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Comparative study on the production of biohydrogen from rice mill wastewater

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ABSTRACT

This study presents the production of biohydrogen from rice mill wastewater. The acid hydrolysis and enzymatic hydrolysis operating conditions were optimized, for better reducing sugar production. The effect of pH and fermentation time on biohydrogen production from acid and enzymatic hydrolyzed rice mill wastewater was investigated, using *Enterobacter aerogenes* and *Citrobacter ferundii*. The enzymatic hydrolysis produced the maximum reducing sugar (15.8 g/L) compared to acid hydrolysis (14.2 g/L). The growth data obtained for *E. aerogenes* and *C. ferundii*, fitted well with the Logistic equation. The hydrogen yields of 1.74 mol H_2/mol reducing sugar, and 1.40 mol H_2/mol reducing sugar, were obtained from the hydrolyzate obtained from enzymatic and acid hydrolysis, respectively. The maximum hydrogen yield was obtained from *E. aerogenes* compared to *C. ferundii*, and the optimum pH for better hydrogen production was found to be in the range from 6.5 to 7.0. The chemical oxygen demand (COD) reduction obtained was around 71.8% after 60 h of fermentation.

Introduction

Excessive dependence on fossil fuels today has led to critical environmental problems. The combustion of fossil fuel contributes more to greenhouse gas and release of toxic gases like CO_2, SO_2, NO_x and other pollutants leading to global warming. For these reasons, the research on the production of alternative fuels has become important. Hydrogen is being projected as a promising fuel, both from the environmental and economic viewpoints, because hydrogen produces only water during combustion, and also offers 2.75 times higher energy yield than hydrocarbon fuels [1]. The major problem encountered in the utilization of hydrogen gas as a fuel, is its non availability in nature (significantly very less concentration) and its production is very expensive. There are various methods available to produce hydrogen, such as the electrolysis of water, photolysis, and thermo-chemical and biological processes, such as photo and dark fermentation [2]. Among the various methods, the biological production of hydrogen has been considered as an effective one, since it consumes less energy and can be carried out at atmospheric temperature and pressure [3]. The biological species used for hydrogen production include *Cyanobacteria*, photosynthetic bacteria, and bacterial species, such as facultative anaerobes (*Enterobacter* sp. and *Citrobacter* sp.) and strict anaerobes (*Clostridium* sp. and rumen bacteria) [2,3]. The biological hydrogen

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production methods include bio-photolysis, photodecomposition, and dark fermentation.

Biohydrogen production from carbohydrate rich waste materials by dark and photo fermentation has been given much attention in the recent past [4,5]. The dark fermentation is carried out under anaerobic conditions with bacterial species such as *Enterobacter* and *Clostridium* species [6,7]. Many food and agricultural industrial wastes contain starch and cellulosic materials, which are rich in terms of fermentable sugars [8,9]. Various industrial wastewaters, such as composite chemical wastewater [10,11], dairy process wastewater [12], food processing wastewater [13,14], domestic wastewater, rice winery wastewater [15], distillery and molasses based wastewater [16,17], cassava wastewater [18] and paper and pulp industry wastewater [19], have been successfully used for the production of biohydrogen.

Chemical and biological pretreatment methods are used for the recovery of fermentable sugars. However, acid hydrolysis assures more sugar recovery and formation of less inhibitor. These inhibitors are not suitable for hydrogen fermentation, since they show negative effects on the hydrogen producing microbes [20,21]. The hydrolysis methods to recover the fermentable sugars vary with different biomass. For example, Datar et al. [22] found acid hydrolysis could recover a large amount of reducing sugars based on the chemical nature of the biomass. The main disadvantages of acid hydrolysis are, the relatively low yield and the excessive formation of by-products. On the other hand enzymatic hydrolysis, the microbial hydrolysis of carbohydrates by *Aspergillus* sp., to fermentable sugar, is one of the possible approaches. Suitable hydrolysis steps and methods or inhibitor production control could reduce the cost, and simultaneously improve the fermentation process [20]. Various kinds of carbohydrates from potato, corn, wheat and rice, proved to be equally good carbon sources for microbial hydrolysis. It is the most abundant utilizable resource in plant biomass, and can be hydrolyzed enzymatically into fermentable sugars.

