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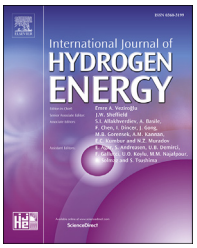

Efficient biohydrogen production via dark fermentation from hydrolized palm oil mill effluent by non-commercial enzyme preparation

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ABSTRACT

Biohydrogen production using palm oil mill effluent (POME) as source of nutrients was evaluated via dark fermentation under mesophilic conditions. The effect of adding a plant enzyme preparation (PEP) extracted from dormant castor bean seeds (Ricinus communis L.) to improve the availability of nutrients from POME was also evaluated and carried out in two different approaches. The addition of PEP in a one step process, a simultaneous hydrolysis and fermentation, resulted in a significant reduction of adaptive phase by approximately 50%. Furthermore, hydrogen yield increased by 14% and hydrogen productivity by 48%. The two step process, which consists in a pre-hydrolysis followed by dark fermentation, promoted a hydrogen yield of 2.58 mmol H_2/g_{DQO}, reduced the adaptive phase by 75% and increased hydrogen productivity by 102% when compared to the original conditions of raw POME fermentation.

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Introduction

Palm oil is an important commodity for developing countries in Southeast Asia, especially Indonesia and Malaysia, which are responsible for 85% of global production [1]. 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 because of its lower cost when compared to other oils [1]. Therefore, in order to supply the global demand for palm oil, its producers are expanding the production. In order to meet the demand, global palm oil production is expected to increase by 6.94%, which represents a total volume of 58.8 millions of tons of palm oil [2]. As a consequence to the increase in palm oil production, the amount of residues generated during the oil extraction process, named POME (palm oil mill effluent) is also increasing substantially. The

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production of one litter of palm oil generates, approximately, 3.5 liters of POME, representing a total annual production of 176.4 millions of tons of this residue. POME is a complex material, containing elevated concentrations of organic matter, oil and greases, as well as suspended solids and it is estimated that the production of 1 ton of palm oil requires 5–7.5 ton of water and more than 50% of that volume is released at the end of extraction [3].

POME can be used as raw material for several biological processes, such as biological hydrogen production. It is a complex material and its composition varies depending on palm oil processing. Generally, POME contains high concentration of organic molecules such as fatty acids, proteins, carbohydrates, nitrogenous compounds, and minerals. This organic matter could be metabolized during dark fermentation process either by pure culture or bacterial consortia in order to produce hydrogen or methane, two fuel gases that can be used as energy vectors [4].

Dark fermentation is one of the several biological processes to produce hydrogen, but it distinguishes itself due to a series of advantages. The main advantages of dark fermentation among biological processes for hydrogen production lies in its light-independence and consequently low energy input requirement, the possibility of employ renewable biomasses as feedstock and faster H_2 production rates [5,6]. It also has the capability of degrading complex organic molecules, and cogenerates methane, acetate, 1,3-propanediol and other secondary metabolites with higher added value [7,8]. During dark fermentation, complex molecules are converted into simpler compounds, as free fatty acids (FFA), simple sugars and amino acids, by using anaerobic sludge as inoculum during the hydrolytical phase of the process.

Acidogenic organisms are capable of metabolizing those compounds into organic acids, mainly acetic and butyric acids, which are consumed during acetogenesis. In this phase, the consumption of organic acids generates CO_2 and H_2. Dark fermentation has a final step, in which H_2 is combined with the carbon dioxide (CO_2) present in the medium and the formation of CH_4 is observed [9–11].

In order to avoid methanogenesis and consequently promote accumulation of H_2, anaerobic sludge should be submitted to several types of pretreatment, such as acid, alkaline or heat before its use as inoculum in dark fermentation aiming the inactivation of methanogenic bacteria [12–14]. Once the inoculum has been pretreated, it can be mixed with POME. As previously mentioned, POME is a very complex material and several authors have reported the hydrolytical stage of dark fermentation as one of the bottlenecks to the feasibility of anaerobic hydrogen production [8,15]. The action of a hydrolytic pool of enzymes could improve the overall process for effluent treatment or biogas production as previously reported in the literature [16–18]. In this work, a plant enzyme preparation (PEP), consisting in a pool of hydrolases obtained from dormant *R. communis* L. seeds with elevated hydrolytical activity, was used [19]. In this manner, PEP is expected to promote the hydrolysis of complex molecules in POME, improving the nutrients availability, therefore reducing the time required for the hydrolysis promoted by bacteria and its adaptive phase.

In this study, PEP was tested under two different conditions: one step and two step process. In the one step process, the biocatalyst was added simultaneously to POME and anaerobic sludge in the fermentor, while in the two step process it was used in a previous hydrolytical treatment for POME. In this sense, the scope of this study was to increase biohydrogen production and productivity in shorter batches through the use of a non-commercial PEP to hydrolyze POME. Three different approaches were used: raw POME fermentation, simultaneous hydrolysis and fermentation (one step process) and separate hydrolysis and fermentation (two step process). It is well known that hydrolysis phase of dark fermentation is one of the processes bottlenecks [20,21]. Several strategies have been used to overcome it, such as inoculum acclimation or physical POME pretreatments. The novelty involved in the present study is based on the association of an enzymatic and microbial process for the production of hydrogen via anaerobic digestion. The use of a non-commercial and low cost enzyme preparation extracted from Ricinus communis strongly affected the required time for hydrolytical and adaptive steps, improving the global process productivity.

Materials and methods

Palm oil mill effluent and seed anaerobic sludge

Raw POME was kindly supplied by Agropalma (a Brazilian oil company). The material was collected from the final discharge point of a palm oil mill in Pará, Brazil and was used as raw material for hydrogen production. POME has brown color, pH around 4.5, 48 g L^{-1} O_2 of chemical oxygen demand (COD), Volatile Suspended Solids of 20.000 mg/L, a total carbon content of 21.700 mg/L, a total reducing sugar of 23.100 mg/L, 1610 mg/L of palmitic acid and 1990 mg/L of oleic acid and it was stored at 4 °C. Inoculum for hydrogen production was obtained from an anaerobic digester in a sewage sludge treatment plant, in Rio de Janeiro, Brazil. The sludge was subjected to an acid pretreatment according to Sá et al., 2013 [22], in order to inhibit methanogenic archaea. Pretreated sludge was diluted to 10,000 mg of volatile suspended solids (VSS) per liter (L) and used as inoculum. Distilled water was used in all the experiments to dilute anaerobic sludge.

Biocatalysts

PEP used to hydrolyze POME was extracted from *R. communis* according to Valladão et al., 2007 [16]. It was kindly provided by the Microbial Biotechnology Laboratory from Federal University of Rio de Janeiro. The pre-hydrolysis step was also tested with five commercial lipases Novozym 435 (immobilized lipase B from *Candida antarctica*), Lipozyme RM IM (immobilized lipase from *Rhizomucor miehei* with higher stearic activity), Lipozyme TL IM (immobilized lipase from *Thermomyces lanuginosus*), Palatase 20.000 L (free lipase from *Rhizomucor miehei* in liquid formulation) and Lipomod 34 MDP (powder lipase from *Candida cylindracea*). The commercial enzymes were provided by Novozymes (Araucaria, Brazil) and Sigma (St. Louis, USA).

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