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Fermentation of lignocellulosic hydrolyzate using a submerged membrane bioreactor at high dilution rates

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Abstract

A submerged membrane bioreactor (sMBR) was developed to ferment toxic lignocellulosic hydrolyzate to ethanol. The sMBR achieved high cell density of *Saccharomyces cerevisiae* during continuous cultivation of the hydrolyzate by completely retaining all yeast cells inside the sMBR. The performance of the sMBR was evaluated based on the ethanol yield and productivity at the dilution rates 0.2, 0.4, 0.6, and 0.8 h^{-1} with the increase of dilution rate. Results show that the yeast in the sMBR was able to ferment the wood hydrolyzate even at high dilution rates, attaining a maximum volumetric ethanol productivity of 7.94 ± 0.10 g L^{-1} h^{-1} at a dilution rate of 0.8 h^{-1}. Ethanol yields were stable at 0.44 ± 0.02 g g^{-1} during all the tested dilution rates, and the ethanol productivity increased from 2.16 ± 0.15 to 7.94 ± 0.10 g L^{-1} h^{-1}. The developed sMBR systems running at high yeast density demonstrates a potential for a rapid and productive ethanol production from wood hydrolyzate.

Keywords: Wood hydrolyzate; Membrane bioreactor; Bioethanol production; Cell retention; IPC membrane panel

1. Introduction

Conversion of renewable lignocellulosic biomass into ethanol is considered as a clean alternative to fossil fuels. Lignocellulosic materials can be hydrolyzed to fermentable sugars using acid or enzymes, prior to fermentation to ethanol. Dilute-acid hydrolysis is fast and inexpensive (Shatalov et al., 2012), but it has two major drawbacks. Firstly, the method is unable to convert all cellulose to sugars (Mosier et al., 2005) and secondly during the hydrolysis, several compounds such as furan, phenolic, and aliphatic acid compounds are formed which are toxic to the subsequent fermenting yeast (Palmqvist et al., 2000).

High yeast concentration in the bioreactor is advantageous when fermenting inhibitory hydrolyzate, then the inherent capacity of yeast to detoxify some of the inhibitors in the hydrolyzate *in situ* can be utilized (Talebnia et al., 2006). The yeast can be retained by different means to achieve a high cell concentrations inside the bioreactor for example by recycling (Brandberg et al., 2005) or immobilizing methods (Purwadi et al., 2008). Cell retention will increase the biomass amount in the reactor, which both improves the sugar utilization and *in situ* detoxification (Purwadi and Taherzadeh, 2008).

One method to retain the cells is to use membrane bioreactors (MBR) in which membranes are used to keep the yeast inside the bioreactor. MBRs are today mostly used for wastewater treatment where they have gained popularity due to their high treatment efficiency, compactness, small footprint and good effluent quality (Katsou et al., 2011, Keskes et al., 2012). But MBRs can also be applied in ethanol production processes. MBRs can make it possible to obtain very high yeast biomass concentration.

As an example, Larfforgue *et al.* (Lafforgue et al., 1987) succeeded in reaching a yeast concentration of 345 g L^{-1} by applying MBR. The major drawbacks of using cell retention by MBRs are e.g. the increased risk of contamination, accumulation of solid particles, and fouling of the membranes. However, studies illustrate many advantages of yeast cultivations performed in MBRs. For example, yeast cells in cultivations performed at high yeast biomass content in MBR can detoxify and survive in a medium containing high levels of the inhibitor furfural, and can produce ethanol rapidly even at a dilution rate of 0.5 h^{-1} (Ylitervo et al., 2013).

Fermentation of toxic dilute-acid hydrolyzate can be troublesome since it contain fermentation inhibitors. In particular, traditional continuous cultivations without cell recirculation or retention has only been successful at low dilution rates (Brandberg et al., 2007, Brandberg and Sanandaji, 2005) if the hydrolyzate has not been detoxified. For example, in continuous cultivations, the yeast was able to ferment a dilute acid hydrolyzate with low toxicity at a dilution rate of only 0.1 h^{-1}, but failed at higher dilution rate of 0.2 h^{-1} (Taherzadeh et al., 2001). Unlike traditional continuous cultivations, the dilution rate in MBRs is not dependent on the growth rate of the yeast (Ylitervo and Franzén, 2013), which gives the advantage that the cells do not have to grow to remain inside the bioreactor. The usage of MBRs can result in both an eliminated washout and a high cell concentration, which is particularly suitable for *in situ* detoxification and fermentation of inhibitory media at higher dilution rates and high productivity (Lafforgue and Malinowski, 1987, Ylitervo and Franzén, 2013). The application of MBRs is therefore interesting when it comes to fermenting wood

hydrolyzate that contains several inhibitors. This fact has not been investigated in any larger extent.

MBR can be constructed with different configurations where the membrane in the system is operated in a cross-flow manner externally to the bioreactor or submerged in the fermentation broth. Since submerged MBRs (sMBR) commonly require less energy to operate in contrast to external cross-flow MBRs (Judd, 2011), a sMBR was developed and used during this investigation.

