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Improvement in biohythane production using organic solid waste and distillery effluent

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ABSTRACT

Biohythane is a two-stage anaerobic fermentation process consisting of biohydrogen production followed by biomethanation. This serves as an environment friendly and economically sustainable approach for the improved valorization of organic wastes. The characteristics of organic wastes depend on their respective sources. The choice of an appropriate combination of complementary organic wastes can vastly improve the bioenergy generation besides achieving the significant cost reduction. The present study assess the suitability and economic viability of using the groundnut deoiled cake (GDOC), mustard deoiled cake (MDOC), distillers' dried grain with solubles (DDGS) and algal biomass (AB) as a co-substrate for the biohythane process. Results showed that maximum gaseous energy of 23.93, 16.63, 23.44 and 16.21 kcal/L were produced using GDOC, MDOC, DDGS and AB in the two stage biohythane production, respectively. Both GDOC and DDGS were found to be better co-substrates as compared to MDOC and AB. The maximum cumulative hydrogen and methane production of 150 and 64 mmol/L were achieved using GDOC. 98% reduction in substrate input cost (SIC) was achieved using the co-supplementation procedure.

1. Introduction

The burning issue of fossil energy depletion and severe environmental problems viz., global warming and greenhouse gas effect has forced the world's scientific community to develop different ways to harness the bioenergy from diversified sources (Ellabban et al., 2014; Ghimire et al., 2015). Hydrogen and methane are the two main gaseous energy carriers which are widely used in the chemical industry. Hydrogen is regarded as the cleanest fuel with zero carbon emission and highest energy content (143 kJ/g) (Das and Veziroglu, 2001; Sharma and Ghoshal, 2015). Methane falls after hydrogen with an energy content 55 kJ/g. It is widely used as transportation fuel in the form of liquefied natural gas because of its higher calorific value and lower CO_2 emissions as compared to oil and coal. In addition, unlike other fuels, methane combustion does not produce any NOx (nitrous oxide) and SOx (sulphur dioxide) which are the major contributors for air pollution (Gaffney and Marley, 2009). So, both hydrogen and methane have independently attracted broad commercial interest and are highly valued. Recently, hythane, a mixture of hydrogen (10–25% by volume) and methane, is garnering growing attention due to its versatile advantages. It acts as an ideal transition fuel for the change to hydrogen because it provides significant reduction in the NOx emission as compared to the natural gas. Unlike hydrogen, it is relatively inexpensive and easy to store (Fulton et al., 2010; Liu et al., 2013).

The conversion of waste into hythane can mitigate the dual problem of environmental pollution and energy crisis. Chemical production of hythane requires costly substrate, is an energy intensive process, and releases hazardous end products which are detrimental to the environment. On the contrary, use of industrial waste for the biochemical conversion into hythane has a potential to use these end products as a substrate for subsequent methanogenesis (Arizzi et al., 2016; Kothari et al., 2012). Dark fermentation is the most efficient biological process for commercial hydrogen production. However, it produces metabolic end-products such as acetate, butyrate, propionate, ethanol and lactic acid which remain unused (Das and Veziroglu, 2001). Therefore, gaseous energy

Abbreviations: A/B ratio, acetate to butyrate ratio; AB, algal biomass; AMC, acidogenic mixed consortium; APHA, American Public Health Association; BOD, biological oxygen demand; CPCB, central pollution control board; COD,, chemical oxygen demand; DDGS, distillers' dried grain with solubles; DE, distillery effluent; GC, gas chromatograph; GDOC, groundnut deoiled cake; FID, flame ionization detector; MDOC, mustard deoiled cake; MMC, methanogenic mixed consortium; VFA, volatile fatty acid; TCD, thermal conductivity detector.

