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Efficiency And Optimization of Bioethanol Production From Bagasse Pretreated By Saccharification And Co-Fermentation Process Using Commercial Cellulase And Saccharomyces sp. To Economic Cost Analysis

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***Abstract*—** The problem of overflowing bagasse in the three southernmost provinces of Thailand has negatively impacted the environmental system. The utilization of bagasse for bioethanol production was one approach that helps in removal and added value. This research aims to study the optimum conditions for the Saccharification of bagasse with commercial cellulase. We found that cellulose was added at 2.5 Units with cellulose, hemicellulose, and lignin content of 72.35 ± 4.36, 11.05 ± 0.95, and 1.12 ± 0.16 percent, respectively. We analyzed the bagasse solution's monosaccharides content that was properly pretreated with High-performance liquid Chromatography using C-18 Column and water as Mobile phase with flow rate at 0.4 ml/min. The result was that the content of Sucrose, Glucose, Xylose, Fructose, and Mannose were 45.25, 36.45, 10.32, 4.32, and 2.36 Percentage, respectively. We studied the optimum conditions for bioethanol Co-fermentation. The preparation of bagasse sugar solution with the initial sweetness of 20 degrees Brix and 10% starter inoculant was more effective than other conditions. The bioethanol content after Co-fermentation was 12.76 percentage at 48 hours. After the distillation for three cycles of such situations, the result was that Bioethanol purity was 32.25, 65.85, and 87.56 percent, respectively. After that, the evaluation of worthiness of bioethanol production in 5 years for community investment was two years in the investment assessment, which could return to the operator. The payback period (PB) was 2.65 years.

***Keywords*—** *Bagasse, Bioethanol Production, Saccharification, Co-Fermentation Process, Saccharomyces sp. , Economic Cost Analysis*

I. INTRODUCTION

Currently, energy is one of the essential factors for production in both business and industrial sectors where energy supply is sufficient and reasonable to meet demand is something that should be done, especially from domestic energy sources.[1,2] But in the past, there was insufficient energy supply from domestic sources. It has to rely on foreign energy sources for more than 60% of the total commercial energy demand.[3-5] Therefore, to ensure that Thailand will still have sufficient energy to meet demand in the future. Therefore, there was one guideline, which was to develop domestic energy sources. The energy source should always be cheap and abundant enough.[3,6] The

renewable energy source from biomass is one of the most likely energy sources. They are easy to find, inexpensive, and contain some nutrients that are likely to be recycled. [7] Therefore, this energy's potential studies should be conducted urgently to reduce the risk of dependence on foreign fossil fuels that are becoming increasingly expensive and scarce.[8] In the energy produced from biomass, The global focus is on ethanol because it can be produced from biological processes. This is caused by the fermentation of biomass conversion from starch into sugar and from sugar to ethanol by some enzymes or chemicals that are used to digest and purify ethanol by distillation and water separation for use as an ingredient in various industries. [8-10]

Most of the world's ethanol production uses two primary raw materials: 1. Sugar such as sugar cane and molasses 2. Flours such as cassava, rice, and corn. We began to have anxiety that Such raw materials may not be sufficient for ethanol production in the future. [11] Sugarcane bagasse was the remnant of a cane trunk that has been removed from the block.[12] Bagasse contains two components: an 82 percent cell and an 18 percent cell wall. It consists of 40 percent cellulose, 29 percent hemicellulose, 13 percent lignin, and 2 percent silica.[13,14] The function of cellulase enzyme activity consists of 2 steps: The first step was Prohydrolytic step as an Anhydroglucose chain that will be swollen. The second stage was Hydrolytic cleavage of the polymer chain. The mechanism of action starts from cellulose to swell up with the breakdown of the hydrogen bond. The resulting interaction with Endoglucanase and Exoglucanase could be free Saccharification while exoglucanase pulls cellobiose molecules from the ends, which are further degraded by β-glucosidase to free glucose.[15-16]

The present study's objective was to Biological pretreatment of bagasse with commercial cellulase enzymes and study optimum conditions for ethanol production with yeast infection *Saccharomyces* sp. TISTR 5091. More than that, the evaluation of worthiness of bioethanol production in 5 years for community investment. To provide supporting information for use in the ethanol production process and alternative use of agricultural waste materials.

II. MATERIALS AND METHODS

A. Material Media and Strain

The sugarcane bagasse was taken from Local market of Yala Province, Thailand. For the sugarcane bagasse, the contents of cellulose, hemicellulose and lignin were determined as described by Van soest [26] were 38.25 ± 2.48%, 24.36 ± 5.54% and 16.85 ± 1.93% respectively.