In the present study, the aim was to investigate the ethanol productivity in a sMBR operated at 30 °C pH 5.0 using toxic wood hydrolyzate at four different dilution rates 0.2, 0.4, 0.6 and 0.8 h^{-1}. The sMBR operation was tested both at low and high yeast concentration by completely retaining the yeast cells in the system by the membrane unit. The sMBR performance was evaluated by studying the ethanol production and productivity, sugar utilization and detoxification of inhibitors present in the wood hydrolyzate.

2. Material and Methods

2.1. Microorganism, medium and hydrolyzate

Saccharomyces cerevisiae CBS 8066, obtained from Centraalbureau voor Schimmelcultures (Delft, The Netherlands) was used throughout this investigation. The strain was maintained on YPD agar plates containing 10 g L^{-1} yeast extract, 20 g L^{-1} soy peptone, 20 g L^{-1} D-glucose, and 20 g L^{-1} agar, and stored at 4 °C until use.

Spruce sawdust was hydrolyzed with H_2SO_4 in order to prepare sugar containing hydrolysate for the cultivations. The wood was hydrolyzed by mixing 450 g of sawdust with 2 L 1.5% H_2SO_4 and heated to 140 °C for 2 h. The moisture content in the sawdust was 12.5%. After hydrolysis, the solid part was separated from the liquid and the latter was pH adjusted with 10 M NaOH to pH 5.0, and filtered through a Whatman filter paper No. 1 to remove all larger particles. Prior to cultivation, the wood hydrolyzate was sterile filtered through a 0.22 μm membrane. The spruce hydrolyzate medium was prepared by mixing sterile spruce hydrolyzate with sterile solutions of nutrients to attain the final concentrations of 1 g L^{-1} yeast extract, 2 g L^{-1} $(NH_4)SO_4$, 5 g L^{-1} KH_2PO_4, 0.4 g L^{-1} $MgSO_4 \times 7\ H_2O$ and 1 mL L^{-1} Antifoam SE-15 (Sigma). During the addition of the nutrients the prepared spruce hydrolyzate was diluted to 95% of its original concentration. The final sugar and inhibitor concentrations in the hydrolyzate medium was determined to be 2.32 ± 0.13 g L^{-1} arabinose, 8.04 ± 0.10 g L^{-1} glucose, 4.57 ± 0.13 g L^{-1} galactose, 16.41 ± 0.96 g L^{-1} mannose, 7.63 ± 0.19 g L^{-1} xylose, 3.21 ± 0.13 g L^{-1} acetic acid, 1.18 ± 0.23 g L^{-1} furfural, 0.47 ± 0.02 g L^{-1} 5-hydroxymethylfurfural, 2.88 ± 0.29 g L^{-1} formic acid and 0.76 ± 0.05 g L^{-1} levulinic acid.

Inoculum cultures of yeast were made in three 500 mL E-flasks with cotton plugs in a water bath at 30 °C and 130 rpm for 24 h. The cultivations were started by transferring one colony of yeast to 200 mL of medium containing the same nutrient concentrations mentioned above together with 50 g L^{-1} glucose as carbon source.

2.2. Analytical methods

The moisture content of the sawdust was determined by balancing and drying triplicate sawdust samples at 105 ℃ for 24 h. Yeast dry-weight concentrations were determined by sampling 1.00 or 1.50 mL liquid from the bioreactor. The yeast samples were centrifuged (9600 × g, 5 min), washed three times with water before drying in preweighted glass tubes at 105 ℃ for 24 h and balanced after temperature equilibration in a desiccator.

Liquid samples to measure sugar, inhibitor and metabolite concentrations in the MBR were taken from the permeate outflow from the membrane units and stored at -20 ℃ until analysis by HPLC (Waters 2695, Waters Corporation, USA). Metabolite and inhibitor concentrations were determined using a hydrogen-based column (Aminex HPX-87H, Bio-Rad, USA) at 60 ℃ with 5 mM H_2SO_4 at a flow rate of 0.6 mL min^{-1} as eluent. A refractive index detector (Waters 2410) and UV absorbance detector at 210 nm (Waters 2486) were used to determine both metabolite and inhibitor concentrations. Sugar concentrations were analyzed using the refractive index detector and a lead-based column (Aminex HPX-87P, Bio-Rad) at 85 ℃ with ultrapure water at a flow rate of 0.6 mL min^{-1} as eluent.

2.3. Submerged membrane units

The schematic configuration of the sMBR is shown in Fig. 1, the total liquid volume in the sMBR was 600 mL. There were three parallelly placed flat sheet membrane panels mounted in an external chamber, and the fermentation liquid was continuously circulated from the bioreactor into the external chamber and back to the bioreactor. The advantage of placing the submerged membrane in an external chamber is that it is easier

to clean (Liao et al., 2006). If the membrane requires cleaning with chemicals when it is placed inside the bioreactor the entire MBR system need to be cleaned at the same time, requiring more chemicals and generating more waste after cleaning. However, the drawback is that the circulation of liquid requires energy.

