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Whey waste as potential feedstock for biohydrogen production

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ABSTRACT

In-house isolate *Clostridium sp*. IODB-O3 was exploited for biohydrogen production using cheese whey waste in batch fermentation. Analysis of cheese whey shows, it is enriched with lactose, lactic acid and protein components which were observed most favourable for biohydrogen production. Biohydrogen yield by IODB-O3 was compared with the cultures naturally occurring in waste solely or in combinations, and found that *Clostridium sp*. IODB-O3 was the best producer. The maximum biohydrogen yield obtained was 6.35 ± 0.2 mol-H_2/mol-lactose. The cumulative H_2 production (ml/L), 3330 ± 50, H_2 production rate (ml/L/h), 139 ± 5, and specific H_2 production (ml/g/h), 694 ± 10 were obtained. *Clostridium sp*. IODB-O3 exhibited better H_2 yield from cheese whey than the reported values in literature. Importantly, the enhancement of biohydrogen yield was observed possibly due to absence of inhibitory compounds, presence of essential nutrients, protein and lactic acid fractions which supported better cell growth than that of the lactose and glucose media. Carbon balance was carried out for the process which provided more insights in IODB-O3 metabolic pathway for biohydrogen production. This study may help for effective utilization of whey wastes for economic large scale biohydrogen production.

1. Introduction

Hydrogen is anticipated as the key alternative to the existing fossil fuel for internal combustion engines. It is basically an energy carrier and has potential for sustainable production from various sources including biological routes. It has been predicted as cleaner energy carrier, because it does not emits green house gases, however produces only water when combusted. Hydrogen exhibits a high calorific value (122 kJ/g) which is about three times higher than gasoline [1]. Hence, hydrogen has huge potential to be developed as an alternative fuel in the near future. 10% blend of hydrogen in natural gas when used as fuels in internal combustion engines is reported to reduce up to 95% green house gas emissions [2]. The global market for hydrogen generation is anticipated to mature with 6.2% increase per year from estimated $87.5 billion in 2011 to $118 billion by 2016 [3]. However, currently, almost all hydrogen is being produced by reforming of natural gas or light liquid hydrocarbons which obviously is not a sustainable proposition.

In the current perspective, among the various routes of hydrogen production, biological ones are much less energy intensive and environment-friendly. In biological hydrogen production processes, dark fermentation is most accepted because it accomplishes waste reduction, energy generation and economic viability of the process. Moreover in the process, complex organic compounds are converted into biohydrogen, volatile fatty acids (VFAs) and CO_2 by fermentative bacteria at ambient temperature and in absence of light which saves lot of energy. However, for economic production of biohydrogen, low cost carbohydrate-rich feedstocks are necessary for making the process more viable and sustainable. Some of the major bottlenecks for the development of biohydrogen production at commercial-scale still remain unachieved and include lower yield and rate of H_2 production, low substrate conversion efficiency, incomplete substrate conversion and its partial conversion into organic acids and CO_2 etc. Therefore, to overcome these challenges industrial effluents such as dairy waste, distillery waste etc which contain low concentration of reducing sugar are found to be attractive for developing economic process for biohydrogen production.

The development of sustainable industrial process for biohydrogen production is challenging. It cannot depend on single feed, therefore other than agro-wastes, industrial wastes such as cheese whey etc could be potential feedstocks for hydrogen production. Whey does not require complex pretreatment process similar to lignocellulosic waste before utilization in biohydrogen production and therefore economically attractive. Few

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studies have investigated the potential of complex whey waste to produce H_2 by studying dark fermentation. By definition, the watery part of milk that is separated from the coagulable part or curd especially in the process of making cheese is known as cheese whey. It contains significant carbohydrate content along with organic acids and is abundantly available as by-product of cheese industry. Therefore it can be an ideal potential feedstock for H_2 production. Recently, increased attention is being paid on dual concept of waste remediation combined with energy production in which utilization of anaerobic bacteria has been greatly exploited. The advancement of biogas fermentation is apparent form growing number of publications which shows increasing academic and commercial interest in recent years. Cheese manufacturing industry generates large amounts of high strength wastewater (whey waste) characterised by high biological oxygen demand (BOD) and chemical oxygen demand (COD) values [4–6]. Such wastes generally contains lactose (45–50 g/L), soluble proteins (6–8 g/L), lipids (4–5 g/L) and mineral salts (8–10% of dried extract). The mineral salts are comprised of predominating NaCl and KCl followed by calcium salts (primarily phosphate) and others. Whey also contains appreciable quantities of lactic acid (aprox. 0.5 g/L), citric acid, non-protein nitrogen compounds (urea and uric acid) and B group vitamins [7,8]. With these attractive properties, few reports have been published in recent years, on utilization of cheese whey as a good source for biohydrogen production. The theoretical maximum hydrogen that can be produced from lactose is 8 mol H_2 per mole lactose [9], however, up to 4 mol yield has been achieved while both acetic and butyric acids are produced. The utilization of cheese whey has also been reported for generation of bioethanol, methane, electricity, food and pharmaceutical products [10–14].

