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Hydrogen Production By *R. Capsulatus* On Dark Fermenter Effluent Of Potato Steam Peel Hydrolysate

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Biohydrogen is a promising energy source since it is clean and renewable. The HYVOLUTION project (EU 6th Framework Programme) is aimed to develop an integrated process in which biomass is utilized for the biohydrogen production in two steps. In the first step biomass is fermented to acetate, lactate, CO_2 and hydrogen by an extreme thermophile (*Caldicellulosiruptor saccharolyticus*). In the second step, acetate and lactate is converted to hydrogen and CO_2 by photofermentation with purple non-sulfur bacteria.
The present work is based on a case study of potato steam peels hydrolysate. The photofermentability of *Rhodobacter capsulatus* was investigated with different adjustments on dark fermenter effluent of potato steam peel hydrolysate. The highest yield (31 mgH_2/gsubstrate.) and productivity (1.13 gH_2/m^3.h) were obtained after buffer, molybdenum and iron addition.
This study showed that PSP hydrolysate as biomass is suitable for the integration of dark fermentation and photofermentation for efficient and sustainable hydrogen production in photobioreactors by phototrophic bacteria.

1. Introduction

There is an increasing demand for hydrogen gas since; unlike fossil fuels it is clean and renewable. Biological hydrogen production is a promising solution since it is environmentally friendly. The HYVOLUTION (EU 6th Framework Programme) is an integrated project which aims to develop a combined process in which biomass is utilized for the production of biohydrogen in two steps. In the first step, a biomass is fermented to acetate, lactate, CO_2 and hydrogen by an extreme thermophile (*Caldicellulosiruptor saccharolyticus*). As biomass, wastes and by-products from the food and agricultural industries are used to reduce high costs of culture media. In the second step, acetate and lactate is converted to hydrogen and CO_2 by photofermentation with purple non-sulfur bacteria. Integrating dark fermentation with photofermentation can significantly increase the hydrogen yield by allowing release of/utilization of complete chemical energy stored in the biomass (Claassen and de Vrije, 2006).
Rhodobacter capsulatus is a gram-negative, purple non-sulfur bacterium, (Imhoff, 1995). It is capable of growing photo-heterotrophically by using light as primary energy

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source and organic compounds as carbon source. Nitrogenase is the key enzyme which fixes nitrogen and catalyzes the production of hydrogen under anaerobic and nitrogen limiting conditions. Oxygen is the main inhibitor and irreversibly destroys the enzyme. High concentrations of ammonium can also inhibit the nitrogenase enzyme by repressesing its expression and inhibition of enzyme activity. The inhibition is reversible and nitrogenase recovers its activity after consumption or removal of ammonium (Koku *et.al*., 2002). In a recent study, *Rhodobacter capsulatus* was used for the phototrophic hydrogen production on dark fermenter effluent of Miscanthus hydrolysate. Some adjustments on effluent such as dilution, buffer and iron addition were found to be necessary for hydrogen production (Uyar *et.al*., 2008).

Potato processing industry produces large amount of products such as French fries and crisps. Potato steam peels (PSP) are a by-product of this industry. The composition of potato steam peels is mainly carbohydrates, protein and organic acids and it seems to be suitable for the two-step biohydrogen production (Claassen *et. al*, 2004). In the present study, dark fermenter effluent (DFE) of potato steam peel hydrolysate was used for the photofermentation of wild type *R. capsulatus* and the effect of buffer, iron, trace element and vitamin addition on hydrogen production performance were investigated.

2. Materials and methods

2.1 Organism and culture

Rhodobacter capsulatus wild type (DSM 1710) used for the photofermentation process, was obtained from Deutsche Sammlung von Mikroorganismen (DSM, Braunsweig Germany).

Two effluents were obtained from Agrotechnology and Food Sciences Group, Wageningen University, Netherlands, after dark fermentation by *Caldicellulosiruptor saccharolyticus* on PSP hydrolysate. The organic acid and ammonium chloride concentrations of effluents were determined by A&F. The DFE of PSP hydrolysate 1 contained 110 mM acetate, 19 mM lactate and 3.1 mM NH_4Cl, and the DFE of PSP hydrolysate 2 contained 102 mM acetate, 28 mM lactate and 4.0 mM NH_4Cl.

All the reactor bottles, trace element solution, iron citrate solution and DFE of PSP hydrolysate were autoclaved. Vitamin solution was filter sterilized. *R.capsulatus,* which was grown on modified BP medium (Biebl and Phennig, 1981), was inoculated (10%) on to 55 mL reactor bottles containing DFE of PSP hydrolysate. The experiments were carried out under sterile conditions.

