Biohydrogen Production from Wastewater by immobilized culture of *Clostridium acetobutylicum* NCIM 2877

Veena Thakur[1], S.K. Jadhav[2]*, K.L.Tiwari[3]

[1, 2*,3-]School of Studies in Biotechnology,
Pandit Ravishankar Shukla University Raipur, Chhattisgarh
*corresponding author –jadhav9862@gmail.com

Abstract: Biological hydrogen is one of the revolutionary finding for the 21st century for being, clean and green energy source. Its production from wastewater with the simultaneous treatment of wastewater is one of the great finding in this area, moreover by the use of immobilized form of bacteria for biological hydrogen production is one of the greatest finding, as after production and treatment the immobilized bacterial cells can be easily removed, without affecting the water quality. In this study production of biohydrogen was carried out by using dairy and rice mill effluent as a substrate by using *Clostridium acetobutylicum* NCIM 2877 in immobilized state and it was found that maximum production was 71.67± 0.88 ml with rice mill effluent and 52.0 ± 0.57 ml with dairy effluent at temperature 35 ^{0}C and 33 ± 1.2 ml and 64.33± 0.67 ml was obtained for rice mill effluent and dairy effluent respectively at 5 pH. And also this process has been found to be an efficient method for the treatment of effluent in different pollution parameters after this fermentative biohydrogen production.

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Key words: Biohydrogen, Fermentation, *Clostridium acetobutylicum* NCIM 2877, immobilized,wastewater.

Introduction:

The world population and consequently energy demands seem to grow following an exponential rate. The impending shortage of energy resources together with the environmental fall off, due to unreasonable use of fossil fuels, has resulted in the search for alternative energy sources which is non polluting and having high energy yield (Winter, 2005) Hydrogen fulfills all the above requirements. Energy density of hydrogen may vary between 120-142 MJ/Kg and one Kg of hydrogen replaces nearly 3.55 l of conventional diesel (Kim *et al.,* 2009), could be directly used to produce electricity through fuel cells. Unlike conventional biofuels, hydrogen produces only water as by product of its combustion.Currently, hydrogen is produced from fossil fuel by different processes such as steam reformation, coal gasification and partial oxidation of heavier hydrocarbons. About 59% of hydrogen is being produced by the above process which increases the production cost as well as green house gas emission. Therefore, hydrogen produced by conventional methods cannot be included as alternative source of energy and other sustainable methods of hydrogen production is becoming inevitable. Hydrogen production from biological waste by the use of microorganisms is called biohydrogen.

Kapadan and Kargi, (2006) categorized major bioprocess for biohydrogen production as bio-photolysis of water by algae, photo-fermentation, dark fermentation, combined dark and photo fermentation. Biohydrogen production by dark fermentation technique is the most suitable method as it is environment friendly and energy saving. Substrate for biohydrogen production must be biodegradable easily available and rich in carbohydrate. Biohydrogen production from waste organic material is nowadays gaining significant importance as it fulfills all the above criteria.

Wastewater consists of different types of carbohydrate, protein, lipids and many other components which can be utilized by micro organisms for the production of biohydrogen. Numerous types of wastewaters are utilized for the production of biohydrogen paper mill, rice mill, dairy effluents, distillery effluent etc (Kapadan and Kargi 2006) Anaerobic treatment of wastewater offers an excellent solution in terms of both energy savings and pollution control. Wastewater from industries basically contains complex organic feed such as carbohydrate, proteins and lipids which upon hydrolysis form simple sugars, amino acids and fatty acids. Wastewater has high amount of chemical oxygen demand (COD) value and is therefore suitable for anaerobic treatment process. Sludge produced during the wastewater treatment also serves as a good substrate for the production of biohydrogen as they are very rich in polysaccharides and proteins. Microorganisms responsible for the production of biohydrogen: Many of the fermentative bacteria are used for the biohydrogen production based on the requirement of processes. For dark fermentation method *Clostridium sps* (Wang and Jin 2009*, Enterobacter, Bacillus, Klebseilla* (Tanisho and Ishiwata, 1994, 1995). *etc* are used. For photo fermentation *Rhodobacter capsulatus, Rhodovulum sulfidophilum, Rodopseudomonos palsutris etc.*

Microorganism which are used for the production of biohydrogen can be use in the free state as well as in the immobilized state as immobilized bacteria cell can be reused. In the present study, rice mill effluent and dairy effluent is used as substrate for biohydrogen production with treatment of wastewater.

