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Biological hydrogen production by extremely thermophilic novel bacterium *Thermoanaerobacter mathranii* A3N isolated from oil producing well

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ABSTRACT

Hydrogen producing novel bacterial strain was isolated from formation water from oil producing well. It was identified as *Thermoanaerobacter mathranii* A3N by 16S rRNA gene sequencing. Hydrogen production by novel strain was pH and substrate dependent and favored pH 8.0 for starch, pH 7.5 for xylose and sucrose, pH 8.0–9.0 for glucose fermentation at 70 °C. The highest H_2 yield was 2.64 ± 0.40 mol H_2 mol glucose at 10 g/L, 5.36 ± 0.41 mol H_2 mol − sucrose at 10 g/L, 17.91 ± 0.16 mmol H_2 g − starch at 5 g/L and 2.09 ± 0.21 mol H_2 mol xylose at 5 g/L. The maximum specific hydrogen production rates 6.29 (starch), 9.34 (sucrose), 5.76 (xylose) and 4.89 (glucose) mmol/g cell/h. Acetate-type fermentation pathway (approximately 97%) was found to be dominant in strain A3N, whereas butyrate formation was found in sucrose and xylose fermentation. Lactate production increased with high xylose concentrations above 10 g/L.

1. Introduction

Hydrogen is considered as one of the most promising alternative energy carrier to fossil fuel in the future. It is clean and environmentally friendly fuel that produces only water when combusted with oxygen. It is a high energy fuel (122 KJ/g) than hydrocarbon fuels [1]. Approximately 95% of commercially produced hydrogen comes from carbon-containing raw materials, primarily fossil in origin [2]. Due to the depletion of fossil fuel and emission of greenhouse gas (CO_2) during conventional hydrogen production process, biological hydrogen production from biomass has been recognized as an eco-friendly, and less energy intensive process to produce hydrogen compared to photosynthetic or chemical processes [3].

Thermophilic anaerobic fermentation processes hold tremendous potential for the forthcoming generations as well as commercial production of hydrogen fuel [4]. The energy required for heating is the main drawback of dark fermentation at high temperatures. However, the biohydrogen fermentation at extremely thermophilic temperatures (over 70 °C) has many outstanding advantages over lower temperature conditions, which could compensate the energy expenses from temperature increased such as higher yields [5], stable continuous production [3], higher production rate [6], better pathogenic destructions [7], higher rate of hydrolysis of complex materials such as household solid waste or manure [8,9]. Further, the extreme thermophilic microorganisms are known to generate low cell densities, which result in rather moderate hydrogen productivities [10].

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Efficient extreme thermophilic hydrogen producing bacteria can be applied for fermentation of high temperature substrates such as hydrolyzates obtained from hydrothermal pretreatment of lignocelluloses or high temperature industrial effluents where the temperature is already high. Thus, additional heating would not be required [11,6]. Moreover, it has been proposed that combination of hydrogen fermentation with methane to produce hythane, which is a mixture of hydrogen and methane [6].

Thermoanaerobacter species might be the efficient dark fermentative bacteria [12]. The dark fermentative hydrogen production is affected by several environmental factors such as initial pH, substrate concentration and fermentation temperature [11,13,14]. It has been observed that the initial cultivation pH affects the hydrogenase enzyme activity while substrate concentration affects the ability of hydrogen producing bacteria to produce hydrogen during dark fermentation [15].The research on dark fermentative hydrogen production at extreme thermophilic temperatures is scanty and yet to be investigated. Further, to the best of our knowledge, *Thermoanaerobacter mathranii* have not been investigated before in detail for the dark fermentative hydrogen production at extremely thermophilic temperatures.

In view of the above, this study investigates the optimum conditions for fermentative hydrogen production by *T. mathranii* strain A3N using different substrates such as starch, sucrose, glucose and xylose in batch operation under extremely thermophilic condition.

