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FERMENTATION OF BANANA FRUIT TO PRODUCE BUTANO

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ABSTRACT

Banana dominates the export and domestic market of Taiwan. According to statistics of the past 5 years, an estimate of 300,000 tons of bananas is produced annually which make its yield the highest among all the pomological products in Taiwan. 10% are found to be below par in the midst of commercialization.

With the widespread of environmental awareness, the concept of energy conservation by recycling is becoming a highly plausible alternative. Butanol has shown great potential to be developed as a renewable energy source .It produces 25% more energy than that of gasoline and yet its properties remain similar to gasoline. Butanol is more miscible with gasoline than ethanol and it does not corrode pipelines easily as ethanol does.

To analyze the yield of butanol, different concentrations (2%, 6%, and 18%) of discarded banana fruit were added to three culture mediums of *Clostridium acetobutylicum*. A pure culture medium without added waste was also prepared as a controlled group. *Clostridium acetobutylicum* will utilize the banana fruit's high composition of carbon, vitamins, minerals and trace metals content. Hence, it is proven that the carbon content has a direct effect on the yield of butanol. In this process, acetone, ethanol and butanol are produced. ABE fermentation is allowed to occur for 120 hours under constant environmental conditions. Finally, gas chromatography was used to isolate and calculate the yield of butanol.

Experimental results showed that fermenting 2% of banana fruit for 120 hours produced the highest yield of 8.78 g / L of butanol and 3.25 g / L of acetone. Culture medium with added banana fruit decreased the formation rate of butanol, but increased the yield of butanol.

However, ethanol yield in all groups showed no significant difference. This means, addition of banana fruit to increase ethanol yield is not significant.

KEYWORDS: ABE Fermentation, Bioresource, Clostridium, Acetobutylicum

INTRODUCTION

In recent times increased fossil fuel use have depleted the Earth's resources. Due to the shortage of fossil fuels, the concept of recycling and reuse are being increasingly employed. Moreover, the exorbitant prices of fossil fuels price and their low availability is making renewable energy an alternative source. When the man power used in agriculture operations was gradually replaced by automation and mechanization, this enabled mass production to occur at much lower cost. For example, banana dominates the export and domestic market of Taiwan. According to statistics of the past 5 years, an estimate of 300,000 tons of bananas is produced annually which make its yield the highest among all the pomological products in Taiwan. 10% are found to be below par in the midst of commercialization. This issue can be resolved using fermentation engineering, to obtain biomass energy which can reduce energy shortages and reuse agricultural waste.

According to the literature, banana fruits have rich carbon sources, and therefore butanol can be yielded with the use of ABE fermentation so there is a direct relationship the carbon source and the amount of butanol formed .In this

experiment batch fermentation was used due to its low cost, and also to explore its effectiveness in fermenting banana fruit using Clostridium acetobutylicum. (Wu Menglong, 2013)

A process that uses bacterial fermentation to produce, metabolites acetone, butanol, and ethanol under anaerobic conditions. ABE fermentation metabolites contain solvents of butyric acid, propionic acid, lactic acid and acetic acid, respectively. The metabolic pathway that was used was named the "butterfly" displacement effect to produce acetone, ethanol and butanol, and produced butanol as the main metabolic pathway. In the early stage of the ABE fermentation engineering, fermentation by batch culture was employed .The concentration of the butonal was more than 1% which will inhibit bacterial growth. However, the skills of engineers improved the way in which fermentation occurred, but the butanol production was still low at 25%. With the help of engineering and technical expertise using two strains of Clostridium tyrobutyricum and Clostridium acetobutylicum with use of continuous and immobilized reactor which can make the butnaol yield up to be 42% which one can maximize the products of hydrogen and butyric acid, another can conversion can be butyric acid into butanol.

