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Comparative evaluation of the hydrogen production by mixed consortium, synthetic co-culture and pure culture using distillery effluent

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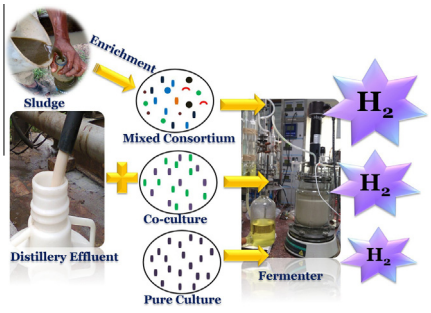
HIGHLIGHTS

- Suitability of consortium over co-culture and pure culture on H_2 production.
- Enrichment of homoacetogens and effect of co-culture on H_2 production.
- Suitability of distillery effluent as a potential substrate for H_2 production.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater comprises of various carbon sources. So, the use of microbial consortium may improve the hydrogen production and organic reduction. The present study deals with biohydrogen production by acidogenic mixed consortia (AMC), synthetic co-culture (*Klebsiella pneumoniae* IIT-BT 08 and *Citrobacter freundii* IIT-BT L139) and pure culture using distillery effluent (DE). Higher hydrogen yield was observed in case of AMC (9.17 mol/kg $COD_{reduced}$) as compared to the synthetic co-culture and pure culture. PCR–DGGE analysis indicated that the consortium was predominated by species closely affiliated to *Clostridium* sp. The average hydrogen production rate was 267 mL/L h. The maximum hydrogen production rate (R_m), hydrogen production potential (P) and lag time (λ) by AMC using DE were 507.2 mL/L h, 3729 m/L and 2.04 h, respectively. Maximum gaseous energy recovery by AMC was found to be higher by 21.9% and 45.4% than that of using co-culture and pure culture respectively.

1. Introduction

The world is craving for the alternative energy resources due to the increasing energy crisis. Moreover, the existing energy resources are non-environment friendly not only due to the emission of greenhouse gases but also unaffordable because of their increasing cost. Therefore, intensive research is going on throughout the world to develop an environmental-friendly technology that could be affordable and accessible.

Abbreviations: CPCB, Central Pollution Control Board; COD, chemical oxygen demand; BOD, biological oxygen demand; BES, 2-bromoethanesulfonate; AMC, acidogenic mixed consortium; VFA, volatile fatty acid; PCR, polymerase chain reaction; EDTA, ethylenediaminetetraacetic acid; EtBr, ethidium bromide; GC, gas chromatograph; TCD, thermal conductivity detector; FID, flame ionization detector; SEM, scanning electron microscopy; DGGE, denaturing gradient gel electrophoresis; DE, distillery effluent; K/C ratio, *Klebsiella* to *Citrobacter* ratio; A/B ratio, acetate to butyrate ratio.

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Gaseous form of energy (fuel) has several advantages over solid and liquid fuels viz. high heat content, easy transportation, controlled combustion (oxidizing or reducing atmosphere, length of flame, temperature, etc.), clean (burn without any shoot or smoke and ashes) and free from impurities found in solid and liquid fuels. Among different gaseous fuels, molecular hydrogen is regarded as the most promising future energy source because of its highest energy content (143 kJ/g) and environmental friendly nature (no CO_2 emission) (Das and Veziroglu, 2001). The advantage of biological hydrogen production is to use a wide variety of waste products as substrate for gaseous energy generation at ambient temperature and atmospheric pressure.

Organic waste/wastewater has very high COD which is detrimental for the environment, but can act as a source for energy generation. It has been reported that 5.2 million tons of solid waste is generated daily worldwide, of which 3.8 million tons comes from developing countries (Modak, 2011). Therefore, utilization of wastes can serve the dual purpose of energy generation and bioremediation. Production of hydrogen has been reported from various organic wastewaters viz. confectionary effluent Ueno et al. (1996), paper mill wastewater (Kádár et al., 2004), starchy wastewater (Wu and Lin, 2004), food wastes (Kotay and Das, 2007), corn syrup wastewater (Hafez et al., 2009), distillery effluent (Kamalaskar et al., 2010) etc. which are potent sources of water pollution due to higher COD. Distillery industry has been listed among top 17 most polluting industries in India by Central Pollution Control Board (CPCB, 2009–2010). It generates 8–15 L of effluent per liter of alcohol produced with a high COD (60–200 g/L) and BOD (25–75 g/L) (Chauhan and Dikshit, 2012).