During filtration gas was sparged continuously around the membrane panels to avoid rapid blocking and fouling of the membranes. For this purpose a linear air pump (model DB15B, Emmecom, Italy) was used. The gas from feeding the gas circulation pump was collected above the liquid level of the reactor and then circulated back again. To avoid extensive foaming antifoam had to be added to the medium. The three membrane panels were developed and produced by the Flemish Institute for Technological Research (Vito NV, Belgium). The panels contained internal permeate channel (IPC) type of membranes prepared from polyethersulfone and polyvinyl-pyrollidone resulting in a hydrophilic membrane with a maximum pore size of 0.3 μm. These are dual-layer membranes with an IPC, interposed to the two membrane layers. The typical property of these IPC membranes is that they are very robust and can withstand high pressure differences during filtration and backwashing. This makes them unique for the sMBR technology (Doyen et al., 2010).

Each panel had a total membrane area of 0.0252 m^2 available for filtration. Before each cultivation, the IPC membrane panels were cleaned using the following procedure: 2% NaOH solution at 50-80 ℃ for 30 min, rinsed with water, thereafter 1% phosphoric acid for 30 min, rinsed with water, and finally disinfected with a 200 ppm NaOCl solution

with a pH >8.5 for at least 30 min and rinsed with sterile water before starting the cultivation.

2.4. sMBR operating conditions

The cultivations in the sMBR were initiated by transferring 600 mL yeast inoculum culture to the bioreactor. To accumulate yeast biomass inside the MBR, glucose (50 g L^{-1}) containing medium was fed with a dilution rate of 0.5 h^{-1} to the cultivation for either 24 h to get a low yeast density or for 45 h to get a high yeast cell density prior to addition of hydrolyzate. The cultivation was performed at aerobic conditions by purging the bioreactor with 400 mL min^{-1} of air to sustain a faster yeast growth. After yeast biomass accumulation, the system was changed to anaerobic conditions by switching the gas purge in the bioreactor to nitrogen gas at 200 mL min^{-1}. Then, spruce hydrolyzate medium was continuously fed into the bioreactor at a dilution rate of first 0.2 h^{-1}. The total liquid volume in the MBR was kept at 600 mL with the aid of a level control, connected to a pump which removed permeate through the membrane unit. The sMBR was operated at pH 5.0, temperature 30 ℃, stirring at 400 rpm and a gas flow of 12 L min^{-1} around the membrane panels to avoid fouling the membranes, the bioreactor was purged with 200 mL min^{-1} of nitrogen gas.

After growing the biomass for either 24 or 45 h with glucose containing medium at a dilution rate of 0.5 h^{-1}, the medium was switched to hydrolyzate medium, the dilution rate was stepwise increased from 0.2 to 0.4, 0.6, and 0.8 h^{-1}, and hold stable at the dilution rate for five retention times. The specified dilution rate was calculated by dividing the reactor volume with the influent medium flow rate per hour. Samples were

taken at least five times at each dilution rate to study the effect upon the increased dilution rate. Yeast dry weight samples were taken at least two times at each dilution rate. During the cultivations the yeast cells were completely retained inside the bioreactor by the membranes, and no cells were bled from the cultivations except when taking yeast dry weight samples. Duplicate sMBR cultivations were performed both at low and high yeast concentrations.

3. Result and discussion

In this work, a sMBR was used to gain a high yeast density inside of the bioreactor. By increasing the cell density in the bioreactor, a more efficient utilization of the substrate can be facilitated. Additionally a high degree of cell retention, will lead to a low specific growth rate, causing an increased in the energy needed only for cell maintenance, which can be positive for the ethanol yield since less synthesis of cell components are required.

To evaluate the toxicity of the used wood hydrolyzate, the combined severity factor for the sawdust hydrolyzate was calculated according to be 2.7 (Tengborg et al., 1998). A combined severity of 2.7 has been reported to give a good yield on mannose present in the substrate and also production of several fermentation inhibitors. This was also confirmed in the level of mannose present in the final hydrolyzate which contained 17 g L^{-1} mannose in contrast to the rater low glucose level of 8 g L^{-1}. A maximum glucose yield is instead gained at a higher combined severity of 2.9-3.4 (Tengborg and Stenberg, 1998).

3.1 Continuous cultivations at low yeast cell density

The wood hydrolyzate used in this study was not detoxified prior to cultivation. The hydrolyzate contained several inhibitors e.g. acetic acid, hydroxymethylfurfural (HMF), furfural, formic acid, and levulinic acid. This hydrolyzate was so toxic that the yeast failed to ferment it in the sMBR at a dilution rate of 0.2 h^{-1} with the low yeast concentration of 12.1 ± 1.2 g L^{-1} (Fig. 2). This yeast concentration normally appears in traditional continuous cultivations. The results illustrate that the ethanol production first increase in the cultivation, subsequently the ethanol concentration in the membrane permeate slowly reduces, and both furan inhibitors i.e. furfural and HMF and sugars started to accumulate in the permeate.

