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Short Communication

Influence of sulfuric acid concentration on biohydrogen production from rice mill wastewater using pure and coculture of *Enterobacter aerogenes* and *Citrobacter freundii*

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ABSTRACT

We report a concise study on the influence of sulfuric acid concentration used in the hydrolysis of rice mill wastewater on biohydrogen production using pure as well as co-culture of *Enterobacter aerogenes* and *Citrobacter freundii*. A higher total reducing sugar release of 14.2 g/L was obtained with 1.5% sulfuric acid (v/v). The effect of pH on cumulative biohydrogen production from 1.5% acid hydrolyzed rice mill wastewater was investigated using pure and coculture of *Enterobacter aerogenes* and *Citrobacter ferundii*. The fermentative hydrogen production using mixed culture showed a maximum hydrogen yield of 1.61 mol/mol sugar. In addition, about 70% reduction in chemical oxygen demand (COD) and higher volatile fatty acids (VFA) production were observed. Modified Logistic, Gompertz and Richards models were used to fit cumulative hydrogen production data whereas Logistic equation was used fit growth data. Richards model showed best fit and Logistic equation gave least error in predicting maximum hydrogen producing potential.

Introduction

The negative environmental impacts associated with fossil fuels prompt the development of improved methods for the production of fuels from renewable and cost effective sources. Moreover, the fossil resources are finite and not carbon-neutral. Hydrogen is a promising alternate and clean fuel and produces only water upon its combustion [1]. Water electrolysis, thermo-chemical methods, and biological processes are the major hydrogen production methods. Among these, biological method is of great interest since it does not require harsh conditions, is cost effective and utilizes wastes rich in carbohydrates as substrate [2–4]. However, the pretreatment of such wastewaters are essential to enhance the hydrogen yield. Importantly, the pretreatment conditions affect the fermentative hydrogen production. More recently, the pretreatment of real textile desizing wastewater by coagulation has been reported to enhance the hydrogen production due to the removal of toxic materials that hinder biohydrogen production [5]. Oragnosolv pretreatment also

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reported to enhance the hydrogen production from rice straw [6].

Microorganisms such as *Bacillus* sp., *Clostridium* sp., *Citrobacter* sp., *Enterobacter* sp., *Klebsiella* sp. and *Rhodobacter* sp. are employed for hydrogen production [7]. The limitations of hydrogen producing microorganisms such as their doubling time, adaptability, survival ability, and thermodynamic limits to microbial metabolism reduce the hydrogen yield much less than the theoretically achievable yield of 4 mol/mol of glucose [8]. Moreover, concomitant production of carbon rich metabolites (organic acids and alcohols) reduce the hydrogen yield much less than the theoretically achievable yield of 4 mol/mol of glucose [9]. The search for potential microorganisms that can acclimatize to a broad range of physiological conditions and utilize diverse substrates is still in progress [2–4].

Availability, cost, carbohydrate content, and biodegradability are the major factors that determine the suitability of the substrate for hydrogen production [10]. Though simple sugars such as glucose, sucrose and lactose are readily biodegradable, the high cost associated with them make agricultural and food industry wastes as more attractive substrates [11]. Recently, raw cassava starch was used as a substrate to produce hydrogen by dark fermentation using mixed-culture and a maximum yield of 1.72 mol H_2/mol glucose was reported [12]. Hydrogen is produced successfully from sewage water, molasses, and dairy industry wastewaters by fermentation [13–15]. In our earlier studies, we demonstrated the biohydrogen production using rice mill wastewater [16,17]. During biohydrogen production using mixed culture in a fixed-bed reactor, hydrogen yield and rate of 2.3 mol H_2/mol glucose and 78 L H_2/L-d, respectively were observed [18].

The acid concentration used during hydrolysis influences the production of hydrogen. Hence this work aims to ascertain the influence of acid concentration on dark fermentative process that was used to treat rice mill wastewater as well as to produce hydrogen. Furthermore, hydrogen production was studied employing pure and coculture of *E. aerogenes* and *C. ferundii* at different operating pH and the cumulative hydrogen production data obtained were fitted with different models.

