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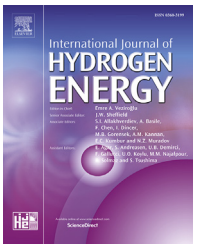

Photofermentative hydrogen production from molasses: Scale-up and outdoor operation at low carbon-to-nitrogen ratio

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ABSTRACT

Photofermentative hydrogen production was carried out under outdoor conditions with a *Rhodobacter capsulatus* strain on molasses, a renewable and sustainable feedstock. An existing photobioreactor design was scaled-up from 9 L to 20 L. The decreased carbon-to-nitrogen (C/N) ratio of 13.0, compared to our previous work, accelerated growth and resulted in a reduced lag period for hydrogen production as well as higher productivities in the exponential phase. However, the low C/N ratio also promoted a high optical density due to growth, limiting light transmission. Still, the maximum productivity was found as 0.47 mol $H_2/(m^3 \cdot h)$, significantly higher than our result with the smaller reactor volume. High rates of production could not be maintained presumably due to the combined effects of cloudy periods, the aforementioned C/N ratio and decreasing pH. These results suggest that the scale-up was successful and there is potential for further improvement using optimal C/N ratio and cell concentration values.

Introduction

Hydrogen is an energy carrier with the highest energy content per mass (142 kJ/g) of any fuel and a promising alternative to fossil fuels. Biological hydrogen production is an environmentally-friendly way of hydrogen production, since it involves mild operating conditions, allows the utilization of renewable feedstock, and results in biodegradable waste. Biohydrogen production methods can be classified into biophotolysis, photofermentation and dark fermentation [1–4]. Among these routes, photofermentation has the added benefit of utilizing the sun as another renewable source, and the metabolic versatility of photofermentative organisms allows the use of complex nutrient media [5,6].

Temperature, pH, substrate type and concentration, C/N ratio and, the intensity and distribution of light in the culture are critical parameters in photofermentative hydrogen production. Optimization of these parameters may lead to higher productivity [7–10]. The optimum temperature was found as 27.5 °C for maximum hydrogen productivity [11]. Growth was observed at a wide range of pH (between 6 and 9), and maximum hydrogen production was achieved at a pH of 7 [12].

Pure substrates such as organic acids and sugars have been utilized in photofermentation studies [9,13,14] but for economically feasible and sustainable operation wastes and side products are preferable. In particular, feedstock based on side-products of sugar factories such as molasses, and thick juice dark fermenter effluent (DFE) have been used in

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photofermentation research [15] and yielded promising results. Molasses and thick juice are mainly composed of sucrose (around 30–60% by weight), while DFE is a mixture of short chain organic acids such as acetate and lactate. Compared to DFE, the direct utilization of molasses eliminates the complexity of the two-stage operation of dark fermentation followed by photofermentation [16–18], and due to the higher hydrogen content of sucrose compared to organic acids, allows a higher theoretical hydrogen yield.

Strains of the purple non-sulfur (PNS) species are known to produce hydrogen [18]. Among these species, *Rhodobacter capsulatus* YO3 (*hup*-) was found to result in the most stable and robust operation outdoors in our previous trials. A common trend observed in all species for production with simple and complex sugars is the continuous decrease in pH during photofermentation [13,16–19] due to the secretion of organic acids, which appears to be intimately linked to the overall photofermentative metabolism [20]. For this reason, the use of buffer solutions and a slightly basic initial pH to offset the later decrease have been recommended and it has been suggested that the sucrose concentration in feed be kept at our below 5 mM, as higher concentrations resulted in rapid acidification [19].

The primary catalyst for photofermentative hydrogen production is nitrogenase and therefore factors that regulate the activity of this enzyme have a strong influence on hydrogen production [21]. Oxygen irreversibly deactivates this enzyme and ammonium inhibits nitrogenase activity when present in the culture medium [22,23]. Non-ammonium nitrogen sources can lead to indirect ammonium inhibition; for instance in *R. capsulatus,* ammonia forms when lactate is exhausted earlier than glutamate, leading to inhibition of nitrogenase [3,24,25].

