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Process kinetic studies of biohydrogen production by co-fermentation of fruit-vegetable wastes and cottage cheese whey

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ABSTRACT

Hydrogen (H_2) is considered as fuel for future and its biological production from renewable feedstocks is very promising. Dark fermentation of fruit-vegetable waste (FVW) and cottage cheese whey (CCW) for the production of H_2 constitutes a promising way for combining energy generation and lignocellulosic waste management. In this work, process kinetics of biohydrogen production via dark fermentation of FVW and CCW by pretreated anaerobic sludge inocula were investigated. To inhibit H_2 consuming methanogens, the effects of various inoculum pretreatment, viz., 2-bromoethanesulfonate, heat-shock, acid, alkali, UV, and ultrasonication on H_2-production were investigated which revealed 2-bromoethanesulfonate, heat-shock and acid-treated inoculum resulted in maximum bioH_2 production and yield of 118.12 ± 1.05, 93.37 ± 1.3, 96.32 mMol/L and 1.66, 1.22 ± 0.01, 1.39 ± 0.02 mMol/gCOD$_{initial}$, respectively. The effects of system initial pH, substrate to inoculum (S_0/X_0) and carbon to nitrogen (C/N) ratio on H_2-production were evaluated which revealed maximum H_2 production and yield could be achieved at pH 7, S_0/X_0 of 10.6 g_{COD}/g_{VS}, and C/N 26.8. Modified Gompertz model and Modified Logistic model were used to define various kinetic parameters pertaining to cumulative H_2-production which showed high R^2 values (≥0.98). The influence of pH on H_2 and ethanol/volatile fatty acids production kinetics were evaluated using Andrew's and Ratkowsky's model showing relatively good R^2 values (≥0.62). Remarkably high production of ethanol (2.43 ± 0.28 mg/L) was noticed alongside H_2 production at pH 7 suggesting that bioethanol can be recovered at the end of fermentative H_2 production. Terminal Restriction Fragment Length Polymorphism and 16s rDNA sequencing revealed dominance of 9 bacterial species such as *Escherichia coli*, *Clostridium butyricum*, *Streptococcus henryi*, and 6 others uncultured bacteroides. This research determined different kinetic parameters for an enhanced H_2 production strategy by co-fermentation of FVW and CCW providing an understanding of process behavior which will in turn help in the upscaling of the H_2-production processes.

Introduction

Energy insecurity and environmental pollution are major concerns faced in 21st century, hence, attention has been drawn to produce cleaner fuel from renewable feedstock. Hydrogen (H_2) is anticipated as the ideal alternative energy carrier for the future owing to its zero-pollution generation and its high energy value of 122 KJ/g which is around 2.75 times higher than the hydrocarbon assorted fuels (Hay et al., 2013; Marone et al., 2014). It has to be produced from various energy sources like natural gases, coal, heavy oil, naphtha, as well as from the electrolysis of water. However, the production of H_2 by using these conventional techniques requires high energy input and emits greenhouse gases (Rahman et al., 2016). Thus, various International Organization and Governing Bodies closely associated with H_2 energy sector such as, International Association for Hydrogen Energy, Fuel Cell & Hydrogen Energy Association, Office of Energy Efficiency and Renewable Energy, Department of Energy have recognized biological H_2 (BioH_2) production by dark fermentation of organic wastes to be a less expensive, less energy demanding compared to aforementioned conventional techniques, and environment friendly process and intend to use this bioH_2 technology in the development of H_2 economy (Gomez-Romero et al., 2014; Gadhe et al., 2015; Ghimire et al., 2015).

Currently in India, H_2 is produced in chemical and fertilizer industries, and although significant work has been done till now in the field of bioH_2 production, the present scope of bioH_2 energy is limited to research, development, and demonstration stage. Therefore, more research

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committed to this field to be followed by scaling up of the technique to pilot and industrial production scale is required (MNRE, 2018b). The legislation pertaining to bioH_2 production in India started back in 2003 after India joining the IPHE (International Partnership for Hydrogen Economy) as founder member. As India moves ahead towards the implementation of Euro 5 and Euro 6 emission norms for automobiles in the coming years, bioH_2 may get placed automatically in the automobile sector to replace fossil fuel large scale power generation. The present emphasis has been on the use of cheap, sustainable, easily available and complex substrate for the production of bioH_2 and more research data is required to develop cost-effective, industrial scale bioH_2 production strategies (MNRE, 2018a).

In India, tonnes of fruit and vegetable wastes (FVW) are generated everyday as the waste product of fruit processing industries, fruit juice vendors, market places and as household waste product. On the other hand, cottage cheese whey (CCW) which is a liquid waste generated after the manufacturing of cottage cheese mostly find their way into nearby stream and thereby increases its biochemical oxygen demand. FVW and CCW are rich in polysaccharides, oligosaccharides, hexoses, and other compounds that easily support microbial growth and bioH_2 production (Gomez-Romero et al., 2014; Argun et al., 2017). Most of the studies involving renewable feedstock have been conducted using mono-digestion process of a single substrate (De Gioannis et al., 2014; Marone et al., 2014; Gadhe et al., 2015). Findings in these studies suggest that bioH_2 production using mono-digestion of single substrate can result in lower yield of H_2 owing to poor buffering capacity of substrates, nutrient limitation or imbalance etc. This drawback can be overcome by using co-digestion of two or more substrate for dark fermentation as suggested by many researchers (Tawfik & El-Qelish, 2012; Gomez-Romero et al., 2014). Co-fermentation of more than one substrate has been reported to increase bioH_2 yield generally by (a) minimizing inhibitory effects of substrate, (b) enhancing buffer capacity of substrate, (c) supplying the missing or inadequately present nutrients, and (d) regulating carbon to nitrogen (C/N) ratio of the substrates (Luostarinen et al., 2009; Gomez-Romero et al., 2014). Moreover, the generation of bioH_2 from waste substrate is subject to a major challenge as many unexplored wastes contain microorganisms that not only hinder H_2 generation but also consume H_2 in their other metabolic processes (methanogenesis) (Hay et al., 2013).