Saccharomyces Sp. TISTR5091 (Ethanol active dry yeast) was sourced waste water from sugarcane factory (Thailand Institute of Scientific and Technological Research)

Yeast Malt Agar (YM Agar) medium was used for maintenance of the cultures. The medium was composed of 5 g Peptic digest of animal tissue, 3 g Yeast extract, 3 g Malt extract, 10 g Dextrose, 20 g Agar and 1,000 ml Distillation water. [15]

B. Saccharification of bagasse with commercial cellulase enzymes

The selected samples were adjusted to pH 5.0, then added 1.25 and 2.5 Unit commercial cellulase enzymes for 60 minutes, then centrifuge at a speed of 9,000 rpm.[23,24] The supernatant was analyzed for the concentration of Reducing sugar by 3,5-dinitrosalicylic acid (DNS) lignocellulose content analysis (Detergent method , Neutral detergent fiber (NDF) Analysis, Acid detergent fiber

(ADF) Analysis and Acid detergent lignin (ADL) Analysis) and Estimated monosaccharide was analyzed by High-performance liquid Chromatography (HPLC) by Column C-18 used water as mobile phase, 0.4 ml/min of flow rate condition [2, 13, 24]

C. The optimum conditions for ethanol production with Saccharomyces sp.TISTR 5091

Take a sample of a selective monosaccharide solution. After that use a substrate was selected for Saccharification And Co-Fermentation Process Fermented by measuring the brix to be approximately 15 and 20 degrees brix, then add yeast inoculum at 5, 10, and 20 percent concentrations Then fermented at room temperature Samples were then collected every 2 hours for 96 hours and analyzed for ethanol, pH and Total of *Saccharomyces* sp.TISTR 5091 Shown in Figure. 1 [17-23]

Figure 1. The Ethanol fermentation with Saccharomyces sp.TISTR 5091 from bagasse that has been treated

D. The evaluation of worthiness of bioethanol production in 5 years for community investment

The best condition of ethanol production process was to select economic calculations such as Net present value (NPV), and Payback Period. They were used Return On Investment= ((Discounted Benefits Discounted Costs))/(Discounted Costs) and Payback Period = Number of years prior to full recovery+((Unrecovered cost at start of year)/(Cash flow during full recovery year)) [22-24].

III. RESULTS AND DISCUSSION

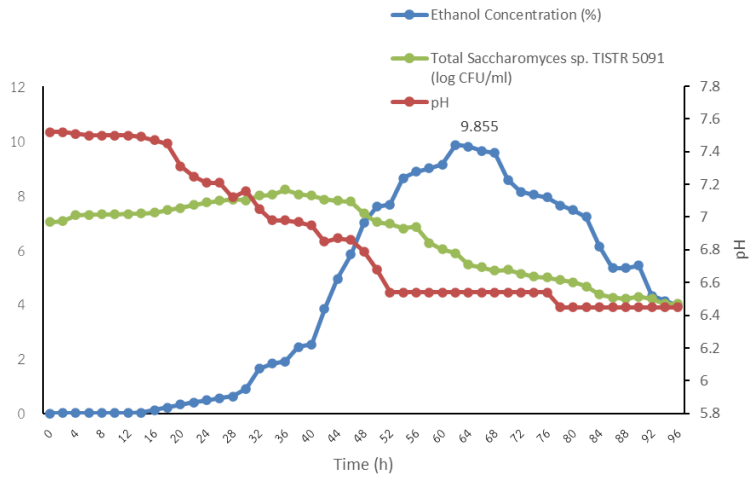
A. The saccharification of bagasse with commercial cellulase enzymes

The bagasse pretreated was adjusted to pH 5.0 and then added 1.25 and 2.5 Unit commercial cellulase enzymes for 60 minutes. Then It centrifuged at a speed of 9,000 rpm, Supernatant was used to analyze the concentration of reducing sugar by 3,5-dinitrosalicylic acid (DNS) method. The result was found that to reducing sugar content were 2,235.65 ± 16.32 and 2,352.32 ± 12.14 ml/ml, respectively. The residue obtained by separation from bagasse solution was found that the lignocellulose content were Cellulose, Hemicellulose and lignin were 65.25 ± 2.36, 14.36 ± 0.85 and 2.65 ± 0.25 percent respectively., for addition cellulase group at 1.25 Unit. And cellulose, hemicellulose and lignin content of 72.35 ± 4.36, 11.05 ± 0.95 and 1.12 ± 0.16 percent, respectively. For the cellulase-added group at 2.5 Unit. shown in Figure. 2 When analyzing the monosaccharides content of the bagasse solution that was properly pretreated with the High- HPLCusing Column C-18 using water as Mobile phase and Flow rate at 0.4 ml /min found that the amount of Sucrose, Glucose, Xylose, Fructose and Mannose was 45.25, 36.45, 10.32, 4.32, 2.36. Percentage, respectively.

