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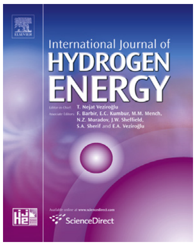

Biohydrogen production from de-oiled rice bran as sustainable feedstock in fermentative process

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ABSTRACT

This research aimed to study the fermentative production of hydrogen using de-oiled rice bran (DRB) as renewable biomass. DRB was obtained by the extraction of oil content of rice bran samples. DRB was then hydrolyzed by dilute H_2SO_4 (1% v/v) to obtain DRB hydrolyzate as substrate for hydrogen generation. The composition of DRB hydrolyzate showed that glucose and xylose formed the main fermentable sugars released from acid hydrolysis of DRB. Hydrogen production was performed by the cultivation of new strain *Clostridium acetobutylicum* YM1 on DRB hydrolyzate in an anaerobic fermentation process. The effect of pivotal operating variables (incubation temperature, initial pH of culture medium and microbial inoculum amount) on hydrogen production was studied using response surface methodology. A second-order polynomial regression model was generated to evaluate hydrogen generation trend under conditions tested. The model analysis revealed the high significance of linear effects of incubation temperature ($P < 0.01$) and inoculum amount ($P < 0.05$) on hydrogen generation. The process model also showed that the quadratic effects of the incubation temperature and pH value were significant at 99% probability level and 95% probability level, respectively. Similar results indicated that the interaction effect between incubation temperature and inoculum amount was highly significant ($P < 0.01$). The regression model suggested that the optimum conditions were an incubation temperature, pH value and inoculum amount of 35.2 °C, 5.5 and 11.6% (v/v), respectively. In order to validate the optimum conditions determined by the model, *Clostridium acetobutylicum* YM1 was cultivated on DRB

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hydrolyzates under optimum conditions determined. Verification experiment showed that a cumulative hydrogen volume of 572.5 ml and a hydrogen yield of 132.2 ml H_2/g total sugars consumed were generated.

Introduction

Fossil fuels have been known as the main source of energy over the last century so that they have contributed to 80.9% of the total energy produced [1]. However, the huge utilization of fossil fuel resources has made detrimental effects on the environment during the last decades, owing to elevated global warming, rising greenhouse gas emission and high air pollution [2,3]. Moreover, increasing concerns about the depletion of fossil fuel sources and abrupt changes in the price of oil have led to a growing trend in the utilization of the renewable and environment-friendly energy sources [4–6]. In this view, hydrogen has been known as a clean alternative to fossil fuels, since it produces only H_2O when it is burned [7,8]. It is noteworthy that hydrogen potentially includes a high content of energy with an approximate value of 112–142 kJ/g, which is 2.75-fold higher than that from fossil fuels [9–12]. Furthermore, hydrogen has high gravimetric energy density compared to any other fuel known with the good compatibility with energy transformation in electrochemical and combustion processes. Hence, hydrogen utilization results in the decrease of releasing carbon-based gasses as the important air pollutants [13].

Sustainable production of hydrogen requires renewable energy resources, which meet increasing demands for energy utilization, environmentally safe technology and low-cost processing. In this regard, biomass has been found as a tremendous feedstock for the production of hydrogen energy [14]. Lignocellulosic biomass is the most abundant feedstock which serves as a low-cost substrate for the sustainable production of hydrogen [8,15]. Energy crops and agricultural residues are known as the main sources of lignocellulosic biomass that can be applied in the clean energy production [16].

Rice is an agricultural product, which makes up the main food for people in many countries, particularly Asian countries such as China, India, Indonesia, Bangladesh, Vietnam, and Thailand. Rice is mainly processed as milled rice with the global production of 480 million tons per annum [17,18]. Paddy rice is an end product of rice harvesting. Paddy rice is composed of husk as an outer layer, rice bran in the middle and white rice at the center of paddy rice [17]. Rice bran contains oil content, which is further processed for the extraction of oil. Rice bran oil is abundantly produced as edible oil in the rice industry [19]. The solid residue left after the extraction of oil from rice bran is known as de-oiled rice bran (DRB). DRB is a lignocellulosic waste, which contains 9% protein, 39% cellulose, 28% hemicellulose and 24% lignin [20]. By the consideration of large production of DRB per annum in the world, DRB represents the low-cost agricultural biomass, which can be considered as a potential source for the production of renewable energy. In the frame of sustainable production of energy, the selection of economically viable feedstocks leads to decrease hydrogen production costs. In this regard, a variety of agricultural biomass has already been used for hydrogen generation such as corn stalk, de-oiled Jatropha, banyan leaves, wheat straw and maize leaves [15,21–24]. However, very little work has been performed to utilize DRB in the production of hydrogen.

At the present time, hydrogen is largely produced by the conversion of fossil fuels to hydrogen, which is resulted to the huge emission of greenhouse gasses [25]. In order to attain sustainable production of hydrogen, the development of cost-effective methods is essential in which renewable resources are used as raw materials. In this context, the production of hydrogen by biological processes has been found as an environmentally friendly approach to clean generation of hydrogen with the lessened energy-intensive operation [2,26]. The different methods have already been used in the biological generation of hydrogen including direct and indirect water biophotolysis, photofermentation, and dark fermentation [27,28]. Among the methods used, fermentative processes are known as the main biological approach for hydrogen production [29].

Dark fermentation is a fermentative process in which heterotrophic microorganisms grow on carbohydrate-based substances to evolve hydrogen gas in anaerobic conditions under the absence of light [25]. Dark fermentation offers superior advantages including the low required time for hydrogen evolution by the fast growth of microorganisms in the bioreactor, lessened energy supply for the system control of hydrogen generation, utilization of low-cost waste with the adverse impacts on the environment, simplicity in the practical experiments and low environmental prerequisite demands [12,27]. The dark fermentation could be run at various temperatures including mesophilic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65–80 °C) and hyperthermophilic (>80 °C) which is depending on the type of microorganism applied [13]. In this view, a variety of bacterial strains have been used in hydrogen evolution such as bacterial genus of *Enterobacter*, *Bacillus*, *Citrobacter* and *Clostridium* [30]. On the other hand, it is well known that hydrogen production in the dark fermentation is affected by the operating process parameters such as incubation temperature, pH value of the culture and microbial inoculums. It is, therefore, imperative to optimize the production conditions for attaining the highest hydrogen evolution [31–33].

Response surface methodology (RSM) is one of the most useful statistical approaches, which is applied for the evaluation of the effects of process parameters on the responses. RSM is also one of the useful methods for the optimization of

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