ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 7 (4): 165-171, 2014
ISSN 1996-3351 / DOI: 10.3923/ajbs.2014.165.171

Effect of Ferrous and Nitrate Ions on Biological Hydrogen Production from Dairy Effluent with Anaerobic Waste Water Treatment Process

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ABSTRACT

Biohydrogen is a clean and environmental friendly renewable energy source. It only produces water after combustion. Anaerobic fermentation process is one of the best suitable technique for the biohydrogen production from industrial wastes. In this study with the consortium of biohydrogen producing bacteria B1 and B4 at the pH 5 and temperature 70°C, the effect of different concentration of ferrous and nitrate ions in the production of biohydrogen from dairy effluent was analyzed. Maximum biohydrogen production at the concentration of 100 mg L^{-1} of ferrous ion during 24 h HRT (Hydraulic Retention Time) was 55 mL with HPR (Hydrogen Production Rate) of 5.729 mL L^{-1} h^{-1} and during 48 h HRT 85 mL of biohydrogen was produced with the HPR of 4.427 mL L^{-1} h^{-1}. This process has been found to be an efficient method for the treatment of effluent in different pollution parameters after this fermentative biohydrogen production.

Key words: Biohydrogen, fermentation, consortium, hydrogen production rate

INTRODUCTION

Hydrogen is a clean and environmental friendly fuel. It has a high energy content of 122 kJ g^{-1} which is about 2.27 times more than hydrocarbon fuels (Chang and Lin, 2004). There are several methods for hydrogen production such as electrolysis of water, auto-thermal processes and steam reforming of hydrocarbons but they are costly due to high energy requirements. But these methods still use electricity derived from fossil fuel combustion (Junyapoon *et al.*, 2011). Hydrogen has several attractive characteristics; it has high translation efficiency, it is recyclable and non-polluting and forms only water after combustion (Krishna *et al.*, 2010). "Biohydrogen" is defined as hydrogen produced biologically, means, with the help of microorganism, most commonly by algae and bacteria by the utilization of biomass. Biohydrogen is a potential biofuel energy obtained from cultivation and organic waste materials. The major processes for biological hydrogen production are bio-photolysis of water through algae, photo-fermentation and dark fermentation of organic materials by bacteria. The fermentation process is attractive than the other methods because it is simple and use organic waste materials as substrate (Kapdan and Kargi, 2006). The basic components of organic waste materials being, carbohydrate, proteins and lipids and the hydrogen yield from carbohydrate fermentation has been reported to be relatively higher than that of lipid (Kim *et al.*, 2012). Mixed culture process are more practical than those using pure cultures because the former are simpler to operate and easier to control and may have a broader source of

Fig. 1: Experimental setup

feedstock, thus mixed cultures are preferred for wastewater treatment (Wang and Wan, 2008a). Iron is an important element which effect activity of hydrogenase and other different enzymes which almost required for biohydrogen production fundamentally (Yang and Shen, 2006). Iron can influence the fermentative hydrogen production by influencing the activity of hydrogenases (Wang and Wan, 2008b). Ni-Fe hydrogenases are widely distributed among bacteria and both nickel and iron have important effects on fermentative biohydrogen yield (Karadag and Puhakka, 2009). Nitrogen is mostly used in both batch and continuous fermentation process conditions. It has been shown that at an appropriate concentration range, nitrogen increases fermentative biohydrogen production (Wang *et al*., 2009). So we have tried to develop easy and cost effective technique for the biohydrogen production with the treatment of effluent and analyze the effect of iron and nitrate in the biohydrogen production.

MATERIALS AND METHODS

Effluent was collected from dairy industry Devbhog Kumhari Chhattisgarh, India. After collection, it was stored at 4°C (Penfold *et al*., 2003; Mohan *et al*., 2007). Before fermentation, for deactivation of unwanted microorganism and methanogenic bacteria, pH of effluent was adjust to 5 and pretreated at 100°C for 1 h. The 400 mL of effluent was taken into the flask and kept the effluent in boiling water bath at 100°C for 1 h (Penfold *et al*., 2003; Fang *et al*., 2006). Consortium of bacteria B1 and B4 isolated from the effluent was inoculated into the substrate with different concentration of ferrous ion ($FeSO_4$) and nitrate ion (KNO_3) as nitrogen source. In this study, the concentration range of 50, 100, 200 and 300 mg L^{-1} of these ions was used. All the experiments repeated three times.

