Evaluation of a one- and two-step process of POME hydrolysis by plant enzyme preparation and sequential production of biohydrogen

Alessandro do Nascimento Garritano[1,2], Érika Cristina Gonçalves Aguieiras[1], Lívian Ribeiro Vasconcelos de Sá[2], Denise Maria Guimarães Freire[1], Viridiana Santana Ferreira-Leitão [1,2]

[1]Federal University of Rio de Janeiro (UFRJ), Department of Biochemistry, 21941-909, Rio de Janeiro, Brazil.
[2]National Institute of Technology (INT), Ministry of Science, Technology and Innovation (MCTI), Biocatalysis Laboratory (LABIC), 20081-312, Rio de Janeiro, Brazil

ABSTRACT

In this study, biological hydrogen production from POME was performed via anaerobic fermentation under mesophilic conditions in shake-flasks. Interference of previous and concomitant addition of a plant enzyme preparation (VE) extracted from dormant castor bean seeds (Ricinus communis L.) to the process was evaluated. The addition of VE in a one-step process promoted a significant effect in the bacterial adaptive phase, reducing it by 50%. Furthermore, hydrogen yield increased by 14% and hydrogen production rate increased by 41%. A pre-hydrolysis of POME with VE, in a two-step process, was also investigated. The pre-hydrolysis step reduced the bacterial adaptive phase duration in up to 75% and improved hydrogen production rate by 93% when compared to the original conditions.

Keywords: Hydrogen, anaerobic fermentation, POME and plant enzyme preparation

INTRODUCTION

Palm oil is a major commodity for developing countries in Southeast Asia, specially Indonesia and Malaysia, which are responsible for 88% of the global production. In the last few years, the demand for that oil has been increasing not only due to its multiple uses (e.g., food industry, biofuels and cosmetics) but also to its lower cost when compared to other oils (AHMED *et al.*, 2015). Accompanying global demand, Thailand, Colombia and, more recently, Brazil are starting to rise as key producers of that market as well (KUSS *et al.*, 2015).

Aiming to supply the demand for palm oil, its producers are augmenting the production and, consequently, the amount of residues generated in the process. Oil extraction generates a highly pollutant residue, Palm Oil Mill Effluent (POME), that cannot be dispensed directly into water bodies (BARCA *et al.*, 2015). POME is a complex and rich residue, which could be used as raw material for bioproducts and biofuels production, in particular, hydrogen.

Hydrogen is a valuable gas, which is often used in the petrochemical industry, but also plays a key role in the food industry (AHMED *et al.*, 2015). Nowadays, most of its production is via steam reforming, a costly and pollutant process. Biological approaches are being developed, as these processes require less energy and are more environmentally friendly (ATIF *et al.*, 2005). Amongst all biological hydrogen producing processes, dark fermentation is one of the most promising due to its higher hydrogen production and the possibility to utilize more complex substrates. One of the main limitations of dark fermentation is its adaptive phase, considerably higher when compared to other processes (BARCA *et al.*, 2015).The scope of this study was to evaluate a new pre-treatment proposal using an enzymatic preparation, named plant enzyme (VE) extracted from dormant castor beans seeds (*Ricinus communis* L.) not only to reduce the bacterial adaptive phase, but also to increase hydrogen yield.

MATERIALS AND METHODS

Wastewater and inoculum pretreatment: Fresh POME was collected from Agropalma Agroindustrial Complex, located at Tailândia, Pará, Brazil. It was maintained in a refrigerator at 4 ºC to prevent degradation. The obtained POME presented a chemical oxygen demand (COD) of 47,2 g O_2/L. Sludge from an anaerobic digester in a sewage sludge treatment plant, in Penha, Rio de Janeiro, Brazil was collected to be used as inoculum. The sludge was submitted to an acid pretreatment according to (Sá *et al.*, 2013), in order to inhibit methanogenic bacteria. Pretreated sludge was diluted to 10.000 mg of volatile suspended solids (VSS) per milliliter (mL).

Plant enzyme preparation (VE) and POME Hydrolysis: VE extracted from *R. communis* according to described in Cavalcanti *et al.*, 2007 and Aguieiras *et al.*, 2014, courtesy of Laboratory of Microbiology and Biotechnology (LaBiM), was used to hydrolyze POME. In a first moment, the hydrolysis was set up to occur along anaerobic fermentation with the addition 0.75% w/v of VE in the reaction media (one-step). POME pre-hydrolysis was also tested with the addition 0,75% w/v of VE to POME, which was incubated for 2 h, according to Serri *et. al*, 2008. After incubation, diluted sludge was added to POME, composing the reaction medium, which was distributed in penicillin flasks. The pre-hydrolysis step was also performed with commercial immobilized lipase Novozym 435 following the same pre-hydrolysis experimental procedure.

