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Batch and continuous thermophilic hydrogen fermentation of sucrose using anaerobic sludge from palm oil mill effluent via immobilisation technique

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ABSTRACT

This study aimed to investigate the feasibility to use immobilised cells on granular activated carbon (GAC) operated in fifteen cycles of repeated batch mode for enhancement of biohydrogen production under thermophilic conditions. The effects of the initial pH (5.0–6.5), sucrose concentration (13–43 mM) and repeated batch cultivation on biohydrogen production from sucrose were investigated using anaerobic sludge from a palm oil mill effluent treatment plant. The cumulative hydrogen production results were fitted into a modified Gompertz equation in order to find the optimum operational conditions. The optimal hydrogen yield (2.8 mol_{H2}/mol_{hexose}) was obtained at an initial pH of 5.5 and sucrose concentration of 13 mM after fifteen cycles of repeated batch. Enriched granular activated carbon (GAC)-immobilised cells from the repeated batch cultivation were used as the immobilised seed culture for anaerobic fermentation of sucrose into hydrogen in continuous operation using a fluidised-bed column reactor (FBCR). The maximum hydrogen production rate (HPR) was found to be 2.7 $mmol_{H2}/L/h$ and the hydrogen yield peaked at 2.8 $mol_{H2}/mol_{hexose.consumed}$ after a hydraulic retention time of 12 h. The main soluble metabolites were identified as acetic acid, butyric acid and ethanol. The hydrogen content ranged from 48 to 50% of the total biogas.

1. Introduction

Hydrogen is an ideal alternative fuel because of its clean, sustainable and renewable characteristics, high energy content and lack of CO_2 emissions from its combustion. The hydrogen gas can be generated through thermochemical, electrochemical or biological processes. Among the different biological processes, anaerobic biohydrogen production by dark fermentation is, technically, more feasible and recognised as environmentally friendly and cost effective. The process, which relies mainly on fermentative indigenous microorganisms and renewable biomass, can also offer prospects of sustainable energy when used in the treatment of organic waste.

Suspended cell culture has been the most frequently used system for H_2 production through anaerobic dark fermentation. Suspended systems allow better mass transfer between microorganism and substrate; however, the difficulty in maintaining a sufficient amount of hydrogen-producing bacterial population in the bioreactor at low hydraulic retention time (HRT) encourages researchers to further explore the attached systems. Immobilisation is one of the most promising attached growth systems that could enhance the density of cells and be stably operated at low HRT. The application of these immobilised cells on an industrial scale is more feasible when operated without sterilisation, such as in a fluidised bed [1], packed bed [2] or up-flow anaerobic sludge bed (UASB) reactors [3]. Several techniques can be applied to entrap cells, such as surface attachment through physical adsorption [1], gel entrapment [4] and self-immobilised approaches [5].

Many types of immobilised carrier materials, such as activated carbon, pumice stone, ceramic ring and so forth, have been studied for their possible applications in biohydrogen production [1,2]. Activated carbon is one of the most studied materials because of its non-toxicity, good adherence to the seed sludge and higher

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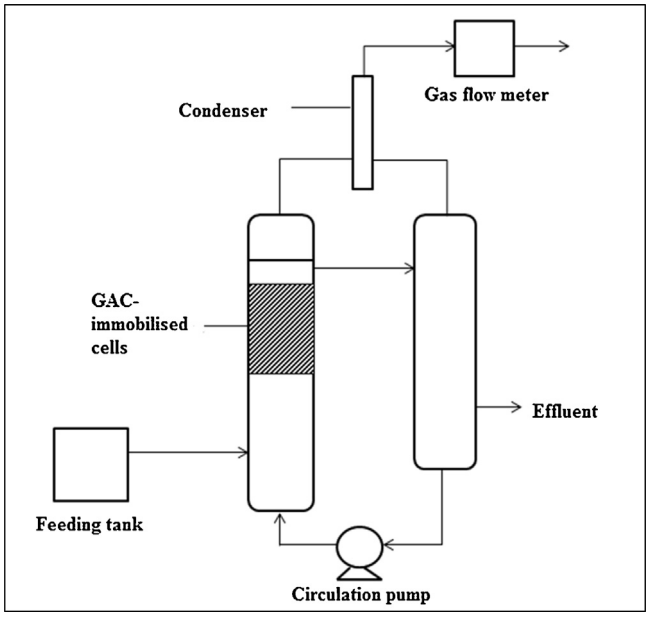

Fig. 1. Experimental set up of the fluidised bed column reactor (FBCR) systems used in this study.

mobility inside bioreactors. In addition, this matrix has excellent mechanical stability and is nearly non-degradable. The use of activated carbon as a support carrier in immobilisation systems are generally well known in the surface attachment approach; however, the operations conducted were mostly under mesophilic conditions [1,6,7]. There are several reports of attached growth cells that operate under thermophilic conditions [2,8,9]. However, to the extent of our knowledge, the use of immobilised mixed cultures on granular activated carbon (GAC) after operating in repeated batch mode under thermophilic conditions has not yet been reported.