It is now well-known that due to the complex interaction of nitrogen sources with nitrogenase, the C/N ratio is critical for hydrogen production [7,25–28]. On the one hand, nitrogen promotes growth, thereby allowing the PNS bacteria to form hydrogen-producing populations earlier. On the other hand, elevated levels of nitrogen (i.e. low C/N ratios) are antagonistic when sustained hydrogen production is the primary goal, due to suppression of nitrogenase responsible for the bulk of hydrogen production. Due to these competing effects, the recommended values in literature for the C/N ratio span a wide range, between 13 and 35with the optimal value varying with the bacterial species, illumination and nutrient sources [7,13,29]. Studies with *R. sphaeroides* have shown that the maximum productivities and lag times for hydrogen production differ for different substrates with the same C/N ratio. On the other hand, the maximum biomass concentrations were nearly the same, indicating the C/N ratio as a dominant factor affecting both growth and hydrogen production [7,30].

Large-scale studies under natural sunlight are crucial for photofermentative hydrogen production to be economically feasible and sustainable and photobioreactor design is critical in this regard. A good design should provide efficient light distribution and mixing, high illuminated surface per ground area, low gas permeability and effective cooling to maintain optimal temperature. In our previous studies, a stacked U-tube photobioreactor design meeting these criteria was proposed. With this design, the illuminated surface area to the ground area ratio was increased to 5:1 [19] compared to horizontal tubular reactors, for which the same ratio is 1:1 [31]. Thus the required ground area, which may constitute as much as 90% of the cost of photofermentative hydrogen production [32] was decreased substantially.

Light intensity and distribution are other critical parameters for photofermentative hydrogen production [33]. The hydrogen production pattern during long term outdoor operation (75 days) was shown to closely follow the daily variation of light intensity [34]. In controlled indoor studies, hydrogen production was reported to increase with light intensity, and reach saturation at 270 W/m^2 [8]. Light penetration for varying depths and dilutions of molasses was analyzed in our previous study, and the radius of the photobioreactor was suggested as 1.5–2 cm accordingly [17].

The aim of the present study was to scale-up and improve the design of the stacked U-tube photobioreactor previously introduced by our group and test the pilot-scale photobioreactor under outdoor conditions with diluted molasses as feedstock. The photobioreactor liquid volume was scaled up from 9 to 20 L by increasing the tube radius from 1.5 to 2 cm and the C/N ratio was decreased from 35 to 13 to reduce the lag period for hydrogen production. To the best of our knowledge this is the largest scale outdoor photofermentative hydrogen production attempt utilizing molasses. The use of the same photobioreactor design, bacterial species and feedstock type enable a meaningful comparison of the results of the present study with the prior study utilizing a 9 L culture volume by Kayahan and coworkers [17].

Materials and methods

Pilot-scale stacked U-tube photobioreactor

The stacked U-tube photobioreactor design, previously introduced by our group [19] was scaled-up from 9 L to 20 L of culture volume. The PBR was built as 4 glass U-tubes vertically connected to each other via 2 manifolds. The inner tube radius is 2 cm, the length of each U-tube 4 m, and the manifold radius is 3 cm. The process flow diagram was illustrated in detail in our previous study [17]. A 30° angle was given to the tubes about their horizontal axes and a slight inclination using blocks (about 10°) from the ground was given to the entire reactor frame to facilitate collection of the produced gas (Fig. 1a). Check valves (1/3 psig) were connected to the manifolds to allow the transfer of the produced gas to the gas collection unit at a fixed pressure. The volume of the gas was measured by the water displacement method at atmospheric pressure. The reactor was cooled by the cooling coils inserted into the manifolds. The glass pieces were fitted together by flange connections (Fig. 1b). The temperature of the liquid culture was maintained below 40 °C with a PID temperature controller by circulating the coolant water at a temperature around 10 °C. The liquid temperature of the bacterial culture was measured by thermocouples inserted into the U-tubes. The recirculation of the liquid culture was achieved by a peristaltic pump.

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