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A pilot-scale study of biohydrogen production from distillery effluent using defined bacterial co-culture

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ABSTRACT

We evaluated the feasibility of improving the scale of hydrogen (H_2) production from sugar cane distillery effluent using co-cultures of *Citrobacter freundii* 01, *Enterobacter aerogenes* E10 and *Rhodopseudomonas palustris* P2 at 100 m^3 scale. The culture conditions at 100 ml and 2 L scales were optimized in minimal medium and we observed that the co-culture of the above three strains enhanced H_2 productivity significantly. Results at the 100 m^3 scale revealed a maximum of 21.38 kg of H_2, corresponding to 10692.6 mol, which was obtained through batch method at 40 h from reducing sugar (3862.3 mol) as glucose. The average yield of H_2 was 2.76 mol mol^{-1} glucose, and the rate of H_2 production was estimated as 0.53 kg/100 m^3/h. Our results demonstrate the utility of distillery effluent as a source of clean alternative energy and provide insights into treatment for industrial exploitation.

1. Introduction

Hydrogen (H_2) is a sustainable, non-polluting source of energy. Owing to its potential use, it has been considered as a prominent future fuel and energy vector. The United States and the European countries have already committed to establish an energy economy based on H_2 from fully sustainable or renewable sources. Based on the national H_2 program of the United States, the contribution of H_2 to total energy market will be ~8–10% by 2025 [1]. The market for H_2 and technologies for its production are potentially huge, and the level of investment into H_2 research has increased significantly in recent years, and H_2 may, in the long-term, replace all other fuels. However, cost is currently the biggest impediment to H_2 fuel production. H_2 is produced from nonrenewable energy sources, such as oil, natural gas, and coal that require expensive energy input [2]. Thus, the development of cost-effective and/or economically viable processes is necessary for increasing the achievable dominance of H_2 as an energy source. As a consequence, scientists are pursuing biological systems as a way to meet increasing energy demand. Biological H_2 production stands out as an environmentally friendly process carried out under mild operating conditions that include fermentation of sugars, photolysis of water and water gas shift reactions [3–6].

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Biological H_2 production is the most challenging and exigent prefecture of renewable energy technology development, especially where it involves the treatment of organic waste. H_2 can also be produced by bacteria from renewable biomass. Several studies on biohydrogen production from various substrates, including wastes [7–10], have been reported at laboratory level. Vatsala and Seshadri [7] have studied the enzymatic hydrolysis of silk-cotton waste into H_2 and volatile fatty acids (VFAs) using *Rhodospirillum rubrm* ATCC 11170. Kapdan and Kargi [8] have published an excellent review on the use of various substrates, particularly simple sugars, corn starch wastes, sweet potato, food industrial wastes, wastewater, and waste sludge, for H_2 production under dark and phototrophic conditions. While, chemical wastewater was used successfully for biohydrogen production at lab-scale [9]. Yu et al. [10] have demonstrated the continuous production of H_2 from anaerobic acidogenesis of a high-strength rice winery wastewater by a mixed bacterial flora at 3 L upflow reactor. Various types of bioreactors with different capacities have also been used for H_2 production, including batch, fed-batch and continuous-flow, stirred tank reactors [11–14]. The concept of waste utilization through a microbial fermentation process represents an ideal alternative technology for coordinating pollutant elimination and energy production. In this sense, biohydrogen is considered an intriguing ''future fuel''. The energy required for aerobic wastewater treatment is huge, but can be reduced through economically viable biohydrogen production processes.

We have been studying H_2 production from various waste materials [15,16]. Among the waste materials examined, distillery effluent was the most suitable substrate for H_2 production [17]. H_2 production from distillery effluent was investigated at lab-scale and pilot-scale [17]. The seasonal variations on the molasses quality, particularly sugar content, sulfur content, and their influences on H_2 production using individual and/or co-culture of *Citrobacter freundii* and/or *Rhodopseudomonas palustris* P2 were investigated [17]. We also reported H_2 production from distillery effluent at 10 m^3 volume and the produced H_2 was subsequently tested for fuel cell application [18]. Here, the present investigation focuses on developing a cost-effective, viable process for producing clean H_2 on a pilot-scale (100 m^3) from distillery effluent. Apart from H_2 as an energy source, the treatment of distillery waste was also accomplished, with a drastic reduction in chemical oxygen demand (COD) and biological oxygen demand (BOD). To the best of our knowledge, this is the first report on H_2 production at 100 m^3 scale.

