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Effect of Ferric Citrate on Biohydrogen Production from Syngas Using *Rhodopseudomonas palustris* PT

Fatemeh Pakpour, Maedeh Mohammadi and Ghasem D. Najafpour

Biotechnology Research Lab, Faculty of Chemical Engineering,
Noshirvani University of Technology, Babol, Iran

Abstract: Biohydrogen production from synthesis gas (syngas) was investigated using a local bacterium isolated from anaerobic sludge of dairy wastewater. The isolated strain *Rhodopseudomonas palustris* PT was able to convert syngas to hydrogen through water shift gas (WGS) reaction. The aim of present study was to determine the optimum concentration of ferric citrate which avoids the cell growth inhibition and improves the activity of enzymes involved in BioWGS reaction. For this purpose, the influence of ferric citrate on cell growth, CO consumption and biohydrogen production was considered. Light and carbon monoxide were used for the first stage of bacterial growth; but throughout the hydrogen production stage, light was eliminated and carbon monoxide supplied the required energy for the cell through the exothermic WGS reaction. The optimum soluble concentration of ferric citrate was achieved at 27 mg/l with 33 mmol/l hydrogen production. It was found that high concentration of ferric citrate (90 mg/l) interfered with cell growth and hydrogen production. It was also found that ferric citrate may affect on enzymatic activity of hydrogenase and carbon monoxide dehydrogenase (CODH) in BioWGS reaction.

Key words: Biohydrogen · Syngas · WGS reaction · Ferric citrate

INTRODUCTION

Hydrogen is an ideal synthetic fuel which leaves water vapor as by-product after combustion [1]. If hydrogen is going to be replaced with conventional petroleum fuels in the near future, it has to be produced in large scale through renewable technologies. The trend towards environmental sustainability and utilization of renewable resources had significantly increased interests in biological ways to produce hydrogen instead of conventional methods. The noticeable advantage of biological hydrogen production is environmentally innocuous process which is performed under mild operation condition [2].

Generally, a mixture of gases are generated from variety of carbonaceous materials in traditional gasifiers, called synthesis gas (syngas) including mainly CO, CO_2 and H_2 [3]. Hydrogen production through BioWGS reaction occurs through hydrogenogenic bacteria which convert CO and H_2O to CO_2 and H_2 according to Equation (1):

$$CO + H_2O \rightarrow CO_2 + H_2 \quad \Delta G = -20.1 \text{ kJ/mol} \qquad (1)$$

Hydrogenogenic bacteria like purple non-sulfur photosynthetic bacteria such as *Rhodopseudomonas palustris, Rhodosprillum rubrum, Rhodocyclus gelatinosus* and *Rubrivivax gelatinosus* CBS are commonly utilized for hydrogen production [4-6]. In biological WGS reaction, the required energy is supplied by electron transferring from CO to H_2O through oxidation of CO to CO_2 [7]. The hydrogenase and CO dehydrogenase (CODH) are two significant enzymes playing important roles in energy generation in the hydrogenogenic metabolism [8]. Hydrogenases catalyze the reduction of protons to hydrogen molecules.

Hydrogen production from syngas requires optimization of several parameters such as type and amount of carbon source, pH of growth medium, temperature and trace elements which affect the activity of the two enzymes responsible for producing hydrogen in the BioWSG reaction [9].

Corresponding Author: Dr. Ghasem Najafpour, Biotechnology Research Lab, Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran.
Tel. /Fax: +98 111 321 0975.

Trace elements are vital for microorganisms. They are needed in minute amounts for appropriate cell growth. Many bacteria use ferric citrate as a trace metal in their media to fulfill their nutritional requirement for iron [10].

In this study, the bacterium *Rhodopseudomonas palustris* PT was isolated from anaerobic sludge of dairy wastewater and was capable to convert CO available in syngas to H_2. The aim of this research was to determine the optimum concentration of ferric citrate as a micronutrient in growth medium for the maximum cell growth of *R. palustris* PT at the growth stage and the highest hydrogen production and CO consumption at the hydrogen production stage.

