Analysis of bio hydrogen production propensity of mixed consortium on food waste– A preliminary study
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ABSTRACT
Biohydrogen production capability of isolated microbial consortium of Clostridium, Pseudomonas and rhodobacter sp. on food waste was studied. Isolation of the bacteria for mixed consortia was carried out with the help of anaerobic chamber. Batch test was conducted to evaluate fermentative hydrogen production of food waste collected from hostel mess and canteens at 35 °C by a natural mixed culture. The experimental results indicate that the initial cultivation of pH markedly affected the hydrogen production. The reduction in chemical oxygen demand was observed from 408.4mg/l to 287.4mg/l with Clostridium, Pseudomonas and rhodobacter sp. COD decreases through the process as the food waste degrades. The production of hydrogen was found to be 49.55 ml of H₂/g COD reduced. This study inveterate the propensity of isolated mixed consortium for biohydrogen production on food waste.

Keywords: bioremediation, petroleum hydrocarbons, soil properties, crop production, phytoremediation, mycorrhizae.

1. Introduction
Demand of energy is increasing continuously due to rapid development in industrialization, population, economic growth, and rising living standards. Therefore, energy production is very important for living things, human and industrial activities (Kahyaoglu et al., 2012). Hydrogen is a clean energy that has a great potential to be an alternative fuel. In nature, some microorganisms with biological processes can produce hydrogen gas. Due to the limited resources and pollutants emission (CO₂, CO, CₙHₙ, SOₓ, NOₓ, ashes, etc.), fossil fuels should be substituted with renewable and non-polluting energy sources (Kim et al., 2004, Nandi et al., 1998))

As a sustainable energy source with minimal or zero use of hydrocarbons and high-energy yield, hydrogen is a promising alternative to fossil fuels. In addition, hydrogen can be directly used to produce electricity through fuel cells (Das and Verziroglu, 2001). Since conventional physico-chemical hydrogen production methods (e.g. water electrolysis or chemical cracking of hydrocarbons) require electricity derived from fossil fuel combustion, interest in biohydrogen production has increased significantly (Levin et al., 2004). Between two biological processes, fermentative processes that use wide range of organic substances are technically simpler than photosynthetic processes (Kim et al., 2004). Carbohydrates are the preferred substrate for fermentative hydrogen-producing bacteria such as Clostridium species. (Kim et al., 2004)
In recent years, some experimental results using sewage sludge (Lin and Lay, 2005), (Chen et al., 2005), (Das and Koaty, 2006) and food manufacturing waste (Kim et al., 2004) were reported. However, food waste might be suitable for fermentative hydrogen production, because it is the carbohydrate-rich and easily hydrolysable waste. Enormous quantities of food wastes are generated from our hostels and canteens. Due to increasing need for hydrogen energy, development of cost-effective and efficient hydrogen production technologies has gained significant attention in recent years. If hydrogen can be produced by anaerobic fermentation of food waste, they would be the important source for hydrogen production due to the amount (Hawkes et al., 2007). The effect of pH on the conversion of glucose to hydrogen by a mixed culture of fermentative bacteria should also be evaluated (Lin and Lay 2004).

The major remaining stumbling block is incomplete substrate conversion and the consequent low yields. There are also a number of other potential advantages of using microbial consortia instead of pure cultures. Microbial consortia address these issues as they have been selected for growth and dominance under non-sterile conditions. As a complex community they are also likely to contain a suite of the necessary hydrolytic activities, and they are potentially more robust to changes in environmental conditions. The use of microbial consortia has indeed been proven useful in reactor systems that yield high volumetric rates of hydrogen production, as discussed above. However, there are also several issues associated with their use(Hallenbeck and Ghosh, 2009). As complex communities, their composition can vary over time, with changes in process parameters and from reactor to reactor, as was shown by molecular (16sRNA) studies (Muyzer et al., 1993). A possible way to overcome this issue might be to construct ‘designer’ consortia with the goal of creating a community of diverse members, each contributing a unique and essential metabolic capacity. The total community metabolic range would be greater than any individual member, while at the same time mutual interdependence would assure stable maintenance of individual members. However, little is known about the complex interactions that occur in natural consortia or how stable synthetic microbial communities could be built. Thus, much additional fundamental work might be required before practically useful synthetic hydrogen-producing consortia could become a reality. It seems that spatial organization within a consortium might be important.

