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Effect of aeration on microbial production of hydrogen from maize stalk hydrolysate

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Abstract : Bio-hydrogen production by dark fermentation of maize stalk hydrolysate was investigated with fed batch operation using a newly isolated facultative strain. Fed-batch production was studied by operating under three different modes, strict anaerobic (N_2 filling), partial aerobic and micro aeration condition. Enhanced production of hydrogen was obtained under micro aerobic condition with a maximum yield of 894.89 ml H_2/L at 42 hr, when compared to strict anaerobic and partial aerobic condition with a yield of 290.08 and 237.95 mlH_2/L respectively. The yield under micro-aeration conditions was found to be almost fivefold higher than the other two conditions. Hydrogen production was further increased by incorporating a microbial electrolysis cell with fermentation effluent as feed.

Keywords: Aeration, Facultative strain, MEC.

1. Introduction

Concerns on green house gas emission and energy security leads to an increased drive for the development of alternative fuels. Hydrogen is a promising and potential biofuel which can be used as an alternative to the predominantly used fossil fuels. Hydrogen is a clean fuel with no CO_2 emission and can be used in fuel cells for generation of electricity[1]. Biological production of hydrogen using wastewater and other biomass as raw materials has been attracting attention as an environmentally friendly process that does not consume fossil fuels[2]. Biological hydrogen production can be carried out either via dark fermentation or photosynthesis[3-6]. Dark fermentation was reported to be the most feasible route of biohydrogen production due to the advantages like high H_2 production rate, rapid bacterial growth rates, low energy demands, minimal pollution generation, operation without light sources, low capital costs and the capability to convert large range of organic wastes[7-9]. Dark fermentative hydrogen production employing different reactor configurations like continuous stirred tank[10], expanded granular sludge bed[11], fluidized bed[12] and trickle bed type reactor[13]. In the present study fed batch fermentation has been used for bio-hydrogen production from maize stalk by dark fermentation. The first step in bio-hydrogen production from biomass is the conversion of lignocellulosic biomass to a solution containing sugar molecules, which is followed by dark fermentation by the isolated bacterial strain for the production of volatile fatty acids (VFAs), hydrogen and CO_2. Batch dark fermentation experiments were carried out using acid hydrolyzed maize stalk and the most suitable conditions maximizing the hydrogen formation rate and the yield were determined. Bioreactors have an edge over shake flasks, as there is better control on process parameters namely; agitation, pH and temperature maintenance. The possibility to enhance the hydrogen productionwas evaluated in a laboratory bioreactor. Fed-batch studies were performed under three different

modes namely strict anaerobic (N_2 filling), partial aerobic and micro aeration condition to check the maximum hydrogen production.

2. Materials and methods

2.1. Microorganism and media

The hydrogen producing bacterial strain isolated from soil rich in decomposed maize stalk was used in this study. The bacterium was grown in medium consisting of glucose, 1.5% (w/v); malt extract, 0.1% (w/v); yeast extract, 0.2% (w/v); peptone, 0.5% (w/v) and NaCl, 0.5% (w/v). It was maintained on agar slants (1.5% agar) using the above composition. Maize stalk hydrolysate equivalent to the concentration of glucose was used as the sole carbon source.

2.2. Acid hydrolysis

The hydrolysate of maize stalk was used as the carbon source for the production of hydrogen. Five gram of the powdered maize stalk was hydrolysed using 100ml of 1% (v/v) sulphuric acid for 75 minutes at 15 psi and 121^{o}C in an autoclave(Hitech equipment, India). The hydrolysate obtained after hydrolysis was filtered using a Whattman No.1 filter paper followed by an ordinary filter paper. The final filtrate was neutralized with 0.5M NaOH. The volume of hydrolysate equivalent to the required amount of glucose is used as the carbon source for the organism.

2.3. Fed batch studies

All fed-batch studies were carried out in a 2.0 liter fermentor equipped with pH, temperature, DO and anti-foam sensors. To start up the fermentation, 1.0 liter production medium with the following composition: glucose, 19.25g/L; peptone, 5.64g/L; malt extract, 1.64g/L; yeast extract, 3.16g/L and NaCl,4.31g/L. After adjusting the pH to 7.0, all the ports were plugged with cotton and the medium was sterilized along with the fermentor vessel. After sterilization, the medium was inoculated with one day pre-grown culture [5% (v/v)] and the fermentation was carried out under controlled conditions (34^{o}C, 7pH). The samples were drawn periodically and analyzed for organic acids and cell mass concentration.

2.4. Microbial electrolysis cell

500ml glass bottles were used for MEC construction. Anode and cathode were made of Type A (3.5 x 4 cm^2) and Type B (4 x 4 cm^2) carbon cloth respectively and were by a J-cloth (4 x 4 cm^2) [14].Anodes used in the MEC were initially enriched with bacteria in a microbial fuel cell using sewage as inoculum. The inoculum was omitted from the MFC, once the voltage output reached above 0.1V.