2. Materials and methods

2.1. Materials

The biochemical characterization kits for bacterial identification were purchased from Hi-Media (Mumbai, India). The genomic DNA isolation kit was procured from Promega (Madison, WI, USA). DNA gel purification kit was purchased from Qiagen (Mannheim, Germany). Primers were synthesized by Cosmotech Co. Ltd., Korea. All other chemicals, unless otherwise indicated, were obtained from E-Merck (Mumbai, India) and/or Sigma–Aldrich (St. Louis, MO, USA).

2.2. Microorganisms and culture conditions

Heterotrophic bacterial strains (HTB) were isolated from the effluent treatment plant, EID Parry (I) Ltd., Nellikuppam, Tamil Nadu, India. Photoheterotrophic bacterial strains (PTB) were isolated from pond soil, Chengalpet, Tamil Nadu, India. Ormerod's minimal medium [19] was used for the regular maintenance of photoheterotropic isolates. To measure the H_2 production efficiency of the PTB isolates, experiments were conducted in serum bottles (125 ml total volume (tv); 100 ml working volume (wv)) at 37 °C, under photoheterotrophic conditions at 7000 lux intensity with an incandescent light source. Malate at 50 mM was used as a carbon source for PTB isolates. Heterotrophic isolates were cultured in H_2 production medium, which contained (per litre): 1.0 g yeast extract, 1.0 g NaCl, 1.0 g sodium thiosulphate and 10 g glucose, under dark anaerobic conditions for 24 h. This medium was fortified with a potassium phosphate buffer (50 mM; pH 7.0), and the experiments were conducted in serum bottles at 37 °C. Before inoculating the seed culture (HTB and/or PTB), the bottles were flushed with argon (Ar) gas (99.9%) for 10 min to ensure that the bottles were completely deprived of oxygen (O_2). Bottles were then sealed with butyl rubber septa and aluminum caps. Isolates were tested for H_2 production efficacy with specific media. The best H_2 producing strains, HTB01, HTB10, and PTB2, were subjected to physiochemical characterization using the methodologies described previously [20].

To further confirm the identification of bacterial isolates, the 16S rRNA genes from the strains HTB01, HTB10, and PTB2 were amplified by using the primers: 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) and sequenced by Cosmotech, Co. Ltd., Korea. Sequence alignment and analysis of similarity of the 16S rRNA genes were performed with CLUSTAL W [21] and DNA baser RC2 (v2.9.97) programs. A similarity search was done in the Ribosomal Database Project (RDP) [22].

To determine the efficiency of the selected microorganism for H_2 production, several experiments were conducted at a lab-scale with 125 ml serum bottles and a glass bioreactor (Bioengineering, KLF, 2000, SW). Axenic strains were acclimatized on distillery effluent in the laboratory under dark anaerobic conditions to grow and produce H_2 for several generations.

2.3. Bioreactors designing, fabrication and operation

The feasibility of H_2 production and effluent treatment was evaluated at the 100 m^3 scale. The lab-scale process of H_2 production was then scaled-up from 1 L to 100,000 L. As shown in Fig. 1, a sequence of bioreactors was constructed for inoculum preparation for the 100 m^3 reactor. The total volumes of the reactors were 0.125, 1.25, 12.5 and 125 m^3, and the working volumes were set from 0.1 to 100 m^3. The height-to-diameter (*H*/*D*) ratio of each reactor was set to 1.28. All the reactors were fabricated using mild steel with epoxy coating to prevent chemical and/or biological corrosion. Temperature and pH were monitored using specialized probes for 10 and 100 m^3 reactors. However, temperature and pH were not controlled. A 10% inoculum was used for each reactor. The