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Biologically pretreated sugarcane top as a potential raw material for the enhancement of gaseous energy recovery by two stage biohythane process

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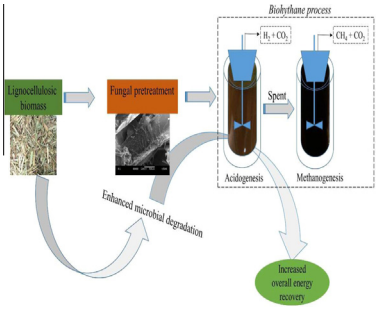
HIGHLIGHTS

- Biological pretreatment showed 60.4% lignin removal from the sugarcane top.
- Confocal microscopic and FTIR analysis confirmed lignin reduction.
- Biohythane production enhanced the gaseous energy recovery up to 37.7%.
- Unstructured model was used to determine the kinetics of product formation.
- Material balance analysis showed the feasibility of the process.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of the present study was to develop a suitable pretreatment method to enhance the microbial degradation of lignocellulosic biomass and to maximize the overall energy recovery by using biohythane process. An efficient and eco-friendly biological pretreatment was used. Maximum lignin removal using biological pretreatment of sugarcane top was 60.4% w/w after 21 d incubation at 28 °C in static condition. Confocal microscopy observation and FTIR analysis confirmed the removal of lignin from sugarcane top. The maximum hydrogen production rate (R_m), hydrogen production potential (P) and lag time (λ) using pretreated sugarcane top were 16.76 mL/g-VS/h, 87.40 mL/g-VS and 3.38 h respectively. The maximum methane production potential using spent medium of dark fermentation was 180.86 mL/g-VS with the lag time of 2.9 d. The overall gaseous energy recovery was 37.7% which is 54% higher than that of the untreated one.

1. Introduction

Exponential increase in world's energy demand and diminishing reserves of non-renewable energy resources are responsible for the development of alternative energy resources. Molecular hydrogen is currently regarded as the most promising future energy source due to its highest energy density among any known fuel (142 kJ/g), clean combustion (no CO_2 emission) and easy conversion into electricity via fuel cells (Ntaikou et al., 2010; Perry et al., 1973). The biological production of hydrogen does not require elevated temperature and pressure and can be produced from different organic wastes. However, a number of challenges must be overcome before biohydrogen can become economically feasible. The primary challenge is the low substrate conversion

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efficiency. In the conventional dark fermentation process, only about 30% of the substrate energy can be recovered (Das and Veziroglu, 2001). The rest of the energy is remained in the spent as volatile fatty acids (VFA) (Hallenbeck and Ghosh, 2009). Consequently, VFA can be further converted to hydrogen employing light or electricity or reduction to methane through anaerobic digestion. The anaerobic digestion is a simpler and well established process as compared to others (Hallenbeck and Ghosh, 2009). The two stage production of biohydrogen followed by biomethane is known as biohythane (Cheng and Liu, 2012; Liu et al., 2013; Pakarinen et al., 2009). The two stage biohythane has the advantage of enhancing energy recovery and chemical oxygen demand (COD) reduction as compared to single stage biohydrogen production.

Feedstock cost is a major determinant for the viability of commercial scale production of biofuel. A consistent and stable supply of raw material is required to maintain the high throughput and profitable. Therefore, it is imperative to use cheapest raw material available in specific region to make the process economically attractive and sustainable for converting biomass to fuel (Vera et al., 2015). Lignocellulosic biomass (mainly from agricultural residues, municipal wastes and forestry sources) is suitable for hydrogen production because of its large-scale availability, low cost, and environmentally benign (Wan and Li, 2010a).

Sugarcane top is the most abundant agricultural residue available in India. The annual production of this residue is approximately 97.8 MMT/Y (matric million ton/year) in which the unused residue of 79.4 MMT/Y is mostly burnt in the field itself (Sukumaran et al., 2010). However, microbial degradation of lignocellulosic biomass is highly influenced by its relatively complex structure, resulting in highly resistant to enzymatic hydrolysis and low cellulose conversion. Therefore, it is necessary to develop a pretreatment process to separate lignin from cellulose and hemicellulose for improving microbial degradation (Travaini et al., 2016).

Physico-chemical pretreatmnet of the lignocellulosic biomass using acid, alkali, stream explosion, microwave, ionizing radiation, hot water or combination require special instrument. It also consumes energy and generates inhibitors, which affect the enzymatic hydrolysis and fermentation processes. Biological pretreatment using metabolites of a microorganism are a promising technique for biofuel production from lignocellulosic biomass. It is eco-friendly, does not require chemicals and also does not release any toxic to the environment (Sindhu et al., 2015). White rot fungi are most effective lignin-degrading microorganisms in nature. It produces lignin degrading enzymes which are responsible for the removal of lignin to expose the cellulose and hemicellulose of lignocellulosic biomass (Ma et al., 2010). These characteristics could be useful in providing an unprotected carbohydrate for subsequent biofuel production. The lignin degradation capabilities of several white rot fungi is due to their extracellular non-specific and non-stereoselective enzyme system composed mainly of laccase, lignin peroxidase (LiP), manganese-dependant peroxidase (MnP) (Dias et al., 2010; Larran et al., 2015; Shirkavand et al., 2016).

