Hydrogen Production From Peach Waste By Thermophilic Bacterium *Thermotoga neopolitana*

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Hydrogen As Fuel

- Hydrogen is one of the answers to foreseeable energy crisis and environmental pollution.
- High conversion efficiency and non polluting nature.
- 50 million metric tons of hydrogen being traded worldwide with a growth rate of 10%.
  - Primarily from fossil fuels.

Hydrogen production by Dark Fermentation

- Hydrogen can be produced by anaerobic bacteria through dark fermentation.
- Anaerobic bacteria capable of hydrogen production includes species of *Enterobacter(Nath et al., 2006)*, *Bacillus(Kotay and Das, 2007)*, and *Clostridium(Ferchichi et al., 2005; Zhang et al., 2006), Thermotoga (Schroder, 1994)*.

Hydrogen production by Dark Fermentation

- Dark fermentation can use various renewable biomass including agriculture waste(Hussy et al., 2005; Logan et al., 2002), municipal waste(Wang et al., 2003), food processing waste(Van Ginkel et al., 2005)
- The amount of hydrogen production from glucose by bacterium is affected by metabolic pathway and end-products.

Stoichiometry

- $C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2$
  $\Delta G = -6.18$ kcal/mol (Thauer, 1976)
- $C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CH_3COOH + 4H_2 + 2CO_2$
  $\Delta G = -51.6$ kcal/mol (Thauer, 1976)
- $C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CH_3CH_2COOH + 2H_2 + 2CO_2$
- $C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2OH + CH_3COOH + 2H_2 + 2CO_2$

Microorganisms used

- Hydrogen-producing microbes have been found in environments with a wide range of temperature, including mesophiles (25-40°C)(Kotay and Das, 2007; Shin et al., 2007), thermophiles (40-65°C), extreme thermophiles (65-80°C), or hyperthermophiles (>80°C) (Jannasch, 1988).

Thermotoga neopolitana

- Originally isolated around the bay of Naples, Italy(Belkin et al., 1986), *Thermotoga neapolitan* is a Gram-negative, rod-shaped, obligate anaerobic, fermentative extreme thermophile surrounded by a bag-shaped sheath-like outer structure called "toga"(Huber et al., 1986).
- *Themotoga neapolitana* accumulated 25-30% hydrogen during its incubation(Schroder, 1994).

Prior Research at Clemson

- Carbon source affected the incubation time of *Thermotoga neapolitana* to produce hydrogen.
- *Thermotoga neapolitana* can utilize different carbon sources ,to produce hydrogen, such as glucose, sucrose, xylan, rice flour, cellobiose, corn starch, starch.

Prior Research at Clemson

- pH and hydrogen partial pressure (pH_2) are two important factors that affect the hydrogen production and bacterial growth.
- When pH decreased to 5.0, it appears to inhibit hydrogen production.
  - (Huber and Hannig, 2006; Ravot et al., 1995; Van Ooteghem et al., 2002).
- Maximum hydrogen pH_2 in this study was 45 kPa.
  - Hydrogen production is inhibited at partial pressures

Effect of pH

- Jannasch et al reported that pH range for growth is between 5.5 and 9(Jannasch et al., 1988). Van Ooteghem also reported that pH of medium dropped from 7.5 to 4.5 within 20hrs.
- The proton concentration affects the yield and rate of hydrogen production and the range of pH favorable to hydrogen production is narrow(Lay, 2000)

Agricultural Wastes- The Answer

- Fermentation of agricultural waste products provides a much cheaper option for production of hydrogen.
- South Carolina –second largest producer of peaches in US.
- 20 million pounds of rotten or spoiled peach waste dumped annually.

Peach Waste As Substrate

- This peach waste has high organic value with 4.6%-9.6% of sugars (Wills et. al., 1986) on wet weight basis.
- Major sugars include glucose, fructose, xylose.

Objectives of study

- Investigate the hydrogen production by *Thermotoga neopolitana* on peach media.
- Investigate unautoclaved peach media for hydrogen production.
- Investigate the effect of pH on hydrogen production using peach media.

Methods Used

- All experiments were done in triplicates using 500 ml serum bottles as batch reactors with 100 ml working volume.
- The media used consisted of 50gms/liter of meshed peaches (on wet weight basis) as carbon source along with nitrogen sources (ammonium chloride, trypticase and yeast extract) and other nutrients.
- The batch reactors were run at 77C for 20 hrs.