In this study, an attempt has been made to produce biohydrogen from rice mill wastewater. Rice milling is the process of removing the husk and part of the bran from paddy, in order to produce edible rice, and this contains carbohydrate, protein and lipid [23]. Parboiled rice production requires large amounts of water for soaking the paddy [24]. The amount of wastewater produced is about 1.0–1.2 L/kg paddy [25] and this wastewater is rich in starch, cellulose and hemicelluloses, and is easily hydrolyzable. It has a high chemical oxygen demand (COD), and is therefore suitable for anaerobic fermentation [26]. In this study, the effect of enzymatic and acid hydrolysis of rice mill wastewater on hydrogen production, using two different microbes such as *E. aerogenes* and *C. ferundii* was studied. The influence of the operating parameters was studied to maximize the performance of the different processes investigated.

Materials and methods

Effluent

Rice mill wastewater was collected from the local rice mill, located in Kanchipuram, Tamil Nadu, India. The collected wastewater was subjected to sterilization in an autoclave at 121 °C for 15 min, to inactivate the non-sporogenic bacteria present in the effluent [27]. Then, the effluent was kept at 4 °C until further use. The physiochemical characteristics of the wastewater are presented in Table 1.

Microorganism

The fungal strain *Aspergillus niger* (MTCC 2208) used in this study was obtained from Microbial Type Culture Collection centre (MTCC), Chandigarh, India. Potato dextrose agar (slant) media were used for the cultivation of *A. niger*, and the slant tubes were incubated at 30 °C for 7 days. After the growth period, strains were serially diluted with distilled water (10^{-1} to 10^{-6}). Each 1 mL of 10^{-1} to 10^{-6} spores/mL dilution of *A. niger* was incubated in potato dextrose agar plates to obtain single isolated colony. Once the spores were formed in the plate, they were transferred into potato dextrose broth, consisting of potatoes infusion 200 g/L and dextrose 20 g/L. The final pH of the medium was around 5.1 ± 0.2. The flasks were incubated in an orbital shaker at 30 °C with 180 rpm. The fungal cells were sub cultured again for 1-2 generation in potato dextrose broth, and then inoculated into the rice mill wastewater. *E. aerogenes* and *C. ferundii* were incubated overnight in nutrient agar (Hi–media, India) at 33 ± 2 °C under anaerobic conditions, and kept at 4 °C as stock culture. Prior to cultivation, *E. aerogenes* and *C. ferundii* were activated by transferring a loop full of stock culture into 100 mL of sterile fresh synthetic medium (pH 7.0) consisting of 2.0 g/L glucose, 1.0 g/L peptone, 0.05 g/L $MgSO_4 7H_2O$, 0.1 g/L KH_2PO_4 and 0.05 g/L $(NH_4)_2SO_4$ [28]. The culture was incubated at 35 ± 2 °C for 24 h at 150 rpm in an orbital shaker.

Acid hydrolysis

Acid hydrolysis of rice mill wastewater was carried out at 121 °C for 60 min in an autoclave. The influence of H_2SO_4 concentration on the performance of hydrolysis was investigated, by varying the concentration from 0.5 to 2.5% (v/v). The hydrolyzate obtained from each acid concentration was inoculated with *E. aerogenes* and *C. ferundii* to analyze the fermentability, and the initial pH was adjusted to 7.0 by adding NaOH (2 M) or HCl (2 M). The samples withdrawn at regular time intervals were centrifuged at 8000 rpm for

Table 1 – Physiochemical characteristics of rice mill wastewater.

Characteristics	Initial values
Chemical oxygen demand (g/L)	18.6
Biological oxygen demand (g/L)	6.9
Total solids (g/L)	49.14
Total dissolved solids (g/L)	24.72
Starch (g/L)	10.2
Cellulose (g/L)	2.1.
Hemicellulose (g/L)	4.2
Total nitrogen (g/L)	3.10
Volatile fatty acids (g/L)	1.92
pH	5.1