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Continuous thermophilic biohydrogen production in packed bed reactor

Shantonu Roy, M. Vishnuvardhan, Debabrata Das *

Department of Biotechnology, Indian Institute of Technology, Kharagpur 721302, India

HIGHLIGHTS

- Continuous H_2 production in whole cell immobilized system was compared with CSTR.
- Suitability of environment friendly support matrix for immobilization of whole cells was explored.
- Pack bed reactor showed higher stability as compared to CSTR at lower HRTs.
- Flow cytometry study showed the influence of recycle ratio on viability of cells.
- Novel approach to find out the effect of NADH/NAD^+ ratio during H_2 production.

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ABSTRACT

The present research work deals with the performance of packed bed reactor for continuous H_2 production using cane molasses as a carbon source. Maximum H_2 production rate of 1.7 L L^{-1} h^{-1} was observed at a dilution rate and recycle ratio of 0.8 h^{-1} and 0.6, respectively which was corresponding to the lowest NADH/NAD^+ ratio. This suggests that the utilization of NADH pool for H_2 and metabolite production might lead to decrement in NADH/NAD^+ ratio. Thus NADH/NAD^+ ratio show inverse relation with hydrogen production. The substrate degradation kinetics was investigated as a function of flow rate considering the external film diffusion model. At a flow rate of 245 mL h^{-1}, the contribution of external film mass transfer coefficient and first order substrate degradation constant were 55.4% and 44.6% respectively. Recycle ratio of 0.6 improved the hydrogen production rates by 9%. The viable cell count was directly proportional to the recycle ratio (within the range 0.1–0.6). Taguchi design showed the significant influence of the feed pH on continuous H_2 production followed by dilution rate and recycle ratio. Thus environmentally friendly and cheaper solid matrix like coconut coir could be efficiently used for thermophilic continuous hydrogen production.

1. Introduction

The demand of energy has increased exponentially due to rapid industrialization and globalization. Non-renewable energy sources like coal, petroleum oil and natural gas provides the major part of the world's energy demand. With the current global energy consumption pattern, the world energy consumption is expected to increase by 50% within 2030 [1]. Hydrogen is considered as a fuel for the "future" because it has the highest energy density of 3042 cals m^{-3} (considering water as a product) [2]. Biological hydrogen production is more promising as compared to other conventional hydrogen production processes due to two reasons: it can use a variety of organic feedstock and the process is less energy intensive [3]. Hydrogen production via biological routes includes photosynthetic hydrogen production, photofermentation, dark fermentation and microbial electrolysis cells [4].

Hydrogen production via thermophilic dark fermentation has favorable kinetics and stoichiometry of H_2 production. Moreover, dark fermentation at higher temperature reduces the risk of methanogenic and pathogenic contamination associated with different organic wastes. Thermophiles also have greater ability to degrade complex and broad variety of substrates and produce fewer types of undesired end metabolites as compared to mesophiles [5]. Hot industrial effluents with high chemical oxygen demand (COD) such as distillery industry and food processing wastewater, are often discharged directly to the environment [6]. This wastewater may be used as substrate for the thermophilic biohydrogen production. Hydrogen production from organic substrates are carried out in continuously stirred tank reactor (CSTR or chemostat) under anaerobic condition [7]. The rate of hydrogen production in chemostat is higher as compared to batch process. Higher dilution rate (short

* Corresponding author. Tel.: +91 9434717424; fax: +91 3222 255303.
E-mail address: ddas.iitkgp@gmail.com (D. Das).

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Nomenclatures

c_s	outlet substrate concentration, g L^{-1}	a_m	surface area per unit mass of dried cell, cm^2 g^{-1}
c_0	inlet substrate concentration, g L^{-1}	K	intrinsic first order substrate degradation constant, h^{-1}
q_p	specific product formation rate, mL g^{-1} h^{-1}	G	mass flux of substrate, g cm^{-2} h^{-1}
H	height of the packed bed, cm	A	surface area, cm^2
Q	volumetric flow rate, mL h^{-1}	ND	normalized deviation
K_p	observed first order substrate degradation constant, h^{-1}	ρ	density of the liquid, g cm^{-3}
K_1	mass transfer coefficient, h^{-1}		

HRT) leads to washout of active cells from the reactor. So, cell recycling is required to maintain the uniformity of the active cell concentration in the reactor for the maximization of hydrogen production [8]. Recycle ratio also influences the mass transfer rates from the bulk liquid to the immobilized microorganisms. The liquid-to-gas mass transfer for poorly soluble gases such as hydrogen and carbon dioxide is also influenced with variation in recycle ratio [9].

