CHEMICAL ENGINEERING TRANSACTIONS Volume 25, 2011
Editors Jiří Jaromír Klemeš, Petar Sabev Varbanov, Hon Loong Lam
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DOI: 10.3303/CET1125159

Efficient Hydrogen Fermentation for 2 - Stage Anaerobic Digestion Processes: Conversion of Sucrose Containing Substrates

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A new designed carrier based bioreactor was used for thermophilic hydrogen fermentation for a 2- stage digestion process. Thick juice, a pre-product of sugar production and molasses, a by- product of it were used as a substrate to compare hydrogen productivity, hydrogen yield and acid spectrum.

Both substrates were efficiently converted to hydrogen and organic acids up to organic loads of 1.75 g/L/h and hydraulic retention times of 10 h. At similar levels of organic load molasses yielded better overall hydrogen production yield (30%), which is contributed to the more complex composition and higher trace element content of molasses. Further products formed were acetate and lactate in a ratio of approximately 70:30, which are preferred substrate for fast and efficient conversion to methane.

1. Introduction

In order to optimize the conversion of biomass in a biogas plant one possibility is to setup a 2 - stage anaerobic digestion process. In the first stage H2, CO2 and fatty acids are the main products. The fatty acids are converted into CH4 and CO2 in the second stage. So the hydrogen fermentation provides not only easy convertible fatty acids for the following steps, but also H_2 which can be used as well. The set up of hydrogen fermentation processes is influenced by a number of parameters, such as type of inoculum, substrate, bioreactor system, organic load, mode of operation, medium composition, temperature and pH (Wang and Wan, 2009). Especially the temperature was shown to be of significant influence. Thermophilic fermentations are superior in terms of hydrogen yield, as compared to fermentations at moderate temperatures (Hallenbeck, 2005). Using (extreme) thermophiles, one mol of glucose can be converted to 4 mol of hydrogen and 2 mol of acetic acid as the main by-product (de Vrije et. al., 2007; Schröder et. al., 1994; van Ooteghem et. al., 2004; Zeidan, 2009), which is the maximum theoretically achievable yield. At mesophilic temperature ranges, however, the average hydrogen yield is only 1–2 mol of hydrogen per mole of glucose (de Vrije et. al., 2007; Kleerebezem et. al., 2007). Thermophilic hydrogen fermentation has 3 major advantages: Higher product yields, sanitation and therefore elimination of pathogenes and avoidance of hydrogen consuming organisms like methanogenes.

Another positive aspect are the better combustion properties due to the hydrogen enriched biogas regarding CO_2 and NO_X emissions.

Please cite this article as: Noebauer S. and Schnitzhofer W., 2011, Efficient hydrogen fermentation for 2 - stage anaerobic digestion processes: conversion of sucrose containing substrates, Chemical Engineering Transactions, 25, 953-958
DOI: 10.3303/CET1125159

This study deals with the thermophilic hydrogen fermentation step in a new bioreactor system with 2 sucrose containing substrates: thick juice, a pre-product of sugar production and molasses, a by- product of it.

2. Materials and methods

A new designed carrier based bed reactor (combined fluidized and trickling bed reactor, CFTB) as shown in Figure 1, with a total volume of 30 L and a liquid volume of 13 L was applied for these experiments. It was operated at a temperature of 75 °C and a pH of 6.5 (adjusted with 2M NaOH). The hydraulic retention times in these tests were 20, 15, 12 and 10 h. These are equal to organic loads between 0.5 and 1.75 g sucrose/L/h (10- 17.5 g/L sucrose in the substrate). The used organisms, C. saccharolyticus (DSM 8903) and C. owensensis (DSM 13100) were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) and were pre- cultured separately at 65 °C and a pH of 6.5 (Zeidan et. al., 2009). The culture was transferred several times till a total volume of 500 mL C. saccharolyticus and 1000 mL C. owensensis was reached. However, after the start up the fermentations were conducted in an auto selective mode.

For the process monitoring gas volume (Ritter Gas counter) and gas composition as well as temperature, pH and Redox were supervised continuously and logged by a data log system (Awite). To determine acids and sucrose in the liquid phase, samples were taken once a day and measured by means of UPLC (Waters).

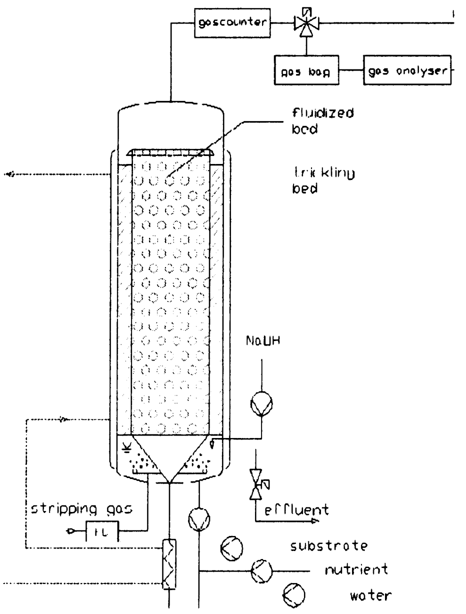

Figure 1: Scheme of the CFTB reactor

3. Results and Discussion

Generally this new bioreactor configuration resulted in an effective immobilization of the hydrogen producing microorganisms providing a very stable process, which could be easily recovered after power failure or leakages. Sucrose in the substrate was completely consumed and converted to H_2, CO_2, acetate, lactate and small amounts of ethanol. Methane was never detected.

