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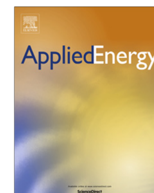

Photofermentive hydrogen production by *Rhodobacter sphaeroides* S10 using mixed organic carbon: Effects of the mixture composition

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HIGHLIGHTS

- H_2 is produced via photofermentation of mixed glucose, xylose and acetic acid.
- Relative amounts of the substrate components affect production kinetics.
- Optimal ratio of the substrate components is identified for H_2 production.
- Most lignocellulosic hydrolysates are made up of the above mixed substrates.

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ABSTRACT

Thermal and acid hydrolysates of oil palm empty fruit bunch and other lignocellulosic substrates contain glucose, xylose, and acetic acid as the main components. In using such hydrolysates for photofermentive production of biohydrogen, the gas yield is highly dependent on the composition of the mixed carbon substrate. Batch photofermentation experiments were used to investigate the effect of the composition of the mixed carbon (glucose G, xylose X, and acetic acid A) on growth and hydrogen production by the bacterium *Rhodobacter sphaeroides* S10. Anaerobic fermentations were carried out at 35 °C under an incident light level of 14.6 W/m^2. The mixed carbon composition strongly influenced hydrogen and biomass production. Depending on the composition of the mixed substrate: the cumulative hydrogen volume ranged from 0.99 to 2.33 L H_2/L medium; the conversion efficiency ranged from 21% to 45%; and the biomass yield on substrate ranged from 0.28 to 0.47 g DCW/g (G + X + A). Based on the conversion efficiency, the optimal substrate for hydrogen production was a mixture of 5 mM glucose, 18 mM xylose and 7 mM acetic acid. This combination gave a cumulative hydrogen volume of 2.33 L H_2/L medium. The hydrogen yield was 3.56 mol H_2/mol mixed substrate and the substrate specific hydrogen production rate was 7.26 mL H_2/g mixed substrate h. The conversion efficiency and the lag period of hydrogen production were 45% and 13 h, respectively.

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1. Introduction

Hydrogen is a potentially renewable and clean fuel that burns to produce only water [1,2]. Of the many processes available for producing hydrogen, biological processes stand out as being environmentally benign and possibly the least energy intensive [3]. Several biological options exist for producing hydrogen [4–6]. Of these, photofermentation by purple non-sulfur bacteria is particularly attractive for potential large-scale applications as it can use a wide variety of waste organic substrates for producing hydrogen with a high substrate conversion efficiency [7–11].

Organic compounds obtained via hydrolysis of widely available lignocellulosic residues [12–14] are promising substrates for inexpensively producing biohydrogen. Although lignocellulosic residues can be fermented directly to hydrogen by some bacteria, the rate of this fermentation is extremely slow as much of the macromolecular substrate is inaccessible to the microorganism [15,16]. A prior hydrolysis of the relatively recalcitrant lignocellulosic substrate to simpler small molecules greatly improves the rate of subsequent production of hydrogen. Oil palm empty fruit bunch (OPEFB) is an abundantly available lignocellulosic residue in certain tropical parts of Asia [14] and can be used to produce biohydrogen

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Nomenclature

Abbreviations
A Acetic acid
CE substrate conversion efficiency (%)
C_P cumulative H_2 volume (L H_2/L medium)
C_S concentration of substrate (mmol (X + G + A)/L medium)
C_X concentration of microbial biomass (g DCW/L medium)
ΔC_P change in cumulative H_2 volume (L H_2/L medium)
ΔC_S change in substrate concentration (mmol (G + X + A)/L medium)
ΔC_X change in biomass concentration (g DCW/L medium)
DCW dry cell weight (g)
DCW_{max} maximum value of dry cell weight (g)
DCW_0 initial value of dry cell weight (g)
G glucose
OPEFB oil palm empty fruit bunch
PHB poly-β-hydroxybutyrate
R_{max} maximum volume specific H_2 production rate (mL H_2/L medium·h)
$r_{s,i}$ carbon (i = glucose, xylose, or acetic acid) consumption rate (mM/h)
SHPR substrate specific hydrogen production rate (mL H_2/g (X + G + A)·h)
t time (h)
X xylose
X_{max} maximum biomass concentration (g DCW/L)
$Y_{P/S}$ hydrogen yield on substrate (mol H_2/mol (X + G + A))
$Y_{X/S}$ Biomass yield on substrate (g DCW/g (X + G + A))