Compared to the early stages of the modified ABE fermentation which can eliminate the solvent acetic acid, lactic acid, propionic acid, acetone, ethanol, isobutanol and only generate the hydrogen gas, butyric acid, alcohol and carbon dioxide, and therefore butanol yield can be increased. Anaerobe which refers to an organism which does not require oxygen for growth there are two types of anaerobes obligate anaerobe and facultative anaerobe. The obligate anaerobes were defined when the growth in non-anaerobic environment which they will be death or spore formation that the reason was under in aerobic environment making cells the superoxide dismutase and catalase lacked causal. On the other hand ,the facultative anaerobes when in the anaerobic environment can available for aerobic respiration ,in otherwise, to do the hypoxic respiration which can let glucose by glycolysis into pyruvic,and then the pyruvic by decarburization to remove the carbon to form the aldehyde and at the same time to release CO2. Among them the aldehyde by glycolysis to produce the NADH2 which revert to form the ethanol and (ATP).(Wu Menglong, 2013)That was higher compared tolerance to strictly anaerobic bacteria, and which can grow in aerobic environment. Unless the products have exception of requests otherwise the products were requested under anaerobic environment for the spindle. In this study, the Clostridium acetobutylicum belonged to obligate anaerobes family which the literature says the formation of endospores help protect itself when in aerobic environment until suitable conditions of anaerobic environment will restore its characteristics, so it was very easy to operate in the experiment. pH value is important as a control parameter to maintain the cells in a suitable environment. When the pH value is too high or too low it inhibits growth and functioning of the cell in the study, the Clostridium acetobutylicum was suitable in pH values between 6.5 to 7. In Anaerobic fermentation processes there are various temperatures needed for growth, these temperatures can be divided into three groups, psychrophils (less than 20℃), mesophils (Between the 20 to 45 ℃),and themophils(more than 45 ℃),respectively. In present study, the Clostridium acetobutylicum belong to mesophils, group and has an annual average temperature of about 30 ℃ which the most suitable for the survival of mesophilic in Taiwan. The most commonly studied bioreactors include batch, fed,-batch,free cell continuous, respectively. Free cell continuous refers to the new culture medium and vessel were exchanged with the same flow, rate and speed simultaneously (at the same time) removed and supplied to the broth such that the broth content of the same in vessel, can increase the nutrient sources and the concentration of the liquid product of the fermentation, pH, cell density, growth rate can remain stable. Based on description above, the nutrient source supplied different with the fed-batch. Batch culture is easy to control which refers the culture medium and cells at the same time to join the vessel until the

end of the fermentation period which has pH value control, air exchange,and no exchange of nutrient sources and materials in vessel. In the present study, batch fermentation has low cost, easy to operate and easy to identify parameters. Based on this study, we examined the pH values, pressure and culture, adding extra banana fruit (single variable) to improve the feasibility of butanol yield. Clotridium acetobutylicum. Clotridium acetobutylicum is belong to obligate anaerobe which grows without oxygen in the environment, and the products of fermentation creates liquid products with acetone ,butanol, ethanol as well as the gas containing hydrogen and carbon dioxide. When in aerobe environment an inhibited cell grows endospores to protect cell itself. Spores are not dead; however, under reactor conditions they are termed inactive, then in the present study, the cells are easy to operate. Glucose, starch and carbohydrates used for generating the solvent have a direct relationship with the carbon source. The carbon source is a major component of the skeletal system of the cells, and can help promote the synthesis of extra-cellular substances. The protein was used as a nitrogen source which helped generate gaseous products such H_2 and CO_2.

(Ji Yi Quan, 2011)