Material and Methods

In the present study, anaerobic fermentation process was carried out with immobilized culture of *Clostridium acetobutylicum* NCIM 2877 for biohydrogen production.

Collection and maintenance of substrate

Effluents for biohydrogen production was collected in sterile bottles. Dairy effluent was collected from Devbhog Kumhari, Chhatisgarh, India and rice mill effluent was collected from Khandelwal Rice mill, Tatibandh Raipur, Chhattisgarh, India. The samples were stored at 4^0 C (Mohan,*et al.,*2007; Penfold, *et al.,*2003; Thakur, *et al.,*2012).

Pretreatment

For the deactivation of hydrogenotrophic methanogens the effluent samples were pretreated at 100^0C for one hour (Mohan, *et al.,* 2007; Fang, *et al.,* 2006).

Laboratory set up for biohydrogen production

A 250 ml conical flask was taken which was connected to another flask containing a 20% KOH for the absorption of CO_2 produced as a byproduct of this process and this flask was connected to a measuring cylinder through a pipe for the collection of liquid displaced, which was the total biohydrogen produced (Zanchetta, *et al.,* 2007).

Immobilization of *Clostridium acetobutylicum* NCIM 2877: 24 hrs old bacterial culture was spinned at 6000 RPM for 10 minutes, supernatant was discarded and pellate was washed with distilled water. This pellate was added to autoclave 3% sodium alginate and dropped into 0.2 molar calcium chloride solution (Thakur *et al.,* 2014 and Wu *et al.,* 2002).

Effect of temperature:

Effect of different temperature between 30-50^0C was studied on biohydrogen production without optimization of initial pH.

Effect of pH:

Effect of different pH between 4-8 was studied on biohydrogen production after optimization of temperature.

Physicochemical analysis of effluent:

Physicochemical analysis of effluent color, turbidity, TSS, TDS, COD and BOD were done before and after fermentation process.

Result and Discussions:

Effect of temperature:

Temperature plays an important role in determining the metabolic activity of bacteria. When effects of different temperature were optimized it was observed that maximum production of 71.67± 0.88 ml was obtained at 35^0C with rice mill effluent. The production gradually decreased with increasing temperature, at 40^0C production was 52.00 ± 0.57 ml and at 45^0C the production was 44.67 ± 0.88 ml (Table1). For dairy effluent maximum of 52.0 ± 0.57 ml production was observed, which initially was 40.2 ± 0.33 ml at 30^0C. Thakur *et al.* (2012) reported best biohydrogen production at 35^0 C and at pH 5.

Effect of pH:

pH is also one of the physical factor effecting the production of biohydrogen from bacteria. A specific bacteria is having an optimum pH for its activity. Different range of pH was studied to optimize the particular pH condition for the biohydrogen producing capacity of bacteria. Effect of pH between 4-7 was studied in both rice mill and dairy effluent for biohydrogen production using the immobilized *Clostridium acetobutylicum* NCIM 2877. Maximum production of 74.33 ± 1.2 ml and 64.33± 0.67 ml was obtained for rice mill effluent and dairy effluent respectively at 5 pH. When the pH was further increased to 6 and 7 the production decreases, 63.33± 0.67 ml and 53.33 ± 2.5 ml respectively for rice bran 51.33 ± 0.67ml and 35.33 ± 0.88ml at pH 6 and 7 respectively for dairy effluent (Table 3). Alalayah *et al.* (2009) reported production of biohydrogen at 37^0C and at pH 6.0 ± 0.2 with *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564).