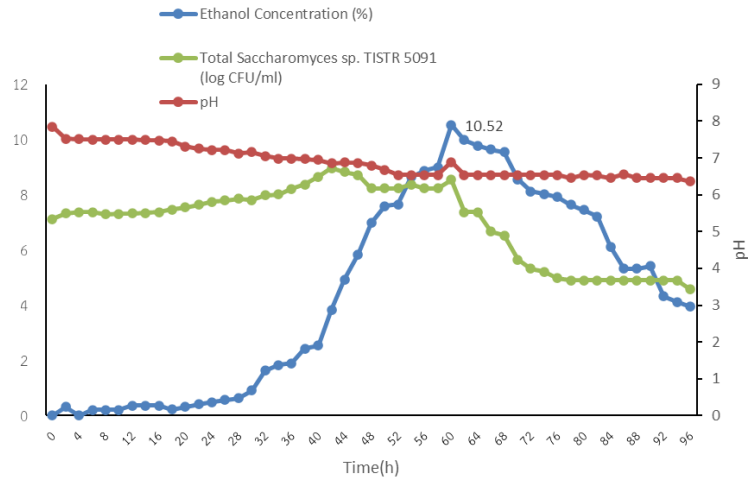
Figure.2 *Characteristics of bagasse that was treated using a was selected and add 2.5 Unit cellulase enzyme for 60 minutes.*

B. The optimum conditions for ethanol production with Saccharomycesm sp. TISTR 5091

A monosaccharide that can be selected The brix was measured to be approximately 15 and 20 degrees, then added *Saccharomyces* sp. TISTR 5091 at concentrations of 5, 10 and 20 percent and fermented at room temperature. The Sampling were collected every 2 hours for 96 hours and analyzed for ethanol, pH and Total *Saccharomyces* sp. It was found that the preparation of sugar solution from bagasse with initial sweetness of 20 degrees brix and the use of the initial inoculant 10 percent was more effective than other conditions. Shown in figure. 3 and 4



(A)



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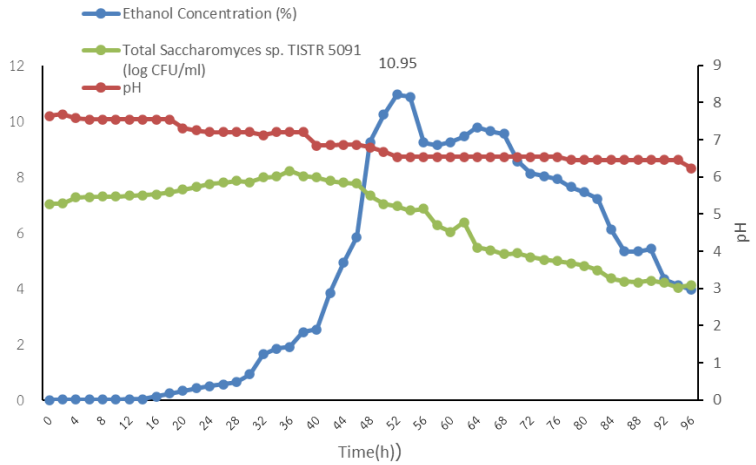
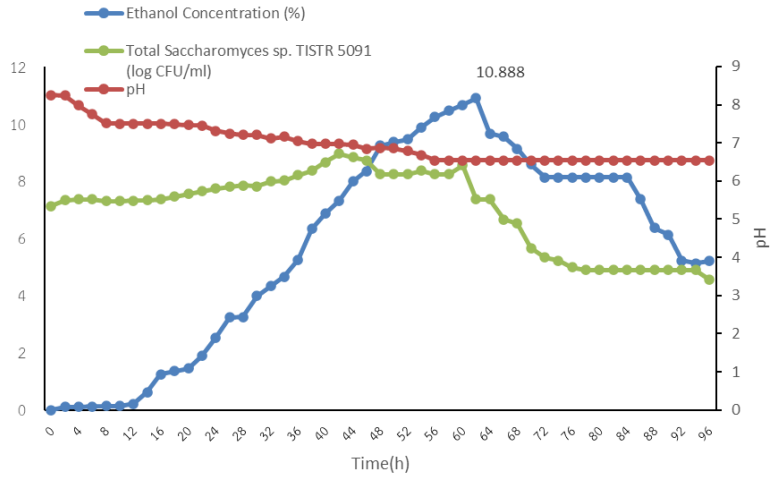
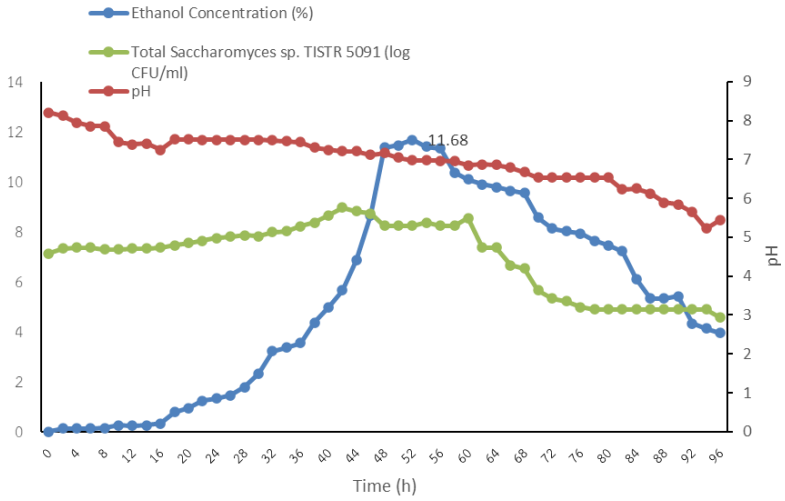


