/year ethanol producing from corn or sorghum (www.ethanolproducer.com).

The first step of the ethanol production from grains (Fig. 1) in the process called dry mills is the milling of the substrate and subsequent liquefaction of the starch. The liquefaction is followed by the hydrolysis or saccharification, which releases the sugar (glucose) monomers into the solution. During the subsequent, or simultaneous fermentation with yeast (*Saccharomyces cerevisiae*), the sugar monomers are converted into ethanol and carbon dioxide. Usually an ethanol concentration of ca. 10% (w/v) is obtained at the end of the fermentation. The fermentation liquid, or beer, is distilled to separate and purify the ethanol, which is then dehydrated to concentrations above 99.7% for fuel applications, according to the European standard EN 15376 (SIS, 2011). In the bottom of the distillation column, the stillage consisting of about 10% TS (total solids), including residual substrate, yeast, and fermentation by-products, is accumulated. Some of the solid particles are removed from the liquid via centrifugation by a decanter and the remaining thin stillage is sent to an evaporator. The centrifugation cake and the resulting syrup from the evaporation are normally mixed to
produce Distillers Dried Grains and Solubles (DDGS). The DDGS, which is principally a protein source as animal feed, plays a crucial role in the overall process economy. More detailed descriptions can be found in the literature, e.g., a recent book chapter by Taherzadeh et al. (2013).

Considering the vast amount of accumulated knowledge gathered from decades of industrial production of the first generation ethanol process, there are very few uncertainties involved in the process, raw materials, and the markets. Thus, even if the process only provides a low rate of return, it comes with relatively low risk, which is mainly based on uncertainties regarding the cost of the feedstock and the price of the products: ethanol and animal feed (DDGS). However, the use of potential human food as feedstock for the process has led to considerable ethical discussions, normally referred to as the “food vs. fuel” debate, with widely diverse and strongly polarized views. The supply of the feedstock can also become a potential limiting factor compared with the potential demand. It is a complex issue that is discussed in its own forum, e.g., cf. Kaye-Blake (2010). This debate also results in propositions for new laws and regulations to push the ethanol plants to direct their expansion away from food-based feedstock, which causes some uncertainties regarding future plans.

2.2. Second generation bioethanol

Second generation ethanol utilizes different types of lignocellulosic materials as substrate. Currently, only negligible amounts of second generation bioethanol are produced in several demo plants around the world that work industrially, but are not yet commercially feasible. At the moment, Borregaard company located in Norway declares to be the largest producer of second generation ethanol with an annual production of 20,000 m$^3$ (Rødsrud et al., 2012). The ethanol is produced from the sugar monomers released as a by-product during their sulfite
process. Historically, more ethanol however has been produced from lignocellulosic feedstock. As an example, during the 1940s more than 30 sulfite mills were in operation in Sweden, all of which included ethanol production (Eklöf et al., 2012), and by the end of the 1980s Soviet sulfite mills had a production capacity of up to 190,000 m$^3$/year (Rabinovich, 2010). Overall, the second generation bioethanol process will most likely be partly similar to the first generation process and current/past processes based on by-products from sulfite mills.

Second generation ethanol processes have technically no issues with feedstock supply, as 7–18 billion tons/year of lignocellulosic biomass is available for human exploitation (Lin & Tanaka, 2006). Instead, the process is currently limited by technical and by economic challenges (the cost of lignocellulosic feedstock, including its transportation, often compete unfavorably with the efficient supply chain of sugar or starch containing raw materials), which although connected can be divided into three groups (Cheng & Timilsina, 2011). The first technical challenge is caused by the recalcitrance of the biomass and thus the need for relatively harsh pretreatments of the feedstock. This harsh pretreatment, in turn, results in the formation of inhibitory compounds, which causes problems during the fermentation. Numerous reviews can be found on the topic, e.g., by Taherzadeh and Karimi (2008). The second challenge is in the production of efficient enzymes to hydrolyze the cellulose, at a cost competitive to the first generation enzymes hydrolyzing starch. Although major improvements have been accomplished by the enzyme manufacturers, reducing the cost of the enzyme to 0.13 USD/L ethanol (Geddes et al., 2011), improvements are still necessary.