Materials and methods

The physiochemical and other characteristics of rice mill effluent were reported in our earlier work [14]. *E. aerogenes* and *C. freundii* were obtained from Microbial Type Culture Collection (MTCC), India and were maintained in 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 $(NH_4)_2SO_4$ at 37 °C under aerobic conditions. Acid hydrolysis of polysaccharides present in rice mill wastewater was carried out in an autoclave at 121 °C for 15–20 min (15 psi) at different sulfuric acid concentrations (0.5, 1.0, 1.5, 2.0 and 2.5% v/v). Samples obtained at different time intervals were centrifuged at 10000 rpm for 10 min and the supernatant was subjected to sugars, acetic acid and furfural analysis. Experimental runs were carried out twice and average values are reported. The experimental methods followed are reported in detail in our earlier work [16]. Fermentative hydrogen production was carried out in a 1500 ml batch reactor filled with 900 ml of pH adjusted (using 2 M NaOH or 2 N HCl) acid hydrolysate and 100 ml of inoculum (*E.aerogenes* or *C. freundii* or its consortium). There was an inlet port for the addition of medium and an outlet port for the collection of biogas. The serum bottles were closed with butyl rubber cocks and then sealed with aluminum caps. The temperature was maintained constant throughout the process at 37 °C. The contents of the bioreactor were mixed thoroughly using magnetic stirrer. The amount of biogas produced, degradation of sugars, volatile fatty acids (VFA) production, and COD reduction were measured by following the methods described in our previous work [16].

Kinetic modeling

The cumulative hydrogen production data were fit with three different sigmoidal models such as modified logistic (Eqn. (1)), Gompertz (Eqn. (2)) and Richards (Eqn. (3)) [19,20].

$$y = \frac{A}{\left(1 + \exp\left[\frac{4\mu_M}{A}(\lambda - t) + 2\right]\right.} \quad (1)$$

$$y = A\exp\left(-\exp\left[\frac{\mu_M\, e}{A}(\lambda - t) + 1\right]\right) \quad (2)$$

$$y = A\left\{\left(1 + \nu\exp(1+\nu)\exp\left[\frac{\mu_M}{A}(1+\nu)\left(1 + \frac{1}{\nu}\right)(\lambda - t)\right]\right\}^{\left(-\frac{1}{\nu}\right)} \quad (3)$$

where y is the cumulative hydrogen production, A is the maximum hydrogen production potential (ml), μ_M is the specific hydrogen production rate (ml/h), e represents exp (1), λ is the lag phase in h, and ν is the shape coefficient.

The growth data were fit with Logistic equation (Eqn. (4)).

$$X = \frac{X_0 \cdot e^{kt}}{1 - \frac{X_0}{X_C}\left(1 - e^{kt}\right)} \quad (4)$$

where X_0 is the initial biomass concentration (g/L), k is the carrying capacity coefficient (h^{-1}), X_c is the carrying capacity (g/L) and t is the time (h).

Results and discussion

The effect of acid concentration on the release of glucose, xylose, and arabinose are presented in our earlier work [16]. With 1.5% acid concentration, highest release of reducing sugar (14.2 g/l), glucose (10.20 g/L), xylose (3.1 g/L), arabinose (0.9 g/L), acetic acid (1.7 g/L), and furans (furfural and 5-hydroxymethyl furfural) (0.19 g/L) were obtained. Acetic acid concentration of 4–10 g/L inhibits the microbial growth besides furfural and in this study, we observed a very low acetic acid concentration [21]. Anburajan et al. [22] studied the inhibitory effect of HMF on continuous hydrogen production using mixed microbial population and observed higher hydrogen production with HMF concentration of less than 2.19 g/L. Kumar et al. [23] demonstrated the hydrogen

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