Methods Used

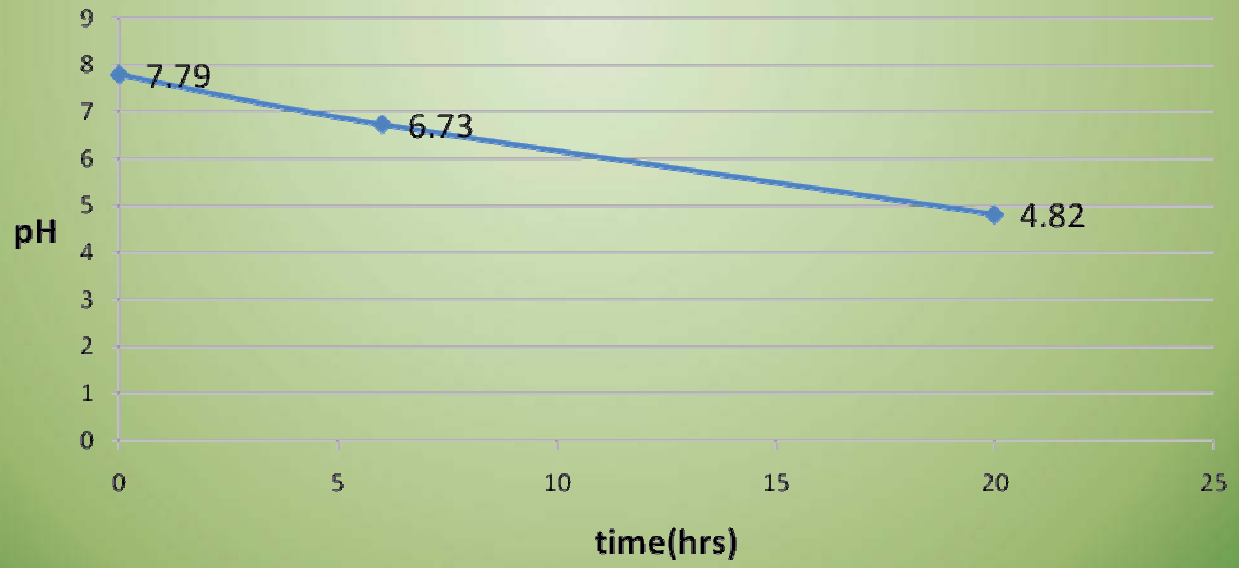
- Hydrogen concentration in the headspace was analyzed through gas chromatography using TCD detector and Argon as the carrier gas on a silica column.

Results

- Using T-test, at 5% level of significance, there was no difference in the mean hydrogen concentration produced after 20 hrs for batch reactors with 5 g/L glucose and reactors with 5 g/L (dry weight basis) peaches as carbon source respectively.

Results

- For the autoclaved media the pH decreased to 4.88 after 20 hrs of incubation. Hydrogen concentration did not increase beyond 20 hrs.



Unautoclaved vs. Autoclaved

Media	[H2] (%) after 20 hrs	Standard Deviation
Unautoclaved	24.74	2.33
Autoclaved	24.33	0.70

Results

- The initial pH of 8 was found to be the best for hydrogen production using non autoclaved peach media. Initial pH of 8.5 was found to be the next best pH.

Initial pH	[H2] (%) after 20 hrs	Standard Deviation
7.5	18.37	3.6
8.0	24.23	0.69
8.5	22.08	0.098

[H2] (%) vs. Initial pH

Results

- [H2] when pH was set back to pH of 7.5 and 6.5 respectively after 12hrs of incubation.

a) There was an approximate increase of 21.2% in hydrogen gas concentration when pH was set back to 7.5 after 12 hrs of incubation.

b) There was an approximate increase of 20.6% in hydrogen gas concentration when pH was set back to 6.5 after 12 hrs of incubation.

Conclusions

- Peach media using peaches as carbon source was found suitable for hydrogen production with hydrogen production of 24%-25% after 20 hrs of incubation at 77C.
- Unautoclaved peach media can be used for hydrogen production on peach media.
- An initial pH of 8 was found best for hydrogen production on unautoclaved peach media.
- pH maintenance is required for hydrogen production on peach media.

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