The biological H_2 fermentation is greatly influenced by numerous factors including cultures and concentration of substrate, cell biomass, pH, temperature nitrogen, phosphorous etc [15,16]. Therefore optimisation of these parameters can remarkably enhance the H_2 yield and rate by anaerobic fermentation [16]. Gadhe et al. [17] have applied multiple response optimisation methodology to improve biohydrogen production from 6 upto 14 mmol g^{-1} COD. Ellis et al. [18] have reported production of hydrogen, ethanol, and a variety of acids by several *Clostridium* species using cheese whey as substrate but production was less than 1 mol-H_2/mol-lactose. Ferreira Rosa et al. [19] have studied the hydrogen production using glucose and cheese whey solely and in combination by a mixed consortium in a continuous reactor system. The cheese whey exhibited the best H_2 yield of 1.9 followed by co-fermentation of the cheese whey and glucose mixture with yields of 1.7 and glucose 1.37 mmol H_2 g^{-1} COD respectively. Utilization of such type of wastes for hydrogen production also offers an extra economic benefit as compared to liquid product in separation process due to inherent phase separation property of gas from liquid culture media.

The aim of the present study was to produce biohydrogen with waste material and test the suitability of whey exploiting in-house isolate *Clostridium sp*. IODB-O3 without or with autoclaving for removal of existing flora in whey. Moreover, comparative analysis of biohydrogen yield by this strain with naturally occurring whey waste flora and in combination with in-house flora was also carried out. *Clostridium sp*. IODB-O3 has exhibited potential for utilization on various substrates for biohydrogen production in previous studies, [20]. Whey was not included in the study and therefore pursued in the present work. Carbon distribution of the process has also been studied for gaining further insight of the process efficiency for feed utilization and hydrogen generation. The study is aimed towards facilitating commercial production of biohydrogen from cheese whey.

2. Materials and methods

2.1. Culture and growth conditions

In-house mesophilic bacterial strain *Clostridium sp*. IODB-O3 isolated from anaerobic waste sludge from Okhla treatment plant Delhi, India [20] was exploited in the current study. The biohydrogen minimal media was prepared with composition expressed as (mg/L): K_2HPO_4- 450; KH_2PO_4- 450; $(NH_4)_2SO_4$- 900; NaCl- 900; $MgSO_4.7H_2O$- 90; $CaCl_2$- 90; Haemin- 0.00049; Yeast extract- 3000; Na_2CO_3- 4000; L-Cysteine.HCl- 500 (reducing agent); Resazurin- 0.6 (redox indicator); Vitamins: biotin- 4.0×10^{-2} and p-Amino benzoic acid- 1.0×10^{-2} and trace elements: $MnSO_4.H_2O$- 3.0×10^{-3}; $FeSO_4.6H_2O$- 8.0×10^{-3} and $CoCl_2.6H_2O$-3.0×10^{-3}. A 10 g/L lactose concentration was supplemented in the media. The minimal medium was set to pH 8.5 with 1 N HCl and 1 N NaOH solution and boiled under nitrogen flush to remove dissolved oxygen until the media turned colourless. The bottles were then sealed with butyl rubber stopper and aluminium cap using crimper. Sealed bottles with media were autoclaved for 15 min at 121 °C and 15 psi pressure. The fermentation conditions for in-house isolate were adopted from previous studies [20].

2.2. Whey waste collection and composition analysis

Fresh cheese whey were collected from local restaurant, domestic and dairy shops in Faridabad, India and stored at 4 °C in the refrigerator. Whey waste sample was centrifuged for 10 min at 8000 rpm, appropriately diluted and filtered using 0.22 μ syringe filter. 1.5 ml filtrate was used for HPLC analysis. The soluble protein of the sample was analysed by Lowry assay method [21].

2.3. Screening of whey waste

The potential of whey wastes were screened and compared to lactose and glucose in the media for biohydrogen production. For this, an experiment was designed in four sets. In first and second set, media was formulated with whey waste B2 (Table 1) with and without centrifugation (10 min at 8000 rpm) whereas in third and fourth sets media comprised of standard lactose and glucose (10 g/L, w/v) respectively. After media sterilization, all the four sets were inoculated with IODB-O3 culture (5% inoculum) and allowed for 24 h of fermentation. Total gas production was recorded and gas as well as liquid samples were analysed as defined in section 2.7.

2.4. Effect of lactose concentration

The effect of lactose concentrations in the media was investigated for best biohydrogen yield for which, lactose concentrations 5, 7.5, 10 and 13 g/L were evaluated in batch fermentation.

Table 1
Composition analysis of various cheese whey samples.

S No	Fraction	Concentration (g/L)		
		B1	B2	B3
1	Lactose	68.0 ± 2.7	38.13 ± 2.2	13.2 ± 3.2
2	Lactic acid	0.4 ± 0.1	2.71 ± 0.7	7.2 ± 1.5
3	Acetic acid	0.2 ± 0.0	0.5 ± 0.1	1.3 ± 0.3
4	Total soluble protein	2.15 ± 0.2	1.70 ± 0.1	1.2 ± 0.1