Thirdly, sufficiently high ethanol concentrations in the beer have to be reached in order to reduce the cost of distillation and wastewater treatment. A goal of 4–4.5% (w/v) is generally considered. This might appear to be a minor issue, but reaching it requires substrate loadings above 15% (Viikari et al., 2012) with subsequent mixing and inhibitor problems.
A number of lignocellulosic materials also release high amounts of pentose sugars during hydrolysis. Corn stover, wheat straw, and switch grass are examples of lignocellulosic materials with xylan contents above 20% on a dry weight basis; more than half of the glucan content in the corresponding materials (Mosier et al., 2005). Since the microorganism of choice, S. cerevisiae, is unable to utilize pentoses, this can become an issue. A plethora of examples of genetic manipulation to overcome this issue exists in the literature (Madhavan et al., 2012). However, although the results are promising, improvements are still necessary. Furthermore, legal issues and consumer opinions regarding the use of genetically modified organisms, especially in Europe, are often overlooked.

3. Process integration
A possible solution to use all the current dry mills for the second generation ethanol production and also decrease the high risk of investing in a new second generation ethanol process is to integrate lignocellulosic ethanol into the current dry mills. In principle, most of the dry mills have access to lignocellulosates produced together with the grains such as straw, corncob, bran, etc. with a relatively low transportation cost. An example of how this process integration could be carried out is depicted in Fig. 2 with two different proposed solutions: (a) integration at the fermentation stage and (b) integration at the fungal cultivation stage (see section 4). In both cases, the first generation process remains mostly unchanged, although not completely unaffected. A larger potential influence on the first generation ethanol process is carried out by the alternative (a), as the inhibitors from the second generation process could enter the fermentor(s). Considering the dilution effect, it is rather unlikely that these inhibitors would disrupt the fermentation. New residuals, such as mainly lignin and undigested cellulose, will also pass through the entire process. Nevertheless, bringing an unknown factor
into the heart of the process is not usually popular for plant managers, which could prevent implementation of the integrated process. If the integration is performed in the later steps, i.e., at the new suggested step “fungal cultivation” (see section 4), the heart of the first generation process would be untouched. This would also minimize the amount of sugar (pentose) rich process streams in use, and thus the risk of unwanted reactions and contamination.

One of the major challenges of the lignocellulosic ethanol processes is obtaining sufficiently high sugar concentrations after the hydrolysis. To a large degree, this is solved by integrating the first and second generation processes, since sufficiently high concentrations are easily reached in the first generation. Thus, lower concentrations of the lignocellulosic feedstock are required, which considerably reduce the problems associated with mixing of the slurry. The lower concentrations will also lead to lower concentrations of inhibitors formed during the pretreatment, resolving the need for detoxification. Other than being less challenging, the pretreatment and hydrolysis will most likely be very similar to any second generation process. Thus, the pretreatment will most likely utilize acids or bases to open up the structure. However, care must be taken because the chemicals have to be chosen so that they do not negatively influence the quality of the animal feed product (DDGS) or produce large amounts of inhibitors. Considering that filamentous fungi have been grown on spent sulfite liquor, which is relatively rich in inhibitors, and used as fish feed without adverse effects to the fish (Bankefors et al., 2011), the latter is probably not become an issue. On the other hand, the choice of chemicals for the pretreatment and even hydrolysis should also be considered. For example, sulfuric acid and dilute-acid processes could be an interesting option for the pretreatment. However, sulfite has limitation in animal feed and it might demand in avoiding sulfuric acid in the pretreatment of the lignocelluloses. Furthermore, the hydrolysis will probably use enzymes and could either be carried out in a separate vessel or together with the
fermentation, and would most likely not influence the quality of the DDGS. Following hydrolysis, the liberated hexoses will be converted into ethanol and CO$_2$ by the fermenting microorganism as usual.

A potential integration of the first and second generation ethanol processes, however, does not solve the problem of how to utilize the pentoses. A possible solution would be to use genetically modified strains of *S. cerevisiae*, especially for the European market legislations; however, negative public opinion may become an issue. Other microorganisms capable of fermenting pentoses into ethanol could also be employed, but they are generally quite sensitive to inhibitors (including ethanol). For instance *Schaefferomyces stipites* (formerly known as *Pichia stipites*) is sensitive to organic acids (Agbobo et al., 2007) and has been found to be inhibited when the ethanol concentration exceeded 30 g/L (Meyrial et al., 1997). This could become an issue, especially on the industrial scale. Co-fermentation of pentoses and hexoses are also yet to be solved. However, the pentoses could also be used for the production of compounds other than ethanol at later stages in the process.

