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Single-stage fermentation process for high-value biohythane production with the treatment of distillery spent-wash

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highlights

- Single-stage hybrid bioreactor was designed and operated for biohythane production.

- Blend of biohydrogen and biomethane (biohythane) which is one of the vital fuels.

- Distillery based spent-wash was used as a substrate for biohythane production.

- Synergistics of VFA production/consumption played vital role for stable production.

- Biohythane from single-stage bioreactor has dual benefit (production and treatment).

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ABSTRACT

The current communication reports the development of a single-stage biosystem for biohythane production from wastewater treatment. A semi-pilot scale bioreactor with 34 L capacity was used for this study. Maximum biohythane production of 147.5 ± 2.4 L was observed after five cycles of operation with production rate of 4.7 ± 0.1 L/h. The biohythane composition $(H_2/(H_2 + CH_4))$ varied from 0.60 to 0.23 during stabilized fifth cycle of operation. During each cycle of operation, higher H_2 fraction was noticed within 12 h of cycle period followed by CH_4 production for rest of operation (36 h). During biohythane production, COD removal efficiency of $60 \pm 5\%$ (SDR, 29.0 \pm 1.9 kg COD_r/m³-day) was also achieved. The synergistic function of volatile fatty acids (VFA) production and consumption during process in hybrid biosystem played vital role on the composition of biohythane. The single-stage biosystem facilitates production of high valued and cost efficient biofuel (biohythane) with fewer controls than individual acidogenic and methanogenic processes.

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1. Introduction

Recent reports documented that the cumulative carbon emissions between 2011 and 2050 should not exceed 1100 GT of $CO₂$ in order to keep the global warming rise below $2^{\circ}C$ throughout the twenty-first century [\(Meinshausen et al., 2009; McGlade and](#page--1-0) [Ekins, 2015\)](#page--1-0). The current global fossil fuel reserves contain three times higher greenhouse gas emissions than this, so the unabated use of all the current fossil fuel reserves is incompatible with a global warming limit of $2^{\circ}C$ ([Raupach et al., 2014; McGlade and Ekins,](#page--1-0) [2015\)](#page--1-0). The report also suggests that, one third of oil, 50% of gas and about 80% of coal reserves should remain unused globally from 2010 to 2050 in order to meet the target of 2° C. In this context, renewable and/or alternative energy not only supports the

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conservancy of existing global fossil fuel reserves to major extent but also compensate the global warming issues.

Biohydrogen (H_2) and biomethane (CH₄) are the two renewable energy carriers with independent, broad commercial interest. CH₄ is being commonly used as compressed natural gas (CNG), which has been regarded as the clean energy carrier in comparison to gasoline or diesel ([Liu et al., 2012](#page--1-0)). However, $CH₄$ is limited by its narrow flammability, slow burning speed and high ignition temperature ([Bauer and Forest, 2001](#page--1-0)), which result in poor combustion efficiency and an intensive energy requirement for ignition of CNG powered vehicles. Interestingly, H_2 perfectly complements the limitations of methane and is an excellent additive to CNG. The mixture of hydrogen and methane is called hythane™ (trademarked; [Eden, 2010](#page--1-0)), HCNG or methagen [\(Ljunggren and Zacchi,](#page--1-0) [2010](#page--1-0)). The suggested H_2 content with CH₄ ranges between 10% and 25% by volume [\(Fulton et al., 2010](#page--1-0)). In comparison, H_2 mass specific heating value of 119,930 kJ/kg is nearly two and half folds