Repeated batch cultivation is a feasible method for improving hydrogen productivity, owing to a reduction in lag phase during fermentation and the reuse of the cell, thus minimising the inoculum preparation [10]. In this study, repeated batch cultivation was applied to grow the hydrogen-producing culture on the surface of a GAC support carrier. The screening of efficient hydrogen-producing bacteria from a mixed culture by repeated batch cultivation has become a suitable strategy to avoid variation in the inoculum, thus maintaining high microorganism growth rates [11,12]. As environmental parameters can exert their influences during the immobilisation process in a repeated batch, it is crucial to study the effect of the pH and substrate concentration at the initial stage of development of the GAC-immobilised cells.

Therefore, the objective of this study was to investigate the effect of pH, substrate concentration and repeated batch cultivation during the immobilisation of mixed microflora onto GAC under thermophilic conditions using a repeated batch technique. The purpose of this study was to provide good fermentation operating conditions as to promote selective microbes to attach on the GAC pore, that to be used for subsequent fermentation in a large-scale bioreactor. The experimental hydrogen production data obtained at various pH values and substrate concentrations were fitted with a modified Gompertz equation to describe the evolution of hydrogen. The optimal parameters obtained from the batch studies were used to enrich the GAC-immobilised cells, and the feasibility of these GAC-immobilised cells were evaluated in a fluidised-bed column reactor (FBCR). Experiments were conducted with continuous operation in sequential batch mode to determine the optimum HRT for maximum hydrogen productivity of the GAC-immobilised cells.

2. Materials and methods

2.1. Source of H_2-producing sludge, carrier support

The mixed microflora for biohydrogen production used in this work was an anaerobic sludge from a previous study and was originally obtained from a sludge pit at a palm oil mill plantation in Pulau Carey, Selangor, Malaysia [13,14]. The sludge was pre-treated by heating at 80 °C for 60 min in a shaking water bath (Model SW22, Julabo, Germany) to inactivate methanogenic bacteria and other non-hydrogen-producing microorganisms prior to batch fermentation.

Granular activated carbon (GAC) was used as carrier to immobilise mixed microflora through surface attachment. The method of the immobilisation onto GAC can be found in our recent publication [14].

2.2. Medium composition

The medium used in batch H_2 fermentation contained 10 g/L (ca. 30 ± 1.5 mM) sucrose as the sole carbon and energy source and supplements, including NH_4Cl (1 g/L), NaCl (2 g/L), $MgCl_2{\cdot}6H_2O$ (0.5 g/L), $CaCl_2{\cdot}2H_2O$ (0.05 g/L), $K_2HPO_4{\cdot}3H_2O$ (1.5 g/L), KH_2PO_4 (0.75 g/L), $NaHCO_3$ (2.6 g/L), cysteine hydrochloride (0.5 g/L), yeast extract (2 g/L), resazurin (0.5 mg/L), trace elements (1 mL/L) (R&M Chemical, UK) [15]. This medium composition was applied for all experiments investigating the effect of initial pH, whereas for experiments investigating the effect of initial sucrose concentration, the sucrose concentration in the medium was set in the range of 13–43 ± 1.5 mM. The optimum initial sucrose concentration obtained from the batch study was used for controlled experiments using a FBCR with same medium.

2.3. Batch hydrogen production with different initial pH values and sucrose concentrations

The effect of different initial pH values (pH 5.0, 5.5, 6.0, 6.5) on hydrogen production was investigated at 60 °C with an initial sucrose concentration of 10 g/L (ca. 30 ± 1.5 mM). The optimal initial pH, which gave the maximum hydrogen production and yield, was further used to study the effect of substrate concentration (13, 23, 33 and 43 ± 1.5 mM) using sucrose. The repeated batch technique was applied to attach the mixed microflora onto GAC as well as to investigate the favourable environmental conditions in the attached system. For that purpose, batch fermentation was carried out with the optimal pH and sucrose concentration and was switched to a repeated batch mode of operation consisting of five successive batches. The experimental procedure is similar to our previous study [14]. However, instead of keeping 10% of spent medium along with GAC-attached cells from previous batch, this study focused on the efficiency of GAC-attached cells alone. Therefore, entire broth was evacuated after five successive batches and GAC-attached cells were refreshed with new media to obtain the kinetic profile of initial pH and initial sucrose concentration.

2.3.1. Determination of optimal pH

To study the effect of the initial pH, the experiment was conducted in a 50 mL serum bottle with a working volume of 25 mL with 10% (2.5 mL) heat-treated POME sludge in the medium. The cells were hosted by adding GAC at a ratio of 1:1 heat-treated POME sludge (mL) to GAC weight (g) in the serum bottle. The effect of the initial pH was studied between pH 5.0 and 6.5 with 0.5 increments using 1 M HCl or 1 M NaOH. The serum bottles were purged with nitrogen gas to create anaerobic conditions, capped with butyl rubber stopper and clamped with an aluminium cap. The serum bottles were incubated at 60 °C in a shaking water bath (Model