Influence of pH and hydraulic retention time on hydrogen and ethanol co-production by dark fermentation in a CSTR with glycerol as substrate.

F. Silva-Illanes[1], E. Tapia-Venegas[1], A. Marone[2], E. Trably[2] and G. Ruiz-Filippi[1]

[1]Laboratorio de Biotecnología Ambiental, Escuela de Ingeniería Bioquímica, Facultad de Ingeniería, Pontificia Universidad Católica de Valparaíso, Brasil 2085, Valparaíso, Chile. (E-mail: fernando.silva.i@mail.pucv.cl; estela.tapia.v@mail.pucv.cl; gonzalo.ruiz@ucv.cl)

[2]Laboratoire de Biotechnologie de l'Environnement, InstitutNational de la Recherche Agronomique. INRA,UR050, Laboratoire de Biotechnologie de l'Environnement F-11100 Narbonne, France. (E-mail: antonella.marone@supagro.inra.fr; eric.trably@supagro.inra.fr)

Abstract
The effect of pH and HRT on hydrogen and ethanol production thought dark fermentation from pure glycerol was studied. Most of the results found in literature belong to batch cultures. In this study aerobic sludge was used as inoculum in a continuous stirred tank reactor (CSTR). A full factorial design with two variables (pH and HRT) and two levels was performed. Results indicate that these variables highly influence, both the hydrogen yield on glycerol (from 0.04 to 0.41 mole H_2/mole gly) and ethanol yield on glycerol (from 0.005 to 0.052 mole ethanol/mole gly), obtaining differences up to an order of magnitude between them in steady state. Also, CE-SSCP fingerprinting analysis was performed, and showed considerable changes in the microbial diversity between different steady states studied, confirming the high effect of these variables over the process.

Keyword
hydrogen; ethanol; dark fermentation; pH; hydraulic retention time, glycerol, CSTR

INTRODUCTION

Hydrogen production via dark fermentation is a promising process, but cheap substrates are necessary to make it economically viable. Biodiesel is the main biofuel produced in Europe, but this process generated as the main byproduct glycerol. Actually, the glycerol surplus is dramatically increasing and the glycerol price is decreasing, so new applications should be developed (Yazdani and Gonzalez., 2007). Hydrogen yields on glycerol obtained in literature for reactor with mixed cultures as inoculum are between 0.05 and 0.41 (mole H_2/mole gly). Furthermore, glycerol degradation by dark fermentation could produce other biofuels of economic interest as ethanol. In general, studies that have employed dark fermentation with glycerol to produce ethanol as byproduct obtain yields between 0.007 to 0.67 (mole ethanol/mole glycerol) (Akutsu et al., 2009 and Seifert et al., 2009). In a continuous system, two important variables in the process are the pH and hydraulic retention time (HRT). This variables have been well studied with other substrate as glucose or starch (Wu et al., 2008; Tapia-Venegas et al., 2013) but not for glycerol, therefore, the correct choice of the setpoints of these operational parameters can improve the process performance and in other hand, is important to study the change in microbial diversity, which may explain the performance of the reactor (Talbot et al., 2008).

The aim of this study was evaluated the effect of pH and HRT on performance of continuous stirred tank reactor (CSTR), in order to improve productivity of hydrogen and ethanol yields on glycerol. Also, we correlating environmental parameters with changes in the microbial populations with single-strand conformation polymorphism (SSCP) analysis.

MATERIALS & METHODS

Experimental design and hydrogen-producing sludge: To evaluate the effect of pH and HRT on the hydrogen and ethanol yield on glycerol, a full factorial design with two variables in two levels with a central point was developed and seven experiment were made between pH 5.5 until 6.5 and HTR 8 until 12 h. The inoculum used was a sludge from a reactor of active sludge with urban wastewater (activated sludge), supplied by the wastewater treatment plant La Farfana, Santiago de Chile. Before starting the continuous system, the inoculum was enriched with aeration at 8.2 ppm (mg O_2 L^{-1}) for 24 h with glycerol as substrate (10 g L^{-1}) (Patent process, **Application N°: 201402319.**)