The reactor bottles were flushed with argon, as the inert gas for obtaining anaerobic atmosphere. They were connected to water filled graduated cylinders by capillary tubes (Uyar *et.al*., 2007). The reactor bottles were illuminated with tungsten lamps, with light intensity of 2500 lux. The experiment was carried out at 30°C in a cooled incubator (Nüve, ES250).

Hydrogen production of *Rhodobacter capsulatus* (DSM1710) wild type, was tested with diluted DFE of PSP hydrolydate 1. In addition to dilution, some other adjustments like individual and mutual additions of buffer, iron, molybdenum and vitamin to DFE of PSP hydrolysate 2 were performed to enhance hydrogen production of wild type *R. capsulatus*. Effluent was adjusted according to composition of the BP medium [22 mM KH_2PO_4 buffer (pH 6.4) 5 mg/l Fe-citrate, trace element solution, 40 µg/l

$NaMo_4.2H_2O$, vitamin solution (0.5 mg/l Thiamin, 0.015 mg/l Biotin, 0.5 mg/l Niacin]. (Biebl and Phennig, 1981).

2.2 Analytical methods

The elemental analysis of DFE of PSP hydrolysate was done in Chemical Engineering Department by an atomic absorption spectrophotometer (Philips, PU9200X), METU, Ankara. Organic acid analysis for acetate and lactate was carried out in Central Laboratory by HPLC (MetaCarb 87H), METU, Ankara. Evolved gas was analyzed by gas chromatography (Agilent Technologies 6890N). The optical densities of bacterial cultures were measured using a spectrophotometer (Shimadzu UV-1201) at 660nm. An optical density of 1.0 at 660nm corresponds to a cell density of 0.55 g dry weight/liter of culture. The pH of the culture medium was measured with a pH-meter (Mettler-Toledo). The light intensity was measured by a luxmeter (Lutron).

3. Results and discussion

In this present study DFE of PSP hydrolysate was used for hydrogen production of *R. capsulatus*. Necessary adjustments were carried out in order to increase the hydrogen yield. Ammonium was used as nitrogen source for dark fermentation. If large amount of ammonium remains in the effluent, it might inhibit nitrogenase activity of *R. capsulatus* and limit hydrogen production during photofermentation (Alexander *et. al*, 1998). For that reason, NH_4 content was reduced by dilution in all treatments of DFE of PSP hydrolysate. Although there was bacterial growth on diluted DFE of PSP hydrolysate 1, pH was fluctuating between 5.5 - 7.0 and no significant hydrogen was produced. These results indicate that further adjustments are necessary to increase hydrogen production. The analysis of DFE of PSP DFE of PSP hydrolysate 2 is compared with BP medium in Table 1. Iron and molybdenum contents were lower than that are in BP medium.

Table 1- Elemental composition of dark fermenter effluent of potato steam peel hydrolysate and BP medium

	BP Medium	DFE of PSP Hydrolysate
Fe (mM)	0.1	0.017
Mg (mM)	2.0	1.43
Ca (mM)	0.3	0.044
Zn (μM)	0.51	7.58
Mn (μM)	0.51	2.3
B (μM)	0.97	6.67
Co (μM)	0.84	0.94
Cu (μM)	0.12	1.06
Ni (μM)	0.084	0.31
Mo (μM)	0.16	0.001

To enhance H_2 production, DFE of PSP hydrolysate 2 was adjusted by the addition of buffer, iron, molybdenum and vitamin, and hydrogen production was tested by wild

type *R. capsulatus*. Max biomass, pH range, yield, productivity, substrate conversion efficiency results are given in Table 2 and acetate and lactate consumptions are given in Figure 1 and Figure 2, respectively.

