Energy Conversion and Management xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Energy Conversion and Management

journal homepage: www.elsevier.com/locate/enconman

Particle size variations of activated carbon on biofilm formation in thermophilic biohydrogen production from palm oil mill effluent

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Thermophilic biohydrogen production
Attached-biofilm
Granular activated carbon
POME fermentation
Renewable energy

ABSTRACT

In this study, we examined the formation of thermophilic microbial biofilm by self-attachment on microbial carrier of granular activated carbon (GAC) in five different micro-pore volumes 0.31, 0.41, 0.44, 0.48, and 0.50 cm^3/g. It was found that the highest hydrogen production rate of 100.8 ± 3.7 mmol H_2/l.d and yield of 1.01 ± 0.07 mol H_2/mol sugar were obtained at 0.44 cm^3/g volume size of GAC. The cellulolytic activity of attached-biofilm was further investigated using POME as a feedstock. The results showed that in all diluted POME substrate, the total sugar consumed by the microbes was found higher than that the amount of soluble monomeric sugar present in the POME medium. It is believe that the microbial biofilm was able to hydrolyse polymeric sugar of cellulosic fibre in the POME by performing enzymatic hydrolysis into simple monomeric sugar. The isolated biofilm bacteria that subjected to 16S rRNA gene analysis presented 99% high homology to the species of *Thermoanaerobacterium thermosaccharolyticum* which were guaranteed to perform a cellulosic degradation activity.

1. Introduction

Hydrogen is known as one of the potential future green and clean energy sources with an energy density of 286 kJ mol^{-1}. The process is widely investigated as hydrogen is one of the alternative renewable energy to replace fossil fuel [1]. Dark fermentation by bacteria is one of the economical and sustainable ways to drive the utilisation of abundant biological wastes to biohydrogen [2]. Asian country such as Malaysia is well related to its big palm oil plantations - an important source of income - has made to the abundant source of wastewater known as palm oil mill effluent (POME). POME's high nutrient content makes it an ideal fermentation substrate for biohydrogen production [3–5] and beneficial in anaerobic treatment process [6].

Lignocellulosic biomass is found as the main plant biomass material in many waste streams such as the food industry and agricultural wastes [7] and it can also be found abundantly in the source of POME. These materials are recognised as excellent sources for a range of fermentable sugars, with their readily available carbohydrate content and useful for hydrogen bioproduction [8,9]. The lignocellulosic fibres is the desired portion of hydrogen production that is built up from long-chain of carbohydrates polymers, containing glucose exclusively in the case of cellulose, a variety of hexoses and pentoses for hemicellulose and lignin [10–12]. Bacterial and fungal species have a tendency to naturally degrade cellulosic biomass to simple monomeric sugars such as glucose and xylose by performing enzymatic hydrolysis and produce cellulolytic enzymes composed primarily of endo-glucanase, exo-gluanase, β-glucosidase, and xylanase [13–16]. The ability of the bacteria to degrade cellulosic biomass and convert it to hydrogen is highly promising in contributing to low wastewater equipment cost, which at the same time helps in utilising these biomass residues as potential resources for biohydrogen production [17].

Fermentation via the high temperature operation known as thermophilic fermentation has been explored and has recently drawn more interest in hydrogen production research [18–20]. This operation involves limited growth of hydrogen consumers like methanogens and homoacetogens that give higher yield due to thermodynamic advantages [21–24]. Also, increased hydrogen productivity rate (HPR) were observed under high temperature operating condition [25].

Lower microbial cell density has, however, been a disadvantage to fermentation at this temperature [3,26–28]. Washout of the cells is often experienced in a suspended system culture, and the microbial population is difficult to be retained in the bioreactor especially at a low hydraulic retention time (HRT) [29,30]. Therefore,

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http://dx.doi.org/10.1016/j.enconman.2016.09.067
0196-8904/

the biological immobilisation approach is of special interest to researchers seeking to enhance cell density especially by the cell attachment method in developing attached-biofilm on the microbial support carrier [30–35].

Support carriers like GAC have been well documented as a support matrix in thermophilic fermentation [35]. The simple attachment procedure on GAC performed effectively at high operating temperatures, coupled with low toxicity, makes GAC an attractive carrier [3]. It has good mechanical strength and inert properties without any additional reaction that might disturb the system. Large area of the GAC particles helps to enhance cell density during the fermentation process [26–28,34–36]. The GAC also has a high surface area and highly porous structure that facilitate to sustain cell viability [37] and colonization density [38,39]. The attachment of microbes to the carrier will increase their cell density over that of suspended free cells, making it more tolerable to environment perturbation, which leads to process stability and consistency of gas production. Moreover, the attachment enables the system to become reusable, and performs higher biological activity [40]. Zhang et al. had reported that the efficiency of hydrogen fermentation is influenced by the amount of biomass retained on the GAC system seems very promising to increase the hydrogen production [41].

Therefore, this study presents the first attempt in investigating the variations of GAC in the form of its volume size as a microbial carrier during biofilm development in thermophilic hydrogen production. The effect of GAC properties on the adhesion and colonization of bacteria through self-attachment immobilisation were investigated using continuous feeding set at 2-days hydraulic retention time (HRT), as found in the early study [35]. The performance of the attached-biofilm developed at optimal carrier size was further investigated and cellulolytic microbes that were able to degrade polymeric sugar in POME were identified. The cellulolytic performance was examined through the total sugar consumed by the bacteria over the soluble monomeric sugars representing complex POME as the fermentation feedstock.

