Influence of operational parameters on biohydrogen and biopolymer production from molasses in a 2-stage process

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Abstract
Polyhydroxyalkanoates (PHA) is a storage polymer inside the cell of bacteria that can be used for the production of biodegradable thermoplastics. This work investigated the feasibility of the biohydrogen and biopolymer production in a continuous 2-stage process from molasses in large lab-scale. In the first stage H_2 production could be established for approximately 170 d without methane production. During acidogenesis lactic, acetic and butyric acids were mainly produced. Bacteria present in the reactor were affiliated to lactic acid bacteria (LAB) and species of the *Clostridium* genus. In the second stage a maximum PHA content of 20.6% TS was reached. As optimal conditions SRT of 8 d and HRT of 2 d were determined. SRT of 5 d and of 12 d resulted to lower PHA yields. Both shorter and longer HRT resulted to lower yields. Increase of OLR to 7.5 g COD/(L·d) did not result to improvement of the PHA content in the cells. The highest PHA accumulation coincided with the proliferation of *Tetracoccus cechii* and *Brevundimonas* sp. The batch addition of pure acetate resulted to PHA accumulation of 36% TS, which was almost 2.5times more than that obtained by the effluent of the biohydrogen reactor. Thus, the suitability of the proven process for PHA accumulation was confirmed and higher PHA production could be achieved if the biohydrogen production pathway could be shifted towards acetate production.

Keywords
Biohydrogen, PHA; molasses, OLR, SRT, HRT

INTRODUCTION

Polyhydroxyalkanoates (PHA) is a storage polymer inside the cell of bacteria that can be used for the production of biodegradable thermoplastics. PHA accumulation within the cells occurs when stress is applied on the biocenosis. This stress may concern the availability of the external substrate,

i.e. transient conditions of feast and famine, the presence of an external electron acceptor, i.e. transient conditions of anaerobic and aerobic milieu, the limitation of nutrients, e.g. nitrogen and phosphorous while excess substrate is present in the bulk (Reis et al., 2003). Volatile fatty acids can be considered as suitable substrates (Reddy et al., 2003), which are abundantly produced during the fermentative biohydrogen production (Hawkes et al., 2007). Aim of the current work was the assessment of the feasibility of the biohydrogen and biopolymer production in a continuous 2-stage process.

MATERIALS AND METHODS

The first stage of the process was used for biohydrogen production by fermentation of molasses. Fermenter seeding and operation for biohydrogen, analytical techniques and bacterial population determination were carried out as previously described (Mariakakis et al., 2011).

The second stage of the process valorised the effluent of the biohydrogen production for biopolymer production in the form of polyhydroxyalkanoates (PHA). For the biopolymer production activated sludge was used as seed sludge. For the selection of biomass able to accumulate PHA two different reactor configurations were initially tested; a Continuous Stirred Tank Reactor (CSTR) and a Sequencing Batch Reactor (SBR). Both had a working volume of 20 L. The optimum configuration was then optimized for its potential to accumulate PHA by varying the Organic Loading Rate (OLR), the Hydraulic Retention Time (HRT) and the Sludge Retention Time (SRT). The total potential of the biomass to accumulate PHA was then tested by addition of acetic acid. All experiments were carried out under aerobic conditions at pH continuously controlled between 7.5 and 7.8, at 20 °C. The duration of the trials at each phase was three times the age of the sludge in order to assume a nearly stationary state can.

RESULTS AND DISCUSSION

Biohydrogen reactor

In figure 1 the reactor operation parameters, the daily biogas and H_2 production and the metabolite concentrations in the reactor for the phases D1 to D7 are presented. Biogas and H_2 production started immediately upon seeding of the reactor. Increase of the OLR at 24.7 g sucrose/(L·d) resulted to the cease of biogas and hydrogen production and the reduction of the metabolite concentration. This problem could be overcome by the reduction of HRT to 1 d on phase D3 and on. During the whole operation of approximately 170 d no CH_4 was detected. Methanogens could be eliminated through the thermal pre-treatment of the seed sludge and the selected operation parameters were sufficient to hinder their proliferation in the system. In all phases except phases D4 and D6 the average H_2 yield was equal or lower than 1 mol H_2/mol hexose. The best results were acquired in phase D4 reaching 1.53 mol H_2/mol hexose for HRT of 1 d and OLR of 36.1 g sucrose/(L·d) and in phase D6 reaching 1.31 mol H_2/mol hexose for HRT of 0.5 d and OLR of 36.1 g sucrose/(L·d).The major metabolites for all phases were lactic, acetic and butyric acid together with ethanol. Propionic and hexanoic (caproic) acid were produced in low quantities and only in same cases. Pentanoic (valeric) acid and butanol were not detected at all. Ethanol was found in various concentrations. The total metabolite COD was in the range of 0.47 to 0.72 of the total soluble COD without the eventual influence of residual sucrose (data not shown). A substantial part of the metabolites could not be monitored. Such may be acetone, propanol, formic and succinic acid.

