INTERNATIONAL JOURNAL OF HYDROGEN ENERGY XXX (2016) 1–11

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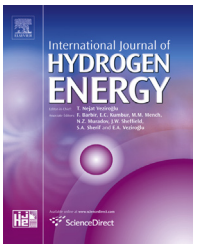

Biofilm formation on granular activated carbon in xylose and glucose mixture for thermophilic biohydrogen production

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ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form
7 May 2016
Accepted 10 May 2016
Available online xxx

Keywords:
Biofilms
Fermentation
Biohydrogen
Thermophiles
Batch processing
Granular activated carbon (GAC)

ABSTRACT

Granular activated carbon (GAC) was used as a support carrier to develop biofilm of Thermophilic biohydrogen producer in an immobilized system of dark fermentation. The optimum ratio of the sludge to GAC loading was investigated in batch fermentation using glucose and xylose mixture as a carbon source. It was found that the highest hydrogen yield of 1.77 mol H_2/mol substrate consumed and hydrogen production rate (HPR) of 2.0 mmol H_2/l.h, were achieved at a sludge-GAC ratio of 1:2. On the other hand, the experiments with suspended culture as a control gave poor performance of hydrogen yield (0.86 mol H_2/mol of substrate consumed) and HPR (0.5 mmol H_2/l.h). The sludge-GAC biofilm was further developed in a sequencing batch feeding mode through controlled acclimatization condition. Stable hydrogen production was achieved after day 40th with consistent HPR of 2.4 mmol H_2/l.h and yield of 1.17 mol H_2/mol substrate consumed with 44.2% of hydrogen. Acetic and butyric acids dominate volatile fatty acids (TVFAs) as a major by-product while ethanol being the only alcohol produced but in a minor amount. This work has proven the possible future of GAC attached biofilm sludge as promising attachment system to achieve consistent hydrogen production even at thermophilic conditions.

Introduction

Hydrogen gas is well-known as a green, clean, high energy fuel without harming the environment [1]. Biohydrogen derived from renewable resources contributes the decrease in CO_2 emissions. With the current proliferation of global economy, production of biohydrogen fuel has emerged as a platform to utilize the abundant biological wastes [2,3]. A thermophilic, dark fermentation (45–65 °C) process has gained attention due

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http://dx.doi.org/10.1016/j.ijhydene.2016.05.092
0360-3199/

to its ability of higher yield that could be due to its thermodynamic advantages, better pathogenic destruction, as well as the thermophilic process limits the growth of hydrogen consumers like methanogens and homoacetogens [3–6]. However, besides the above advantages, lower cell density remains the drawback of fermentation at the thermophilic temperature [7–14].

Good transfer of nutrient into microorganisms can be achieved via free suspended cell culture. However, at high hydraulic pressure and low hydraulic retention time (HRT), the microbial population in the bioreactor is hard to maintain as washout of the cells is often experienced [15,16]. Therefore, many research efforts have been made to increase the cell density by using physical and biological immobilization approaches onto support matric as an alternative approach to the suspended cell systems [16–26]. There are three types of immobilization cell systems in biohydrogen production being introduced so far including surface attachment [16,18,19,27,28], self-flocculation [22,29–33] and gel entrapment [20,21,24,25] approaches. Of all the methods, surface attachment approach becomes the most popular and frequently sought by researchers in dark hydrogen fermentation [34]. Attached cell immobilization is more advantageous than suspended cell since the system is more tolerance towards environment perturbation, process stability, reusable, higher biological activity. Moreover, this system can operate at higher dilution rates without biomass washout from the reactor [35,36].

In many types of support matric used in developing the attached-biofilm, granular activated carbon (GAC) has been well documented as a support matric in thermophilic fermentation. GAC has high surface area, inertness, low toxicity, and good mechanical properties, which are suitable for high-temperature fermentation [37–40]. Its character of highly porous structure also helps to sustain cell viability which served excellently in microbial colonization, where the fermentative bacteria can grow freely inside the porous structure and on the surface of the carrier material, forming a biofilm [41].

In our preliminary work, the focus on implementing the advantages of GAC as support material in thermophilic fermentation were done on glucose and xylose mixture, as the mixture of carbon source that mostly composed in lignocellulosic residue. As the first attempt in investigating the ability of the microbial culture to self-attach and retain on the surface of GAC, the efforts were made to create high cell density that able to grow and produce good hydrogen production. The studied parameters were subjected to the best GAC-sludge ratio obtained in batch fermentation to form the attached-biofilm, that in the end be used in a continuous hydrogen production.

Material and methods

Microorganism, the GAC carrier and medium conditions

The source of microorganism was obtained from the sludge pit of palm oil mill effluent (POME) located at Sime Darby Plantation, West Oill Mill, Pulau Carey, Selangor, Malaysia. The sludge containing mixed culture was subjected to heat treatment process at 80 °C for 60 min to inhibit the growth of methanogenic population. The granular activated carbon (GAC), made from coconut shell, was purchased from Concepts Ecotech Sdn Bhd, Malaysia. Initially, the GAC were sieved to obtain a particle size of 2–3 mm as shown in Fig. 1. The characteristics of the GAC were summarized in Table 1.

The prepared synthetic nutrient contains a mixture of glucose and xylose (as carbon source); and other components that prepared in deionized water such as NH_4Cl 1 g l^{-1}, NaCl 2 g l^{-1}, $MgCl_2.6H_2O$ 0.5 g l^{-1}, $CaCl_2.2H_2O$ 0.05 g l^{-1}, $K_2HPO_4.3H_2O$ 1.5 g l^{-1}, KH_2PO_4 0.75 g l^{-1}, $NaHCO_3$ 2.6 g l^{-1} and yeast extract 2 g l^{-1}. The medium composition was taken from Wu et al. [42] with slight modification. The initial pH of the culture medium was pH 6.0 and cultivated in a water bath shaker at 60 °C and 200 rpm for 48 h.

Selection of ratio of microbial sludge to GAC loading

The sludge added to the medium was chosen at constant amount of 10% (v/v), which 800 ml of final culture volume were prepared in a 1 l bioreactor. The amount of sludge suspension (in ml) to clean GAC loading (in g) was varied as shown in Table 2.

Biofilm development through cell acclimatization on GAC

The microbial sludge was acclimatized in a system operated at sequencing batch and the experiments were run at HRT of 2 days in 800 ml working volume. The feed medium initial pH was set at pH 6. The acclimatization were conducted in a water bath shaker (model SW22, 230 V/50–60 Hz) at 200 rpm and 60 °C. Sequencing batch operation was carried out by the removal of 50% of culture medium and replacing with new medium after each cycle. The process cycle was continued until hydrogen production rate achieved consistently. The samples of fermentation products were taken at each cycle and sent for analysis.

Characterization of the biofilm

The cell biomass adhered on the GAC were measured to quantify volatile suspended solid (VSS) according to the standard protocol by APHA [43]. The density of the GAC was

Fig. 1 – Granular activated carbon with particle size of 2–3 mm as microbial support carrier.

Please cite this article in press as: Jamali NS, et al., Biofilm formation on granular activated carbon in xylose and glucose mixture for thermophilic biohydrogen production, International Journal of Hydrogen Energy (2016), http://dx.doi.org/10.1016/j.ijhydene.2016.05.092