Efficient biohydrogen production from whey using a pilot scale carrier based bioreactor system

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Abstract
Thermophilic hydrogen production from whey, a by-product of cheese production, was studied using a 600 L continuous carrier based bioreactor system. The reactor was operated at hydraulic retention time between 20 h and 15 h resulted in organic loading rates from 0.75 to 1.0 g/h/L. It achieved a maximum volumetric hydrogen production rate of 322 mL/h/L, which is much higher than the actual output of a lab-scale stirring tank reactor used as reference. The maximum hydrogen yield of the pilot plant reactor was 1.9 mol H_2/mol hexose, which is also significantly higher than the reference system. The gas-phase hydrogen content exceeded 60% H_2, sugar was converted completely. The fermentation turned out to be very stable, therefore higher organic loading rates are definitely achievable.

Keywords
Biohydrogen, carrier based bioreactor system, whey

INTRODUCTION

Hydrogen, an energy carrier with very high energy content, can be provided by different chemical or biotechnological processes. One of those processes is the dark fermentation of sugar. The dark fermentation is influenced by a number of parameters, such as type of inoculum, substrate, bioreactor system, organic loading rate, 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 et. al.), 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.

This study dealed with the thermophilic hydrogen fermentation in a carrier based bioreactor system with whey, a lactose rich by-product of cheese production in comparison with sucrose containing substrates.

MATERIALS AND METHODS

A new designed pilot scale carrier based bed reactor (combined fluidized and trickling bed reactor, CFTB), made of stainless steel with a total volume of 600 L and a liquid volume of 280 L was tested. A 2 L stirring tank reactor was used as reference. Both were operated at a temperature of 60 °C and a pH of 5.5 (adjusted with 20% NaOH). Initial lactose concentration in the bioreactors was set to 10g/L. Anaerobically digested sludge from a municipal waste water treatment plant was used as an inoculum. The sludge was sieved, heat treated at 105°C for 2h and cooled down to room temperature before inoculating.

The hydraulic retention times achieved in these experiments were 20 h and 15 h, respectively. These correspond to organic loading rates between 0.5 and 1.0 g lactose/L/h (10- 15 g/L lactose in the feed). For the process monitoring gas volume (Ritter gas counter) as well as temperature, pH and ORP were supervised continuously and logged by a data log system (Awite and Sartorius respectively, Germany). To determine acids and sucrose in the liquid phase, samples were taken once a day and measured by means of UPLC (Waters).

Batch digestion tests at 60°C and 70°C were carried out according to VDI 4630 with loading rates between 2% and 6% lactose.

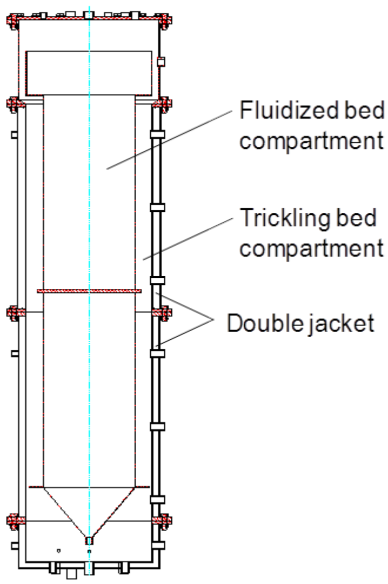

Figure 1. Bioreactor configuration of the 600 L pilot scale carrier based system

RESULTS AND DISCUSSION

Whey turned out to be a very promising substrate and resulted in high H_2 yields also achieved in batch digestion tests. The batch tests showed that the hydrogen yield can be nearly doubled at a temperature of 60°C in comparison to those at 70°C as seen in Table 1. It should also be mentioned that the gas production rate of the fermentation at 60°C was terminated within 48 h, whereas the fermentation at 70°C obtained a much lower gas production rate resulted in overall fermentation time of 192h. The fermentation temperature in the reactors was set accordingly.

Table 1. Results of batch digestion tests with different whey concentrations

Lactose concentration	H_2 yield at 70 °C (NL/kg lactose)	H_2 yield at 60 °C (NL/kg lactose)
2%	88.8	216.5
4%	55.7	135.4
6%	19.05	114.2

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. Lactose in the substrate was completely consumed and converted to H_2, CO_2, acetate, lactate and small amounts of ethanol. Methane was never detected.
The H_2 -productivity in the reference fermentation increased from 110 to 304 mL/L/h according to the organic load as can be seen in Table 2. The hydrogen yield increased from 1.51 mol/mol hexose to 1.76 mol/mol hexose corresponding to 47- 66% of the theoretical maximum. The carrier based reactor obtained higher productivities between 162 mL/h/L and 276 mL/h/L at hydraulic retention times of 20 h and 15 h. Hydrogen productivity and yield at lower hydraulic retention times could only be calculated at this time, the data based on a similar fermentation with sucrose containing substrates.

Table 2. Achieved hydrogen productivity and yield during whey fermentation in a carrier based bioreactor (CFTB) in comparison to a reference system (CSTR)

HRT (h)	Lactose input (g/L)	Hydrogen productivity		Hydrogen yield	
		CFTB (mL/h/L)	CSTR (mL/h/L)	CFTB (mol H_2/mol hex)	CSTR (mol H_2/mol hex)
20	15	162	110	1.51	1.54
15	15	276	163	1.93	1.72
12	15	345*	236	1.85*	1.98
10	15	414*	304	1.76*	2.13

* calculated according to a similar fermentation with sucrose containing substrates

Lactose was converted to 3.9 ±0.3 g/L acetate, 2.13 ±0.6 g/L lactate and 0.95 ±0.4 g/L butyrate during the CFTB fermentation dependent to the organic load as shown in Table 3. Sucrose was completely converted. Acetate concentration in the liquid increased at 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. Similar results could be obtained in former fermentation conducted with sucrose containing substrates.

Table 3. Average metabolite concentrations calculated from whey fermentations in a carrier based bioreactor

HRT (h)	Acetate (g/L)	Lactate (g/L)	Butyrate (g/L)
20	3.8	2.3	0.4
15	4.2	0.9	2.5

CONCLUSION

These tests revealed that lactose based substrates can be efficiently converted to H_2 and acids which is comparable to previous studies (Ferchichi 2004 et. al., Yang 2007 et. al.). However, the new designed bioreactor system showed a much better fermentation performance regarding to hydrogen production rate and hydrogen yield than the standard stirring tank reactor. Furthermore it ensures a very stable fermentation process, both due to effective immobilization of biomass. Although the reached hydrogen productivities and yields were more than acceptable, additional fermentations at shorter HRT and higher organic loading rates will be performed to confirm the results.

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