The best opportunity for late utilization of pentoses is most likely after the separation of most of the solids from the stillage, i.e., the thin stillage (Fig. 2). However, a dedicated process step solely for pentose utilization in an integrated first/second generation process is not likely to be economically optimal due to the relatively low concentrations. Still, unfermented substrate (including carbohydrate polymers), dead yeast cells, and metabolites are likely to remain in relatively large quantities in the thin stillage as well. Therefore, a method to utilize both pentoses and the other residues is needed. Furthermore, since the animal feed product DDGS plays a crucial role in the process economy of existing first generation plants, its quality must not be compromised. This significantly reduces the number of potential solutions, as the
microorganism essentially has to be food-grade to avoid damage to the environment or the animals eating the feed.

4. Fungal cultivation and pentose utilization

A proposed solution to the utilization of unfermented substrate without compromising the quality of the DDGS is to use food-related strains of Zygomycetes and Ascomycetes filamentous fungi. Potential strains include \textit{Rhizopus} sp. isolated from tempe; \textit{Fusarium venenatium} used for the production of Quorn; \textit{Aspergillus oryzae} from e.g., sake fermentation; \textit{Neurospora intermedia} isolated from oncom (fermented food based on left-overs in Indonesia); and \textit{Monascus purpureus} used for the production of red rice. All of these strains have been confirmed to grow on mostly wheat-based thin stillage in aerobic conditions, resulting in the production of 11-19 g/L fungal biomass and 0.9-4.7 g/L ethanol (unpublished data). The fungal biomass can then easily be separated from the liquid due to its filamentous nature and dried. The ethanol will remain in the fermented broth, which is sent to the evaporators. The volatile ethanol will naturally join the outgoing steam, which is condensed and sent back into the process as is currently done in the first generation plants. Thus, no additional process steps will be required to separate the ethanol.

For pentose utilization and second generation processes, the focus among these filamentous fungi has been on the Zygomycetes. The research was initiated by Taherzadeh et al. (2003) with the use of sulphite liquor from the paper pulp industry as a substrate for \textit{Rhizopus}, and has been ongoing since then. Noteworthy, publications for the use of food related Zygomycetes include the works by Millati et al. (2005), Ferreira et al. (2012), and Wikandari et al. (2012). The general trend has been that while the ethanol yield from xylose is most often limited (ca. 0.2 g/g), the production of fungal biomass has been more promising (ca. 0.35 g/g).
These ethanol yields from xylose can also be considered close to what is achievable, since all the evidence suggests that Zygomycetes follow the general fungal pathway (c.f. Chiang and Knight (1960)), resulting in an imbalance among the redox carriers. Without access to oxygen, it is not possible for the cells to correct this imbalance, which prevents anaerobic fermentation of xylose by these fungi. Thus, the need for aeration adds a natural limitation to produce ethanol, especially in industrial scale, which prevents the required micro-adjustments in the oxygen level for obtaining a high ethanol yield. The production of fungal biomass, which is the best in aerobic conditions, can probably still be optimized from pentoses by adjusting the process parameters and the feed composition. However, in general utilization of pentose sugars by fungi is a slower process than of hexose sugars and has not been reported at high hexose concentrations for these filamentous fungi.

Considering that utilization of xylose for biomass production requires aerobic conditions, aeration has to be considered an important factor. This is also true for the Ascomycetes strains. Aeration is also a crucial factor to decompose carbohydrate polymers in the thin stillage; metabolites from the fermentation and infections such as glycerol, lactic acid, and acetic acid; low concentrations of unfermented sugars such as xylose; and yeast cells lysis products. All of these compounds either require oxygen to be utilized by the fungi or the utilization is considerably enhanced by oxygen. Many of the compounds also need to be degraded enzymatically in order to be accessible to the fungi. Zygomycetes, however, are known to be able to produce e.g., amylases, cellulases, proteases, and lipases and can thus utilize most substrates (Ferreira et al., 2013). Similar enzyme production by different Ascomycetes is also very well known, including enzymes for more uncommon reactions (Zelinski & Hauer, 2002). Since the production of enzymes increases the energy expenditure
of the cells, good access to ATP generating processes is required. This further increases the importance of aeration.