Measurements of the colony forming units (CFU) levels after cultivations with low yeast concentration (36 or 47 h) showed that the viability of the yeast in the sMBR was below 1×10^4 CFU mL^{-1}. This demonstrates that the hydrolyzate had a great negative impact on the yeast viability when the yeast was cultivated at a low yeast cell density of about 10-12 g L^{-1}.

3.2 Continuous cultivations at high yeast cell density

Continuous cultivations of the hydrolyzate was successfully conducted at high yeast density where the employed sMBR contained > 50 g L^{-1} yeast at the dilution rates 0.2, 0.4, 0.6 and 0.8 h^{-1}. During cultivations at high yeast density, the yeast *in situ* detoxified some inhibitory compounds present in the hydrolyzate like e.g. furfural and HMF to less inhibitory compounds, as shown in Table 1. It is well known that the furan aldehydes,

furfural and HMF, is converted by the yeast to less inhibitory alcohols at anaerobic conditions (Liu et al., 2004). The cells *in situ* detoxification of furan aldehydes is performed by alcohol dehydrogenase (Modig et al., 2002). Additionally, *Sacchaomyces cerevisiae* is able to detoxify phenolic inhibitory compounds which are often toxic for the yeast even at low concentrations (Larsson et al., 2000). By maintaining a high cell concentration in the sMBR the inhibitor to cell ratio can be kept low aiding the continued detoxification of convertible inhibitors.

In the performed cultivations the detoxification of both furfural and HMF was very high and therefore the levels of furfural and HMF were close to 0 g L^{-1} even at a dilution rate of 0.8 h^{-1}. Conversion of furfural was 98% at a dilution rate of 0.2 h^{-1} and stayed at 97% even when the dilution rate was increased to 0.8 h^{-1}. Furfural conversion was faster than the conversion of HMF, as also earlier studies shown (Almeida et al., 2007). Larsson et al. (Larsson et al., 1999) suggested that the reason for the lower conversion rate of HMF because of is its lower membrane permeability (Palmqvist and Hahn-Hägerdal, 2000). HMF has also been shown to cause less inhibition at the same amount as furfural, and is therefore considered to have a less toxic effect on growth and fermentation. HMF conversion was 89% at the lowest dilution rate and reduced to 70% at the highest dilution rate.

During cultivations at this high yeast cell density, the yeast dry weight was measured several times throughout the entire cultivation (Fig. 3). Both viability and yeast dry weigh were stable or indicated that the yeast was growing somewhat during the

cultivation. No reduction in viability was observed even when wood hydrolyzate was fed with a dilution rate of 0.8 h^{-1} for 5 retention volumes.

Probably the yeasts capacity to reduce the level of toxic compounds in the fed medium to low concentrations made the yeast able to maintain the inhibitory concentration at levels which the cells could cope with and continue ethanol fermentation. The maintained ethanol fermentation is clearly illustrated for all dilution rates in Fig 4, which shows the ethanol levels during the entire cultivations. What may occur at intermediate yeast cell concentrations between 12 - 50 g L^{-1} is most probable that the yeast would first be able to ferment the wood hydrolyzate at a low dilution rate but fail at a higher dilution rate, because of too low detoxification capacity and high inhibitor to cell ratio. As the yeast cell concentration would be increased inside the sMBR the yeast would be able to withstand higher amounts of inhibitors and thereby higher hydrolyzate dilution rates, as was also shown in the study.

At the highest dilution rate there is, however, a tendency that the ethanol concentrations start to decrease. Nevertheless, the sugar utilization was measured to 86.8 ± 1.0% (calculated on glucose and mannose) after 5 retention times at 0.8 h^{-1} dilution rate, see Table 1. The sugar concentrations of the hexose sugars glucose, mannose and galactose during the cultivations are shown in Fig. 5. Some inhibitors such as acetic acid, levulinic acid and formic acid stabilized at a constant value and did thereafter not change during the cultivation. The weak carboxylic acids are not detoxified by the yeast at anaerobic conditions. However, they are also known to be harmful for the yeast cells and inhibit cell growth (Beales, 2004). In the sMBR cell growth in not crucial as long as

an enough cells have been accumulated before adding hydrolyzate medium. This since the cells can be completely retained inside the bioreactor by the membrane.

The yeast strain *S. cerevisiae* CBS 8066 used in this study is a laboratory strain which is not very tolerant to the inhibitors present in hydrolyzate, this has been shown in several other investigations without cell retention where the yeast failed to ferment wood hydrolyzate at a dilution rate above 0.1 h^{-1} (Purwadi et al., 2007, Talebnia and Taherzadeh, 2006). By increasing the yeast density in the cultivation, this study shows that even this non-tolerant yeast could cope with the applied hydrolysate. Probably if a more tolerant yeast strain would be applied in the sMBR the yeast can endure even hydrolyzate medium containing more inhibitors.