EXPERIMENTAL APPARATUS AND METHODS

Materials

In this study, we use the North banana which named “Musa Tai Chiao No.1” introduced from the South of China is common specie in Taiwan. First, we made the medium with Beef extract (0.4g), peptone (0.4g), NaCl (0.2g), glucose (0.2g), yeast extract (0.12g), CH_3COONa (0.12g), soluble starch (0.04g) and agar (0.72g) which was added to 40 ml deionized water contained in a 100 ml screw-capped Pyrex bottle. The medium was sterilized at 121℃ for 10 minutes then cooled to room temperature to provide an anaerobic environment. When above process, is completed the strains were added for 12–16 hours in an anaerobic environment at 35~37℃ and pH values of 6~7. The process was repeated so that the cells can be domesticated which lead to them becoming stable. Then the domesticated strains were usedwhen it was stable for a fermentation period of 120 hours at a temperatures of 35~37℃ and pH values 6~7 ,the batch culture medium composed of 1L total volume of the different concentration of total volume 2%, 6% and 18% of the bananas fruits (used the heterogeneous machines crushed), glucose (70g), CH_3COONH_4 (2.2g), Yeastextract (1.5g), K_2HPO_4(0.5g), KH_2PO_4 (0.5g), $MgSO_4 \cdot 7H_2O$ (0.2g), $MnSO_4 \cdot 7H_2O$ (0.01g), $FeSO_4 \cdot 7H_2O$ (0.01g) and NaCl (0.01g) in anaerobic environment (N_2,100%).

Figure 1: The Clostridium acetobutylicum BCRC10640 strains stored at 4————————————C in distilled
Which came from the Food Industry Research and Development Institute

Analyses

Solvents were determined by gas chromatography (SHIMADZU GC-2014) using flame ionization detector and 4M x 3mm stainless steel column (SHINCARBON ST 80/100 mesh). The broths were analyzed (solvent ABE) per 24h for setting centrifuge for 13000rpm for 10min,and then the supernatant was filtered through a 0.22 µm filter by injecting the samples into the gas chromatography, with three replications being done.

RESULTS AND DISCUSSIONS

After 24 hours of fermentation of the medium which had no banana fruit, it was found to have higher reaction rates, which likely caused bacterial activity to be inhibited. When the cells generated metabolites there was a sharp decrease in the ph values. When an 18% concentration of banana fruit was used the magnitude of the ph value changed.slowly because the higher banana fruits concentration of the medium the lower decomposition of the reaction rate.

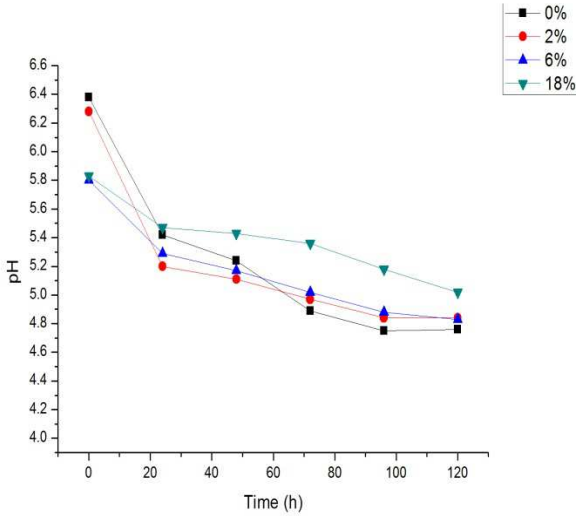

Figure 2: Graph Showing Added Different Concentration of Banana Fruit the pH Value From ABE fermentation

The non-adaptation of cells to the environment made the acetone yields non-significant within 0-24hours. However in the logarithmic growth phase the cells started to produce a large volume of acetone. Within the 120 hours we detected that when a 2% concentration of banana fruits was added to the fermentation broth, a yield of3.25g/L.

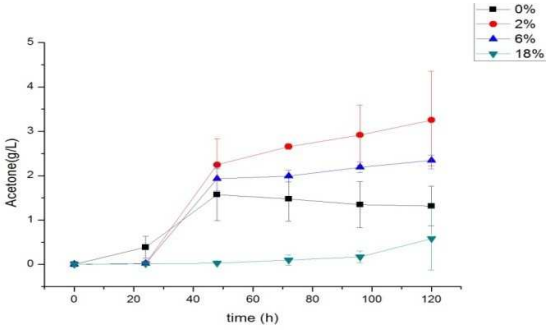

Figure 3: Graph Showing Added different Concentration of Banana Fruit the Acetone Yield from ABE Fermentation

Within the 0-24 hours the adapted cells did not generate a significant amount of butanol. But in the logarithmic growth phase of 48 hours the cells released the amount of undissociated acid, which generated the metabolites and balanced the ph. In the experiment results showed that when adding 2% concentration of banana fruit the amount of butanol present in the broth was 8.78 g / L within 120 hour time frame.

