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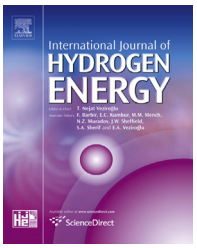

Single stage hydrogen production from cellulose through photo-fermentation by a co-culture of *Cellulomonas fimi* and *Rhodopseudomonas palustris*

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ABSTRACT

Biohydrogen production from cellulose by a bacterial co-culture is a potentially promising approach for producing bioenergy from a low cost substrate. The use of a cellulolytic bacterium, *Cellulomonas fimi*, permits cellulose conversion and the in situ production of substrate for growth and hydrogen production by the photosynthetic bacterium *Rhodopseudomonas palustris*. Response surface methodology (RSM) with a Box-Behnken design (BBD) was used to examine variations in the key parameters: substrate (cellulose) concentration, yeast extract concentration and the microorganism ratio (*Rps. palustris*/*C. fimi*). For the co-culture of *R. palustris* and *C. fimi* the highest hydrogen production (44 mmol H_2/L) was achieved at the highest substrate concentration (5 g/L); however, the highest hydrogen yield (3.84 mol H_2/mol glucose equivalent) was observed at the lowest cellulose concentration and highest microorganism ratio. High COD removal efficiencies, over 70%, were achieved over a wide range of conditions and were positively affected by the concentration of yeast extract.

Introduction

Energy scarcity and concerns about environmental degradation are driving research into sustainable energy generation. A wide variety of possible waste streams are potentially in use in terms of bioenergy production [1] and waste disposal from wastewater [2,3] using pure or co-culture of phototrophic organisms under anaerobic conditions. Biological hydrogen production from cheap and abundant materials, such as cellulosic wastes, is one approach of interest which couples bioenergy production and waste disposal [4,5]. Photo-fermentation is a light driven process performed by diverse genera of purple non-sulfur photosynthetic bacteria,

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e.g. *Rhodobacter* and *Rhodopseudomonas*, which are able to consume organic acids [6], sugars [7,8] and a variety of agricultural and industrial wastes [1,9–11] for biological hydrogen production.

Although a great deal of research in this area has already been carried out, the process is not as yet practical. The economic viability of such a process could be increased by the use of low cost substrates as lignocellulosic feedstocks, which are abundant and available worldwide [4]. However, the challenge for using this type of substrate for biological hydrogen production is the achievement of reasonable yields of soluble substrates, which can be converted to hydrogen. Acid hydrolysis or enzymatic pre-treatment of the raw material have been used to breakdown cellulose [12]. However, these present several disadvantages including the production of inhibitory compounds, such as furfural, during acid hydrolysis, or the requirement of high enzyme loads, with significant costs, to achieve significant yields of cellulose conversion [12].

Therefore, the possibility of using cellulolytic bacteria, such as *Cellulomonas fimi* and *Clostridium thermocellum*, is an interesting alternative for cellulose conversion into easily fermentable sugars to be further consumed by hydrogen-producing bacteria [12–16]. Furthermore, the co-culture of microorganisms, which breaks down cellulose with one and converts the newly created substrate to hydrogen with another, allows for the in situ production of substrate for synergistic hydrogen production. Thus, this process employs complementary strains where the capability of one supports the activity of the other [17–19]. On the other hand, the challenges in using co-culture of two strains, being different metabolic types, often have quite different media requirements. Therefore, there is a need for careful evaluation of culture conditions to find a composition that is suitable for both microorganisms, cellulolytic and hydrogen-producing bacteria, at the same time.

Recently, hydrogen production from cellulose has received more attention and higher hydrogen yields has been reported by co-cultures of cellulolytic and dark fermentative hydrogen-producing microorganisms [13,16,18]. However, because of the incomplete oxidation of sugars during dark fermentation, reduced compounds, such as organic acids, are generated, producing a dark fermentative effluent (DFE) which would require additional treatment before discharge. Therefore, co-cultures of cellulolytic and photosynthetic bacteria can be an interesting approach for producing hydrogen from cellulosic carbon sources, one potentially allowing simultaneous hydrogen production and COD removal [21]. An increase in hydrogen production from sugarcane bagasse was observed with a system which incorporated cellulolytic, dark fermentative and photosynthetic bacterial strains [12]. Here *C. fimi* releases sugar for dark fermentation by *Enterobacter aerogenes*, which produces hydrogen and organic acids. The organic acids are used by *Rhodopseudomonas* for additional hydrogen production.

The diversity of cellulolytic and hydrogen-producing strains are available suggests that they could be successfully used to convert cheap and abundant material, such as cellulose, into biohydrogen, thus addressing concerns about environmental degradation and fossil fuels scarcity. However, the use of co-cultures necessitates the valuation of optimal culture conditions. Several operational parameters are crucial for maximal production of hydrogen from both dark and photo-fermentative bacteria. These include; pH, temperature, and carbon and nitrogen source, among others [22,23]. The type of substrate and its concentration have been evaluated extensively for both dark and photo-fermentation [11,14,24]. However, since the ranges assessed vary largely, there is little consensus about optimum substrate concentration with an apparent divergence in the optimum ranges for total H_2 production and maximum yields [15].

Besides substrate type and its concentration, nitrogen source also plays an important role in hydrogen production efficiency. Although NH_4^+ has been commonly used in dark fermentation, it inhibits nitrogenase activity and, thus, should not be used as a nitrogen source for photo-fermentation [22]. Glutamate is often used in photo-fermentation studies. In one report, yeast extract has been reported to stimulate growth and enhance hydrogen photo-production [25]. On the other hand, it has also been reported that its addition (0.01–0.04%) was without a positive effect or, at higher concentrations (0.1%), actually caused a slight suppression of hydrogen production by a co-culture of *Cellulomonas* and *Rhodopseudomonas* [26]. The negative effect of yeast extract on hydrogen production by photosynthetic bacteria is probably related to suppression of nitrogenase activity [27]. Nevertheless, since the addition of yeast extract (YE) had a significant positive effect on cellulose degradation and hydrogen production in dark fermentation [13,14], its addition should be better evaluated in a system aiming to produce hydrogen from cellulose with co-cultures of cellulolytic and photosynthetic bacteria. Furthermore, since it has also been reported that hydrogen yields for both dark and photo-fermentation are affected by the carbon to nitrogen ratio, with the optimal ratio varying among different microorganisms [21,28,29], it is also worthwhile to assess the effect of the carbon to nitrogen ratio on hydrogen production by co-cultures.

In addition, it would seem desirable to evaluate the ratio of cellulolytic and hydrogen-producing microorganisms used in a co-culture to produce hydrogen from cellulose in order to avoid limited availability of substrate for hydrogen production [13]. Thus, some previous research noted that an increase in the ratio of hydrogen-producing bacteria to cellulolytic bacteria had very little effect on hydrogen formation, probably due to substrate limitation [13,26].

Thus, the main aim of the present study was to evaluate single-stage hydrogen production from glucose [7,30] by co-cultures of *C. fimi* and *Rhodopseudomonas palustris*, a co-culture system which could allow the direct use of cellulolytic substrates, without previous pre-treatment, along with potentially increasing the overall hydrogen yield and COD removal. The effect of key parameters, cellulose and yeast extract concentration, and microorganism ratio, were evaluated by using experimental design methods based on statistical modeling.

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