MATERIALS AND METHODS

Microorganism: *Rhodopseudomonas palustris* PT was isolated from anaerobic sludge of dairy wastewater and was used in this study. The strain *R. palustris* PT was rod-shape and Gram-negative. It was grown anaerobically as a non-sulfur photosynthetic bacterium. Cells were cultivated for 48 h (growth stage) and then shifted to dark conditions (hydrogen production stage) for production of hydrogen. The cultures were exposed to tungsten light (1000 lux); illumination was used as an energy source in the growth stage. In the presence of tungsten light, large amounts of purple pigments were produced.

The microorganism was grown on sodium acetate in 125 ml serum bottles. The medium contained $NaCH_3COO$, 1.5 g; yeast extract, 2g; NH_4Cl, 1.5 g; $MgSO_4.7H_2O$, 0.2 g; $CaCl_2.2H_2O$, 0.07 g; KH_2PO_4, 0.6 g and K_2HPO_4, 0.9 g. Trace element solution of Pfennig and Lippert [11] (9 ml), B vitamin solution including nicotinamide, 0.2 g; nicotinic acid, 0.2 g and thiamine HCl, 0.4 g (9 ml) while distilled water were added to 1000 ml. The concentration of ferric citrate in the media was varied in different experiments (10, 27, 60 and 90 mg/l). The mentioned chemicals and vitamins were obtained from either Sigma-Aldrich (USA) or Merck (Darmstadt, Germany). A 30 ml medium was distributed into each serum bottle. After autoclave, the serum bottles were purged with syngas containing CO, CO_2, H_2, Ar: 60, 10, 20, 10 % through two needles on the serum bottle septum for gas inlet and outlet. Argon was selected as internal standard for gas analysis.

The serum bottles were maintained at 30°C and 200 rpm in a shaker incubator. At the growth stage, illumination was provided by 200 W tungsten light bulbs to supply 1000 lux light intensity. After 48 h of incubation, when the cell growth was in the exponential phase, light was removed in order to initiate the hydrogen production stage. Gas samples were taken every 12 h at hydrogen production stage (after 48h) and the liquid samples were withdrawn every 12 h to determine the cell concentration.

Analytical Methods: Gas chromatograph (Agilent Technologies, USA) equipped with thermal conductivity detector (TCD) was used for gas analysis. A GC column 15' x 1/8" (2.1mm ID), stainless steel Carboxen 1000 (Supelco) with 60 /80 mesh was used. The column temperature was initially maintained at 40°C for 5 min followed by increasing the temperature at a rate of 20°C/min until it reached 220°C. The carrier gas (helium) flow rate was set at 30 ml/min. A gastight syringe (Hamilton, USA) was used to inject 0.6 ml of samples obtained from the headspace gas phase of the serum bottles. The injector and detector temperatures were set at 150°C and 200°C, respectively.

The cell concentration of the culture was determined by the cell optical density readings at a wavelength of 660 nm by means of a spectrophotometer (2100series UNICO, US) and data were collected and compared with a standard calibration curve.

RESULTS AND DISCUSSION

Cell Growth Rate: Figure 1 shows the cell growth of *R. palustris* PT under different ferric citrate concentrations (10, 27, 60 and 90 mg/l). These data demonstrate that an increase in concentration of ferric citrate in the medium from 10 to 27 mg/l improved the cell density; however, increase of ferric citrate concentration to beyond 27 mg/l decreased the cell density. Ferric citrate (iron) in ample amounts is toxic to cells and probably imposed inhibitory effect on cell growth at high concentrations. The maximum cell density of 1.2 g/l was achieved in the liquid phase at 27 mg/l of ferric citrate. The cell growth experimental data were modeled using Logistic growth equation in which the cell dry weight (x) is calculated as follows [4, 12]:

$$X=\frac{x_0 e^{\mu_m t}}{1-\left(\frac{x_0}{x_m}\right)\left(1-e^{\mu m t}\right)} \quad (2)$$

where x_0 is the initial bacterial cell concentration (g/l), t is time (h) and μ_m is the maximum specific growth rate (h^{-1}) and x_m is the maximum cell density (g/l). The results of curve fitting and parameters of this model are summarized