2.5. Analytical methods

The total gas produced during fermentation was measured regularly by water displacement method using a graduated aspirator bottle. Hydrogen content in the total gas was analyzed by a gas chromatogarph (AIMIL-NUCON 5765, Mumbai, India) using a steel column of 2.0 m (1/4 in. inside diameter) filled with Porapak Q (50/80 mesh) and equipped with a thermal conductivity detector. Nitrogen was used as a carrier gas at a flow rate of 20 ml/min. The temperatures for the injector, the oven and column were set at 150℃, 80℃ and 200℃ respectively.

Organic acids present in the fermented medium were analyzed by Shimadzu HPLC which is equipped with 4µm Hydro-RP column (250 x 4.6 mm) at ambient temperature. 20mM potassium phosphate (K_2HPO_4) adjusted to a pH of 2.9 was used as the mobile phase with a flow rate of 0.7 ml/min. Organic acids like oxalic, tartaric, formic, pyruvic, and citric acid were quantified in this method using an ultraviolet detector. The stock solutions were prepared by dissolving 1g or diluting 1ml of the acid in 100ml of HPLC grade water. The working standards were prepared by diluting 1ml of the stock solution using 100ml of HPLC grade water. From the working standard 5, 10, 15 and 20µl were taken and made up to 20µl using HPLC grade water and injected for analysis. Standard graphs were prepared by plotting the area obtained against the respective volume of the working standard. The sample collected was centrifuged and 20µl of the supernatant was injected for analyses with a run time of 20min. Concentrations of various acids were obtained from their respective standard graphs.

3. Result and discussion

3.1. Under strict anaerobic condition (N_2 filling)

In this mode of fermentation, nitrogen gas is filled before the start of fermentation to remove O_2 present in the headspace. Hydrogen yield and the concentration of other metabolites were recorded at regular time intervals and are given in Table 3.1. The results indicate that, the percentage hydrogen present in the accumulated gas increases constantly during the course of fermentation. The maximum percentage of hydrogen reaches to 41.6% and resulted in the hydrogen yield of 237.95 ml H_2/L. The yield obtained in this mode of fermentation is 1.03 fold higher than the maximum yield obtained in batch fermentations. To understand the mechanism of metabolite synthesis pathway, the volatile fatty acids synthesized in the fermentation was analyzed using HPLC.

The formic and pyruvic acid concentration plays an important role in the hydrogen synthesizing mechanism. In general, the hydrogen production pathway follows the conversion of glucose to pyruvic acid. The pyruvate is then converted to formate and acetyl Co-A which in turn breaks down as hydrogen and carbon-dioxide.In this mode of fermentation, the extracellular concentration of pyruvate and formate were found to be slightly in higher level. This may lead to switch-off the expression of gene responsible for the synthesis of pyruvate and formate. Thus, the low hydrogen yield may be due to the transport of these major metabolic intermediates to the cell external in the hydrogen production pathway.

Table 3.1The levels of hydrogen yield and the other metabolites synthesized in fed-batch under strict anaerobic condition

Time (h)	Total gas evolved (ml/L)	%H_2 Content	Hydrogen yield (ml H_2/L)	Metabolite concentration (µg/ml)				
				Oxalic	Tartaric	Formic	Pyruvic	Citric
6	280	2.96	8.29	-	-	-	-	-
12	310	7.74	23.99	-	-	-	-	-
18	332	12.6	41.83	2.3	5.98	0.3	1	0.25
24	365	19.1	69.72	3.15	5.4	0.7	0.812	0.9
30	417	25.1	104.67	-	-	-	-	-
36	475	32.6	154.85	-	-	-	-	-
42	550	41.2	226.6	3.95	5.15	0.72	0.75	0.92
48	572	41.6	237.95	4.75	3.1	1.04	1.50	0.32

3.2. Under partial aerobic condition

In this mode of fed-batch study, the fermentation was allowed to continue with the available dissolved oxygen present in the medium. The optimum fermentation conditions of pH 7.0, temperature 34.5^{o}C and the maize stalk hydrolysate level of 64% [equivalent to 1.5 % (w/v) glucose level] were used. Hydrogen yield and the concentration of other metabolites were recorded at regular time intervals and are given in Table 3.2.The results indicate that, the percentage hydrogen increases constantly during the course of fermentation. The maximum percentage of hydrogen reaches to 51.8% and resulted in the hydrogen yield of 290.08 ml H_2/L. The yield obtained in this mode of fermentation is 1.06 fold higher than the maximum yield obtained in batch fermentations.