Experimental setup: Fermentation process was performed in small anaerobic batch fermentation flask. Temperature 70°C and agitation was maintained through hot plate magnetic stirrer. This was connected through a pipe to another flask containing 10% KOH for the absorption of CO_2 produced during fermentation process along with H_2. During fermentation process, hydrogen produced by bacteria was measured through the liquid displacement technique (Zanchetta *et al*., 2007; Thakur *et al*., 2014) (Fig. 1). Different changes in the physiochemical properties of effluent before and after fermentation were analyzed with reference to different parameters (Thakur *et al*., 2012, 2014).

RESULTS

Biohydrogen production at different ion concentrations: Present study deals with production of biohydrogen at pH 5, temperature 70°C and at various concentration of ferrous and nitrate ions. It was found that at the concentration of 100 mg L^{-1}, the production of biohydrogen was maximum in both ferrous and nitrate ions but the better results were found in the ferrous ion. At different concentration of ferrous ion, the production of biohydrogen at 50 mg L^{-1} during 24 h, HRT (Hydraulic Retention Time) was 40±0.58 mL with the HPR (Hydrogen Production Rate) of 4.166±0.06 mL L^{-1} h^{-1} and during 48 h, HRT 60±1.15 mL of biohydrogen was produced with HPR of 3.125±0.04 mL L^{-1} h^{-1}. At 100 mg L^{-1} concentration, 55±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 5.729±0.06 mL L^{-1} h^{-1} and during 48 h, HRT 85±0.58 mL with HPR of 4.427±0.03 mL L^{-1} h^{-1} biohydrogen was produced. At 200 mg L^{-1}, 45±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 4.687±0.06 mL L^{-1} h^{-1} and during 48 h HRT 70±1.15 mL of biohydrogen was produced with HPR of 3.645±0.07 mL L^{-1} h^{-1}. At 300 mg L^{-1}, 35±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 3.645±0.06 mL L^{-1} h^{-1} and 55±0.58 mL of biohydrogen was produced during 48 h HRT with HPR of 2.864±0.02 mL L^{-1} h^{-1} (Table 1, Fig. 2 and 3).

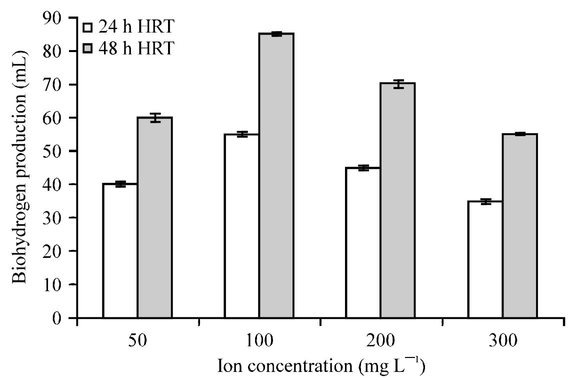

Fig. 2: Biohydrogen production analysis from consortium of biohydrogen producing bacteria at different ferrous ion concentration at 70°C and pH 5

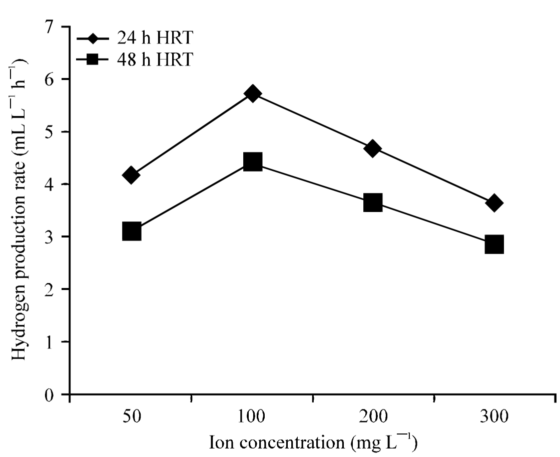

Fig. 3: Hydrogen Production Rate (HPR) at different ferrous ion concentration at 70°C and pH 5

Table 1: Biohydrogen production analysis at different ferrous ion concentration. Biohydrogen production analysis from consortium of biohydrogen producing bacteria under different ion concentration (Ferrous) at temperature 70°C in pH 5.0

Ion concentration (mg L^{-1})	HRT (h)	Biohydrogen production (mL)	HPR (mL L^{-1} h^{-1})
50	24	40±0.58	4.166±0.06
	48	60±1.15	3.125±0.04
100	24	55±0.58	5.729±0.06
	48	85±0.58	4.427±0.03
200	24	45±0.58	4.687±0.06
	48	70±1.15	3.645±0.07
300	24	35±0.58	3.645±0.06
	48	55±0.58	2.864±0.02

HRT: Hydraulic Retention Time, HPR: Hydrogen Production Rate

Table 2: Biohydrogen production analysis at different nitrate ion concentration. Biohydrogen production analysis from consortium of biohydrogen producing bacteria under different ion concentration (Nitrate) at temperature 70°C in pH 5.0

