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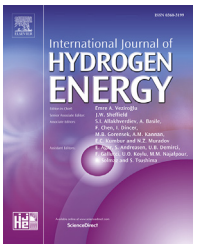

Biohydrogen production by extracted fermentation from sugar beet

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ABSTRACT

In the study, the production of biohydrogen by extracted fermentation from sugar beet was evaluated. Effects of initial amount of sugar beet, biomass and particle size of sugar beet on biohydrogen formation were investigated. The hydrogen (H_2) gas was predicted to be 78.6 mL at initial dry weight of sugar beet 24.6 g L^{-1} and H_2 yield was calculated as 81.9 mLH_2 g^{-1}TOC while biomass concentration (1 g L^{-1}) and particle size (0.3 cm) were constant. The peak H_2 gas volume was predicted to be 139.9 mL at the low particle size of 0.1 cm. Hydrogen gas production potential was predicted as 143.6 mL h^{-1}. The peak value of 197.9 mLH_2 g^{-1}TOC was obtained with particle size of 0.1 cm when dry weight of sugar beet and initial amount of biomass was kept constant at 24.6 g L^{-1} and 1 g L^{-1}, respectively.

Introduction

The world's energy consumption provided from fossil fuel, nuclear fuel and renewable resources increased with increasing global population [1,2]. Recently, there has been a large increase in international agreements to use of renewable energy source such as H_2 [3]. Hydrogen is considered an alternative fuels because of clean oxidation products generation after burning and its high-energy content (122kj kg^{-1}) [4]. However, many challenges are known to shift from fossil fuels to H_2 energy.

Currently, many technologies can be used in the production of H_2. Biological processes are a favorable method because of simplicity of process and different feed material utilization especially wastes [5]. Dark fermentation (DF) is the most promising way to biohydrogen production [4,6]. Dark fermentation for H_2 production could be improved by many strategies [7]. Substrate is converted to volatile fatty acid (VFA), CO_2 and H_2 during the DF process by suitable heterotrophic and facultative anaerobes [8]. In theory, 4 mol H_2 can be obtained for a mol glucose in acetic acid pathway and yield of H_2 decreased by formation of alcohol, biomass and other VFAs [9]. It's reported that some pre-treatment methods like heat shock, acid or alkaline treatment, sonication, freezing and thawing, etc. can be used to eliminate H_2 consuming bacteria to some extent. The heat-treated anaerobic sludge was found to be the most effective culture in the literature [10]. Pre-treated sludge is usually used for the selection of spore forming anaerobic bacteria and removal of methanogens that consume H_2 gas. Clostridia species present in heat-treated anaerobic acidogenic culture can ferment carbohydrates to produce H_2 gas, volatile fatty acids (VFA), CO_2 [7,8,11,12].

The cost, carbohydrate content and biodegradability is the first step for selection of raw material [4]. Sweet sorghum

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[13], sugar beet [14], carrot pulp [15], Jatropha seeds [12] in category of energy crops were used for raw materials. Sugar beet is a cheap and easily accessible material for biohydrogen fermentation [16]. It is more advantageous to use instead of other substrate sources because of its high amount of readily fermentable sugar, nutrient and mineral contents [14,17]. Fermentable sugar in form of monomeric and dimeric is suitable for production of biohydrogen. The materials containing starch such as potato peels, wheat bran and wheat grains and containing cellulose-hemicellulose such as carrot press cake, barley straw and wheat straw require enzymatic or chemical hydrolysis process to obtain fermentable sugar [16]. As a result of the sugar processing beet-pulp and wastewater are formed. Additionally, molasses is by-product. Ozgur et al. [18] reported that biohydrogen yields of 13.7 mol H_2 mol^{-1} sucrose, which was lower as compared to theoretical value, was obtained from molasses by sequential dark and photo fermentation. Generally, studies focus on biohydrogen formation from sucrose that obtained from hot water extraction of sugar beet or waste products of sugar processing. For example, Dhar et al. [19] investigated H_2 formation from beet juice by integrated microbial electrolysis cell and DF. The yields of 3.2 mol H_2 mol^{-1} glucose with 84 mLH_2 h^{-1} from sugar beet juice was reported. Hussy et al. [14] investigated pulped and water extract of sugar beet as substrate for biohydrogen production. Hydrogen yields were 0.9 mol H_2 mol^{-1} hexose for pulped sugarbeet and 1.7 mol H_2 mol^{-1} hexose for water extract of sugar beet in the continuous experiments. However, the dry matter is about 5% pulps and about 75% sugar at the end of hot water extraction process. Typical sugar beet contains 25% dry matter and 75% water. Sucrose content of sugar beet juices is varying between 15 and 18% and according to the dull substance weight of sugar beet contains around 75% sucrose [20]. The pulp is insoluble in water and consists of cellulose, hemicellulose, lignin etc. The solubilizing of organic contents from beet-pulp can be increased by applying pre-treatment method such as thermal-alkaline method. Ozkan et al. [21] reported that the maximum H_2 production (87.7 mLH_2 g^{-1} COD) was obtained at 4.5 g L^{-1} of initial COD from sugar industry wastes. In other work, maximum biohydrogen production yield of 115.6 mLH_2 g^{-1} COD was obtained with alkaline pretreated beet-pulp [22]. Direct fermentation of sugar beet has not been investigated [14].