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higher than CH₄ (50,020 kJ/kg) [\(Bauer and Forest, 2001](#page--1-0)). Addition of small amount of $H₂$ significantly extends the lean flammability range of CH4 ([Bauer and Forest, 2001; Porpatham et al., 2007; Liu](#page--1-0) [et al., 2012](#page--1-0)). About sevenfold increase in the burning speed of a H_2 flame (265–325 cm/s) over CH₄ results in shorter burning time ([Bauer and Forest, 2001; Porpatham et al., 2007; Liu et al., 2012](#page--1-0)). A proper blend of the advantages of both H_2 and CH₄, can transition the technical fossil fuel-based society model to a terminal hydrogen-based society [\(Bauer and Forest, 2001; Das et al., 2000;](#page--1-0) [Fulton et al., 2010; Liu et al., 2012; Venkata Mohan, 2009\)](#page--1-0). Hythane has been used commercially as vehicle fuel in the USA and India [\(Das et al., 2000; Eden, 2010\)](#page--1-0), has also received much commercial attention in transportation sector ([Liu et al., 2012\)](#page--1-0). The increasing market for CNG vehicles due to shortage of gasoline/diesel and the pressure to reduce GHG emissions, are found to stimulate the development of clean and sustainable fuels [\(Liu](#page--1-0) [et al., 2012\)](#page--1-0).

At present physical/chemical methods via adding H_2 to CH₄ are practiced. Two separate gas channels with quantitative control of the gas flow rates would be required for the desired $H_2/(CH_4 + H_2)$ ratio. These approaches are mostly not sustainable due to their dependence on fossil fuel base. However, the recent advantages in bioenergy research, allows the use of biological process, i.e., darkfermentation for the production of both these gases using abundantly available and renewable, waste, as a feedstock ([Monlau](#page--1-0) [et al., 2015; Jia et al., 2014; Lee et al., 2014; Pasupuleti et al.,](#page--1-0) [2014; Sarkar et al., 2013; Mohankrishna et al., 2010; Venkata](#page--1-0) [Mohan et al., 2007a,b, 2009](#page--1-0)). A two stage dark-fermentation process was reported to produce H_2 and CH₄ (mixture also called as biohythane; [Cavinato et al., 2011; Liu et al., 2012\)](#page--1-0) from biomass/ bio-waste ([Venkata Mohan et al., 2008; Lu et al., 2009; Ljunggren](#page--1-0) [and Zacchi, 2010; Mohanakrishna and Venkata Mohan, 2013\)](#page--1-0). This biological method guarantees the regulation of H_2 /(CH₄ + H₂) ratio by adjusting the conditions of microbial fermentation and also accounting for waste remediation simultaneously. Although, this two-stage fermentation has advantages, operation of two bioreactors was economically less viable due to financial, energy and manpower constrains.

In this study an attempt was made to produce biohythane in a single-stage biosystem using spent-wash as feed stock, integrating wastewater remediation as integral part. There are approximately 295 distilleries in India which are producing 2.7 billion liters of alcohol and generating 40 billion liters of spent-wash annually ([Tapas et al., 2002](#page--1-0)). The technologies currently used by these distilleries for treatment of spent-wash are anaerobic digestion for CH4 generation followed by aerobic activated sludge system prior to disposal. It has been observed that almost all distilleries have adopted anaerobic digestion as industry standard practice for the first stage treatment of raw spent-wash. The distillery spent wash has potential to produce approximately 1100 million cubic meters of biogas [\(Venkata Mohan et al., 2005, 2009; Nataraj et al., 2006\)](#page--1-0) making it a potential feedstock for biohythane production.

2. Methods

2.1. Anaerobic consortium

Anaerobic consortium acquired from a full scale anaerobic bioreactor was used as a parent inoculum. The consortium was sieved to separate the coarse material and the resulting thick sludge (3.6 g VSS/L) was washed thrice (saline buffer; 5000 rpm, 20 $^{\circ}$ C) and enriched in designed synthetic wastewater (DSW; glucose – 3 g/L; NH₄Cl - 0.5 g/L, KH₂PO₄ - 0.25 g/L, K₂HPO₄ - 0.25 g/L, $MgCl_2 - 0.3 g/L$, CoCl₂ – 25 mg/L, ZnCl₂ – 11.5 mg/L, CuCl₂ – 10.5 mg/L, CaCl₂ – 5 mg/L, MnCl₂ – 15 mg/L, NiSO₄ – 16 mg/L, FeCl₃ – 25 mg/L) under anaerobic microenvironment at pH 7.0, prior to inoculation into bioreactor.