Table 3.2The levels of hydrogen yield and the other metabolites synthesized in fed-batch under partial aerobic condition

Time (h)	Total gas Evolved (ml/L)	%H_2 Content	Hydrogen Yield (ml H_2/L)	Metabolite concentration (µg/ml)				
				Oxalic	Tartaric	Formic	Pyruvic	Citric
6	262	4.3	11.27	-	-	-	-	-
12	275	13.7	37.68	-	-	-	-	-
18	293	21.4	62.7	1.23	3.25	2.04	1.125	0.375

24	326	34.2	111.49	2.5	4.9	1.80	0.812	-
30	375	40.4	151.5	-	-	-	-	-
36	487	43.1	209.9	-	-	-	-	-
42	546	51.1	279.0	3.75	6.7	-	0.531	-
48	560	51.8	290.08	-	6.3	-	0.719	-

In this mode of fermentation, the extracellular concentration of pyruvate and formate were found to be in higher level. This may lead to switch-off the expression of gene responsible for the synthesis of pyruvate and formate. Thus, the low hydrogen yield may be due to the transport of these major metabolic intermediates to the cell external in the hydrogen production pathway.

3.3. Under micro-aeration condition

Oxygen supply was varied in this mode of fermentation with manual adjustment in order to maintain the hydrogen production at the optimum level throughout the fermentation period. Oxygen was supplied at a rate of 1 lpm (litre per minute) for 30 seconds in every 12h. The results indicate that, the maximum hydrogen yield of 894.89 ml H_2/Lwasreached at a fermentation time of 42h. The yield obtained in this mode of fermentation is 4.74 fold higher than the maximum yield obtained in batch fermentations.

In this mode of fermentation, the extracellular concentration of pyruvate and formate were less when compared to the concentrations of other two fermentation conditions. This may switch-on the expression of gene responsible for the synthesis of pyruvate and formate. Thus, the high hydrogen yield may be due to the presences of these major metabolic intermediates inside the cell in the hydrogen production pathway.

Table 3.3The levels of hydrogen yield and the other metabolites synthesized in fed-batch under micro-aerobic condition

Time (h)	Total gas evolved (ml/L)	%H_2 Content	Hydrogen yield (ml H_2/L)	Metabolite concentration (µg/ml)				
				Oxalic	Tartaric	Formic	Pyruvic	Citric
6	1035	1.9	19.67	-	-	-	-	-
12	1095	4.1	44.90	-	-	-	-	-
18	2200	4.1	90.20	1.7	2.6	0.6	0.281	-
24	2675	8.9	238.08	-	0.5	1.11	0.343	-
30	4210	11.4	479.94	-	-	-	-	-
36	4750	14.95	710.13	-	-	-	-	-
42	6370	13.80	879.06	-	-	0.24	0.37	-
48	6415	13.95	894.89	5.9	-	-	0.28	-

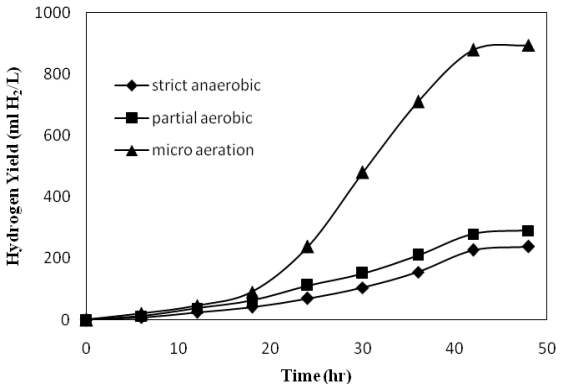

Fig 1 -Hydrogen yield profile in fed-batch

3.4. Hydrogen production using MEC

The fermentation effluent obtained by centrifugation at the end of fermentation is used as a feed for MEC. To maintain anaerobic condition, MEC reactor was sparged with nitrogen gas for 10 minutes. Anode enriched

with microbial consortium was placed at an applied voltage of 0.6V. Production of hydrogen was observed right after the placement of anode in all the three cases and the yield was found to be higher for MEC with effluent from strictly anaerobic (198 ml H_2/L) followed by partial aerobic (179 ml H_2/L)and micro aeration (27 ml H_2/L) mode of fermentation. Production increased continuously up to third day and no further increase observed thereafter.

4. Conclusions

Hydrogen production and the volatile fatty acids synthesized by the newly isolated strain under strict anaerobic, partial aerobic and micro-aeration conditions were investigated. A maximum hydrogen yield of 894.89 ml H_2/L was obtained for fermentation with micro-aeration, which was 4.65 and4.52 times higher than that under strict anaerobic and partial aerobic conditions. The concentrations of formate and pyruvate were found to be lower in micro-aeration condition were the hydrogen yield was higher. Combined hydrogen production from fed-batch and MEC were found to be 435.95, 469.08 and 921.89 ml H_2/ml respectively for strict anaerobic, partial aerobic and micro aeration mode of fermentation. Hydrogen production under strict anaerobic condition was increased to 1.83 times using MEC.

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