Greek letters
μ specific growth rate (h^{-1})
μ_{max} maximum specific growth rate (h^{-1})
λ lag period of hydrogen production (h)
η_{actual} moles of hydrogen produced (mol H_2)
η_{theory} theoretical number of moles of hydrogen that would be produced from a given quantity of the substrate (mol H_2)

after hydrolysis as previously shown [17,18]. Various methods can be used to hydrolyze OPEFB. OPEFB hydrolysis by heat and acids produces mostly glucose, xylose and acetic acid [17]. The latter is formed via deacetylation of hemicellulose [19]. The production rate and yield of hydrogen from a cellulosic hydrolysate depend strongly on the relative proportions of its three principal components, i.e. glucose, xylose and acetic acid. Depending on the specific scheme of hydrolysis, the concentrations of glucose, xylose and acetic acid in the hydrolysate vary. The hydrolysate of OPEFB fiber may contain roughly between 3.9 and 10 mM glucose; from 39 to 157 mM xylose; and from 8.5 to 41 mM acetic acid [17]. For example, Rahman et al. [20] reported a composition of 196 mM xylose, 13 mM glucose and 21 mM acetic acid.

Although much effort is underway for understanding and engineering of metabolic pathways involved in the production of hydrogen [21–26], the need for suitably formulating a mixed substrate remains for attaining optimal production kinetics. In the presence of multiple sugars such as glucose and xylose, bacteria generally consume glucose preferentially and leave the other substrate in the medium for later use [27–29]. Moreover, the acetic acid in the hydrolysate typically has an inhibitory effect on hydrogen production [8,30]. For example, Kim et al. [8] showed that the bacterium *Rhodobacter sphaeroides* strain KD131 produced less than 1/3 of the hydrogen in an acetate medium compared to in a succinate medium. In some media, the bacterium preferentially produces the energy storage polymer poly-β-hydroxybutyrate (PHB) rather than hydrogen [30]. Thus, suppression of PHB formation improves hydrogen production.

Potentially, the composition of the OPEFB hydrolysate can be modified by selecting the conditions of the hydrolysis, or by supplementing with some of the pure components, so that the production of hydrogen is maximized. The cost of producing hydrogen by using mostly the carbon substrates in the hydrolysate, with the minimal necessary supplementation with the pure components, is likely to be much lower than if pure commercial substrates were exclusively used to formulate the desired mixed substrate. For adjusting the composition, the most appropriate composition of the mixed substrate needs to be identified. This work reports on the effects of the composition of the mixed carbon (glucose, xylose and acetic acid) on growth and hydrogen production by photofermentation with the bacterium *R. sphaeroides* strain S10. The various compositions of an OPEFB hydrolysate were simulated by mixing the pure components of glucose, xylose and acetic acid. The most appropriate composition of the hydrolysate for maximizing the production of hydrogen was thus identified.

2. Materials and methods

2.1. The bacterium, starter culture and media

The photosynthetic bacterium *R. sphaeroides* S10 was used for hydrogen production [17]. This bacterium had previously been shown to grow and produce hydrogen on mixed organic carbon derived from acid–thermal hydrolysis of OPEFB [17,18]. The starter culture was grown in a 175-mL sealed flat-bottle that contained 160-mL of the GM medium. The bottle was incubated at 35 °C for 36 h under light (7.3 W/m^2, 100 W tungsten lamps). The cells were harvested by centrifugation (5590×g, 10 min), washed twice with 0.85% (w/v) NaCl and then resuspended in MO3 medium containing the desired mixture of organic carbon sources, to an optical density of 1.0 at a wavelength of 660 nm. This starter culture was used to inoculate fresh MO3 medium containing an identical mixture of organic carbon sources such that the inoculum constituted 10% of the final volume.

The GM medium used for preparing the starter culture contained the following (g/L) in distilled water: L-glutamic acid 3.8, DL-malic acid 2.7, yeast extract 2.0, ammonium sulfate 0.8, KH_2PO_4 0.5, K_2HPO_4 0.5, $MgSO_4{\cdot}7H_2O$ 0.2, $CaCl_2{\cdot}2H_2O$ 0.053, $MnSO_4{\cdot}5H_2O$ 1.2×10^{-3}, thiamine-HCl 1.0×10^{-3}, nicotinic acid 1.0×10^{-3} and biotin 1.0×10^{-5}. The pH of the medium was adjusted to 6.8 by using 1 M HCl or NaOH solutions. The medium was then sterilized at 121 °C for 15 min and cooled.

The MO3 medium was used for hydrogen production experiments. The medium contained (per L) the following: L-glutamic acid 735.6 mg, mixed carbon (a specified mixture of pure glucose, xylose and acetic acid) 30 mM, KH_2PO_4 0.6 g, K_2HPO_4 0.9 g, yeast extract 0.3 g, $MgSO_4{\cdot}7H_2O$ 2.46 g, $CaCl_2{\cdot}2H_2O$ 75 mg, $Na_2MoO_4{\cdot}2H_2O$ 1.45 mg, $FeSO_4{\cdot}7H_2O$ 11 mg and EDTA 0.02 g [17]. The medium was adjusted to pH 7.0 before autoclaving at 121 °C for 15 min.

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