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Optimization of photo-hydrogen production based on cheese whey spent medium

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ABSTRACT

Cheese whey wastewater diluted to 10 g lactose/L was initially subjected to dark-fermentation by *Enterobacter aerogenes* MTCC 2822, and the VFAs-rich spent medium (acetic acid 1900 mg/L, butyric acid 537 mg/L, and traces of propionic acid) was subjected to photo-fermentation through enrichment by Ni^{2+} (0–8 μmol/L), Fe^{2+} (0–100 μmol/L) or Mg^{2+} (0–15 mmol/L) in batch mode by *Rhodopseudomonas* BHU 01 strain. The maximum cumulative H_2 production (144 ml) and yield (58 mmol) was obtained at 4 μmol Ni^{2+}/L. Likewise, Fe^{2+} (60 μmol/L) resulted in maximum cumulative H_2 production (139 ml) and yield (56 mmol). Nevertheless, 6 mmol of Mg^{2+} did not significantly affect H_2 production (110 ml) or yield (44 mmol); the latter value in close proximity with the control (37 mmol). The concomitant reduction in COD was maximum (15.61%) for 4 μmol Ni^{2+}/L, followed by 15.33% for 60 μmol Fe^{2+}/L, and the least for 6 mmol Mg^{2+}/L (14.5%). The observations suggest the role of Fe^{2+} and Ni^{2+} in regulation of nitrogenase and hydrogenase, while that of Mg^{2+} mainly in the biosynthesis of photopigment bacteriochlorophyll (Bchl).

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1. Introduction

The ever increasing energy demand with the resultant depletion of fossil fuels and environmental pollution attracts global attention to look for the alternative energy sources that are renewable and eco-friendly. Hydrogen, as a fuel produces water as the main product with no CO_2 emissions, thus considered as a clean and viable alternative fuel. Hydrogen is produced by many methods but biological H_2 production processes are less energy intensive than chemical or electrochemical routes [1,2]. Several reports reveal that sequential dark- and photo-fermentation systems relatively efficient in H_2 production, and offer certain merits over the single dark- or photo-fermentation step [3–5]. A combination of dark- and photo-fermentation is theoretically capable of producing the maximum possible yield of 12 mol H_2/mol glucose [6]. This indicates that the system holds promise in being the effective option for H_2 production from the economical view point. The trace metals such as Ni, Fe, Mg, Mo, and Ca are essential for growth/metabolism of most microorganisms. The lack of trace elements such as Mo and Fe inhibited H_2 production while their supplementation favoured the process in *Rhodobacter sphaeroides* O.U 001 [7]. The concentrations of trace elements in the fermentation medium also affect H_2 production as low Ni concentrations promoted H_2 production in *Rhodopseudomonas faecalis* RLD 53 contrary to inhibitions at elevated concentrations [8]. Also, the type and concentration of trace element vary depending on the organism as the Fe threshold concentration suppressive to H_2 production by *R. sphaeroides* was significantly higher than in case of *Clostridium butyricum* [9]. Among the trace elements, Ni, Fe and Mg play important role in biological H_2 production [8–15]. Fe and Ni are the

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components of several enzymes (hydrogenase and nitrogenase) involved in H_2 production while Mg ions are one of the most abundant elements within microorganisms, and the photosynthetic pigment bacteriochlorophyll (Bchl) contains Mg^{2+}. A few reports on the effects of these ions on photo-fermentative H_2 production are available [8,9,14,15], but none on the optimization of trace element concentrations in the spent medium of dark-fermentation for its subsequent photo-fermentation.

The use of defined media for bacterial growth and maintenance is ideal, however, their large-scale consumption for biohydrogen production is challenging owing to the cost involved. The use of various domestic, agricultural and industrial wastes rich in organics for biohydrogen production makes the scale up process cost-effective with the added advantage of effective removal of the organic load [16]. It is here that we adopted raw cheese whey wastewater for biohydrogen production. Cheese whey contains 4.6% of lactose, 1.2% crude protein, 0.6% ash, 0.3% fat, 5–8% total solids and 92.7% of water [17], and is the ideal substrate for fermentative H_2 production [18–23].

The main aim of the present study was to find out the optimum concentration of selected trace elements (Ni^{2+}, Fe^{2+}, and Mg^{2+}) for photo-biohydrogen production by *Rhodopseudomonas* BHU 01 strain from the nutrient-poor and VFAs-rich spent medium resulting from dark-fermentation of cheese whey wastewater by *Enterobacter aerogenes* MTCC 2822.