Most MBRs applied for ethanol production has used glucose as carbon source (Chang et al., 1993, Kang et al., 1990). A sMBR has also been utilized by Lee et al. (Lee et al., 2000) to ferment a stem pretreated and enzymatically hydrolyzed wood hydrolyzate. However, enzymatically treated wood hydrolyzate is usually less toxic than dilute acid hydrolyzate and additionally the study was conducted at a relatively low dilution rate of 0.22 h^{-1}.

Purwardi et al. (Purwadi and Brandberg, 2007) reported successful fermentation of a wood hydrolyzate at a dilution rate of 0.52 h^{-1} by using a flocculating yeast strain of *S. cerevisiae* CCUG 53310. This flocculating yeast is, however, known for its inhibitor tolerance and can easily be sedimented and recycled back to the bioreactor to get a high yeast density of up to 50 g L^{-1} (Purwadi and Brandberg, 2007). One drawback of using

flocculating yeast is the mass transfer problem. Since sugars need diffuse into the yeast flocks'. Unlike suspended yeast the flocculating yeast generally require approximately twice the time to complete the fermentation at anaerobic conditions (Purwadi and Brandberg, 2007).

4. Conclusions

By using a sMBR to reach a high yeast density of above 50 g L^{-1} dilute acid, wood hydrolyzate could be successfully fermented at high dilution rates of 0.2, 0.4, 0.6 and 0.8 h^{-1}. The sMBR could be used to completely retain all yeast cells in the system during cultivations performed at 30 °C and pH 5.0. The viability of the yeast stayed stable at around 0.8 billion CFU mL^{-1} even when the dilution rate was increased stepwise to 0.8 h^{-1} for 5 retention volumes. Glucose and mannose utilization remained above 85% in the used MBR even at a dilution rate of 0.8 h^{-1}. The maximum reached volumetric ethanol production in the continuous cultivations was 7.94 ± 0.10 g L^{-1} h^{-1} and was achieved at a dilution rate of 0.8 h^{-1}.

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Supplementary graph

Supplementary graph showing the membrane panels with an IPC can be found, in the online version.

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Figure captions

Fig. 1 sMBR configuration, straight lines denote liquid streams and dashed lines denote gas streams. The different compartments are; (1) bioreactor with circulation loop to and from container with membrane, (2) IPC membrane panels, (3) container with membrane panels, (4) flask for collect of water droplets and condensate in gas, (5) gas tight container where (6) linear gas pump is placed to circulate the gas (and keep the system anaerobic).

Fig. 2 Concentration of metabolites, yeast dry weight, furfural and HMF, and hexose levels during anaerobic continuous cultivations of wood hydrolyzates at low yeast concentrations at a dilution of 0.2 h^{-1}. Error bars indicates the standard deviation for two separate cultivations.

Fig. 3 Concentration of yeast dry weight and yeast viability during anaerobic continuous cultivations of wood hydrolyzates at different dilution rates from 0.2, 0.4, 0.6 and 0.8 h^{-1}. Error bars indicates the standard deviation for two separate cultivations.

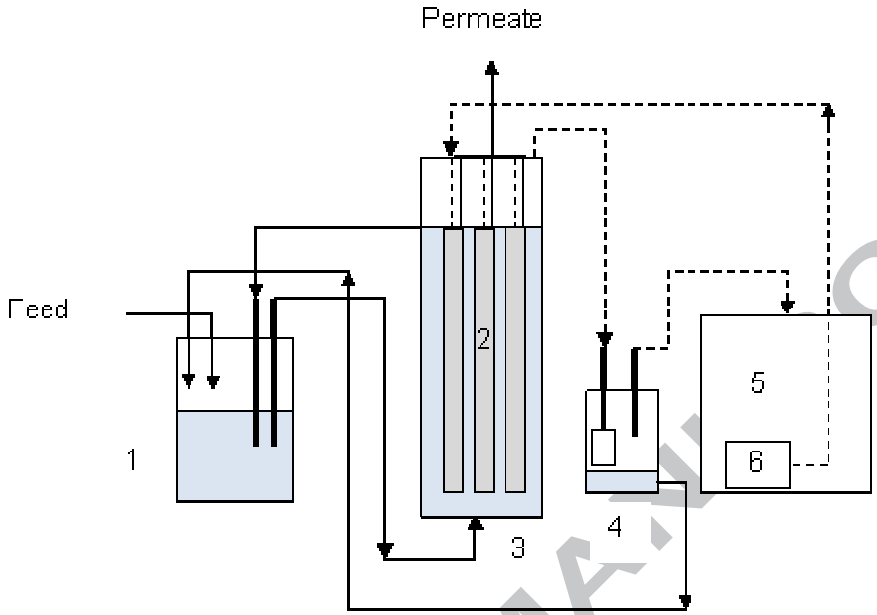
Fig. 4 Concentration of the metabolite ethanol and glycerol during anaerobic continuous cultivations of wood hydrolyzates at different dilution rates from 0.2, 0.4, 0.6 and 0.8 h^{-1}. Error bars indicates the standard deviation for two separate cultivations.