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recovery can be increased up to 65–70% by using the spent media for subsequent methane production (Schievano et al., 2014). Different organic wastes viz., potato wastes (Zhu et al., 2008), ethanol stillage (Luo et al., 2011), food waste (Lee et al., 2010), wheat straw hydrolysate (Kongjan et al., 2011) and garbage slurry and paper waste (Uneo et al., 2007) have been used for the two stage biohythane production in a Mesophilic conditions. Khongkliang et al. (2015) have reported a high hydrogen and methane yield of 81.5 and 310.5 L/kg COD, respectively using thermophilic consortium. Among different waste substrates, organic wastewater including distillery effluent has high organic load which is detrimental for the environment (Lin et al., 2012). According to Central Pollution Control Board (CPCB, 2010), India, distilleries are ranked among the top 17 heavily polluting industries of the country. Annually, 40 billion litres of effluent (spent wash) is disposed from distilleries. The distillery wastewater, owing to its huge organic load, is an ideal substrate for the biohydrogen production. It comprises of high COD (60,000–120,000 mg/L) and BOD (45,000–60,000 mg/L) (Pant et al., 2007; Krishnamoorthy et al., 2017). It contains very high amounts of potassium, calcium, chloride and sulphate. However, the distillery effluent lacks most of the essential micro nutrients such as Fe, Mg, P, Cu, and Zn (Mohana et al., 2009; Basu, 1975). They serve as cofactors of the different enzymes involved in the fermentation pathway. Therefore, the supplementation of such micronutrients is essential for the improvement of the biohydrogen yield (Karadag et al., 2010; Mishra and Das, 2014). Reports show that the supplementation of pure nitrogen and mineral salts to the distillery effluent results in the increased cost of the biohydrogen production process (Da Silva et al., 2014; Saharan et al., 2011). This problem can be solved by supplementing inexpensive nutrient sources.

Distillers' grain with solubles (DDGS) is a rich source of proteins and minerals (Li et al., 2002). DDGS is made by blending distiller's dried grains (DDG) with solubles. The water and solids remaining after distillation of ethanol are called whole stillage. Whole stillage is comprised primarily of water, fiber, protein, and fat. This mixture is centrifuged to separate coarse solids from liquid. The liquid, called thin stillage, goes through an evaporator to remove additional moisture resulting in condensed distiller's solubles (syrup) which contains approximately 20–40% (w/w) proteins and 30% dry matter. The coarse solids are called distiller's dried grains (wet cake) and contain 35% (w/v) dry matter and 60% (w/w) protein (Ratanapariyanuch et al., 2016). The sources of DDGS are mainly wheat, maize and corn. DDGS has been used for different purposes viz., specific protein extraction (Gupta et al., 2016; Chatzifragkou et al., 2016), production of liquid hydrocarbons (Taneeru and Steele, 2015), bio-oil production (Lei et al., 2011), chemical hydrogen production (Tavasoli et al., 2009) and photo fermentative hydrogen production (Sargsyan et al., 2016). However, the use of DDGS as a supplement for fermentative biohydrogen production has not been explored.

DDGS, DOC are rich source of nitrogen and minerals and used as another co-substrate owing to its high production rate of 253 million tons oil seeds per year (Barnwal and Sharma, 2005). After extraction of the oil, the de-oiled cakes can be used for the biohydrogen production. DOC has been used for bioenergy generation in the form of bio oil (Volli and Singh, 2012), biodiesel (Sánchez-Arreola et al., 2015), biogas (Barik and Murugan, 2015) and biohydrogen (Kumar et al., 2015) production.