3.1 Thick Juice

In the first fermentation run thick juice was used as a substrate and different hydraulic retention times (HRTs) of 20, 15, 12.5 and 10 adjusted. Additionally the sucrose content of the substrate was varied between 10 g/L and 17.5 g/L, which correspond to organic loads between 0.5 and 1.75 g/L/h.

In these tests hydrogen productivities between 3.24 and 28.26 mmol/L/h as well as hydrogen yields between 2 and 5.3 mol/mol sucrose were reached as shown in Figure 2. In general the average hydrogen productivity was increasing with increasing organic load. The hydrogen yield increased as well with increasing organic load to reach its maximum at an organic load of 1 g/L/h. However the hydrogen yields stayed nearly constant at a level of 4.9 ±0.25 mol/mol sucrose. This indicates that even higher loads at shorter retention times could be applied without influencing the degree of conversion negatively. This could be due to the effective immobilisation of hydrogen producing organisms in the system and their adaption to the substrate and environmental conditions.

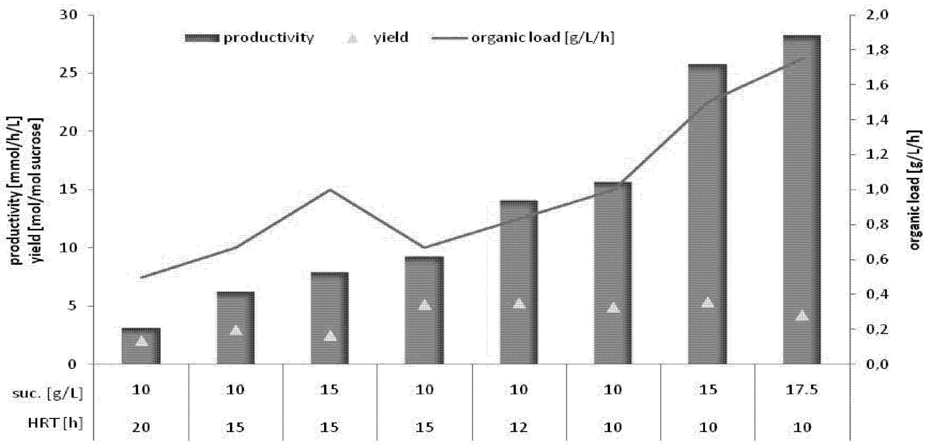

Figure 2: Average hydrogen productivity and hydrogen yield during thick juice fermentations in a carrier based bioreactor at different hydraulic retention times

A common tendency was obvious: The slower the variation of the HRT or organic load, the better the sugar conversion. This can be especially seen at the third data point of Figure 3. At this point the organic load was raised rapidly, by increasing the sugar content, but the hydrogen productivity did not increase to the same extent. At a later state the substrate could be easily converted at the same organic load level (sixth data point in Figure 3). This effect of overloading the process is also visible in the value of the hydrogen yield.

The above described effect might indicate furthermore, that the reduction of HRT is the better way to increase the organic load than to increase the sugar concentration in substrate feed.

Tab. 1: Average metabolite concentrations calculated from thick juice fermentations using a co-culture of C. saccharolyticus and C. owensensis

HRT [h]	Acetate thick juice [g/L]	Lactate thick juice [g/L]
20	4.98	2.07
15	4.75	1.71
12	5.82	1.81
10	5.64	1.28

Sucrose in the medium was converted to 4.9 - 5.8 g/L acetate and 1.3 – 2.1 g/L lactate dependent to the organic load (Table 1). Except for the mentioned case of overloading the process, sucrose was completely converted. Acetate concentration in the liquid increased with shorter retention times, indicating a better conversion of the substrate. The formation of lactate, which was always found in the medium, showed, that a partial product inhibition took place, even if the content decreased at shorter HRTs.

3.2 Molasses

In the second long term fermentation run molasses was used as a substrate and HRTs of 20, 15, 12.5 and 10 h were adjusted, corresponding to a stepwise increase of the organic load.

The H_2 - productivity increased from 12.4 to 21.3 mmol/L/h with molasses according to the organic load as can be seen in Figure 4. The hydrogen yield stayed nearly constant - similar to the thick juice fermentation - at around 6.7 - 7.7 mol/mol sucrose corresponding to 83 – 96% of the theoretical maximum.