The major criteria for the selection of waste materials to be used in bio-hydrogen production are the availability, cost, carbohydrate content and biodegradability. Simple sugars such as glucose, sucrose and lactose are readily biodegradable and preferred substrates for hydrogen production (Kargi and Kapdan, 2005). However, pure carbohydrate sources are expensive raw materials for hydrogen production. The present study on “production of biohydrogen from food waste with the help of mixed consortia” was undertaken to isolate and identify hydrogen producing microorganisms by performing biochemical tests to confirm whether the mixed culture contains hydrogen producing bacteria from pond water. The production of biohydrogen from food waste is also studied.

2. Materials and Methods

2.1. Enrichment and isolation of anaerobic isolates

Basal medium was prepared with the following composition (g/l) KH$_2$PO$_4$ - 0.33; MgSO$_4$.7H$_2$O - 0.33; trace solution – 1ml; FeSO$_4$- 0.5 ml was added to the basal medium. To
100 ml of basal medium, 10 ml of polluted pond water was added. The medium was placed under light at room temperature at 30°C and 100 rpm such that no air bubbles enter. After 48 hours the medium becomes turbid creamy white, it is streaked onto plates which are incubated anaerobically inside the anaerobic glove box for the cultivation of strictly anaerobic bacteria. When streaking plates for enrichment there was addition of another 1.0 g of sodium succinate and 1.0 g of yeast extract per liter of enrichment medium. Since anaerobic bacteria take longer time to grow, it was allowed to incubate for 48-72 hours. If the medium is kept for a longer time it turns brown and finally black showing the presence of hydrogen sulfide.

2.2 Identification and sequencing of anaerobic isolates

After the growth of the colonies after 48hrs, 16s r-RNA gene sequencing was done to identify the isolates. The sequence analysis was done by using XL Genetic analyzer (Applied Biosystems, USA), Sequence Scanner, Version 5.2 (Applied Biosystems, USA). Many other biochemical tests were also done to identify the bacteria as hydrogen producing anaerobic bacteria. For this purpose gram staining, hydrogen sulfide production test and sugar fermentation tests were carried out (APHA standards, 1995) and (Loewus, 1952). The COD was measured by using K$_2$Cr$_2$O$_7$ open – reflux method (Pitwell, 1983).

2.3. Reactor setup

Biohydrogen production experiment was carried out in batch mode by using glass serum bottles as reactor. The working volume of the bottle was 200 ml. After the mixing of food waste with inoculum (pond water) the headspaces of the bottles were flushed with N$_2$ gas for 2 hours and the bottles were tightly sealed using open-top screw caps with rubber septa. The bottles were then placed in a magnetic shaker at 35°C and 150 rpm while one of the outlets were connected to graduated water displacement system which contains saline solution at ambient pressure and room temperature was used to know the volume of gas diffused through water to know the amount of biohydrogen produced. Biogas production volume was determined by water displacement method. This setup was kept for 72 hours. Samples were collected timely from the reactor for the estimation of pH, COD, and temperature. Production of gas was studied after 72 hours.

2.4. Biohydrogen production

Food waste was collected from our University hostel mess. It contained cooked vegetables mainly rice and potato to increase the source for starch. Cooked food was preferred over raw vegetables because it is already hydrolyzed so that degradation is easy. Food waste is grinded by using blender such that the size of the food particles is approximately 1-2 mm. The experiment was carried out in 200ml serum bottle. After adding the inoculum, it was preheated at 115°C for 2 hours so that methanogenic bacteria may die. The mixing ratio of food waste and inoculum was mixed in 1:1 ratio while the blank contained only inoculum and water. pH was adjusted between 6.8-7.2 by adding either 1M NaCl or 1M KCl. Temperature was also monitored continuously.

The effluent from the reactor was collected timely for estimation of COD reduction. The gas mixture consisting of CO$_2$, H$_2$ and other gases was allowed to pass through 40 %w/v KOH solution for selective absorption of CO$_2$ (Das and Koaty, 2006) The filtered gas was collected in a graduated water displacement system containing saline solution at ambient pressure and
3. Results and Discussion

3.1 Enrichment and isolation of anaerobic isolates

Growth of microorganisms was seen after 48 hours of incubation. First the inoculation medium became turbid white and then turned brown and finally black. After streaking on the agar plates colonies were developed which appeared spongy white which confirmed the production of hydrogen sulphide.