Fig. 5 Hexose concentration during anaerobic continuous cultivations of wood hydrolyzates at different dilution rates from 0.2, 0.4, 0.6 and 0.8 h^{-1}. Error bars indicates the standard deviation for two separate cultivations.

Table 1. Summary of yields, ethanol productivity, sugar assimilation and inhibitor conversion during anaerobic continuous cultivation of toxic wood hydrolyzate at different dilution rates.

1 **Tables and Figures**

2



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4 Figure 1

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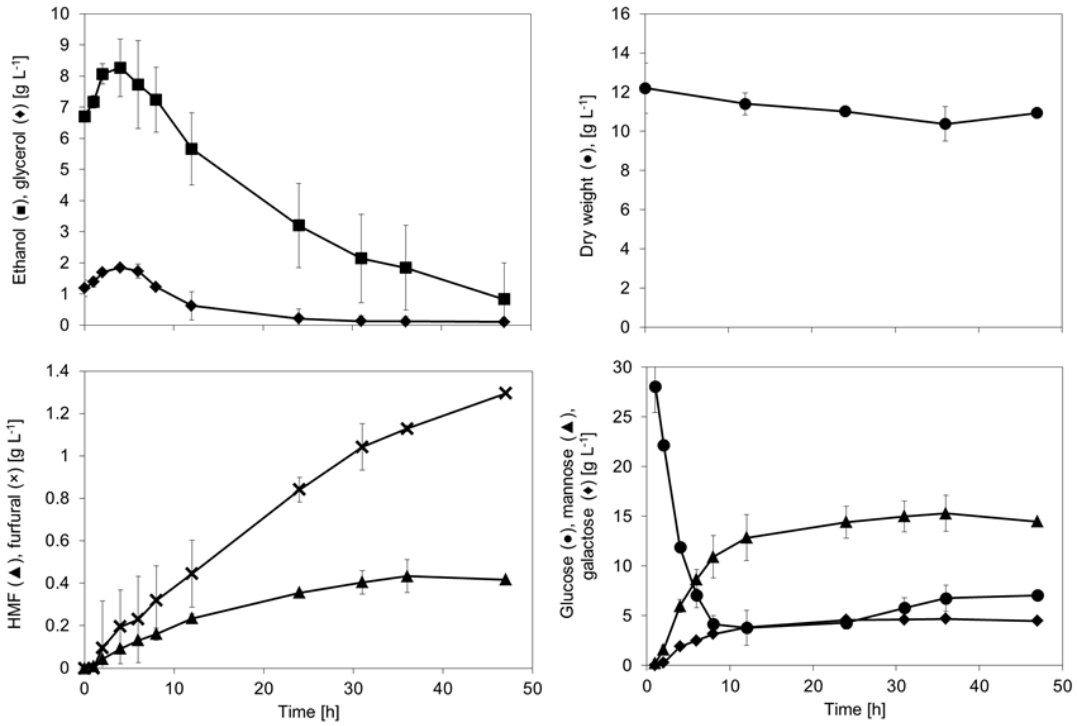