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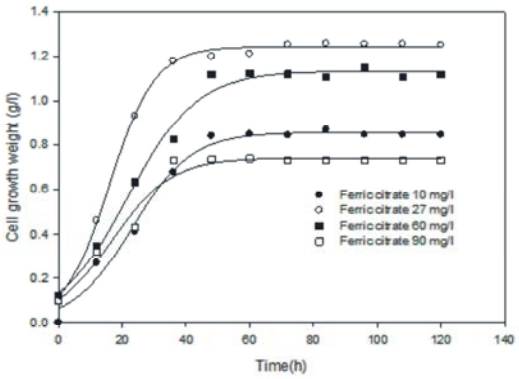

Fig. 1: Cell dry weight of *R. palustris* PT using various concentrations of ferric citrate

Table 1: Parameters of the Logistic growth model and hydrogen production efficiency of *R. palustris* PT

	Ferric citrate concentration (mg/l)			
Cell growth	10	27	60	90
Logistic parameters				
x_0 (g/l)	0.0646	0.1194	0.1345	0.0806
μ_m (h^{-1})	0.1083	0.1401	0.0921	0.1143
x_m(g/l)	0.8566	1.2450	1.1353	0.5524
R^2 (%)	98.59	99.8	98.9	97.93
Hydrogen production				
Y (%)	78.21	85	80.02	75.31
R^2 (%)	99	98.67	96.96	97.41

in Table 1. The results show that the regression coefficient (R^2) values for the Logistic model in all cases were above 0.98, indicating reasonable agreement between the experimental data and the curves predicted by the model.

Hydrogen Production: *R. palustris* PT as a hydrogenogenic bacterium was employed to oxidize CO to CO_2 and produce H_2 from water. Figure 2 (a) shows the consumption of CO in the gas phase in absence of light (hydrogen production stage). The abatement of CO concentration in the gas phase corresponded to the cell population growth in the liquid phase. The minimum level of CO oxidation was monitored at 90 mg/l of ferric citrate; the conversion of CO reached to 37 %. The maximum CO conversion was 68 % at 27 mg/l of ferric citrate. A 1.82-fold decrease was found in CO conversion with an increase of ferric citrate concentration from 27 to 90 mg/l. Once the light was removed (after 48 h), the activation of hydrogenase enzyme started along with simultaneous CO consumption. Hydrogen production is shown in Figure 2 (b). The minimum hydrogen production was related to concentration of 90 mg/l of ferric citrate in the liquid medium. Low cell density in the growth stage led to poor hydrogen production at 10 and 90 mg/l. The concentrations of 27 and 60 mg/l of ferric citrate