Cultivation of filamentous fungi is not without challenges. Mixing can particularly become an issue due to the broth viscosity caused by the filamentous nature of the cells (Gibbs et al., 2000). The fungi may also attach to the equipment inside the reactor such as baffles and impellers (Byrne & Ward, 1989). There are two possible ways to counteract this phenomenon. One is to adjust the process conditions and try to control the growth morphology. For instance, pellets (small beads consisting of intertwined hyphae) can be formed if the conditions are controlled (Nyman et al., 2013) to reduce the broth viscosity. However, growth in the form of pellets instead of free mycelia/clumps has been shown to both increase and decrease the metabolite yields, depending on the strain and the metabolite. Thus, growth in the form of pellets is not always beneficial. The other way to solve the problem is to adjust the cultivation vessel to fit the growth of the filamentous fungi. For instance, air-lift and bubble-column type reactors have been performing well for fungal cultivations on the thin stillage in aerobic conditions (unpublished data). The common factor between these two types of reactors is that they lack internal moving parts, and the mixing is achieved via the aeration process. This also has the benefit of a relatively low energy demand for the mixing.

5. Benefits of biomass production

Although the first generation ethanol production is a well-known process with few uncertainties, it is still very dependent on the raw material cost and the selling price of ethanol and DDGS. Even though the market values of both the raw material and the ethanol have a strong correlation with the price of fossil fuel, individual fluctuations still occur (cf. The
World Bank (2013) and Alternative fuels data center (2013)). Since the profit margins are relatively small, these fluctuations represent a considerable risk to the process economy.

One way to decrease the impact of substrate/production price fluctuations is to follow the biorefinery concept and produce more than one product. Edible Zygomycetes or Ascomycetes fungal biomass have the potential to fulfill this role as an additional product. The fungal biomass could either be used to improve the quality of the DDGS, or be sold separately. The first alternative has the advantage of being relatively easy to implement. The second alternative has the potential advantage of providing the highest price. This can mainly be attributed to the high protein content (>50%), which makes it potentially useful as a fish feed component. The fungal biomass would then replace part of the fishmeal (Bankefors et al., 2011) or be added as an extract (Bhandari et al., 2002). In both cases the fish consumed the feed and grew well. Alternatives to fishmeal is of particular interest since it has more than quadrupled in price from January 2000 to April 2013, ending with an average price of 1,849 USD/ton (The World Bank, 2013). The demand is also likely to remain high, as more and more fish are produced in aquacultures. Production of fungal biomass is also advantageous since it will utilize substrate that is challenging to use for bioethanol production (Section 4).

Fungal biomass could also find other uses. Some strains are known to produce valuable lipids (Bellou et al., 2012), which could be extracted from the biomass and sold as e.g., dietary supplements. The lipid contents are also high, close to 30 % have been observed for some species (Kavadia et al., 2001). Low-grade fatty acids could instead be used for e.g., biodiesel production. If Zygomycetes are cultivated, the cell wall fraction of the biomass could be used as a source of chitosan, or be used to produce a bio-based superabsorbent (Zamani, 2010).
However, all these applications require additional process steps after the harvesting and their economic benefit is unknown.

Cultivation of filamentous fungi provides benefits other than an additional product; there are also process related advantages such as easy separation of the produced mycelium. A major potential advantage can be found in the evaporators, which have the challenging task of removing as much water from the thin stillage as possible. Fouling, in particular, and the viscosity of the liquid can be major obstacles in the process. By reducing the total amount of suspended solids and organic compounds in the liquid, the severity of these obstacles could be decreased. This could allow more water to be removed in the evaporators and less in the driers. It could also allow more of the thin stillage to be sent back into the process as back-set, which would directly decrease the load on the evaporators and the driers.

6. Conclusion

Integration of second and already existing first generation ethanol processes is an attractive way to reduce the investment costs and risks compared to a standalone second generation processes. However, since most of today’s ethanol production is based on starch and thus dependent on by-products sold as e.g., animal feed to be economically feasible, the integration cannot adversely affect these by-products. This severely limits the possible ways to utilize the pentose sugars released from the lignocellulosic feedstock. The proposed solution is to use edible Zygomycetes and Ascomycetes filamentous fungi, which are naturally capable of utilizing pentoses, but also other unfermented substrates left after distillation.
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References


Figure 1: Process outline for a first generation ethanol process (top) and second generation ethanol process (bottom).
Figure 2: An integrated first and second generation ethanol process. The integration could occur at the fermentation step (top) or at the proposed fungal cultivation step (bottom).
Graphical abstract
Highlights

- Investment in 2\textsuperscript{nd} generation ethanol plants is expensive and high risk
- The 2\textsuperscript{nd} generation ethanol can be integrated into 1\textsuperscript{st} generation plants using fungi
- Animal feed is an important byproduct not be challenged in this integration
- There are ascomycetes and zygomycetes suitable for this purpose