Figure 2

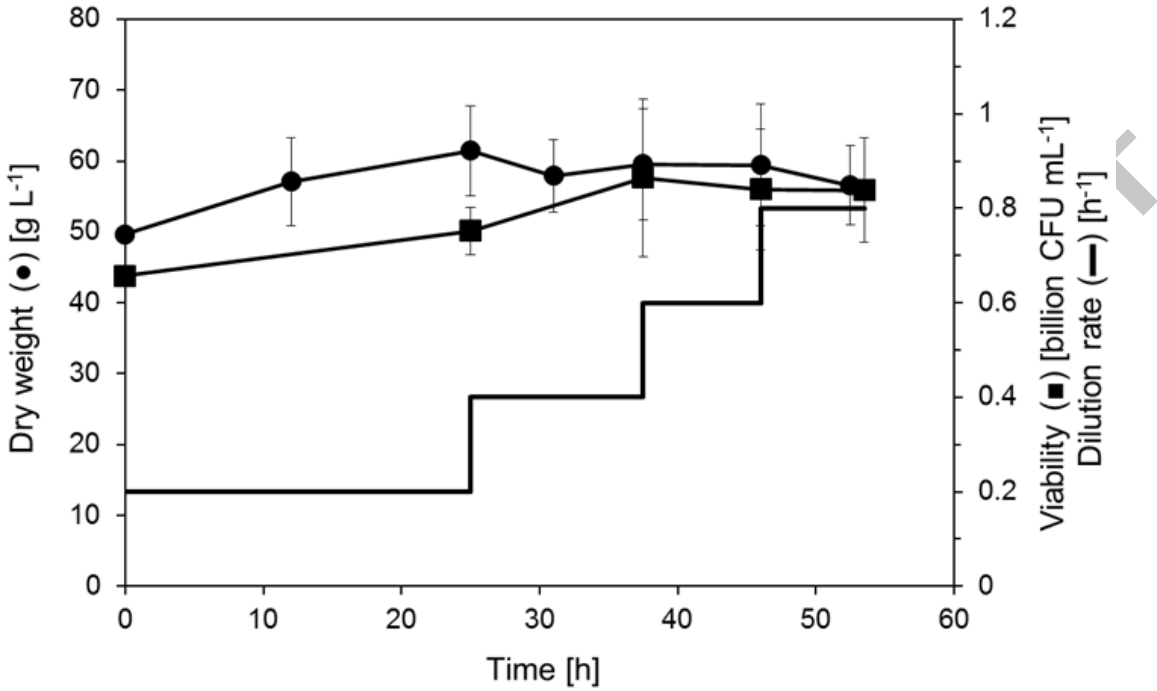

Figure 3

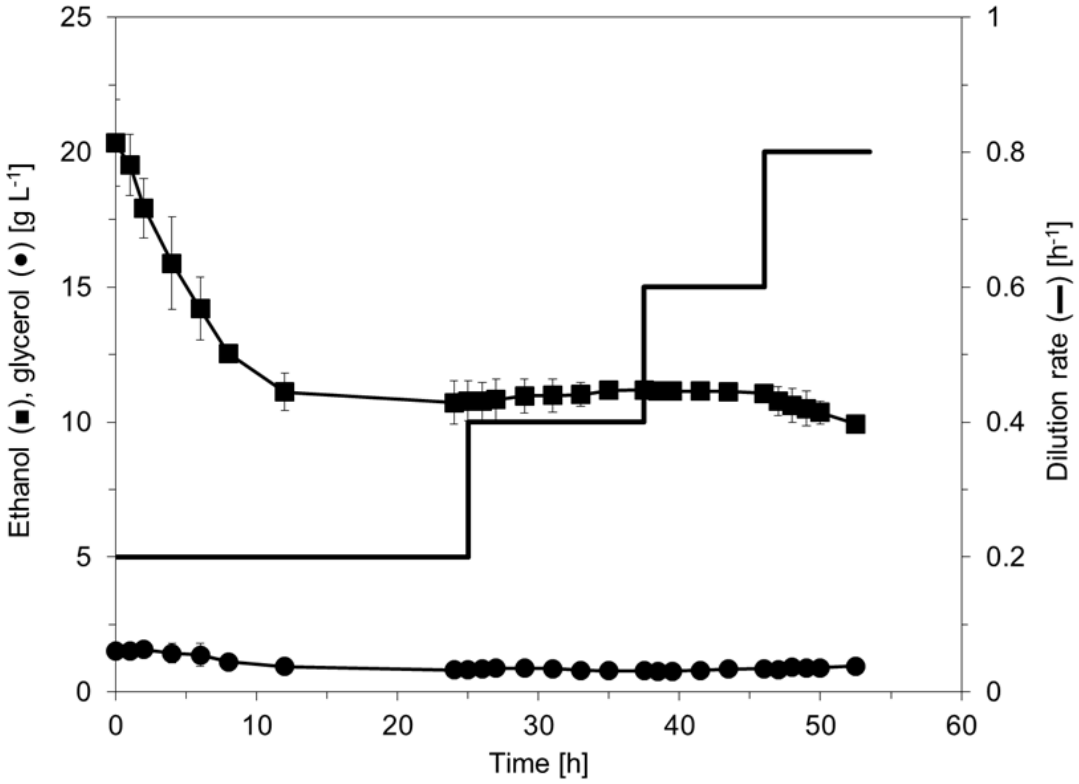

Figure 4

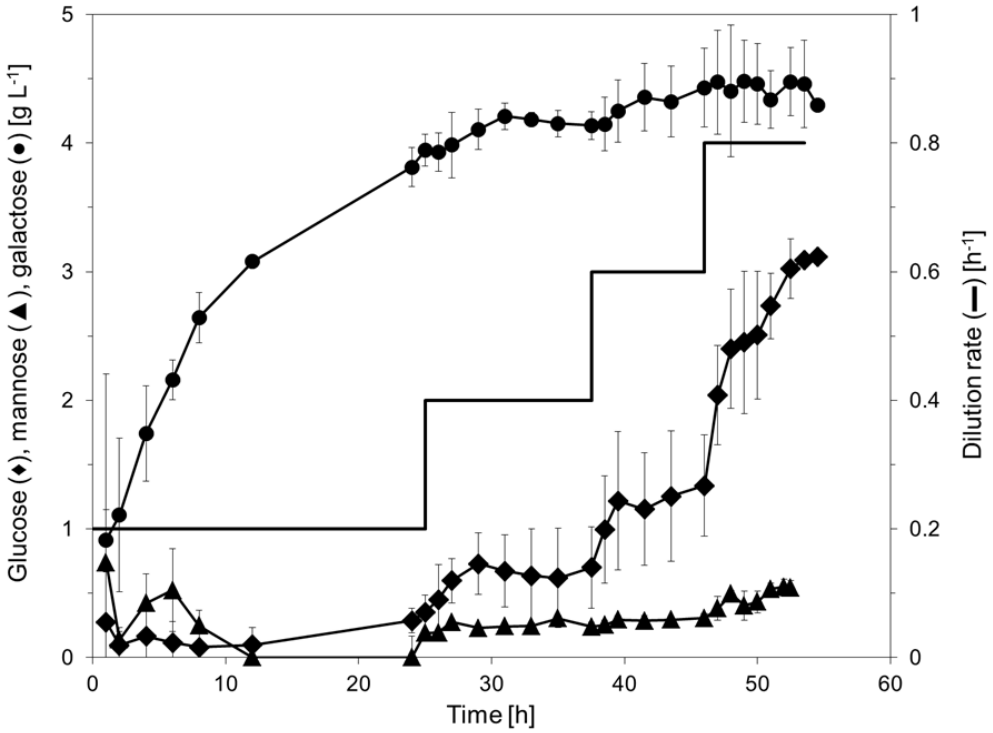

Figure 5

1 Table 1

					Sugar assimilation (%)		Conversion (%)	
D[a] (h^{-1})	$Y_{Ethanol/s}$[b] (g g^{-1})	$Y_{Glycerol/s}$[b] (g g^{-1})	Q_p[c] (g L^{-1} h^{-1})	Q_{sp}[d] (g g^{-1} h^{-1})	Hexoses[e]	Mannose and glucose	Furfural	HMF
0.2	0.42 ± 0.03	0.032 ± 0.007	2.16 ± 0.15	0.035 ± 0.006	85.2 ± 0.7	97.8 ± 0.6	98.3 ± 1.0	89.0 ± 5.9
0.4	0.44 ± 0.01	0.031 ± 0.003	4.47 ± 0.07	0.076 ± 0.011	83.5 ± 0.7	96.6 ± 1.4	98.0 ± 1.0	82.2 ± 7.1
0.6	0.45 ± 0.01	0.035 ± 0.000	6.66 ± 0.16	0.113 ± 0.014	80.2 ± 0.7	93.7 ± 2.3	98.0 ± 0.9	78.2 ± 7.0
0.8	0.44 ± 0.01	0.043 ± 0.002	7.94 ± 0.10	0.141 ± 0.012	74.2 ± 0.4	86.8 ± 1.0	97.4 ± 1.1	70.0 ± 0.3

2 [a]All results are based on values after 5 retention volumes at the specific dilution rate
3 [b]Yields are based on consumed hexoses i.e. glucose, mannose and galactose
