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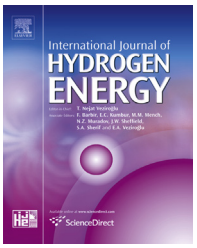

Investigation of the effects of initial substrate and biomass concentrations and light intensity on photofermentative hydrogen gas production by Response Surface Methodology

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ABSTRACT

Biohydrogen, which can be produced by dark fermentation and photofermentation processes, is a renewable and clean approach for hydrogen production. In this study, it was aimed to determine the operational conditions which satisfy the highest photofermentative hydrogen production rate in batch reactors. To that purpose, the effects of initial substrate concentration, initial volatile suspended solids (VSS) concentration and light intensity on photofermentation process, and their interactive effects were investigated by using Response Surface Methodology (RSM). The photofermentative process was followed by using pure strain of purple non-sulfur (PNS) bacteria: *Rhodobacter capsulatus* DSM 1710. RSM results revealed that the highest hydrogen production rate of 1.04 mmol/$L_{reactor}$·h can be obtained when acetate concentration, initial *R. capsulatus* concentration and the light intensity values were 35.35 mM, 0.27 g VSS/L and 263.6 W/m^2 (3955 lux), respectively. Optimum Substrate/Initial biomass concentration ratio (S/X_o) was found as 7.7 g acetate/g VSS (8.3 g Chemical Oxygen Demand/g VSS).

Introduction

Energy is an essential requirement and a key element for the development of the world. Due to the increase in energy demand and depletion of fossil fuels, the terms of alternative and renewable energies become more of an issue for sustainable development in future. As well as energy crisis, global warming and environmental policies to reduce CO_2 emissions stimulate to find new clean energy resources [1]. The winds, tides, waves and solar radiation are among the energy sources which generate renewable, environmental friendly and, therefore, sustainable solutions over the long term. Waste and biomass are also viewed as sustainable energy sources. By way of waste-to-energy technologies, it can be possible to convert waste materials to useful energy forms like hydrogen (biohydrogen), biogas, bioalcohol, etc. [2].

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Because it is replaceable with fossil fuels, hydrogen fuel gains more importance in recent times. Hydrogen is a potential non-carbon energy source which does not contribute to harmful oxides of carbon, nitrogen, sulfur, etc. like fossil fuels. Thus, hydrogen is considered as an alternative clean and green fuel source. Because of carbon free and environmental friendly quality, hydrogen is thought as a future fuel and energy carrier, and it is expected to play an essential role in future energy systems [3].

Hydrogen can be produced from fossil fuels by way of gasification of coal, and steam reforming of natural gas. Hydrogen can also be produced by electrolysis of water and via biological means [4]. The biological hydrogen production can be procured by biophotolysis, dark fermentation and photofermentation [5]. Among all the technologies employed, the biological hydrogen production via dark fermentation and photofermentation, which are achieved by different bacteria, offer an opportunity to utilize renewable resources. Photofermentative hydrogen production can be obtained from renewable resources such as sunlight, water and biomass. Small organic acids are decomposed to hydrogen and water by photosynthetic bacteria such as purple non-sulfur (PNS) bacteria in photofermentative hydrogen production [6]. Compared to biophotolysis, photofermentation is advantageous for favoring large scale production and due to its capability to use wide variety of substrates both for growth and biohydrogen production [7]. Photofermentative H_2 production is also superior to dark fermentative H_2 production in terms of high hydrogen yield values [8]. Hallenbeck [9] states that, despite of the high substrate conversion yields, there are some drawbacks of photofermentation such as low volumetric hydrogen production rate, which prevents practical application. On the other hand, dark fermentation is superior to photofermentative H_2 production in terms of H_2 production rates [8]. Therefore, studies which combine and alleviate the dominant/significant properties of photofermentation and dark fermentation are promising to improve the total energy production in a sequential dark- and photo-fermentation system. This study, therefore, focuses on improvement of photofermentative H_2 production rate as an attempt to develop the second-stage of sequential systems.

Photofermentation is affected by some environmental and nutritional factors such as carbon and nitrogen sources, the carbon to nitrogen ratio, temperature, pH levels and light intensity [10]. Temperature and light intensity are two of these factors which strongly affect the hydrogen production by PNS bacteria [11]. Optimal temperature for PNS bacteria to grow and produce hydrogen is between 30 and 35 °C [12]. Moreover, optimum temperature for nitrogenase enzyme, which catalyzes the hydrogen production, is reported as 30 °C [13,14]. Furthermore, the rate of hydrogen production increases with increasing light intensity and reach saturation at 270 W/m^2 [15].

As seen from literature, photofermentative H_2 studies investigate the effects of one parameter or separate effects of more than one parameter on photofermentative H_2 production [10,15,16]. Yet, the optimum value found for one parameter may not necessarily result in the highest H_2 production, when there are other potential parameters affecting the influence of this predetermined parameter. For example, light intensity and cell concentration are two important parameters influencing the feasibility of the H_2 production by PNS bacteria [13]. However, cell concentration might affect the distribution of the light intensity in the reactor, and, in turn, the H_2 production. Therefore, when there is more than one parameter or factor affecting the production rate or yield, their potential interactions should be also taken into consideration. One way of researching this combined (interactive) effect can be achieved via experimental design methods such as Response Surface Methodology (RSM). To our knowledge, the studies investigating this combined effect of parameters on photofermentative H_2 production are very limited [11,17]. In addition, these studies only focus on two parameters. Therefore, the objective of this study was to investigate the individual and combined (interactive) effects of three important factors, i.e. the initial substrate and biomass concentrations and the light intensity on photofermentative H_2 production by RSM. Initial substrate concentration (i.e. acetate) might affect the biomass growth, which might further affect the distribution of light intensity; thus, eventually the H_2 production. This study is, therefore, of significance for investigating the combined effect of these three important parameters, and, in turn, the operational conditions leading to the highest hydrogen production rate. It was also aimed in this study to determine the optimum initial substrate concentration to initial biomass (i.e. volatile suspended solids, VSS) concentration ratio (S/X_o) resulting in the highest photofermentative H_2 production. S/X_o value is an important design parameter in many biological systems [18], yet has not been investigated so far in photofermentative H_2 production studies. Determination of optimum S/X_o ratios to decrease the productions of both biomass and bacteria-mediated inert chemical oxygen demand (COD) is necessary [19], which might affect the distribution of light intensity and, in turn, H_2 production. This study is the first to determine the optimum S/X_o value resulting in the highest hydrogen production.

Materials and methods

Bacteria and culture media

Rhodobacter capsulatus DSM 1710 (obtained from Deutsche Sammlung von Mikroorganismen, DSM, Braunschweig, Germany) was grown in a modified media of Biebl and Pfennig by photoheterotrophically [20]. The inoculum growth media was composed of 20 mM acetic acid and 10 mM sodium glutamate as carbon and nitrogen sources, respectively. In order to use as hydrogen production media, different concentrations of acetic acid and sodium glutamate were added to basal media according to the experimental design of RSM. Hydrogen production media consisted of either 20 mM, 40 mM or 60 mM acetic acid concentrations. Sodium glutamate concentrations were determined as 1.33 mM, 2.67 mM and 4 mM for the corresponding 20 mM, 40 mM and 60 mM acetic acid concentrations, respectively (resulting in a C/N ratio of 15). The pH of both media was adjusted to 6.4, so that after autoclaving and further inoculation, the pH in the reactor could be kept at a tolerable range of 6.8–7.4 during hydrogen production by PNS bacteria [21]. Both media were sterilized by an autoclave.

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