2.2. Distillery spent-wash

The wastewater was composed of good amount of carbon load (COD, 126 g/L) with reasonably good biodegradability (BOD/ $COD \sim 0.50$). The wastewater was acidic in nature (pH, 3.83) due to the presence of volatile fatty acids (VFA, 28.25 g/L) and mainly composed of acetic acid (H_{AA} , 12.14 g/L), butyric acid (H_{BA} , 5.44 g/L) and propionic acid (H_{PA} , 12.14 g/L). The wastewater also contained sulfate (3 g/L) and total dissolved solids (TDS) of 112 g/L.

2.3. Single-stage bioreactor and operation

A semi-pilot scale bioreactor (120 cm (length) \times 20 cm (diameter)) fabricated using 'Perspex' material was designed with total/working volume of 34/18 L and gas holding capacity of 4.5 L (head space). The bioreactor was filled with coir pith (50% v/v; void fraction, 0.18) as a fixed bed material to support the growth of anaerobic consortium. The coir pith was positioned at the center and in concentric manner with the core of the cylindrical chamber. The bioreactor was fabricated using leak proof sealing material along with proper inlet and outlet arrangements. The biosystem was operated at mesophilic temperature $(30 \pm 2 \degree C)$ conditions in up flow mode with the velocity of 0.214 m/h and recycle to feed ratio of 8 day⁻¹. Prior to start up, the bioreactor was filled with anaerobic consortia (20% of the total volume) along with designed synthetic wastewater (DSW, 18 L) to aid biofilm formation on the supporting medium (coir pith bed) at an organic loading rate (OLR) of 6 kg CD/m^3 -day by adjusting the substrate pH to 6.0, using concentrated ortho-phosphoric acid (30%) and/or 1 N NaOH. Effluent from the bioreactor was collected from the bottom and biogas generated during the operation was collected by water displacement method through an outlet provided at the top of the bioreactor. After obtaining steady performance with DSW, bioreactor was shifted to spent-wash and operated with an OLR of 7.94 kg COD/m^3 -day for five consecutive cycles. Fed-batch experimental protocol was employed to evaluate the biohythane production with a cycle retention time of 48 h. Prior to feeding, the wastewater pH was also adjusted to 6. At the beginning of each cycle, immediately after withdrawal of effluent (earlier sequence), a predefined volume (18 L) of spent-wash was fed to the reactor during fill phase and the reactor volume was circulated in closed loop at recirculation rate (recirculation volume to feed volume ratio) of 8 during the reaction phase, to achieve homogeneous distribution of the substrate as well as uniform distribution of requisite consortia along the reactor depth. Peristaltic pump was used to regulate the feed, recirculation, and decant operations.

2.4. Analysis

The biogas compositional analysis was evaluated by gas chromatograph (NUCON 5765) using thermal conductivity detector (TCD) with $1/800 \times 2$ m Heysep Q column employing nitrogen as carrier gas. The injector and detector were maintained at 60° C each and the oven was operated at $40 °C$, isothermally. The biogas was quantified with the calibration gas (Spangas & Equipments Ltd., India). Separation and estimation of VFA was carried out by high performance liquid chromatography (HPLC; Shimadzu LC20AD) operated under optimized conditions (UV–Vis detector; C18 column-reverse phase column – 250×4.6 mm and 5 m particle size; flow rate – 0.6 mL/min; wave length – 210 nm; mobile phase - 40% of acetonitrile in 0.001 N $H₂SO₄$ (pH 2.5-3.0); sample injection $-20 \mu L$). Chromatograms were compared with 1 ppm standards (acetic acid, butyric acid, propionic acid, formic acid,

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