Hydrogen production using POME as raw material: A $POME_{COD}$:$sludge_{SSV}$ = 2:1 relation was found to be effective for the fermentation medium. The medium pH was adjusted to 6,5. A total volume of 45 mL of medium was poured into penicillin flasks. The flasks were purged with N_2 to ensure an anaerobic medium and were incubated under 35 ºC and 150 rpm. Hydrogen production was monitored along 28 h and biogas samples were collected every 4 h. All the tested conditions were made in triplicate.

Analytical method: The gas was collected in gasometrical ampoules and was analyzed in an Agilent Technologies 3000A Micro GC with a thermal conductivity detector (TCD). The carrier gas used was nitrogen. The standard curve of hydrogen was plotted using standard hydrogen gas. The chromatographic columns used were HP-PLOT U (3 m x 0.32 mm x 30 mm) and HP-PLOT Molecular sieve 5A (10 m x 0.32 mm x 12 mm).

RESULTS AND DISCUSSION

Effect of VE addition into the reaction media: Maximum hydrogen production rate increased approximately by 41%, while total yield increased by 14% in this one-step process. Figure 1 shows the differences between hydrogen production rate and yield in the absence (figure 1A) and presence (figure 1B) of VE.

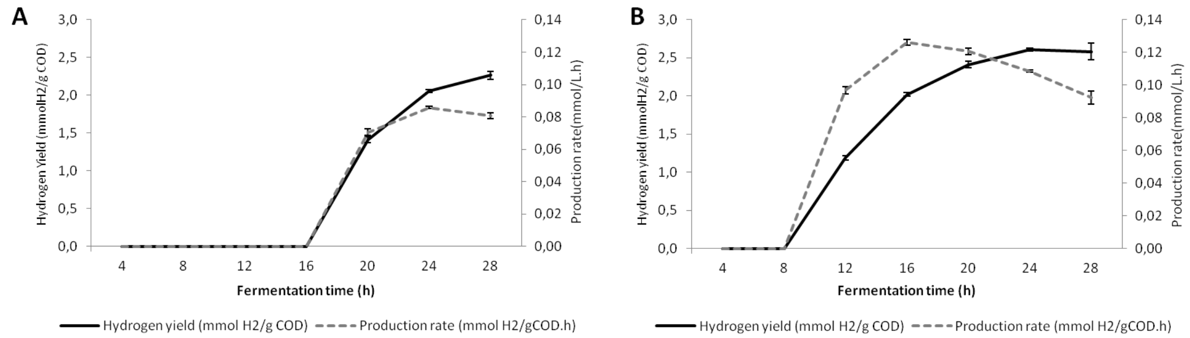

Figure 1: Hydrogen yield and hydrogen production rate profiles over 28 h of fermentation a) without VE and b) with 0.75% w/v VE (one-step process). The experiments were conducted at 35 ºC, pH 6.5 and 150 rpm.

Bacterial adaptive phase was reduced by, approximately, 8 h when VE was added. It is known that the acid lipase from *R. communis* seeds is rich in hydrolyses, which are able to break complex molecules present in POME, accelerating the fermentative process (Cavalcanti *et al*., 2007, Aguieiras *et al*., 2014). The higher yield could be explained by previously unavailable nutrients, which became assimilable after enzymatic hydrolysis. The bacterial consortium would be able to assimilate the hydrolysis product and convert it into hydrogen.

<u>Effect of POME pre-hydrolysis with VE and comparison with Novozym 435:</u> Maximum hydrogen production rate increased approximately 93% (figure 2) when compared to POME fermentation without VE addition (figure 1A) and 37% when compared to the one-step process (figure 1B). Total hydrogen yield increased by 14% when compared to POME fermentation without VE addition, a similar increase to the obtained in the one-step process. As POME is rich in oils and greases, an experiment with commercial lipase Novozym 435 was also performed to determine if lipases present in the VE were responsible for adaptive phase reduction. Figure 2 shows the production profile for pre-hydrolyzed POME over 28 h and the maximum productivity for the four experiments in 12 h of fermentation.

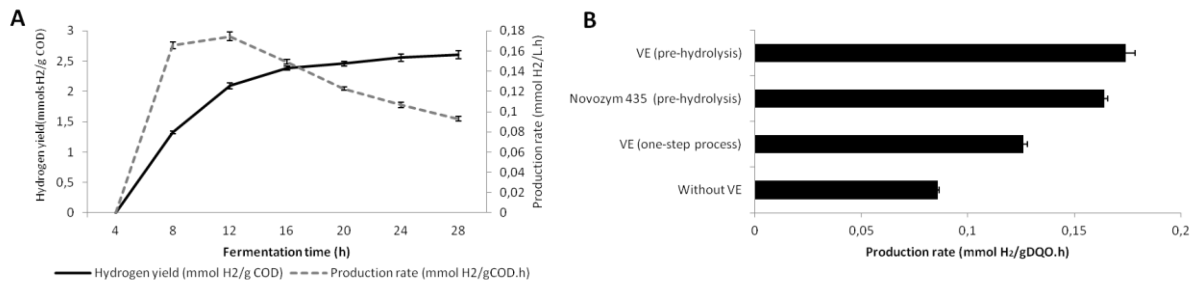

Figure 2: a) Hydrogen yield and hydrogen production rate profile over 28 h of fermentation with pre-hydrolyzed POME using 0.75 w/v of VE and b) Maximum productivity comparison of the four experiments performed.