4 [c]Volumetric ethanol productivity
5 [d]Specific ethanol productivity
6 [e]Sugar assimilation in % of hexoses i.e. glucose, mannose and galactose
7

Highlights

► A novel submerged membrane bioreactor (sMBR) was used to retain the yeast.

► During cultivations the sMBR contained high yeast concentrations of around 60 g L^{-1}.

► Fermentation of toxic hydrolyzate was successful at a dilution rate of up to 0.8 h^{-1}.

► High sugar utilization was attained even at higher dilution rates.

► A maximum ethanol productivity of 7.9 g g^{-1} h^{-1} was achieved at the dilution 0.8 h^{-1}.

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3 **Graphical abstract**

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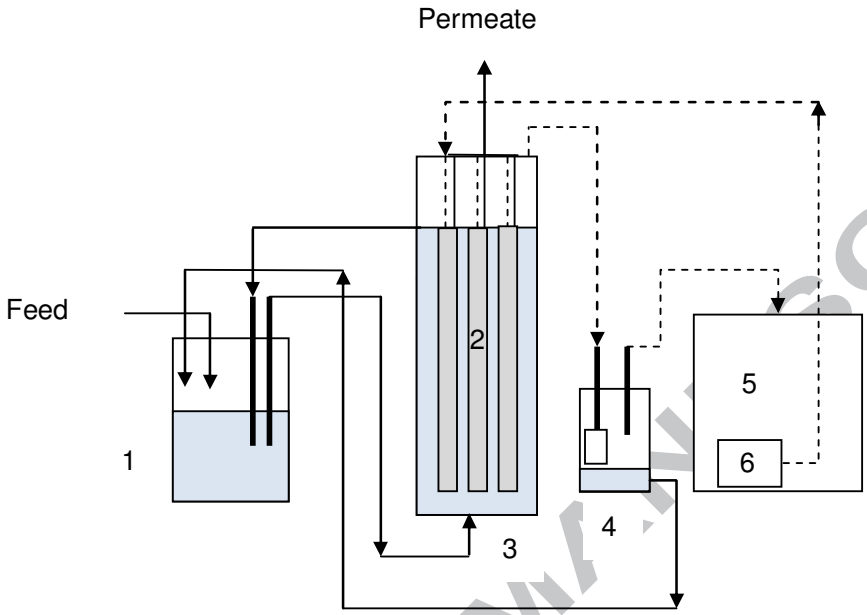

(1) Bioreactor
(2) IPC membrane panels
(3) Container with membrane panels
(4) Flask for collect of water droplets and condensate in gas
(5) Gas tight container
(6) Linear gas pump