3.2. Identification and sequencing of anaerobic isolates

Gram staining procedure was carried out and the microscopic slide was viewed under 40X and 100X which showed pink colored bacteria proving that the mixed consortium contained more number of colonies of gram negative bacteria.

While performing the hydrogen sulphide production test brisk occurrence of bubbles (oxygen+ water) took place which clearly showed the presence of anaerobic bacteria. Sugar fermentation test is also carried out to check the ability of the mixed consortium in fermenting the available carbohydrates in the food waste. The mixed consortium fermented large amount of starch which was seen by the color change from red to yellow. Little amount of glucose and sucrose were also fermented. After 48hrs of incubation the color in Durham tubes changes red to orange and then yellow due to the fermentation of carbohydrates. Here maximum amount of starch is fermented.

The anaerobic isolates were identified by genomic sequencing of the bacteria with forward and reverse 16s rRNA sequencing by using NCBI – Nucleotide BLAST. The result showed was a mixed consortium in the form of an electrophorogram which showed most similar nucleotides with the sample. When run in BLAST query sequences, 93% of identity was found with pseudomonas. With Clostridium species 75% identification was seen. And with rhodobacter species 63% identification was observed. Hence the mixed consortium that was isolated consisted of pseudomonas, clostridium and rhodobacter species. The isolated anaerobic bacteria are renowned for their hydrogen production propensity in former reported works. Hence, the attempt was to study their abilities in consortium for biohydrogen production.

3.3 Biohydrogen production and its analysis

Hydrogen gas was produced with constant optimization of temperature, pH and Chemical Oxygen Demand. The data is given as follows.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.30</td>
<td>42.1</td>
</tr>
<tr>
<td>Food waste</td>
<td>6.80</td>
<td>37.2</td>
</tr>
</tbody>
</table>
From table 1 it was observed that optimal pH (5-8) and temperature (29-40˚ C) was maintained throughout the process. From table 2 it was observed that for control the reduction in COD is not very significant but for food waste it decreases significantly. The decrease in COD suggests that food waste degrades more readily and produces more amount of gas.

There is a specific range of temperature and pH that has to be maintained throughout the process for better yield of hydrogen. Different waste materials have been successfully used in different processes for the hydrogen generation. Starch based waste has great potentiality for the H₂ production pH value in the range of 6.8-7.2 is considered to be most favorable for both hydrolyzed and raw starch (Chen et al., 2001). Other parameters like COD was also measured for control (without food waste) the COD values do not decrease much hence showing lesser production of hydrogen. On the other hand inoculum with food waste showed very big difference proving that hydrogen production was more with food waste. COD for both initial and final samples was measured to know the extent of degradation.

The total volume of gas produced was calculated as 49.55 ml of H₂/g COD reduced. The hydrogen yield from this study (49.55 ml H₂/ g COD reduced) has been found to be distinctively higher than by using heat –treated sludge which shown similarity to those of anaerobic spore forming bacteria, *Clostridium sp.*, on food waste (Kim et al., 2004) and by the mixed consortium of *Enterobacter cloacae*, *Citrobacter freundii* and *Bacillus coagulans* on sewage sludge (41.23 ml H₂/ g COD reduced) (Das and Koaty, 2006). However, it was lower compared to the yield obtained from co-digestion of food waste and sewage sludge (122 ml H₂/ g COD reduced (Kim et al., 2004). Nevertheless, it was found that the addition of food waste to the seed sludge enhanced hydrogen production as the hydrogen production was the highest at the moderate reaction rate and fermentation efficiency (Kim et al., 2004). Also the addition of inoculum to food waste would be beneficial in field-scale operation. Mixed consortium is more beneficial for hydrogen production than pure cultures and there are less chances of contamination and less sterile conditions can be used.

4. Conclusion

This preliminary study analyzed the hydrogen producing capability of isolated mixed consortia on food waste. The mixed consortium had shown better biohydrogen production capability than pure culture. The further studies will be carried out to strongly errand the effect of consortium by comparing with individual isolates, to analyze the composition of food waste, technical optimization of various process parameters to improve the yield of biohydrogen production and analysis of biohydrogen production by gas chromatography.
5. References