Organic wastewater can be used as a potential feedstock for the biohydrogen production process. Mixed consortium harbors a variety of microorganisms (Roy et al., 2012). Hydrolytic enzymes produced by different microbes may help in the efficient utilization of various complex substrates present in wastewater. Biohydrogen production has been reported using mixed consortium (Mohan et al., 2008; Roy et al., 2012), various co-cultures and pure cultures using DE. The DE used in this study is derived from starch based feedstock (Mishra and Das, 2014). Most of the reports regarding hydrogen production are based on cane molasses derived DE (Vatsala et al., 2008). However, to the best of our knowledge, no report is available for comparing the biohydrogen production potential of mixed consortium, synthetic co-culture and pure culture using starch based DE. The present study deals with a comparative analysis of hydrogen production potential of enriched acidogenic mixed consortium (AMC), synthetic co-culture (*Klebsiella pneumoniae* IIT-BT 08 and *Citrobacter freundii* IIT-BT L139) and pure culture (*K. pneumoniae* IIT-BT 08) using DE. It also throws light on the gaseous energy recovery (as hydrogen) of the process.

2. Methods

2.1. Collection and characterization of distillery effluent

Rice based DE was collected from IFB Agro Industries Ltd., West Bengal and used in the present investigation. Characteristics of DE was determined using the standard methods (APHA, 1998) as reported by Mishra and Das (2014). The carbohydrate composition of DE was analyzed by using HPLC and starch content was estimated by iodine test.

2.2. Enrichment of acidogenic mixed consortium

Anaerobic sludge collected from IFB Agro Industries Ltd., Kolkata has been used as seed culture in the present study. For enrichment, 20 mL of the collected slurry has been inoculated into 60 mL of anaerobic media (glucose: 10 g/L; yeast extract: 0.34 g/L; NH_4Cl: 0.84 g/L; K_2HPO_4: 0.234 g/L; KH_2PO_4: 0.136 g/L; $MgCl_2{\cdot}6H_2O$: 0.084 g/L; $FeCl_3$: 0.05 g/L, Na_2SO_4: 0.5 g/L, cysteine HCl (1 g/L), 6.5 pH, 40 mM of 2-bromoethanesulfonate (BES) and vitamins solution (DSMZ medium No. 141, German Collection of Microorganisms and Cell Cultures) in 100 mL serum bottle for 24 h. Anaerobic conditions has been maintained by sparging the media with nitrogen gas for 30 min (Roy et al., 2012).

2.2.1. Single parameter optimization for hydrogen production using acidogenic mixed consortium

Initial pH, operational temperature and suitable substrate concentration do play an important role towards hydrogen production (Ginkel et al., 2001). In the present study, above mentioned process parameters were studied for the improvement of hydrogen production. The experiments were performed in 100 mL serum bottles (working volume of 80 mL) under strict anaerobic conditions. Hydrogen production by AMC was studied in media containing glucose (10 g/L), yeast extract (2 g/L) and tryptone (10 g/L).

2.2.2. Development of acclimatized acidogenic mixed consortium using DE

To develop acclimatized AMC, glucose media was fortified with DE at varying concentration viz. 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% (v/v). Culture was transferred from lower to higher concentration of DE after every 12–14 h.

2.3. Microorganism and culture conditions for pure strains

The isolates used in the present study were *K. pneumoniae* IIT-BT 08, *C. freundii* IIT-BT L139 and *Bacillus coagulans* IIT-BT S1 (Kumar and Das, 2000). These strains were grown overnight aerobically at 37 °C in NB medium (Himedia) in an incubator shaker (New Brunswick Scientific, NJ, USA) at 180 rpm. Malt extract (10 g/L), yeast extract (4 g/L) and glucose (10 g/L) was used for hydrogen production. The CFU count technique was used to estimate the number of active cells present in the inoculum.

2.4. Experimental conditions

The batch fermentation was performed in 500 mL double jacketed reactors (working volume of 400 mL) at 37 °C for 12–14 h at 180 rpm. DE was centrifuged at 10,000 rpm for 10 min to remove the suspended solids. The supernatant was collected, autoclaved and used for fermentation studies. Nitrogen gas was sparged to create an anaerobic environment. The gas produced during batch fermentation was passed through 40% (w/v) KOH solution for selective absorption of CO_2. The remaining gas was collected in a gas collector by displacement of 10% (w/v) saline at normal temperature and atmospheric pressure. The fermentation broth was analyzed to find out the biomass concentration, substrate consumption, and the total volatile fatty acid (VFA) production. The batch experiment was continued until hydrogen production ceased. Composition of the gas and volatile fatty acids (VFAs) were done by Gas chromatography. All the samples for VFA analysis were drawn during the maximum hydrogen production phase.

2.5. Hydrogen production study with pure culture, synthetic co-culture and mixed culture using distillery effluent

Batch fermentation for hydrogen production by AMC, synthetic co-culture and pure cultures (*K. pneumoniae* IIT-BT 08, *C. freundii* IIT-BT L139) under optimized parameters were performed in a controlled fermenter (New Brunswick Scientific, NJ, USA) with working volume of 2 L. The modified Gompertz equation was used to