The metabolites of the hydrogen fermentation process such as VFAs (volatile fatty acids) are also recycled back to the reactor. This might affect the physiological state of the microorganisms in the reactor. Little information is available on the physiological state of the microorganisms in the reactor during the hydrogen production process. Fluorescent dyes combined with flow cytometry are used to find out the physiological state of microorganisms at a particular substrate concentration [10]. These studies indicate that the quantification of the total bacterial population (both viable and nonviable cells) by using flow cytometry is a rapid and accurate estimation technique.

Hydrogen production by using immobilized cell has several advantages as compared to suspended cells [11]. Cell wash out problem in suspended cells reactor is overcome by using immobilized whole cells [12]. Increase in hydrogen production by immobilized whole cell reactor is due to higher substrate conversion efficiency and increase in mean cell residence time [13]. The majority of whole cell immobilization techniques are based on adsorption or entrapment phenomenon. The demerits associated with gel entrapment of cell are degradation of the gel matrix on prolonged operation and limitations of nutrient and metabolite mass transfer. On the contrary, natural adsorption of cells on matrix is a simple and inexpensive technique. Such technique illustrates minimum internal mass transfer resistance and relatively cheaper to implement [14]. Immobilized whole cell systems are considered for hydrogen production in granular reactors, fixed-bed reactors, fluidized bed reactors and up-flow anaerobic sludge blanket reactors (UASB) [15]. There are some limitations of using UASB reactors such as low stability of hydrogen forming granules and mass transfer resistance.

Currently, a very few reports are available on the improvement of continuous hydrogen production processes in packed bed reactor system under thermophilic conditions [16]. Hydrogen production in packed bed reactor is significantly influenced by influent pH. During continuous hydrogen production, pH inside the reactor ranges from 4.8 to 5.5. It is very difficult to maintain the uniformity of pH in the immobilized whole cell reactor due to improper mixing. Feed with higher pH can negate the pH drop inside the reactor to some extend [13,17]. Increase in recycle ratio improves hydrogen production as it reduces the mass transfer resistance [18]. Study of continuous hydrogen production in packed bed reactor using thermophilic isolate (*Thermoanaerobacterium thermosaccharolyticum* IIT BT ST1) was reported in our previous study [13] where high rate of hydrogen production was observed at low HRTs.

In the present study, continuous hydrogen production was performed using immobilized whole cell (thermophilic mixed culture) bioreactor with coconut coir as solid matrix. A kinetic model was proposed to study the effect of different flow rates on mass transfer and substrate utilization and the results were validated with experimental data. Effect of recycle ratio on hydrogen production was also evaluated. Flow cytometry was used to study the effect of recycle ratio on physiological state of suspended cell concentration inside the reactor. Taguchi design was employed to study the influence of feed pH, dilution rate and recycle ratio on continuous H_2 production in packed bed reactor. To the best of our knowledge, this is the first report showing changes in reducing equivalent content ($NADH/NAD^+$) and viable cell count during continuous hydrogen production in packed bed reactor.

2. Materials and methods

2.1. Continuous hydrogen production by packed bed reactor

An enriched thermophilic mixed culture dominated by *Thermoanaerobacterium* sp. was used in this study [19]. The media used in the present study comprises of cane molasses (1.5% w/v) as carbon source along with Na_2HPO_4 (4.2 g L^{-1}), KH_2PO_4 (1.5 g L^{-1}), NH_4Cl (1.95 g L^{-1}), $MgCl_2$ (0.18 g L^{-1}), yeast extract (2.0 g L^{-1}), cysteine HCl (1 g L^{-1}), vitamins solution (DSMZ medium No. 141, German Collection of Microorganisms and Cell Cultures) and final pH was adjusted to 6.5.

A cylindrical glass column reactor packed with coir having working volume of 350 mL and over head space of 150 mL was used for continuous hydrogen production [13]. A quasi-steady state was observed at each dilution rate with respect to steady state values of H_2 evolution rate, glucose and cell mass concentration in the effluent. The experiments were repeated at different flow rates to get maximum hydrogen production and sugar utilization. The reactor was purged initially with sterilized N_2 to create anaerobic conditions. The temperature of the reactor was maintained at 60 °C and HRT (hydraulic retention time) was decreased from 10 h to 1 h. Recycle ratio was varied from 0.4 to 1.0 by using peristaltic pumps.

2.2. Study of effect of recycle ratio by using flow cytometry

Flow cytometry based studies have provided quantitative estimation of individual cell phenotypes. This technology has been applied in our present work to estimate the live and dead cell percentages after recycling of effluents into the reactor at different ratios. Briefly, cells collected from different recycle ratios were stained with two fluorescent dyes, CYTO C for live cell membrane staining while PI stained dead cells. 10,000 events were analyzed in the FACS cytometry and the percentage of live and dead cells was calculated by drawing quadrants and using the following equations [20].

$$\%\,\text{dead cells} = [\text{Quadrant 2 events}/(\text{Quadrant 2} + \text{Quadrant 4 events})] \times 100 \quad (1)$$