Table 2- Summary of the results of *R. capsulatus* on adjusted DFE of PSP hydrolysate

Adjustments	***Max Biomass gDW/L***	***pH range***	***Yield $mgH_2/g_{substrate}$***	***Productivity $g/(m^3.h)$***	***Substrate conv Efficiency* (%)***
Buffer	1.20	6.6-7.6	5	0.08	3.6
Buffer + Fe	1.23	6.6-7.4	18	0.57	14
Buffer + Mo	1.26	6.6-7.3	18	0.52	13
Buffer + Fe + Mo	1.21	6.6-7.3	31	1.13	24
Buffer + Fe + Mo+ Vit	1.12	6.6-7.3	28	1.00	22

**Efficency%=(Mole H_2 produced*100)/(Theoretical H_2 produced from initial moles of substrates)*

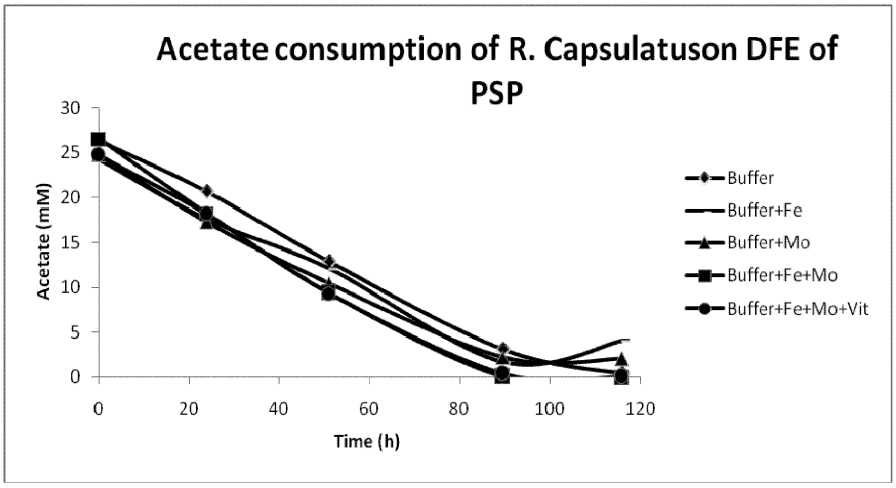

Figure 1- Acetate consumption by R. capsulatus on adjusted DFE of PSP hydrolysate

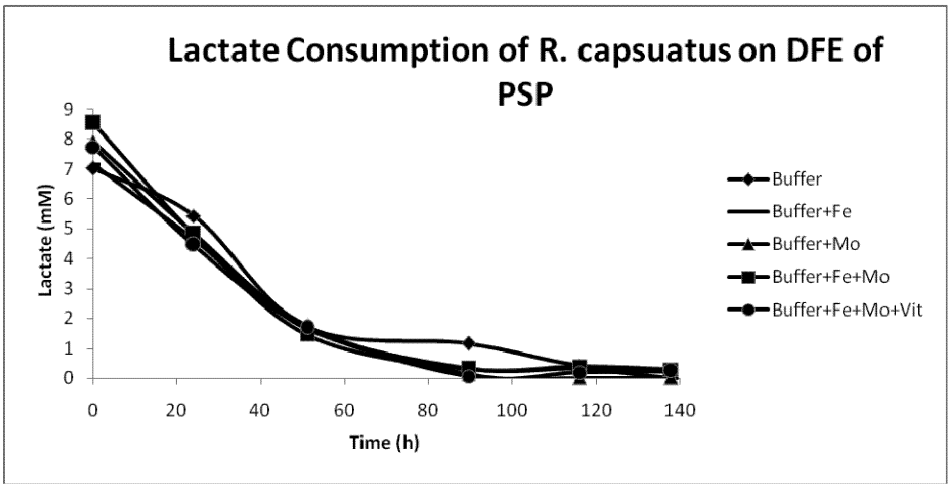

Figure 2- Lactate consumption by R. capsulatus on adjusted DFE of PSP hydrolysate

Buffer addition resulted in a stable pH (6.6-7.5) and doubled the cell dry weight. Fang *et. al.* (2005) has also reported that for an efficient growth and hydrogen production pH should be kept stable during photofermentation. Both Fe and Mo addition improved hydrogen productivity from 0.08 to 0.5 $gH_2/(m^3.h)$ and yield from 5 to 18 mgH_2/g substrate. It should be emphasized that co-addition of Fe and Mo increased the productivity and yield values by 117% and 72%, respectively. Fe and Mo are found in the structure of the nitrogenase enzyme (Kars et al., 2005) therefore their co-existance in the medium enhanced the activity of the nitrogenase enzyme. There was no further increase in hydrogen production of *R. capsulatus* wild type after vitamin solution addition. The presence of vitamins such as biotin, niacin, and thiamin in the culture medium does not enhance hydrogen production directly (Koku *et. al*., 2003).

4. Conclusion

This study showed that DFE of PSP hydrolysate can be utilized for hydrogen production by photofermentation. Adjustments such as addition of buffer, iron and molybdenum are strongly recommended for higher hydrogen productivity and yield.

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