2. Materials and methods

2.1. Cultivation of seed mixed culture

Mixed culture of POME-sludge was collected from a sludge pit of processing mill located at Pulau Carey, Selangor, Malaysia. The microbial sludge was subjected to a heat treatment process at 80 °C for 60 min to inactivate the growth of methanogenic population before it being used as inoculum in the fermentation. The 10% (v/v) sludge was cultivated in a 200 ml synthetic medium inside a 250 ml modified Scott Duran bottle with subsequent flushing using mixed N_2 gas at a flow rate of 1 L/h. The fermentation mixture was adjusted to pH 6.0 and then kept in a 60 °C water bath shaken, 200 rpm for 48 h.

2.2. Synthetic medium

The synthetic wastewater composition was slightly modified from Angelidaki and Sanders [42], consisted mainly of glucose and xylose as carbon source, and contained (per litre of deionised water): NH_4Cl 1 g, NaCl 2 g, $MgCl_2{\cdot}6H_2O$ 0.5 g, $CaCl_2{\cdot}2H_2O$ 0.05 g, $K_2HPO_4{\cdot}3H_2O$ 1.5 g, KH_2PO_4 0.75 g, $NaHCO_3$ 2.6 g, and yeast extract 2 g [42].

2.3. Analysis of sugar composition in POME

Sugar composition in POME was determined using the National Renewable Energy Laboratories standard method [43]. Prior to samples analysis, a series of dilution were done with distilled water at 20% increment on POME. The determination of different types of soluble monomeric sugars (SMS) was subjected to direct HPLC analysis, while total monomeric sugars (TMS) was subjected to acid hydrolysis [43]. The TMS analyses represented the amount of total sugars available in POME, which including the SMS and the complex sugars intact in cellulosic fibres (cellulose and hemicellulose).

Acid hydrolysis was used to obtain TMS in POME. After POME was diluted using distilled water at every 20% dilution increment, each samples were subjected to complex sugar degradation into monomeric sugar using 72% sulphuric acid. The mixture was autoclaved at 121 °C for an hour. The samples were prepared in triplicates and analysed using the HPLC.

The volume of 72% H_2SO_4 required for acid hydrolysis was calculated as follows [43],

$$V_{72\%,H_2SO_4} = \frac{\left[(C_{4\%} \times V_s) - \left(V_s \times [H^+] \times \frac{98.08\ g\ H_2SO_4}{2\ moles[H^+]}\right)\right]}{C_{72\%}} \quad (1)$$

where $V_{72\%}$ is the volume of 72% acid (ml), V_s is the initial volume of sample (ml), $C_{4\%}$ is the concentration of 4% w/w H_2SO_4, 41.0 g/l, $C_{72\%}$ is the concentration of 72% w/w H_2SO_4, 1176.3 g/l, $[H^+]$ is the concentration of hydrogen ions (moles/l).

2.4. GAC of different particle sizes as microbial carriers

The effect of different GAC-particle sizes as the best microbial support carrier on the development of attached-biofilm in thermophilic biohydrogen production was investigated. The characteristics of the GAC are shown in Table 1. The GAC carrier was a coconut shell origin that was supplied by Concepts Ecotech Sdn Bhd, Malaysia.

The fermentation experiments using five different size of GAC labelled as A, B, C, D and E, were placed in a modified lab-scale glass bioreactor. All the experiments have a similar set-up, and shown in Fig. 1. The temperature was controlled at 60 °C using hot water circulation from a water bath. The pH was maintained at 6 by adding 6 M KOH or 6 M HCl. The gas meter, MilliGascounter® (Ritter, Germany) is used to measure the biogas generated from the exhaust of that connected from the gas condenser.

The fermentation experiments were carried out using 200 cm^3 synthetic wastewater as a feedstock, with 10% GAC and 10% inoculum added into a 250 cm^3 bioreactor. The systems were acclimatized through continuous feeding mode 2-days HRT until consistent hydrogen production achieved. The profile of daily cumulative biohydrogen produced over fermentation time was plotted and analysed.

2.5. Characterisation of biofilm formation

The density of the clean GAC (g/l) was calculated by measuring the weight of the GAC (g) over the occupied volume (l). The biofilm formation was calculated based on the cell biomass adhering to the GAC, which was determined by measuring the volatile suspended solid (VSS) according to the standard protocol by APHA [44]. Therefore, the clean GAC was subjected to VSS determination to ensure that all bacterial growth on the surface had been removed [35]. The ratio of the attached biomass present on GAC compared to clean GAC was calculated as shown in Eq. (2).

$$\text{Weight of attached-biofilm (g cell/g GAC)} = \frac{\text{VSS of attached biomass on the GAC surface (g/l)}}{\text{Density of clean on the GAC (g/l)}} \quad (2)$$

Please cite this article in press as: Jamali NS et al. Particle size variations of activated carbon on biofilm formation in thermophilic biohydrogen production from palm oil mill effluent. Energy Convers Manage (2016), http://dx.doi.org/10.1016/j.enconman.2016.09.067