Bacterial adaptive phase was reduced by, approximately, 12 h when compared to the initial fermentative conditions (figure 1A) and, by 4 h, when compared to the one-step process (figure 1B). The hydrogen yield did not increase any further than the one obtained in the one-step process, suggesting a complete hydrolysis of nutrients metabolized by the enzyme in both experiments. The observed hydrogen production rate was similar to the process carried out in the absence of VE. The results obtained are summarized in Table 1, which shows the differences between the pretreatments tested.

Table 1: Comparison between experiments without VE, with VE in a one-step process and with a previous hydrolysis of POME using VE.

Parameter	Without VE	With VE (One-step process)	With VE (Pre-hydrolysis)
Adaptive phase (h)	16	8	4
Maximum yield ($mmolH_2/gCOD$)	2.26 ± 0.05	2.58± 0.02	2.56 ± 0.05
Maximum productivity ($mmolH_2/gCOD.h$)	0.086 ± 0.001	0.127 ± 0.001	0.174 ± 0.004
Percentage increase	-	41%	93%

The increase of nutrients availability promoted by lipase action might be responsible for a shorter adaptive phase, as the hydrolysis step mediated by bacteria was deeply facilitated. In that manner, it is possible to suggest that the cellular machinery is mainly focused in synthesizing DNA and cellular division proteins, accelerating bacterial growth(Zhang, 2005). The obtained results, when compared to the literature, evidentiate the effectiveness of the pre-hydrolysis treatment, as reported in Table 2.

Table 2: Yield and productivity of hydrogen obtained from POME anaerobic fermentation.

Substrate	Culture/Process	Highest yield (L-H_2/L POME)	Highest productivity ($L H_2$/L POME/h)	Reference
POME	Mixed/batch	1.32	0.144	(RASDI *et al.*, 2012)
POME	Mixed/batch	4.70	0.124	(ATIF *et al.*, 2005)
POME	Mixed/fed-batch	2.25	0.112	(ATIF *et al.*, 2005)
POME	Mixed/shake-flask	2.42	0.201	This study

CONCLUSIONS

Higher hydrogen yields and productivity were obtained using POME as raw material when compared to the literature. The addition of VE improves the hydrolytic phase of fermentation, reducing bacterial adaptive phase and increasing hydrogen production. A pre-hydrolysis step might be performed to further increase the production rate.

REFERENCES

AHMED, Yunus *et al.* Production of biogas and performance evaluation of existing treatment processes in palm oil mill effluent (POME). **Renewable and Sustainable Energy Reviews** v. 42, p. 1260–1278 , 2015.

ATIF, A. a Y *et al.* Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. **International Journal of Hydrogen Energy** v. 30, n. 13-14, p. 1393–1397 , 2005.

BARCA, Cristian *et al.* Anaerobic biofilm reactors for dark fermentative hydrogen production from wastewater: A review. **Bioresource Technology** v. 185, p. 386–398 , 2015.

SÁ, Lívian Ribeiro Vasconcelos Pentoses, hexoses and glycerin as substrates for biohydrogen production: An approach for biofuel integration. **International Journal of Hydrogen Energy** v. 38, n. 7, p. 2986–2997 , 2013.

KUSS, Vivian Vicentini *et al.* Potential of biodiesel production from palm oil at Brazilian Amazon. **Renewable and Sustainable Energy Reviews** v. 50, p. 1013–1020 , 2015.

RASDI, Zatilfarihiah Kinetic analysis of biohydrogen production from anaerobically treated POME in bioreactor under optimized condition. **International Journal of Hydrogen Energy** v. 37, n. 23, p. 17724–17730 , 2012.

SERRI, N a; KAMARUDIN, ABDUL RAHAMAN, S N. Preliminary studies for production of fatty acids from hydrolysis of cooking palm oil using C.Rugosa Lipase. **Journal of Physical Science** v. 19, n. 1, p. 79–88 , 2008.

ZHANG, Yi-Heng Percival; LYND, Lee R. Cellulose utilization by Clostridium thermocellum: bioenergetics and hydrolysis product assimilation. **Proceedings of the NAS of the USA** v. 102, n. 20, p. 7321–5 , 2005.