(C)

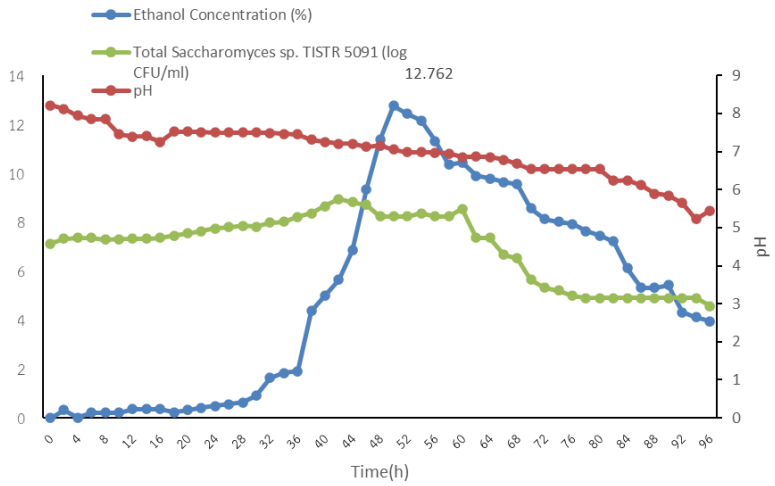
Figure.3 Ethanol Production, pH and Total *Saccharomyces* sp.TISTR 5091 were A) 5 percent starter, B) 10 percent starter, and C) 20 percent starter, and bagasse solution selected has value of 15 degrees Brix.



(A)



(B)



(C)

Figure.3 Ethanol Production, pH and Total *Saccharomyces* sp.TISTR 5091 were A) 5 percent starter, B) 10 percent starter, and C) 20 percent starter, and bagasse solution selected has value of 20 degrees Brix.

The Figures 3 and 4, it was found that using an initial fraction of 15 degrees brix with an initial inoculation of 5 percent yielded 9.855 percent of ethanol at 62 hours. It was found that the ethanol content was reduced. Likewise, pH will decrease from weak base to weak acid approximately from 8.00 to 6.00. *Saccharomyces* sp. TISTR 5091 was initially used for 7 log CFU / ml. The total of bacteria will grow until the peak will then be reduced. But there will be more cell mass in the fermentation tank Which the working mechanism is like in every state of the experiment from the study, it was found that the optimum conditions for further distillation were The 20-degree brix with the 20% initial inoculum was able to produce 12.762% ethanol at 48 h after three distillation cycles of these conditions. The purity of ethanol was 32.25, 65.85 and 87.56 percent respectively.

C. The evaluation of worthiness of bioethanol production in 5 years for community investment

The evaluation of worthiness of bioethanol production in 5 years for community investment was two years in the investment assessment, which could return to the operator. The payback period (PB) was 2.65 years under the calculate of economic equation.

IV. CONCLUSIONS

The optimum conditions for the Saccharification of bagasse with commercial cellulase. We found that cellulose was added at 2.5 Units with cellulose, hemicellulose, and lignin content of 72.35 ± 4.36, 11.05 ± 0.95, and 1.12 ± 0.16 percent, respectively. Gave a reducing sugar 2,235.65±16.32 mg/ml The bagasse analyzed solution's monosaccharides content that was properly pretreated with HPLC using C-18 Column. The result was that the content of Sucrose, Glucose, Xylose, Fructose, and Mannose were 45.25, 36.45, 10.32, 4.32, and 2.36 Percentage, respectively. The bioethanol content after Saccharification and Co-fermentation was 12.76 percentage at 48 hours. as 20 degrees Brix and 10% starter inoculant was more effective than other conditions. After the distillation for three cycles of such situations, the result was that Bioethanol purity was 32.25, 65.85, and 87.56 percent, respectively. After that, the evaluation of worthiness of bioethanol production in 5 years for

community investment was two years in the investment assessment, which could return to the operator. The payback period (PB) was 2.65 years.

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