The investigation on eubacterial 16S rDNA showed mostly lactic acid bacteria (LAB) of the phylum *Firmicutes* as closest relatives to the detected DGGE bands. Through the clostridia specific primers it was possible to allocate several bands to different clostridia species. Most of the bands could be affiliated to the species *C. butyricum*, *C. tyrobutyricum* and *C. ljungdahlii*. The co-

existence of LAB together with H_2 producing bacteria could be observed resulting to high lactic acid production rates and medium to low H_2 yields.

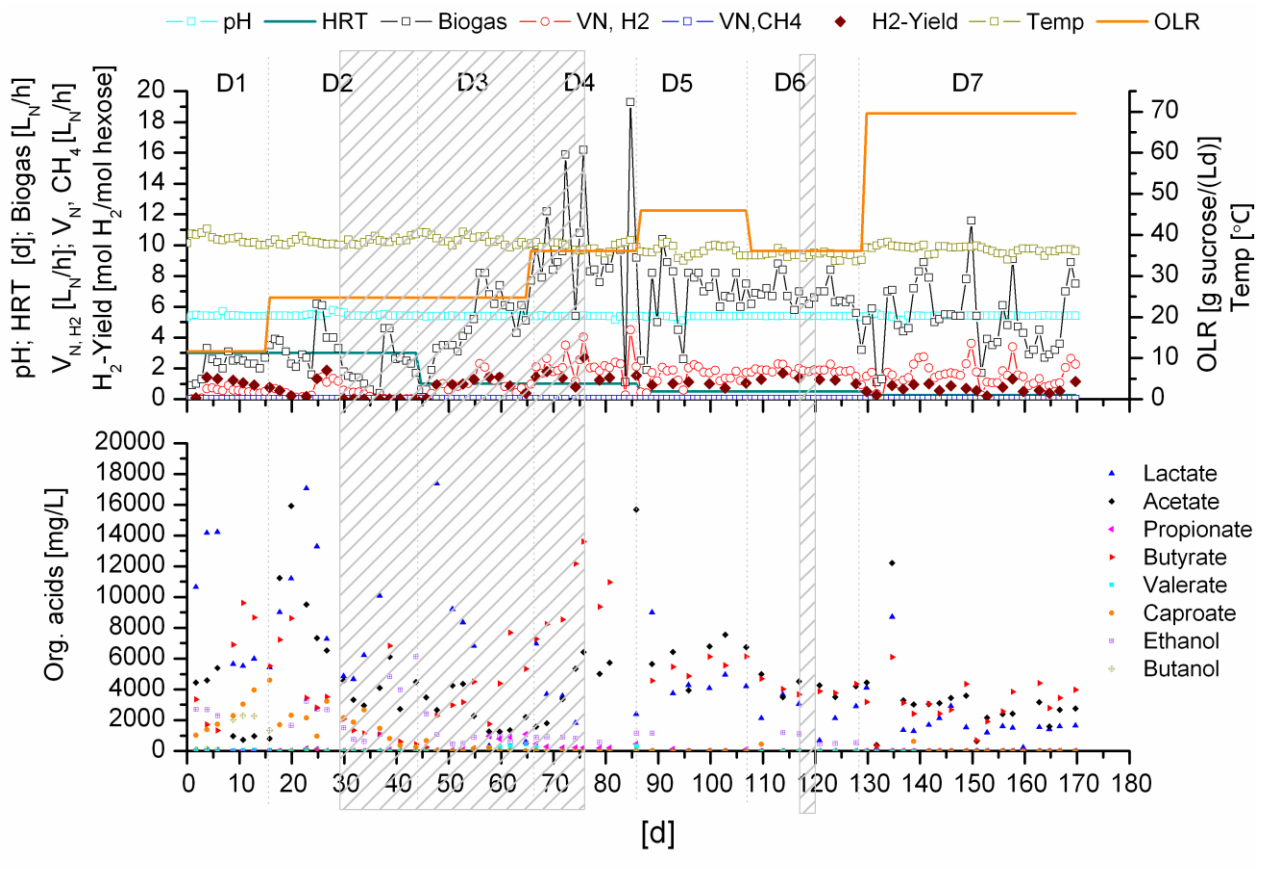

Figure 1: Biohydrogen production; reactor operation parameters, biogas, H_2-, CH_4-production, H_2-yield and organic acid concentrations. Shaded areas correspond to the days in which the effluent was used as substrate for biopolymer production