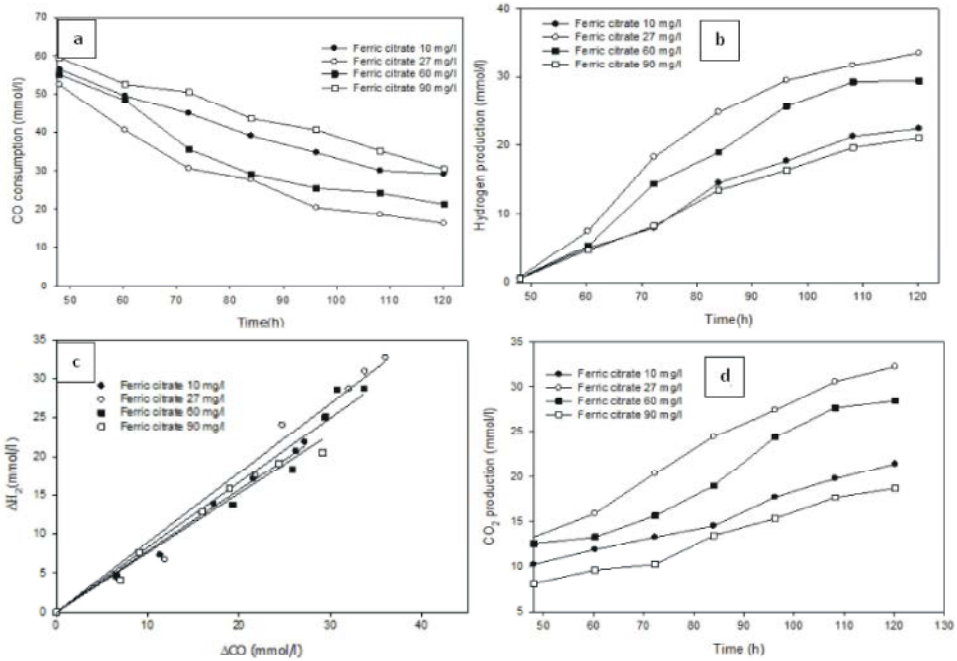

Fig. 2 (a): CO consumption; b) hydrogen production; c) hydrogen production efficiency in the BioWGS reaction and d) CO_2 formation by *R. palustris* PT in the gas phase of bioreactor at different initial ferric citrate concentrations

Table 2: Comparison of hydrogen production results in the batch fermentation by two strains of *R. palustris*

Bacteria	Substrate	Time(h)	Temp. (°C)	Culture mode	Cell density(g/l)	CO conversion	Hydrogen production rate	Ref.
R. palustri PT	CO/ CO_2/ H_2/Ar60/10/20/10	120	30	Batch	1.12	XCO=68%	0.93 mmol H_2/ l.h	Presentstudy
R. palustri P4	CO/$N_2$20/8010g sucrose	100	30	Batchanaerobic	1.2	XCO=60%	3.1 mmol H_2/l.h	[17]
	CO/$N_2$20/8010g sucrose	100	30	Batchaerobic growth	3.2	XCO =60%	1.9 mmol H_2/l.h	[17]
	CO/ $N_2$20/80	100	30	Batchanaerobic phototrophic	2	XCO=60%	2.2 mmol H_2/l.h	[17]

substantially increased the activity of hydrangeas and CODH enzymes. The maximum hydrogen production was 33 mmol/l with 27 mg/l of ferric citrate.

R. palustris PT catalyzed the BioWGS reaction to produce molecular H_2 and CO_2 as mentioned in Equation (1). The hydrogen production efficiency is acquired from the incline of the curve in Figure 2 (c). The maximum efficiency of hydrogen production was observed at 27 mg/l of ferric citrate which was equal to 85 %. The efficiency of hydrogen production for 60 and 90 mg/l ferric citrate dropped to 80.02 and 75.31 %, respectively. Inhibition of the two enzymes (hydrogenase and CODH) responsible for catalyzing the BioWGS reaction was probably due to increasing the ferric citrate concentration to 90 mg/l [14]. Figure 2 (d) illustrates the total content of CO_2 in the gas phase which was produced as a result of carbon monoxide oxidation in the WGS reaction. The generated CO_2 in this reaction should be eliminated from syngas by absorption to supply pure hydrogen [7]. The formation of CO_2 followed the same trend as that of biological hydrogen production [13].

In this study, the syngas composition was very similar to the syngas composition of an obtainable gasifier. Yeol Jung *et al*. [15] isolated a bacterium, *R. palustris* P4 from an anaerobic wastewater sludge to catalyze the WGS reaction and P4 strain was cultivated under mixed gas containing only carbon monoxide (20 %) and an inert gas, though mentioned that the strain was capable to oxidize the CO available in the syngas. The less hydrogen production in this investigation in comparison to the hydrogen production of the reported P4 strain could be related to the higher percentage of CO (60 %) in the syngas. High concentrations of CO could led to the inhibition of hydrogenase enzyme activity [16]. Use of high concentration of CO was the major advantage of this investigation. A comparison between the results of this work and the literature is presented in Table 2.

CONCLUSION

R. palustris PT as a local bacterium was suitable for catalyzing the BioWGS reaction and this strain was capable for converting the carbon monoxide existing in syngas to hydrogen. Hydrogen production started at the beginning of the stationary phase in the growth stage. Addition of ferric citrate at concentrations of 10, 27, 60, 90 mg/l had a considerable impact on the cell growth, CO consumption, hydrogen production and CO_2 production. The desirable concentration of ferric citrate as a trace element was 27 mg/l which is resulted in production of 33 mmol/l of hydrogen. An optimum of trace element concentration in the fermentation medium should certainly improve enzyme activities for hydrogen production.

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