Design and Development of a Simultaneous Saccharification and Fermentation-Distillation Apparatus

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Abstract *- Bioethanol is a form of quasi-renewable energy produced from common agricultural crops like cassava, corn, potato and sugarcane. In order to improve the conversion process, Simultaneous Saccharification-Fermentation process was developed. Saccharification and fermentation are done simultaneously in order to decrease enzymatic end-product inhibition thus producing a better yield. Such process involves the use of bioreactor for controlling parameters like temperature and pH where enzymes can perform its best. In this study, Simultaneous Saccharification Fermentation-Distillation apparatus was designed and developed mainly for the production of bioethanol from cornstarch. The design was based on the idea that there existed so many studies about biomass conversion to bioethanol, but still the country's modern technology could not suffice the completion of such bioreactor. Design considerations were brought up to enhance the performance of the apparatus. The efficiency of the prototype was measured by comparing the %ethanol obtained from the prototype to that from the laboratory scale. Results from the laboratory experiment and the prototype testing were 6.48%, 6.76% and 7.43%, 9.53% respectively. The mean percent ethanol of the prototype exceeded the mean percent ethanol of the laboratory sample thus proving its efficiency. Temperature ranges during saccharification and fermentation process was resolved thus improving both the yield and process efficiency. Also, the manual laboratory experiment was a laborious and uncontrolled one while the prototype was easy to use and had the capacity to measure and control all the important parameters of the process.*

Keywords *– bioethanol, bioreactor, fermenter, simultaneoussaccharificationfermentation process*

INTRODUCTION

Fuels and energy sources that are made from organic by-products or naturally occurring, living organisms are known as biomass fuels or simply biofuels. According to Republic Act No. 9367, also known as the Philippines's "Biofuels Act of 2006", the State should develop and utilize indigenous renewable and sustainably-sourced clean energy sources to reduce dependence on imported oil; and ensure the availability of alternative and renewable clean energy without any detriment to the natural ecosystem, biodiversity and food reserves of the country [1].

The Philippines embraced the development of biofuels hoping to achieve future energy security, augment farmers' income, and generate rural employment. The member economy also hoped to position itself as a leading biofuels producer in the region. The main challenge facing the industry is the availability of feedstock and the processing facilities to meet the demand of the government's National Biofuels Program [2].

As the country sought to end its dependence on oil, alternative sources of fuel were being examined. Bioethanol has been developed as both an additive and an alternative to fuel. It can be produced either from petroleum bases or from sources known as biomass which include wood, grass, grains, or indigestible plants. Sugarcane and corn both contain readily assessable sugars are popular fuel sources because very little processing needs to be done in order to prepare the feed for fermentation. Another alternative source for biofuel is from cellulosic biomass. Crops like switch grass, poplar trees, and straw as well as waste from paper mills or livestock such as cattle can be converted into ethanol [3].

In an effort to improve the process of ethanol conversion, industry created a process called Simultaneous Saccharification and Fermentation (SSF). SSF combines the hydrolysis step and the fermentation step in order to make the conversion process more efficient. Glucose produced from hydrolysis are to be fermented immediately. Thus, the concentration of the glucose remains low thereby allowing the hydrolysis process to continue without significant inhibition. However, hydrolysis and fermentation both required specific temperature and pH ranges for optimal operation [3]. Any sudden change of temperature and pH during the process could kill or retard the reaction. Those factors affecting the process were hard to maintain. Thus, a need of a bioreactor was highly recommended by most studies. A bioreactor is an apparatus, like a large fermentation chamber, for growing organisms such as bacteria or yeast under controlled conditions. Bioreactors are used in the biotechnological production of substances such as pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic waste [4].They provide the following facilities for the process such as contamination free environment, specific temperature maintenance, maintenance of agitation and aeration, pH control, monitoring Dissolved Oxygen (DO), ports for nutrient and reagent feeding, ports for inoculation and sampling, fittings and geometry for scale up, minimize liquid loss and growth facility for wide range of organisms [5].

A lot of countries boasted of their own bioreactors. Each contained almost the same principles but differ in the process equipment used. That was because of the diverse advancement of electronics and technology. Here in the Philippines, there existed so many studies about biomass conversion to bioethanol, but still its modern technology could not suffice the completion of such bioreactors.

This study aimed for the design and development of prototype Simultaneous Saccharification Fermentation-Distillation apparatus for the production of bioethanol. The proponents wanted an apparatus, which would be easy to use and with the capacity to measure and control all the important parameters of the process. Also, the proponents tried to resolve a variety of problems regarding specific temperature ranges during saccharification and fermentation process in order to improve both yield and process efficiency. Furthermore, the proponents wanted to overcome the main challenge facing the industry regarding feedstock and processing facilities and to meet the demand of the government's National Biofuels Program.