Biopolymer production

Table 1 shows the results of the various experimental phases for the determination of the appropriate mode of operation of the reactors, the optimization of the operation parameters and the validation the optimum conditions with the defined substrate acetate are compiled. It can be seen that the SBR operation yielded higher levels of PHA than the CSTR. The further optimization of the process was conducted in a SBR. In the optimization of the process, the maximum PHA content of 20.6% TS was reached. As optimal conditions SRT of 8 d and HRT of 2 d were determined. SRT of 5 d and of 12 d resulted to lower PHA yields, although the higher yields obtained at SRT of 8 d may be attributed to the very low phosphate concentrations which has been reported to promote PHA accumulation within the cells (Reddy et al., 2003). In the experiments with the SRT of 12 d the highest yield was achieved for HRT of 2 h. Both shorter and longer HRT resulted to lower yields. Increase of OLR to 7.5 g COD/(L·d), in order to increase the quantity of the organic acids did not result to further improvement on the PHA accumulation. The yield obtained is much higher than that obtained in a previous work that coupled fermentative biohydrogen production and aerobic PHA production. Ntaikou et al. (2009) used olive oil mill wastewater for biohydrogen production in a 500 mL CSTR and achieved a maximum PHA content of 8.94 % of the TS content when they used the effluent for PHA accumulation in a SBR with a working volume of 750 mL. Despite the scale-up of the process in this work higher PHA contents were achieved. The batch addition of pure acetate at an OLR of 2.5 g COD/(L·d) at the biomass of SBR2 resulted to PHA accumulation of 36% TS, which was almost 2.5times more than that obtained by the effluent of the biohydrogen

reactor. Thus, the suitability of the proven process for PHA accumulation was confirmed and higher PHA production could be achieved if the biohydrogen production pathway could be shifted towards acetate production.

Table 1: Biopolymer production; reactor configuration, operational conditions, nutrient concentrations maximum achieved PHA content and COD elimination

		Duration	HRT	SRT	OLR	Org. acids[4)]	NH_4^+-N	PO_4^{3-}-P	max PHA	COD-elimin.
		[d]	[d]	[d]	[gCOD/(L·d)]	[Cmmol/L]	[mg/L]	[mg/L]	[%TS]	[%]
Operation	SBR1[1)]	57	2	5	2.5	37	59.7	85.9.	17.4	88
	CSTR1[1)]	57	5	5	2.5	47	30.1	249.0	12.5	--
Optimization	SBR2[1)]	72	2	12	2.5	53	95.7	28.7	15.6	90
	SBR3[1)]	44	5	12	2.5	116	43.0	123.0	7.6	88
	SBR4[1)]	28	1	12	2.5	116	11.6	44.0	12.8	80
	SBR5[2)]	28	2	8	7.5	121	148.0	0.3	17.8	--[3)]
	SBR6[2)]	28	2	8	2.5	40	65.2	0.2	20.6	--[3)]
Validation	Batch	--	(2)	(12)	0.7	--	--	--	7.2	--
(Acetate)	Batch	--	(2)	(12)	2.5	--	--	--	36.0	--

[1)]Phases D2-D4
[2)]Phase D6
[3)]Addition of antifoam agent resulted to COD increase
[4)]End concentration in the biopolymer reactor at the maximization of PHA content

The population analysis indicated that two types of bacterial species became more abundant over time, *Tetracoccus cechii* and *Brevundimonas* sp.. Both genera were reported to be able to PHA (Blackall et al., 1997; Silva et al., 2007; Kessler and Palleroni, 2000). *Tetracoccus cechii* is commonly present in wastewater treatment plants (Blackall et al., 1997).

CONCLUSION

The downstream coupling of biopolymer production in the form of PHA to biohydrogen by fermented molasses has been proven to be possible. However, there is still potential for optimization with regard to the increase of acetate concentration during biohydrogen production.

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