Further, algal biomass was considered as another co-substrate for biohythane production because of its high protein and mineral content. Besides being rich nutrient source, it has several other advantages viz., higher productivities, use of non-arable land and harvest solar energy. The low lignin content (<2%) of algae like *Coleochaete* and some of the filamentous fresh water algal species surpasses the energy intensive de-lignification step enabling faster substrate utilization during fermentation (Ververis et al., 2007; Montingelli et al., 2015; Maurya et al., 2016). Excess algal growth (algal bloom) in natural water bodies causes eutrophication which leads to the death of the aquatic plants and animals. Cleaning of algal bloom needs additional man power and energy. Utilization of such wastewater grown algal biomass for biohythane production can save energy and cost of the process (Chiu et al., 2015). These substrates have been used as major or sole nutrient source for biohydrogen production. The study on the effect of fortifying such solid organic wastes (rich in nitrogen and minerals) with a liquid waste containing high carbohydrate load such as the distillery effluent has not yet been fully investigated. In the present study, algal biomass (AB), distillers dried grains with solubles (DDGS), mustard deoiled cake (MDOC) and groundnut deoiled cake (GDOC) as a co-substrate were considered for the improvement of the biohythane production. Emphasis has been laid in reducing the substrate cost besides achieving waste remediation in a two stage biohythane production process.

2. Materials and methods

2.1. Microorganism and culture conditions

2.1.1. Cultivation of acidogenic mixed consortium

Acidogenic mixed consortium (AMC) was developed from the anaerobic sludge collected from IFB Agro Industries Ltd., Kolkata (Mishra et al., 2015). The AMC was maintained in a broth media containing glucose (1% w/v), tryptone (1% w/v) and trace amount of $FeSO_4$ at 37 °C and pH 6.5 with regular subculturing.

2.1.2. Cultivation of methanogenic mixed consortium

Methanogenic mixed consortium (MMC) was developed from anaerobic sludge collected from IFB Agro Industries Ltd., Kolkata. It was used as an inoculum for the second stage methane production. The consortium was maintained in the media (per Litre) containing acetic acid 2.0 g; butyric acid 1.0 g; NH_4Cl 0.84 g; K_2HPO_4 0.234 g; KH_2PO_4 0.136 g; NaCl 0.6 g; $MgCl_2{\cdot}6H_2O$ 0.084 g; $CaCl_2{\cdot}2H_2O$ 0.006 g; $FeCl_3$ 0.05 g; Na_2SO_4 0.5 g; trace elements solution 10 mL (Per Litre: nitrilotriacetic acid 1.5 g; $MgCl_2{\cdot}6H_2O$ 2.476 g; $Mn(CH_3COO)_2{\cdot}4H_2O$ 0.655 g; NaCl 1.0 g;$CoCl_2{\cdot}6H_2O$ 0.106 g; $CaCl_2{\cdot}2H_2O$ 0.10 g; $ZnCl_2$ 0.18 g; $CuCl_2{\cdot}2H2O$ 0.007 g; $AlCl_3$ 0.0056 g; H_3BO_3 0.01 g; $Na_2MoO_4{\cdot}2H_2O$ 0.01 mg; $NiCl_2{\cdot}6H_2O$ 0.025 mg; $Na_2SeO_3{\cdot}5H_2O$ 0.30 mg); and vitamin solution 10 mL (Per Litre: folic acid 2.0 mg; pyridoxine - HCl 10.0 mg; riboflavin 5.0 mg; nicotinic acid 5.0 mg; D-Ca-pantothenate 5.0 mg; vitamin B_{12} 0.10 mg; p-aminobenzoic acid 5.0 mg). The consortium was maintained in its active form by sub-culturing at 35 °C and initial pH of 7.8 after every 15 d of interval.

2.2. Collection and characterization of different organic solid wastes/wastewater

Rice grain based distillery effluent, collected from IFB agro, West Bengal, India, was used in the study. The distillery effluent was characterized in our previous work (Mishra and Das, 2014).

The AB was collected from a fresh water pond, Jakpur, West Bengal, India in the month of March. Leaves and other such materials were manually separated and the residual clay/soil particles were removed by washing. The powdered algal biomass was used as a co-substrate for the hydrogen production. DDGS was collected from IFB agro, West Bengal. The GDOC and MDOC were collected from a local market in Kharagpur, West Bengal, India. The physico-chemical characteristics were determined using standard protocols APHA (1995) (Table 1).

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