Reactor, variable controlling, continuous feed and analysis: A 2L reactor was used (working volume) at 37° C. The pH was monitored and controlled by ODIN (INRIA) and the addition of NaOH 1M. HRT was maintained varying the flow of the feed pump. The biogas flow was measurement with a Ritter MilliGascounter® gasometer. For continuous feed, a synthetic medium used contained per liter 10 g of pure glycerol and macro and micronutrients (Tapia-Venegas et al., 2013). The hydrogen and ethanol yield on glycerol was calculated by the moles of these compounds produced per the fed glycerol. This values were evaluated during 20 HRT of reactor operation in steady state (steady state was considered when the hydrogen yield on glycerol was unchanged more than 30%). Hydrogen percentage in biogas was measured using a Perkin Elmer 500 (Whaltam) gas chromatograph with a thermal conductivity detector (TCD). The metabolites (volatile fatty acids, glycerol and 1,3-propanediol and ethanol) were measured using an HPLC (Biorad HPX-87-H column) and also ethanol was measured by GC (Perkin Elmer 500) with flame ionization detector (FID) and a Wide Bore Equipy 1, supelco 30x53´´ column. The biomass was measured by volatile suspended solids (gravimetry).

RESULTS

Hydrogen and ethanol yield on glycerol

pH and HRT had an effect on hydrogen and ethanol yields, differences, even than an order of magnitude were observed (Table 2). An decrease of pH and a increase of HTR has a hydrogen yield increase on the range studied. The higher hydrogen yield was 0.41 mole H_2/mole gly and is similar to higher yield reported in literature for mixed cultures (0.41 mole H2/mole gly) (Seifert et al., 2009). pH and HRT are also very influential on ethanol yield, however there would be no clear correlation. The best result was 0.05 mole ethanol/mole gly, but is low compared with the maximum yields reported in literature for this mixed cultures (0.67 mole ethanol/mole gly) (Temudo et al., 2008). Before long, others metabolite produced will be measured by HPLC.

Table 2. Hydrogen and ethanol yields on glycerol in all full factorial design experiments

		Hydrogen yield (mole H_2/mole glycerol)		
	TRH	8 h	10 h	12 h
pH				
5.5		0.13		0.41
6			0.21	
6.5		0.17		0.04
		Ethanol yield (mole ethanol/mole glycerol)		
5.5		0.01		0.05
6			0.005	
6.5		0.05		0.02

Microbial analysis

Community structure at steady state by SSCP was studied. Figure 1 shows the Community structure of original inoculum (before enrichment) and one sample in steady state for every experiment made.

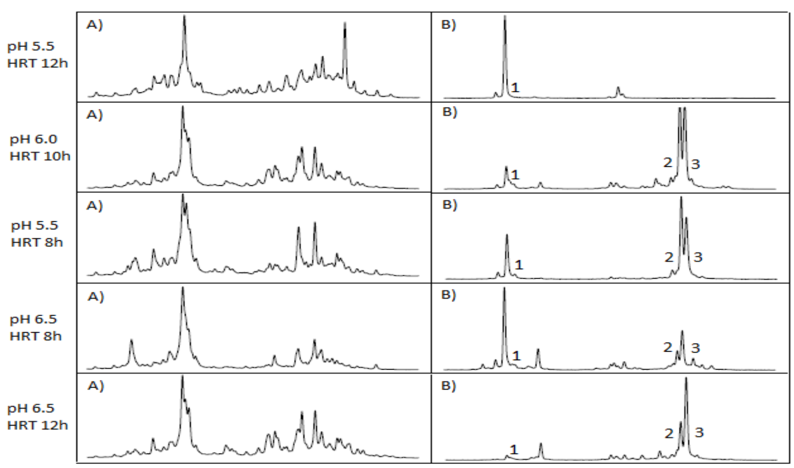

Figure 1: SSCP analysis for every experiment. “A” and “B” are points before enrichment and in steady state respectively. 1,2 and 3 are OTUs (operational taxonomic units).

The inoculums were similar in all the experiments (Figure 1 A). In contrast, SSCP profiles of the steady state of the experiment were different from each. In figure 1B, the OTUs “1”, “2” and “3” indicate the dominant and subdominant microorganisms. The best hydrogen yield is obtained at pH 5.5 and HRT of 12h, where OTU “1” is the dominant peak. Experiments with pH 6 HRT 10h and pH 6.5 HRT 8h have similar hydrogen yields, and OTUs “2” , “3” and “1”are dominants and but at different proportions, which indicate a possible competences and/or relations between these OTUs. Similar to that observed with experience with the lower H_2 yield (pH 6.5 and HTR 12 h).

CONCLUSION

pH and HTR have a significant influence on hydrogen and ethanol yield in glycerol and also in the microbial community relative abundance, in a continuous system (CSTR). The best H_2 and ethanol yields were obtained at pH 5.5 and HRT 12h. The operating parameters can determined different proportions of three dominants OTUs which in turn could be related the hydrogen and ethanol yields.

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