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PHOTOFERMENTATION OF THE CASHEW APPLE BAGASSE HYDROLYSATE FOR BIOHYDROGEN PRODUCTION BY *Rhodopseudomonas palustris* CGA009: INFLUENCE OF SUBSTRATE CONCENTRATION

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ABSTRACT

The production of biofuels, such as H_2, with the simultaneous reduction of organic waste, will have an immense impact on both society and the industrial production sector. Therefore, the present study aimed to evaluate the H_2 production using the enzymatic hydrolysate from cashew apple bagasse (CAB-HE) and the phototrophic purple non-sulfur bacteria, *Rhodopseudomonas palustris* CGA009. The effects of supplementation with carbon and nitrogen sources in the H_2 production were evaluated. The initial glucose concentration of CAB-HE was standardized at 20 g/L. *R. palustris* CGA009 was able to assimilate 81 % of the glucose present in the CAB-HE supplemented with 5 g/L peptone, resulting in a productivity of 10.3 mmol H_2/L, indicating that peptone increases improved the hydrogen production, due to this compound being a source rich in amino acids and inorganic salts, reaching a yield of 0.33 molH_2 /mol glucose. In the bioprocess using CAB-HE supplemented with 5 g/L sodium acetate there was a consumption of 69 % glucose, resulting a low productivity, and using 3 g/L potassium nitrate, there was a consumption of only 26 % glucose, without a significant production. Thus, it is concluded that CAB-HE has a significant potential to be used as culture medium for bioH_2 production by photofermentation process.

Keywords: Hydrogen. Photofermentation. Cashew apple bagasse. Nitrogen source.

1 INTRODUCTION

Energy is one of the basic requirements of human society and civilization. Non-renewable energy sources such as oil, coal, and others are used as the primary source of energy for industrialization, modernization, and lifestyle improvement, and the demand for energy has increased[1]. Fossil fuels represent around 80% of global energy demand and are largely responsible for the intensification of greenhouse gases and climate change[2]. The growth of the human population has led to an increasing demand for energy, which by 2030 is expected to increase by up to 50%[3].

In this scenario, renewable energy sources stand out for being a promising alternative for reducing carbon emissions and greenhouse gases (GHG) released when using fossil fuels[4]. Examples of alternative energy sources are hydroelectric, geothermal, wind, solar, and biofuels. Hydrogen (H_2) appears as one of the possible alternatives to replace fossil fuels, as it has an energy capacity 2.75 greater than oil, and during its combustion it does not emit carbon dioxide (CO_2)[5], and making it an attractive option amid the current scenario.

The use of agro-industrial waste, as substrates for biohydrogen production, emerges as an important approach to overcoming energy problems. Biohydrogen (H_2), obtained from low-cost renewable biomass and agricultural waste of a lignocellulosic nature, presents some benefits, reducing waste that in the vast majority of cases does not have a correct destination, is highly nutritious in carbohydrates, and fuel production clean[6].

The Brazilian Northeast stands out in the production of cashews apple, a fruit that generates a high volume of waste after processing, which in turn can represent a potential raw material for the production of various bioproducts based on biotechnological processes. Cashew apple bagasse (CAB) has been studied to obtain hydrogen, [6] obtained promising results using CAB hydrolysates through the dark fermentation process. In this context, the present study evaluated the BioH2 production using the enzymatic hydrolysate from CAB as a culture medium for photofermentation process using *Rhodopseudomonas palustris* CGA009.

2 MATERIAL & METHODS

Raw material – Cashew apple bagasse: The lignocellulosic raw material used to produce hydrogen was the cashew apple bagasse (CAB), which was provided by Agroindustrial Cashew Cooperative of Ceará - Brazil. For use, the bagasse was washed three times with water and dried in a Tecnal TE-397/4 oven (Tecnal, Piracicaba, SP, Brazil) at 60 °C for 24 h. Subsequently, the CAB was crushed and sieved to standardize the size of 20-80 mesh (0.177-0.841 mm). After these steps, the standardized material was stored at room temperature (25 °C).

Pretreatment of cashew apple bagasse: The CAB was pretreated according to the best conditions obtained in studies carried out by Rocha et al [9]. The acid pretreatment was performed using diluted sulfuric acid (0.6 mol/L) and 20% (w/v) CAB in an autoclave at 121 °C for 30 min. After, the mixture was cooled and filtered using filter paper for separation of the solid (CAB-H) and liquid fraction. The solid fraction was washed three times with distilled water and again pretreated using sodium hydroxide and a solid load of 20% (w/v) at 121 °C for 30 min. After, the solid fraction was separated and dried in an oven at 60 °C for 24 h, crushed, and standardized the particle size for 0.25-0.84 mm. The solid resulting of this pretreatment was named CAB-OH and it was enzymatically hydrolyzed.