The major advantage that can be achieved by using acidogenic mixed culture is that there is no need of sterilization of substrates thereby reducing overall process cost (Ghimire et al., 2016; Kumari & Das, 2017). In order to inhibit methanogenesis from the mixed culture inocula, various inoculum pretreatment viz. heat treatment, chemical treatment, freezing/thawing have been proposed (Akutsu et al., 2009; Kumari & Das, 2017). Nevertheless, the use of non-sterilized substrates will definitely add other microorganisms than those present in the inocula, thereby modifying the fermenting microbial community in the system (Gomez-Romero et al., 2014). Therefore, challenge is to prevent methanogenesis in large scale dark fermentation processes to produce cleaner bioH_2.

The kinetic parameters of bioH_2 production describe the performances of the dark fermentation process and vary over wide range depending upon the inoculum microbial community and the type of substrates being fermented. Getting better insights about the kinetic behaviour of microbial community fermenting a rich substrate such as organic wastes is a prerequisite as each H_2 producing microbial community has its own H_2 production dynamics which can foretell the bioH_2 productive capability of that particular microbial community growing on the particular substrate. The kinetic model such as modified Gompertz model (MGM) and modified Logistic model (MLM) have been widely used to describe the dark fermentative bioH_2 production which helps to define the specific functions of parameters thereby influencing H_2 yield and helping in process design for sustainable bioH_2 production (Wang & Wan, 2009b; Pasupuleti & Venkata Mohan, 2015).

As mentioned above, since use of single substrate for dark fermentation poses constraint of nutrient imbalance and limitation as well as poor buffering capacity leading to low yield of H_2, a novel technique of co-fermentation of two substrate has been proposed and analyzed in the present study. Most of the study reported so far has involved studying the suitability of a given substrate for H_2 production and resultant H_2 yield and productivity. However, in real practice organic wastes will contain more than one type of wastes, hence, it is important to perform a proper kinetic analysis which will help in the upscaling of dark fermentation process employing more than one substrate. Therefore, the present study involved a rigorous and thorough investigation of the kinetics of bioH_2 production by the co-fermentation of two common wastes in India, FVW and CCW. Different unstructured comprehensive kinetic models viz., MGM, MLM, Andrew's model (AM), Ratkowsky's model (RM) were used to describe the kinetic behavior of the dark fermentative bioH_2 production (Wang & Wan, 2009a, 2009b). The effects of system redox conditions on the kinetics of bioH_2 and volatile fatty acids (VFA) production were also determined by Andrew's model (AM) and Ratkowsky's model (RM). Six different inoculum pretreatment viz., 2-bromoethanesulfonate treatment, heat treatment by microwave, ultrasonication, UV ray treatment, and acid and alkali treatment methods were adopted to evaluate the effects of inoculum pretreatment for better yields of H_2. The microbial profile analysis of the pretreated anaerobic sludge was also performed using *T*erminal *R*estriction *F*ragments *L*ength *P*olymorphism (T-RFLP) instead of denaturating gradient gel electrophoresis (DGGE) (Osborne, 2014) and 16s rDNA amplification by PCR, followed by its sequencing.

Materials and methods

Chemicals

All the chemicals used in this study were of analytical and HPLC grade and were purchased from Sigma Aldrich (USA), Himedia (India), Merck (India) and SRL Pvt. Ltd. (India). 99.99% pure H_2 standard for GC was purchased from Sam Air Products & Equipment, India. Other HPLC grade standards for Acetic acid, ethanol, butyric acid, propionic acid etc. were purchased from Merck (India) and SRL Pvt. Ltd. (India).

Substrate preparation and characterization

FVW were collected from a local cafeteria in Durgapur (23.55° N, 87.32° E) India. Fruit and vegetable wastes used as substrate in this study generally consisted of peels and refuses of a wide range of typical Indian fruits (citrus fruits, pomegranates, banana, apple, mangoes, guava, pears, pineapple, papaya etc.) especially after extraction of juice (25% *w/w*), and refuses of green leafy vegetables such as cabbage, cauliflower, spinach etc. (35% *w/w*), as well as peels of potato, carrots etc. (40% *w/w*). The CCW used in this study was obtained from a local cottage cheese manufacturer and vendor. The whey sample was filtered through Whatman Grade 1 filter paper (pore size 11 μm) and stored at 4 °C until used.

The FVW were milled in a domestic electrical blender without adding water in order to homogenize the sample. The slurry thus produced was sieved through a No. 12 mesh (1.68 mm diameter) stainless steel sieve. Substrate for dark fermentation did not receive any kind of pretreatments and was prepared by mixing different proportions of the FVW slurry and CCW as to achieve desired C/N ratios used in different set of experiments. Finally, the mixtures of FVW and CCW were diluted into 0.1 M phosphate buffer (pH 7) (25% *v/v*) and used for fermentation.

The physicochemical characterization of FVW and CCW was done in terms of total solids (TS), volatile solids (VS), total inorganic carbon (TOC), chemical oxygen demand (COD), pH etc., using standard methods of APHA (APHA, 2005).