Physicochemical analysis of effluent before and after biohydrogen production:

Effluent samples when brought to the laboratory were first analyzed for different physicochemical properties. Initially it was observed that color of rice mill effluent was brown, turbidity was 280.0 NTU, TDS was 1100.0 mg/l, TSS was 892.0 mg/l and BOD was 350 mg/l. After fermentation it was observed that turbidity reduced to 178.00 NTU, TDS was 650.00 mg/l, TSS was 101.00 mg/l and BOD was 59.00 mg/l COD was reduced from 1590.0 mg/l to 405 mg/l (table 4). This indicates that effluent can be treated simultaneously with biohydrogen production process. Similarly when dairy effluents were analyzed before and after fermentation process it was observed that initially the colour of the water sample was milky which turned to creamish, turbidity was 177.0 NTU which became 158.0 NTU, TDS, TSS, BOD and COD initially were 950.0 mg/l, 2092.0 mg/l, 370mg/l, and 1060 mg/l respectively. All the values get reduced after the fermentation process. Paul *et al.* (2014) also reported in the improvement of dairy effluent after the biohydrogen production process.

Table 1. Biohydrogen production from rice mill effluent at different temperature.

S. no.	Temperature (^{0}C)	Rice mill effluent (mean production ± S.E ml)	Initial pH± SE	Final pH
1	30	54.33 ± 1.2	5.3 ± 0.05	4.2 ± 0.03
2	35	71.67 ± 0.88	5.2 ± 0.03	3.6 ± 0.14
3	40	52.00 ± 0.57	5.4 ± 0.03	4.1 ± 0.05
4	45	44.67 ± 0.88	5.1 ± 0.03	3.8 ± 0.05

Table 2. Biohydrogen production from dairy effluent at different temperature.

S. no.	Temperature (^{0}C)	Dairy effluent (Production ml ± SE)	Initial pH ± SE	Final pH
1	30	40.2 ± 0.33	4.3 ± 0.05	3.2 ± 0.03
2	35	52.0 ± 0.57	4.9 ± 0.03	3.6 ± 0.14
3	40	43.67 ± 1.20	4.4 ± 0.03	3.9 ± 0.05
4	45	34.33 ± 0.33	4.1 ± 0.03	3.5 ± 0.05

Table 3. Effect of different pH on biohydrogen production.

S. No	pH	Rice mill effluent (Production ml ± SE)	Dairy effluent (Production ml ± SE)
1	4	47.67± 0.89	41.00 ± 0.57
2	5	74.33 ± 1.2	64.33 ± 0.67
3	6	63.33 ± 0.67	51.33± 0.67
4	7	53.33 ± 2.5	35.33± 0.88

Table 4. Physicochemical analysis of rice mill effluent and dairy effluent before and after biohydrogen production.

S.No.	Physicochemical parameter	Rice mill effluent		Dairy effluent	
		Before fermentation	After fermentation	Before fermentation	After fermentation
1.	Color	Milky	creamish	Brown	Yellowish
2.	Turbidity	177.0 NTU	158.00 NTU	280.0 NTU	178.00 NTU
3.	TDS	950.0 mg/l	450.00 mg/l	1100.0 mg/l	650.00 mg/l
4.	TSS	2092.0 mg/l	681.00 mg/l	892.0 mg/l	101.00 mg/l
6.	BOD	370.0 mg/l	86.00 mg/l	350.0 mg/l	59.00 mg/l
7.	COD	1060.0 mg/l	125.00 mg/l	1590.0 mg/l	405.00 mg/l

Conclusion:

Wastewater from the food industries contain loads of organic wastes which when discharged to the water bodies, causes serious environmental problems. Due to high organic content these can be used as a best source for biohydrogen production. Dairy effluent and rice mill effluent was taken as substrate in the present study. Best production was observed at 35^0 C 71.67 ± 0.88 for rice mill effluent and 52.0 ± 0.57 for dairy effluent and at 5 pH the production were 74.33 ± 1.2 and 64.33 ± 0.67 for rice mill effluent and dairy effluent respectively. Improvement in the physicochemical properties has also been observed for the effluent samples after biohydrogen production.

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