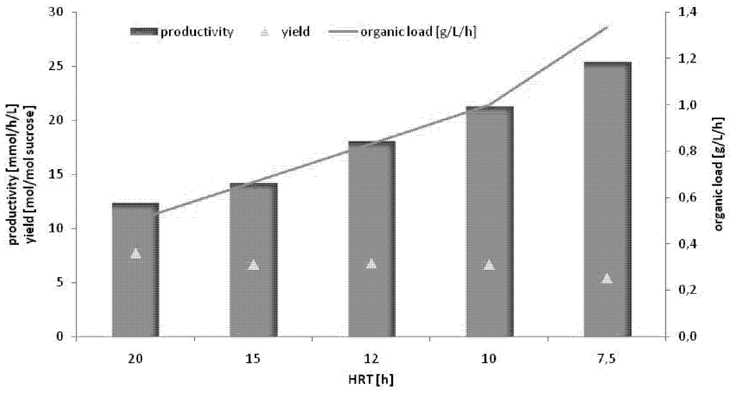

Figure 3: Average hydrogen productivity and hydrogen yield during molasses fermentation in a carrier based bioreactor at different hydraulic retention times

Sucrose in the medium was converted to 3.7 - 4.6 g/L acetate and 1.6 – 2.0 g/L lactate dependent to the organic load (*Table 2*). As stated before the formation of lactate showed, that a partial product inhibition took place, in contrast to thick juice fermentation the level was increasing at shorter retention times.

Table 2: Average metabolite concentrations calculated from molasses fermentations using a co-culture of C. saccharolyticus and C. owensensis

HRT [h]	Acetate molasses [g/L]	Lactate molasses [g/L]
20	3.66	1.63
15	4.06	1.82
12	4.20	1.72
10	4.59	2.02

3.3 Comparison

The hydrogen productivity and yield obtained from sucrose containing substrate were generally higher using molasses than thick juice which can be seen in Figure 4, though less acetate was produced. This was most probably due to the composition of the different substrates. Molasses contains additional proteins and amino acids which can be beneficial for hydrogen formation. Since the content of these additional components was not known, it was not taken into account for calculation of hydrogen productivity and yield. The yields obtained might be apparently higher, since the data were referred to the sucrose content inserted in the system.

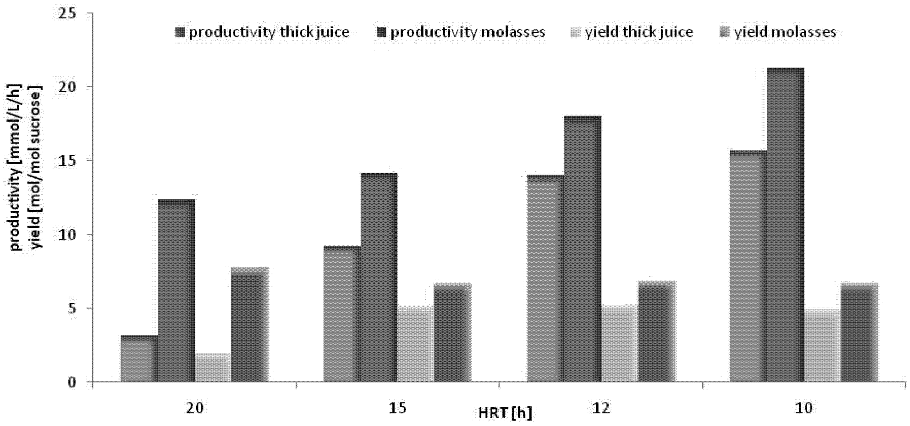

Figure 4: Comparison of the hydrogen productivity and yield of fermentations using thick juice and molasses as a substrate in a carrier based bioreactor

Molasses was selected as a substrate for hydrogen production before. Ren et al., 2006 obtained a maximal hydrogen productivity of ca. 10 mmol/L/h in a 2 m3 CSTR type bioreactor at mesophilic conditions using seed sludge from waste water treatment plant. In another study it was found that thermal pretreatment of molasses at 70 °C for 10 min lead to an up to 40 % increased hydrogen production rate, even so the obtained figures were rather low (6 mmol/g volatile suspended solids/d, Lin et al., 2006). This finding supports the approach to apply extreme thermophilic processes for efficient hydrogen production from sucrose containing substrates.

4. Conclusion

These tests revealed that sucrose based substrates can be efficiently converted to H_2 and acids. Although the reached hydrogen productivities and yields were more than acceptable with both substrates, additional fermentations with shorter HRT and higher organic loads will be performed with molasses which is more complex and needs therefore less additional nutrients.

Acknowledgement

This work was performed in the context of the integrated project "HYVOLUTION" which is funded by Framework Program 6 of the EC.

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