Enzymatic Hydrolysis: The enzymatic hydrolysis of the CAB-OH was carried out in 100 mM sodium citrate buffer (pH= 4.8) at 45° C and 150 RPM for 72 h using 8% w/v CAB-OH and the commercial enzymatic extract of *Trichoderma reesei* ATCC 26921 (Sigma Aldrich, Brazil) with an enzyme load of 15 FPU/$g_{cellulose}$[10]. After, the material was heated at 100 °C for 10 min to denature the enzyme and centrifugated at 5000 RPM for 20 min. The fraction liquid was named enzymatic hydrolysate of cashew apple bagasse (CAB-HE), filtered and adjusted to pH 6.8 for use as culture medium in the photofermentation.

Microorganism and culture conditions: The microorganism used in the present work was the *Rhodopseudomonas palustris* CGA009 bacterium, obtained by the Tropical Research and Technology Foundation - André Tosselo. For the preparation of inoculum, the cells were cultured in medium (composed of 10 g/L yeast extract, 1 g/L bibasic potassium phosphate, and 0.5 g/L magnesium sulfate, pH 6.8), at 30 °C under anaerobic conditions and illumination with tungsten lamp (2000 lux) for 48 h. The cell concentration was measured and used in the biohydrogen production.

Hydrogen production process: The photofermentation of CAB-HE by *Rhodopseudomonas palustris* was carried out in a 250 mL reactor (Brand Schott Duran®) using an operating volume of 200 mL. The CAB-HE was inoculated with 10% v/v inoculum with a cell concentration of 0.8 g/L. Anaerobic conditions were created by purging sterile nitrogen gas at 0.5 L/min for 5 min into the flask. The systems were maintained at constant agitation of 100 rpm and a temperature of 30 °C in the light presence (tungsten lamp at 2000 lux). The initial glucose concentration from CAB-HE was 68 g/L of glucose, and for the bioprocesses, it was diluted to 20 g/L of glucose and the initial pH was adjusted to 6.8. Supplementations with carbon and nitrogen sources (5 g/L peptone, 5 g/L sodium acetate, and 3 g/L potassium nitrate) were evaluated. At pre-defined times, liquid aliquots were removed and analyzed. The gas was collected in bags, measured the gas volume, and analyzed by gas chromatography (GC).

Analytical methods: The liquid samples were analyzed to determine glucose consumption and metabolic formation by High Performance Liquid Chromatography (HPLC) using a Thermo Finnigan Surveyor system (Thermo Fisher Scientific, San Jose, CA, USA) equipped with a refractive index detector and with a Supelco 610 H column. The solution of 0.1% v/v phosphoric acid (H_3PO_4) was used as the mobile phase at a flow rate of 0.5 mL/min and the analyzes were conducted at 30 °C. The injection volume of the samples was standardized at 20 µL.
The composition of the gaseous fraction was determined by GC using a Shimadzu Chromatograph, model GC-2010 ProAF, with thermal conductivity detector, using a Carboxen 1010 capillary column, with a length of 30 m and an internal diameter of 0.53 mm. During analyses, the temperatures of the injector, column, and detector were maintained at 200 °C, 30 °C, and 230 °C, respectively. Argon was used as carrier gas at a rate of 6 mL/min.

3 RESULTS & DISCUSSION

The hydrogen production by *R. palustris* predominantly occurs under illuminated and anaerobic growth. The supplementations of the CAB-HE with nitrogen sources and sodium acetate (carbon source) for the biohydrogen production were evaluated and the cell growth and glucose consumption are shown in Fig. 1