Extracted fermentation (Ex-Ferm) was firstly applied to produce ethanol from sugar cane [23]. According to results, the concept of Ex-Ferm for ethanol fermentation is technically feasible. It's reported that sugar consumption was above 98% depending on the yeast strain for Ex-Ferm cycles [23–28]. Ex-Ferm is a process that extraction and fermentation process were carried out simultaneously in a substrate - water suspension. In the Ex-Ferm process, microorganisms grow on sugar beet particles and suspended in the liquid during the DF. Sugar is extracted from the solids to the liquid medium. Sucrose in liquid and dry matter are exploited by microorganism and converted to H_2 gas. Utilization of organic matters in sugar beet can be enhanced by extracted fermentation without pre-treatment methods [29,30].

There are many studies on biohydrogen production obtained from energy crops feedstock such as sugar beet. Generally, biohydrogen production was obtained from beet juice with pre-treatment in the literature. However, there is still high amount of sugar available in the beet pulp. With this method, more sugar can be consumed by the microorganism without pre-treatment from beet pulp. The study shows that the utilization of sugar, which can be obtained from the solid substrate without pre-treatment, can be enhanced. Substrate concentrations, biomass concentrations and particle size were carried out in the batch experiments.

Material and methods

Inoculum

The mixed culture was taken from anaerobic treatment plant's acidogenic phase of PakMaya Yeast Industry. In order to obtain spore forming-hydrogen producing bacterial culture, heat-treatment was applied and the culture was incubated using growth media containing glucose of 10 g L^{-1} for 2 days at 37 °C [31].

Experimental setup and procedure

Fresh sugar beet was used as a source of carbon. It was obtained from sugar factory named as Konya Şeker in Turkey. Particle size of sugar beet was prepared as cubes in the required dimensions according to the experimental conditions. Particles according to their moist weight were chopped into small particles and used in the experiments. Experimental results were given according to dry weight of sugar beet to make it comparable. $(NH_4)_2SO_4$ and KH_2PO_4 were added into medium in order to obtain C/N/P ratio of 100/2/0.5. The fermentation media also included 0.05 g L^{-1} $MgSO_4$ and 0.025 g L^{-1} $FeSO_4 \cdot 7H_2O$ and 0.1 g L^{-1} cysteine $HCl \cdot H_2O$. Three sets of extracted fermentation experiments were done at mesophilic conditions (37 °C). Batch experiment was performed at five different amounts of sugar beet between 5 and 60 g L^{-1} (2.05–24.6 g dry weight L^{-1}) while initial biomass concentration was 1 g L^{-1} and particle size was constant at 0.3 cm. Then, the total biomass concentration was varied between 0.25 g L^{-1} and 2 g L^{-1} while sugar beet amount was 60 g L^{-1} (24.6 g dry weight L^{-1}) and particle size was constant at 0.3 cm. In variable particle size experiments, particle size of sugar beet was varied from 0.1 to 1 cm while sugar beet amount was 60 g L^{-1} (24.6 g dry weight L^{-1}) and biomass concentration of 1 g L^{-1}. Batch experiments were carried out in 250 mL bottles (Isolab) with 150 mL fermentation volume (pH 6.8 ± 0.3). Each bottle was charged with sugar beet particles, fermentation medium and inoculated culture. Control experiments without biomass were conducted in parallel. Experiments were conducted in duplicate and the results deviated less than 5% from the average.

Analytical methods

The acid-phenol spectrometric method was used for sugar analysis in liquid [32]. Measurement uncertainty associated with the calibration result and repeatability was calculated as ± 1.02 for 20 gL^{-1} of total sugar (k = 2). Total organic carbon

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