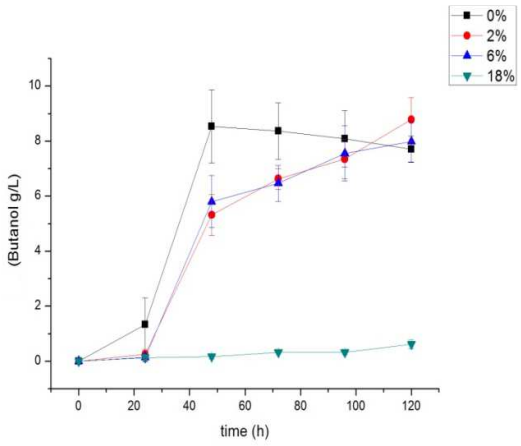

Figure 4 : Graph Showing Added Different Concentration of Banana Fruit the butanol Yield from ABE Fermentation

When the 6% concentration was used results showed that an ethanol yield of 0.415g/l was obtained and when compared to the standard a yield of 7% less than yield 6% concentration was obtained, this turned out be a actual yield of 0.386g/l, and when compared to the other samples the lower ethanol yields proves to be less economical due to the fact that if further purified very small amounts of ethanol maybe obtained

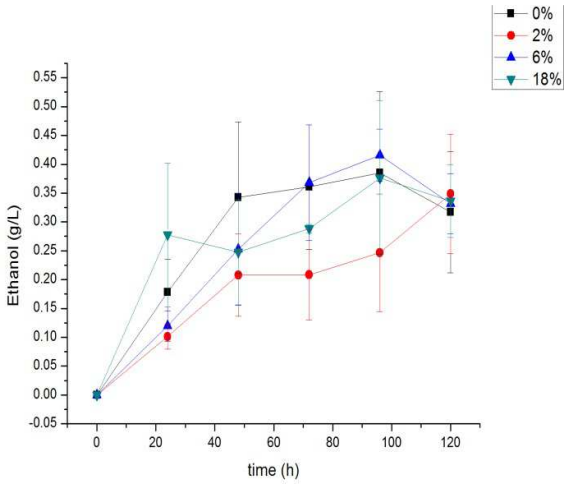

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Figure 5 : Graph Showing Added Different Concentration of Banana Fruit the ethanol Yield from ABE Fermentation

From the figure 5, the fermentation final time of 120 hours obtained the highest yield of the solvents butanol8.78g/L and acetone and3.25g/L, respectively and in 96 hours of the highest yield of ethanol obtained was 0.415g/L. The experiment results showed, adding optimum amount of bananas fruits in to medium, increased the yields of acetone and butanol, but an increase in ethanol yield was not significant.

Table 1: Comparison of Solvent in ABE Fermentation from Added Different Bananas Fruit Concentration

Added proportion of bananas fruit	Initial pH	Final pH	Acetone(g/L)	Butanol(g/L)	Ethanol(g/L)
0%	6.38	4.76	1.57	8.53	0.385
2%	6.28	4.84	3.25	8.78	0.348
6%	5.80	4.83	2.34	7.98	0.368
18%	5.83	5.02	0.58	0.93	0.376

CONCLUSIONS

Proper use of plants and agricultural waste for butanol is feasible route to replace fossil fuels. Culture medium with added banana fruit decreased the formation rate of butanol, but increased the yield of butanol. If the fermentation time lengthens until 120 hours, adding highest concentration of banana fruits will result in highest butanol yield. Based on the experiment results, in the future using continuous fermentation and increased use of banana fruits which maybe have considerable results and can have a higher processing efficiency for agricultural waste. Banana fruits are low cost compared to the pseudo stem it means the pseudo stem would be costlier for the conversion of cellulose into a carbon source, because banana fruit have trace metals that could be used for cell growth. However, because of pseudo stem high yield and high efficiency process banana is a potential source for producing butanol.

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