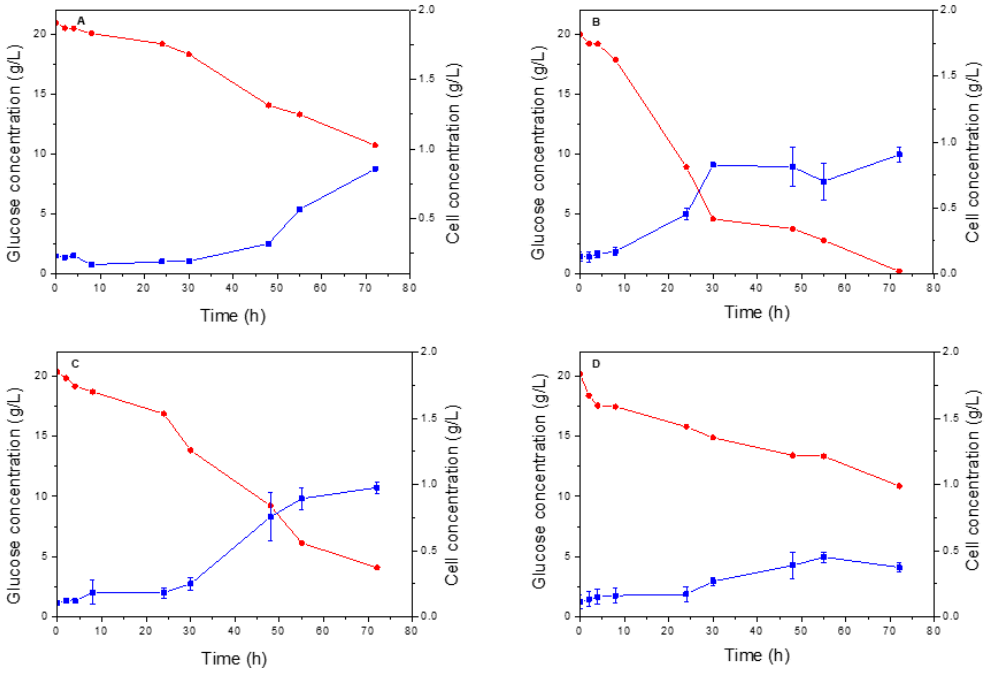

Figure 1 Concentration of cell growth (●) and glucose (■) as function of time, of liquid phase analyzed during the photofermentation by *R. palustris* at 30 °C, 100 rpm, under a tungsten lamp using: (A) CAH-HE without supplementation; (B) CAB-HE supplemented with 5 g/L peptone; (C) CAB-HE supplemented with5 g/L sodium acetate, and (D) CAB-HE supplemented with 3 g/L potassium nitrate.

The photofermentation without supplementation reached a cell concentration of 0.85 g/L, however the latency phase time was longer than the others, indicating the need for supplementation. The highest cell concentrations obtained in the processes using CAB-HE supplemented with peptone and sodium acetate, with a value of 0.90 g/L and 0.97 g/L, respectively. The great result obtained with peptone can be explained as it is a source of carbon and nitrogen, rich in amino acids and inorganic salts. On the other hand, CAB-HE supplemented with nitrate only reached a cell growth of 0.37 g/L.

Table 1 presents the final pH, glucose consumption, metabolites produced (acetic and lactic acids, and ethanol), and the results of the gas phase produced by *R.palustris* during photofermentation at 30° C and 72h of process using CAB-HE. The highest consumption of glucose in the medium was obtained in the bioprocess using CAB-HE supplemented with peptone. The peptone is a source of carbon and nitrogen, rich in amino acids and inorganic salts, and this composition influenced positively the metabolism of *R. palustris*, in which the microorganism metabolized all glucose in 72 h (Figs. 1.B). However, in the CAB-HE process supplemented with nitrate (Fig. 1.C) only 26% of the glucose was consumed and in process conducted without supplementation (Fig. 1 A), the bacterium consumed 48%. These results can be explained by the decrease in the pH, interfering in the optimal condition of the microorganism.

Table 1. Glucose consumptiom,metabolic formation and gas phase produced by *R.palustris* during photofermentation at 30° C and 72h of process using CAB-HE.

Supplementation in the culture medium	Liquid phase					Gas phase				
	Final pH	Glucose consumption (% w/w)	Acetic acid (g/L)	Lactic acid (g/L)	Ethanol (g/L)	Biogas (mL)	H_2 % in gas	Hydrogen yield ($mol_{H2}/mol_{glucose}$)	Volumetric (mL_{H2}/L)	Production rate ($mL_{H2}/L.h$)
Without supplementation	5.0	48.95	0.96	0.39	0.28	0	0	0	0	0
Peptone	5.0	81.15	3.9	0.05	2.08	583	24.79	0.330	0.24	10.0
Sodium acetate	4.7	69.5	5.6	0.55	2.5	400	1.70	0.012	0.017	0.43
Nitrate	6.3	26	1.78	0.26	0	130	0.08	0	0	0

Table 1 shows that the bioprocess supplemented with peptone produced the highest H_2 productivity by promoting the nitrogenase enzyme-mediated metabolic pathway. Acetic acid, lactic acid and ethanol were the main byproducts of fermentation that were produced during the pathways that produce hydrogen. The lowest H_2 productivity was explained by the increased production of acetic acid in the CAB-HE medium supplemented with sodium acetate, which also showed the greatest pH decrease (6.8 to 4.7).

4 CONCLUSION

The hydrolysate produced by the enzymatic hydrolysis of cashew apple bagasse supplemented with peptone was found to be a viable culture medium for biohydrogen production by *Rhodopseudomonas palustris* CGA009, with a yield of 0.33 molH2/mol glucose. This biofuel has the potential to lower greenhouse gas emissions.

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