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Potential of biohydrogen production from effluents of citrus processing industry using anaerobic bacteria from sewage sludge

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ABSTRACT

Citrus crops are among the most abundant crops in the world, which processing is mainly based on juice extraction, generating large amounts of effluents with properties that turn them into potential pollution sources if they are improperly discarded. This study evaluated the potential for bioconversion of effluents from citrus-processing industry (wastewater and vinasse) into hydrogen through the dark fermentation process, by applying anaerobic sewage sludge as inoculum. The inoculum was previously heat treated to eliminate H_2-consumers microorganisms and improve its activity. Anaerobic batch reactors were operated in triplicate with increasing proportions (50, 80 and 100%) of each effluent as substrate at 37 °C, pH 5.5. Citrus effluents had different effects on inoculum growth and H_2 yields, demonstrated by profiles of acetic acid, butyric acid, propionic acid and ethanol, the main by-products generated. It was verified that there was an increase in the production of biogas with the additions of either wastewater (7.3, 33.4 and 85.3 mmol L^{-1}) or vinasse (8.8, 12.7 and 13.4 mmol L^{-1}) in substrate. These effluents demonstrated remarkable energetic reuse perspectives: 24.0 MJ m^{-3} and 4.0 MJ m^{-3}, respectively. Besides promoting the integrated management and mitigation of anaerobic sludge and effluents from citrus industry, the biohydrogen production may be an alternative for the local energy supply, reducing the operational costs in their own facilities, while enabling a better utilization of the biological potential contained in sewage sludges.

1. Introduction

Energy plays a key role in the progress of human civilizations, and it has become increasingly vital to support the technological and globalized world nowadays. According to estimates from the International Energy Agency (EIA, 2011), between 2008 and 2035 there will be an increase of 53% on the energy consumption, with an average annual growth of 1.6%. However, this growth would not be followed by conventional energy sources such as oil, coal, and natural gas, as these reserves are estimated to deplete until the year of 2050 (Goyal et al., 2008). Recent projections (EIA, 2011) also reported that renewable energy is the fastest-growing energy source in the world, which may increase on an average rate of 3% per year from 2010 to 2035, reaching 14%.

About 200 billion tons of lignocellulosic biomass have been generated worldwide by the primary agricultural sector (Guo et al., 2010). Brazilian agroindustry occupies an area of 28,840,726 ha, producing about 597 million tons of residues from several crops per year (sugarcane, corn, rice, soybean, cassava, wheat, coconut, and citrus) (Ferreira-Leitão et al., 2010).

Regarding citrus crops (oranges, lemons, grapefruit, and mandarins), they are among the most abundant crops in the world, being orange the most typical one, accounting for about 82% of the global citrus crop production (Ferreira-Leitão et al., 2010). The processing of citrus is based mainly on juice extraction, but these fruits are also used to produce several derivatives, either in the chemical industry for the production of flavonoids, essential oils, biofuels, limonene, and pectin (Pourbafrani et al., 2010), or in food industry for canning, sweet and soluble dietary fiber production (Ferreira-Leitão et al., 2010 and Marín et al., 2007).

Brazil is the main citrus producer country in the world. The overall orange production reached 16.9 million tons in 2014, which

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represents 33% of the worldwide production. Within these statistics, São Paulo State is the most representative, with 12.3 million tons (73%). In the same period, the total amount of citrus waste generated from orange processing by Brazilian industries was about 8.4 million tons (USDA, 2015).

Citrus wastes consist of peels (60–75%), segment membranes (30–35%), and seeds (10%) (Crawshaw, 2001 and Wilkins et al., 2007), which are mainly composed by highly fermentable carbohydrates. Thus, the disposition of these wastes in landfills, besides being costly, can increase the production of leached and methane, causing severe environmental impacts (Negro et al., 2016).

Usually, after drying and pressing, this solid residue is used to produce the citrus pulp pellets, employed as a supplement for cattle feed, which is not a cost effective solution (Awan et al., 2013; Ferreira-Leitão et al., 2010 and Lohrasbi et al., 2010).

Second-generation ethanol (2G), through residues of pulp and citrus bagasse processing, may be a promising and profitable alternative (Awan et al., 2013; Lohrasbi et al., 2010; Pourbafrani et al., 2010 and Widmer et al., 2010) to the management and energy recovery from residues generated in agroindustry. Meanwhile, similar to sugarcane bioethanol production (Moraes et al., 2014), significant amounts of vinasse are produced in either first (1G) or 2G-ethanol producing processes.

This effluent needs further treatment due to its high content of organic matter and nutrients, besides heaving low pH and high corrosivity. Such properties might cause several environmental impacts if it is improperly disposed, including water and groundwater pollution, toxicity for aquatic organisms, proliferation of vectors for diseases, as well as greenhouse gases emissions during its degradation in soil (Christofoletti et al., 2013). Instead of harmful, the surplus organic load may turn the effluents of citrus industry into promising substrates for hydrogen generation through dark fermentation process.