2. Materials and methods

2.1. Microorganisms and growth conditions

E. aerogenes MTCC 2822 (from IMTECH, Chandigarh, India) was used to generate spent medium containing volatile fatty acids (VFAs) from cheese whey (10 g lactose/L). The bacterium (hereafter *E. aerogenes*), was maintained in the growth medium containing (per litre) as prescribed by Microbial Type Culture Collection and Gene bank, IMTECH, Chandigarh (India): beef extract, 0.5 g; yeast extract, 1 g; peptone, 5.0 g and NaCl, 0.5 g. The growth medium pH was adjusted to 6.8, and bacterium grown at 30 °C in a gyratory incubator shaker (200 rpm).

Rhodopseudomonas BHU 01 strain was adopted for photo-fermentation of *E. aerogenes* spent medium. The organism was grown photo-heterotrophically in the Ormerod medium containing (per litre): KH_2PO_4, 0.6 g; K_2HPO_4, 0.9 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.075 g; $FeSO_4 \cdot 7H_2O$, 0.012 g; EDTA, 0.02 g; yeast extract, 2 g; trace element solution 1 ml [24]. Trace element solution (per 100 ml) contained H_3BO_3, 280 mg; $MnCl_2 \cdot 4H_2O$, 185 mg; $NaMoO_4 \cdot 2H_2O$, 75 mg; $ZnSO_4$, 24 mg; $CuSO_4 \cdot 5H_2O$, 40 mg. The organism was grown anoxically at 34 °C with the incident tungsten light (8.5 W/m^2) at a distance of 30 cm. The initial pH of the growth medium was maintained at 6.8. Bacterial cells were collected by centrifugation (4000 rpm, 20 min), washed twice in Mili Q water and finally suspended in the spent medium enriched with previously stated medium components except the carbon source for pre-adaptation of the cells and initialization of H_2 production activity.

2.2. Characterization of whey

Fresh raw cheese whey from a local dairy, had a pH of 4.87, COD 93 g/L, total sugars 43 g/L (as lactose), and total suspended solids (TSS) 6.7 g/L. Cheese whey was subjected to heating (85 °C, 30 min) to eliminate lactic acid bacteria, and stored at 4 °C until use.

2.3. Generation of spent medium, dark-fermentation

Fresh raw cheese whey waste water was diluted to final lactose concentration of 10 g/L with Mili Q water and supplemented with (per litre) beef extract, 0.5 g; yeast extract, 1 g; peptone, 5.0 g and NaCl, 0.5 g. The pH of the medium was adjusted to 6.8. Fermentation was carried out in 120 ml serum bottles with the working volume of 100 ml at 30 °C and agitated (200 rpm) in triplicate sets. Each set was inoculated with 10% (v/v) of overnight grown *E. aerogenes* cells in the medium initially sparged with N_2 (99.9%). The culture pH was not controlled during fermentation. The spent medium from the serum bottles was collected separately for estimation of acetic acid, butyric acid, propionic acid and other parameters.

2.4. Photo-fermentation

Rhodopseudomonas BHU 01 cells pre-adapted to spent medium, as stated previously, were used as the starter inoculum for photo-fermentation of the spent medium. The 48 h old culture grown in the spent medium supplemented with medium components (except carbon source), was the source of inoculum. For inoculation of each experimental set, 10 ml culture was withdrawn from the source with the help of a sterilized syringe and centrifuged (4000 rpm, 20 min), washed twice in Mili Q water to remove the medium components, and used as the inoculum. The spent medium obtained after dark-fermentation was centrifuged and filtered through 0.2 μ cellulose filter and supplemented with the same strength of ingredients as applicable to routine bacterial growth stated earlier except the carbon, Ni^{2+}, Fe^{2+}, and Mg^{2+} source. The trace elements Ni^{2+} ($NiCl_2$), Fe^{2+} ($FeSO_4 \cdot 7H_2O$) or Mg^{2+} ($MgSO_4 \cdot 7H_2O$) were individually added to the fermentation medium. The concentration of $NiCl_2$ ranged from 0 to 8 μmol/L, $FeSO_4 \cdot 7H_2O$ from 0 to 100 μmol/L and $MgSO_4 \cdot 7H_2O$ from 0 to 15 mmol/L. H_2 in the gas mixture was collected by water displacement and channelled through a needle inserted on the top to pass through a 200 ml solution of KOH (30%) for selective absorption of CO_2. H_2 production rate corresponds to the amount of H_2 produced (mL)/volume of substrate (spent medium) (L) × time (h), and H_2 yield to amount of H_2 produced (mmol)/amount of substrate (spent medium) consumed (L).

2.5. Analytical methods

Total sugars (as lactose) were estimated by phenol–sulphuric acid method of Dubois et al. [25]. Samples were centrifuged (12,000 rpm, 10 min) and COD and TSS estimated according to the standard methods [26]. Gas samples were taken out into a 250 μL pressure lock gas tight syringe (Hamilton) and analysed by gas chromatograph (Varian CP 3800) in TCD mode as described earlier [27], and also by gas monitor (UNIPHOS 290,

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