2. Materials and methods

2.1. Sample collection and isolation of the strain A3N

Oil water mixture samples (97% water, 3% oil) were collected from oil producing well (well No KD 269) in Nawagam, Ahamadabad, India into 50 mL of anaerobic sterilized serum bottles containing 1 mL of 2% Na_2S during April, 2010. The reservoir temperature was 87 °C and salinity of formation water was 15 g/L. Reservoir pressure was 700 psi.

2.2. Isolation of the strain A3N

Samples (0.1 mL) were inoculated in liquid anaerobic basal medium consisting of (g/L): NH_4Cl 0.5; Yeast extract 5; K_2HPO_4 0.25; KCl 0.002; $MgCl_26H_2O$ 0.125; NH_4HCO_3 0.4; Peptone 1; $NH_4H_2PO_4$ 0.4; NaH_2PO_4 0.5. trace element 1 mL, vitamin solution 1 mL [16]. Sucrose (10 g/L) was used as sole carbon and energy source. All the culture bottles were incubated at 70 °C for 3–5 days and sub cultured in same medium after 3 days of incubation. Serial dilution technique was used to obtain pure cultures. In order to be sure to obtain a pure isolates, serial dilution steps were repeated several times. All the sub cultures and diluted cultures were incubated at 70 °C under atmospheric pressure. Cells were observed under a light microscope (Olympus, Japan) and pure isolate A3N was routinely cultivated in basal medium.

2.3. Identification of strain using 16S rRNA gene sequence

Genomic DNA from the pure isolate A3N was extracted as reported [17]. The 16S rRNA gene sequence was amplified by PCR using MicroSec full Gene Kit (Applied Biosystems, UK) as per the manufactures instructions. Amplified product was sequenced using the DyeDeoxy Terminator Cycle sequencing Kit (Applied Biosystems, UK) as directed in the manufacturer's protocol with an automatic Genetic analyser (Model 300; Applied Biosystems, USA).

The nucleotide sequence of 16S rRNA gene of the isolate A3N was compared with other related sequences available in GenBank using BLAST programme [18]. Further, the nucleotide sequence of isolate A3N was aligned with closely related sequences found in GenBank, using CLUSTAL W, and pair wise evolutionary distances were computed using Jukes–Canter Model [19]. Phylogenetic analysis was performed using MEGA version 4. Confidence in the tree topologies was evaluated by re-sampling 100 bootstrap trees [20].

2.4. Study on the growth properties of the strain A3N

Effect of different temperatures (40, 45, 55, 65, 70 and 80 °C), NaCl concentrations (0–7%) (w/v) and different carbon sources (5 g/L glucose, fructose, sucrose, xylose, xylan, ribose, raffinose, mannose, arabinose, xylan, rhamnose, galactose, cellobiose, dextrose, lactose, maltose, sorbitol, molasses, starch and cellulose) on the growth of the isolate A3N were studied in anaerobic liquid basal medium. All the growth experiments were performed in triplicate, using 60 mL serum bottles containing 30 mL of anaerobic basal medium.

2.5. Optimization of hydrogen production in batch fermentation

Batch dark fermentation studies were performed in 125 mL anaerobic bottles with working volume of 10 mL of liquid anaerobic basal medium aforementioned. The inoculum acquired during exponential growth phase was added at 0.5% (v/v) by using disposable syringe. The inoculum was adapted to the each substrate by subculturing several cycles in batch cultures. Unless noted otherwise as a variable, initial starch, sucrose, glucose and xylose concentrations were kept at 10 g/L.

Influence of different initial pH levels for the hydrogen production on different substrates (starch, glucose, xylose and sucrose) was studied. The initial pH levels ranging from 5.5 to 9.5 with 0.5 increments were selected for this study. The pH of the medium was adjusted with 1 M HCl or 1 M NaOH prior to dispensing the medium into the 125 mL serum bottles.

Further, effect of initial substrate concentration on hydrogen production was investigated in batch experiments as described above. Optimal pH levels obtained for the maximum hydrogen production from each individual substrate were used as initial culture pH. Initial substrate concentrations tested were 5, 10, 15, 20 and 25 g/L for each individual substrate.

The obtained optimal initial cultivation pH and initial substrate concentration which gave the maximum hydrogen