Hydrogen is considered a promising energy source for the future due to its renewability, as well as for its clean end of usage. It has greater energy contents per unit of weight (142.35 kJ g^{-1}; 2.75 times) (Khamtib and Reungsang, 2014) in comparison to hydrocarbon fuels (Hu et al., 2013), and since water is the only by-product generated by its combustion, hydrogen is an alternative energy source more sustainable than fossil fuels (Guo et al., 2010). However, to make it competitive with conventional energy carriers, ensuring its sustainable benefits, further technological advancements (Kumar et al., 2016) as well as the improvement of practical and scientific knowledge are essential.

Dark fermentation is an environmental feasible process due to its simultaneous waste treatment and hydrogen production and is advantageous because of its high production rate with a low energy input (Liu et al., 2011), and because of the versatility in the use of carbohydrate-containing substrates as agricultural wastewater, food waste, domestic wastewater, industry wastewater, among others (Hu et al., 2013 and Khamtib and Reungsang, 2014). It may be a suitable alternative for energy production in small-scale from industrial plants with highly available and low-cost biomass (Das and Veziroglu, 2008), providing a low-cost local energy supply.

Tropical countries like Brazil, with average annual temperatures around 25 °C, favor the activity of hydrogen-producing communities during anaerobic fermentation and offer an opportunity to investigate the hydrogen productions potential (Maintinguer et al., 2015) through various substrates without requiring a significant energy input.

The clean energy production may represent an interesting alternative to the management of effluents from citrus industries that, up to now, it has not been performed. Therefore, the aim of the present study was to evaluate the potential reuse of different effluents (wastewater and citrus vinasse), generated in large amounts by the citrus processing industry in Brazil, as substrate for biological production of hydrogen, by employing the sewage sludge as inoculum source.

2. Material and methods

2.1. Inoculum source and adaptation conditions

The inoculum was obtained from full-scale UASB (Upflow Anaerobic Sludge Blanket) reactors used to treat the sanitary sewage of São José do Rio Preto (20°49′13″S 49°22′47″W, São Paulo State, Brazil), a city of 442,500 inhabitants.

The anaerobic granular sludge is a suspension with 2.5% of suspended solids and pH 6.8. After the collection, it was inoculated in natura (20% v/v) in anaerobic batch reactors (100 mL of total volume) containing 50 mL of culture medium, (PYG: glucose, 10 g L^{-1}; peptone, 5 g L^{-1}, yeast extract, 5 g L^{-1}, and meat extract, 5 g L^{-1}; pH 7.0) and 50 mL of the headspace filled with N_2 (100%). The reactors were maintained at 37 °C for 7 days, and the resulting biomasses were subjected to heat treatment (100 °C for 10 min) to inactivate the methanogenic archaea (H_2 consumers) and select endospore-forming anaerobic bacteria involved in H_2 production, such as *Clostridium* sp (Maintinguer et al., 2008).

2.2. Enrichment of hydrogen-producing bacteria

Before tests of hydrogen production, the cellular purification of the heat-treated inoculum was performed through serial dilutions (1/10) in anaerobic batch reactors containing a new sterile PYG media in pH 5.5, (at 37 °C). Even after the heat treatment, it is appropriate to maintain this pH value (Fang and Liu, 2002) to avoid methanogenesis.

The hydrogen production and the absence of methane in headspace of reactors were confirmed by chromatographic analysis, after 72 h of incubation.

The hydrogen-producing bacteria (10 mL of the cellular suspensions) were inoculated in triplicate of batch reactors (2 L of total volume), containing 1 L of a culture medium (Del Nery, 1987) with the following composition (expressed in mg L^{-1}): fructose (5000), peptone (1000), urea (40.0), and 2.5 mL L^{-1} of solutions A, B, C, and D, which are: A – $NiSO_4 \cdot 6H_2O$ (0.50); $FeSO_4 \cdot 7H_2O$ (2.5); $FeCl_3 \cdot 6H_2O$ (0.25); $CoCl_2 \cdot 2H_2O$ (0.04); B – $CaCl_2 \cdot 6H_2O$ (2.06); C – SeO_2 (0.14); D – KH_2PO_4 (5.36); K_2HPO_4 (1.3); $Na_2HPO_4H_2O$ (2.76).

In addition, 2.5 mL of solutions of B_{12} vitamin (0.04 g L^{-1}), p-amino benzoic acid (0.04 g L^{-1}), and biotin (0.01 g L^{-1}) were added to supplement the synthetic medium (Maintinguer et al., 2008), and the initial pH was adjusted for 5.5. The synthetic medium and the vitamin solutions were previously sterilized through filtration in a 0.22 μm membrane. The headspace of the reactors were filled with N_2 (100%) and they were maintained at 37 °C for 72 h. After this period, the cellular suspension of enriched consortia was separated by centrifugation (9000 rpm at 4 °C, for 10 min) and the resulting biomass was used as inoculum in batch tests with citrus effluents.

2.3. Substrates for hydrogen bioproduction

Two effluents from citrus processing industry were employed as a substrate for H_2 production: the raw wastewater and citrus vinasse. They were provided by one of the leading companies of citrus juice production, located in the city of Matão (21°36′12″S 48°21′57″W), São Paulo State, Brazil.

The wastewater represents the liquid residue of the entire production process, including the steps of juice extraction, concentration, as well as the production of derivatives from citrus bagasse.

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