Ion concentration (mg L^{-1})	HRT (h)	Biohydrogen production (mL)	HPR (mL L^{-1} h^{-1})
50	24	35±0.58	3.645±0.06
	48	50±0.58	2.604±0.03
100	24	50±0.58	5.208±0.06
	48	70±0.58	3.645±0.03
200	24	43±0.58	4.479±0.06
	48	60±0.58	3.125±0.03
300	24	35±0.58	3.645±0.06
	48	45±0.58	2.343±0.03

HRT: Hydraulic Retention Time, HPR: Hydrogen Production Rate

At different nitrate ion concentrations, the production of biohydrogen at 50 mg L^{-1} during 24 h HRT, the production of biohydrogen was 35±0.58 mL with the HPR of 3.645±0.06 mL L^{-1} h^{-1} and during 48 h HRT 50±0.58 mL of biohydrogen was produced with HPR of 2.604±0.03 mL L^{-1} h^{-1}. At 100 mg L^{-1} concentration, 50±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 5.208±0.06 mL L^{-1} h^{-1} and during 48 h HRT 70±0.58 mL with HPR of 3.645±0.03 mL L^{-1} h^{-1} biohydrogen was produced. At 200 mg L^{-1}, 43±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 4.479±0.06 mL L^{-1} h^{-1} and during 48 h HRT 60±0.58 mL of biohydrogen was produced with HPR of 3.125±0.03 mL L^{-1} h^{-1}. At 300 mg L^{-1}, 35±0.58 mL of biohydrogen was produced during 24 h HRT with HPR of 3.645±0.06 mL L^{-1} h^{-1} and 45±0.58 mL of biohydrogen was produced during 48 h HRT with HPR of 2.345±0.03 mL L^{-1} h^{-1} (Table 2, Fig. 4 and 5).

Physiochemical changes results: Bacteria have been found to have an excellent property to treat waste water. Therefore different physiochemical properties of dairy effluent, both before and after fermentation, were measured at the maximum biohydrogen production ion concentration of ferrous and nitrate. Before fermentation, it was found that the colour of effluent was milky due to the presence of milk and milk products, smell was rotten, turbidity of the effluent was 546 NTU, EC was 656 μS, TDS was 346.2 ppm, DO was 6.6 mg L^{-1}, BOD was 2.4 mg L^{-1}, COD was 8 mg L^{-1} and pH of effluent was 5 which was the working pH (Table 3).

After the fermentation, satisfactory results of effluent treatment in the presence of both ferrous and nitrate ions was found. After fermentation in ferrous ions, the physiochemical changes were

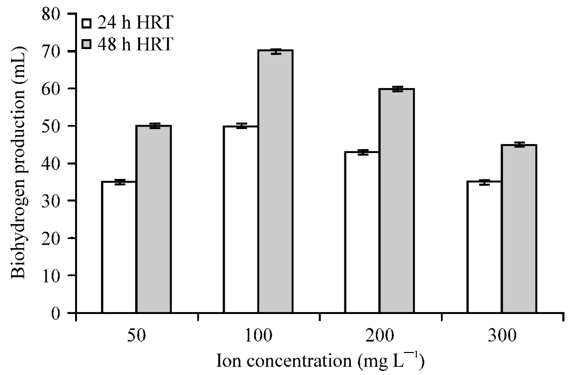

Fig. 4: Biohydrogen production analysis from consortium at biohydrogen producing bacteria of different nitrate ion concentration at 70°C and pH 5

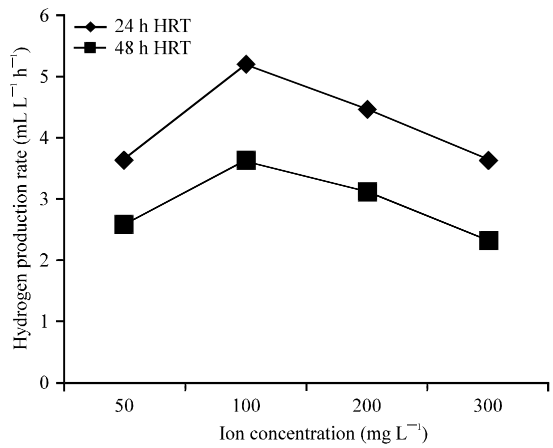

Fig. 5: Hydrogen Production Rate (HPR) at different nitrate ion concentration at 70°C and pH 5

Table 3: Physiochemical changes of dairy effluent before and after fermentation process