So, the purpose of the present study was to investigate the potentiality of fungal pretreatment process for the degradation of lignin present in sugarcane top. *Pleurotus pulmonarium* MTCC 1805 was used for delignification of sugarcane top. This was followed by the anaerobic digestion of fungal-pretreated sugarcane top by using acidogenic and methanogenic mixed microbial consortia to produce H_2 and CH_4 respectively. Furthermore, unstructured model were used to describe the kinetics of hydrogen and methane formation. In addition, the mass and energy analysis of the biohythane production process was also carried out. The present study is an attempt for the first time to find out the suitability of the biologically pretreated sugarcane top for the maximization of gaseous energy recovery using biohythane process.

2. Materials and methods

2.1. Materials

The sugarcane top used in the present study was collected from the local market of IIT Kharagpur. It was washed properly under tap water, air dried, grinded and sieved through a 3 mm screen before storing at room temperature prior to use. The white rot fungus *Pleurotus pulmonarium* MTCC 1805 was procured from Microbial type culture collection (MTCC), Chandigarh, India. The culture was maintained on potato dextrose agar (PDA) plates at 4 °C. The acidogenic and methanogenic mixed consortia used for biohythane fermentation process were developed from cow dung (Kumari and Das, 2015).

2.2. Fungal pretreatment

Fungal pretreatment of sugarcane top was performed in 250 mL Erlenmeyer flasks containing 10 g of sugarcane top with moisture content of 70% (w/w). Erlenmeyer flasks containing wet sugarcane top were autoclaved at 121 °C for 15 min prior to inoculation. The inoculum was prepared by inoculating two dick cut from the margin of active fungal plate into Erlenmeyer flask containing 100 mL potato dextrose broth (PDB). The flask was kept at 28 °C and 150 rpm in incubator shaker for 5 d. After 5 d, 2 mL of supernatant was used as fungal inoculum. Control was prepared by using 2 mL distilled autoclaved water in place of the culture. Pretreatment was carried out at 28 °C under static condition for 21 d. The samples were drawn every third day for enzymatic and compositional analysis.

2.3. Enzyme extraction and assay

50 mL buffer (0.05 M citrate buffer, pH 4.8) was added to soak the fermented sugarcane top and kept at 25 °C for 6 h in incubator shaker. The crude filtrate was separated and kept at −20 °C for the determination of enzymatic activity. The pretreated sugarcane top was washed with tap water and oven dried at 60 °C till to constant weight. The dried biomass was further used for chemical composition analysis and biohythane production.

All enzymatic assays were performed in triplicates using a UV–Vis spectrophotometer (LAMBDA 25, Perkin Elmer, Singapore). Laccase activity was determined by oxidation of ABTS (2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) at 470 nm ($e = 46{,}900\ M^{-1}\ cm^{-1}$) (Liu et al., 2013). The reaction mixture (3 mL) comprised of 0.1 mL of sample, 0.5 mL of 10 mM ABTS and 2.4 mL sodium citrate buffer (0.1 M, pH 3.5). Lignin peroxidase (LiP) activity was determined by oxidation of veratryl alcohol at 310 nm ($\varepsilon = 9300\ M^{-1}\ cm^{-1}$) (Bak et al., 2009). The reaction mixture contained 400 μL citrate buffer (0.1 M, pH 3.0), 400 μL sample, and 200 μL veratryl alcohol solution (2 mM). The reaction was initiated by addition of 0.1 mL H_2O_2 (4 mM). MnP activity was determined by oxidation of $MnSO_4$ at 270 nm ($\varepsilon = 11{,}590\ M^{-1}cm^{-1}$) (Boer et al., 2004). Reaction mixture (4 mL) contained 3.7 mL citrate buffer (0.1 M, pH 4.5), 0.1 mL substrate $MnSO_4$ (10 mM), and 0.1 mL sample, and the reaction was initiated by adding 0.1 mL H_2O_2 (4 mM). One unit of enzyme activity (laccase, LiP, and MnP) was defined as the amount of enzyme required to produce 1 μmol product per min at required temperature. The activity of each enzyme was measured as U/mL. U/g of untreated biomass was calculated by multiplying the activity of the enzyme with the total volume of the crude extract followed by dividing with the total dried sugarcane top (Wan and Li, 2010b). Cellulase, xylanase and β-glucosidase activity were determined using National renewable energy laboratory (NREL) standard protocol (Adney and Nrel, 2008).