		After fermentation	
Test	Before fermentation	Ferrous ion	Nitrate ion
Colour	Milky	Turbid	Turbid
Smell	Rotten	Slight milky	Slight milky
Turbidity	546 NTU	285 NTU	253 NTU
EC	656 μS	475 μS	347 μS
TDS	346.2 ppm	255 ppm	243 ppm
DO	6.6 mg L^{-1}	4.4 mg L^{-1}	6.3 mg L^{-1}
BOD	2.4 mg L^{-1}	2.6 mg L^{-1}	3.2 mg L^{-1}
COD	8 mg L^{-1}	3.2 mg L^{-1}	4.8 mg L^{-1}
pH	5	4.4	5.16

found as, colour of effluent was turbid, smell was slight milky, turbidity was 285 NTU, EC was 475 μS, TDS was 255 ppm, DO was 4.4 mg L^{-1}, BOD was 2.6 mg L^{-1}, COD was 3.2 mg L^{-1} and pH of effluent was 4.4, pH was decrease due to formation of acid (Table 3).

After fermentation in nitrate ions, the physiochemical changes were found to be much better than ferrous ion as, colour of effluent was turbid, smell was slight milky, turbidity was

253 NTU, EC was 347 μS, TDS was 243 ppm, DO was 6.3 mg L^{-1}, BOD was 3.2 mg L^{-1}, COD was 4.8 mg L^{-1} and pH of effluent was 5.16 (Table 3).

After fermentation, it was clearly showed that the effluent was treated in both the ions at the concentration of 100 mg L^{-1}. In nitrate ions, the treatment was better than the ferrous but with the production of biohydrogen and treatment of effluent the ferrous ion showed better results.

DISCUSSION

Related results at different concentrations of iron for biohydrogen production have also been found by Karadag and Puhakka (2009). Hydrogen production has been found increasing with increased concentration range of 25-50 mg Fe^{2+} L^{-1} and remained stable at 100 mg Fe^{2+} L^{-1}. They found lowest and highest average of hydrogen production yield of 0.66 and 1.13 mol H_2 mol^{-1} glucose at 0.5 and 50 mg Fe^{2+} L^{-1}. Hydrogen production yield remained constant with further increasing the concentration of iron. They conclude that the optimum concentration of iron was 50 mg Fe^{2+} L^{-1} for biohydrogen production and hydrogen production yield was enhanced upto 71%.

Wang and Wan (2008a) found similar results with nickel ions at 35°C and initial pH 7.0, Ni^{2+} was able to enhance the biohydrogen production rate with increasing Ni^{2+} concentration from 0-0.2 mg L^{-1} and enhanced hydrogen production potential and hydrogen yield with increasing Ni^{2+} concentration from 0-0.1 mg L^{-1}. They found maximum hydrogen production potential of 288.6 mL and the maximum hydrogen yield of 296.1 mL g^{-1} glucose at the Ni^{2+} concentration of 0.1 mg L^{-1}.

Wang *et al.* (2009) found similar result that the degradation rate of substrate, hydrogen production potential, hydrogen yield and hydrogen production rate increased with increasing the nitrate concentration from 0-0.1 g L^{-1} of N, while decreased with increasing the concentration of nitrate from 0.1-10 g N L^{-1}. They obtained maximum hydrogen production potential of 305.0 mL, maximum hydrogen yield of 313.1 mL g^{-1} glucose and maximum average hydrogen production rate of 13.3 mL h^{-1} at a nitrate concentration of 0.1 g N L^{-1}.

CONCLUSION

Industrial effluent is a good substrate for the production of renewable biohydrogen. Biohydrogen is the good alternative source of fossil fuel. Fermentative bacteria have capable to produce biohydrogen from industrial effluent. At pH 5, temperature 70°C and at the concentration of 100 mg L^{-1} of ferrous ions, the production of biohydrogen was maximum during 24 h HRT, the biohydrogen production was 55±0.58 mL with HPR of 5.729±0.06 mL L^{-1} h^{-1} and during 48 h HRT 85±0.58 mL of biohydrogen was produced with the HPR of 4.427±0.03 mL L^{-1} h^{-1}. It was found that the biohydrogen production rate (HPR) was maximum during the HRT of 24 h. Bacteria are having good properties of the effluent treatment, satisfactory results have been found in the treatment of effluent after the fermentation and biohydrogen production, the quality of effluent was improved at various parameters. This technique is efficient for the production of biohydrogen with effluent treatment and it is cost effective and eco friendly also and it does not get any harm to our environment.

ACKNOWLEDGMENT

The authors gratefully acknowledge Pandit Ravishankar Shukla University and School of Studies in Biotechnology, Raipur, Chhattisgarh, India for providing space and other requirements for this study.

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