This study is of great importance to the society especially to industries for it would open doors of opportunity to government and private sectors to explore more input indigenous materials to get the desired results. This development would help in further evaluation of different feed stocks locally available for its conversion to bioethanol with further emphasis on maximizing the yield.

OBJECTIVES OF THE STUDY

The primary objective of the study was to design and develop a Simultaneous Saccharification Fermentation (SSF) apparatus mainly for the production of bioethanol. Specifically this study aimed to present the system design of the SSF apparatus taking into considerations the apparatus specifications and dimensions, system components and controls like temperature and pH measurement and control, distillation set up, and material and energy requirements. In addition, this study also aimed to test the prototype yield and efficiency through the comparison of the %ethanol result from the said prototype with that of the laboratory process. Lastly, it aimed to present the overall cost of the developed SSF apparatus and to provide an instructional manual for future users.

MATERIALS AND METHODS

This study used the project development method of research. It was divided into three parts: (1) design and development of the prototype Simultaneous Saccharification Fermentation apparatus for the production of bioethanol; (2) preliminary testing and evaluation, performance evaluation of the efficiency of the SSF apparatus in terms of %ethanol which was done at the National Institute of Microbiology and Biotechnology (BIOTECH), University of the Philippines, Los Baños, comparison of performance to that of the laboratory scale and; (3) formulation of instructional manual.

Design Specification

The prototype developed was a Simultaneous Saccharification Fermentation apparatus. Its major function was to produce bioethanol from different biomass. The first step undertaken in the development of the prototype was the design development and design computations. It is mainly composed of two vessels; the SSF vessel and the distillation vessel. The saccharification and fermentation process were performed in the SSF vessel and the distillation process was performed in the distillation vessel. The pH indicator regulated the pH of the slurry while the motor combined with the pitched blade agitator controlled the agitation and mixing of the slurry. The heating system in each vessel provided the heat on the equipment and the temperature controller regulated the temperature required for the SSF and distillation operation.

Table 1. Summary of SSF apparatus Components and Description

Parts	Specification/ Description
SSF vessel	A cylindrical with conical bottom made of stainless steel and maximum of 22. 045 L capacity
Top plate	Stainless steel with 3 reserved ports. Thickness=5mmDiameter=0.3m
CO_2 Outlet	Stainless steel with plastic hose connected into lime water vessel
Motor	1/4 hp motor attached at the top plate
Agitator	Stainless steel blades, upper is paddle type and the lower is pitch-blade agitator
Distillation vessel	stainless steel jacketed with heating coils and 10 L capacity
Auxiliary devices and parts	
• Temperature controller	
○ Range	0 to 100°C
• pH controller	
○ Range	0 to 14 pH
○ Resolution	0.01 pH
○ Accuracy	±0.01pH

Several designs were considered before ending up to the last and final assembly. Stainless steel was used as the primary materials for construction of the body of the SSF apparatus and for the clamps and holders. The final design was evaluated in terms of the availability of materials, cost, and the difficulty of fabrication.

Before equipment fabrication and assembly, the necessary materials were gathered and processed based on the design specifications. Auxiliary devices such as temperature and pH indicator control were also acquired.

Accurate control of the environment was the key to have an optimal yield in SSF processes. To provide an optimally controlled environment for saccharification and microbial fermentation processes, various control system were required for heating, agitation and pH control [6].

Table 2. Summary of SSF apparatus Components and Specifications

Component	Dimension
SSF inner vessel	Diameter = 0.25 m Height = 0.50 m Thickness = 2 mm
SSF outer vessel	Diameter = 0.34 m Height = 0.50 m Thickness = 1.5 mm
Conical bottom	Diameter = 0.25m Height = 0.13m Thickness = 2 mm
Distillation inner vessel	Diameter = 0.20 m Height = 0.25 m Thickness = 2 mm
Distillation outer vessel	Diameter = 0.27 m Height = 0.25 m Thickness = 1.5 mm
Conical bottom	Diameter = 0.20 m Height = 0.05 m Thickness = 1.5 mm

Cost Estimation

The factors that affected the cost of the equipment includes the type of material to be used on the equipment, the quantity and availability of the materials, the design specification of the equipment and the fabrication of the equipment.

Table 3. Bill of Materials

ITEM	**QTY**	**UNIT PRICE**	**COST (Php)**
Digital pH controller	1	15,200	15,200.00
¼ hp Induction Motor with frequency inverter	1	11,000	11,000.00
Heating system with temperature controller	1	35,600	35,600.00
Fabrication including materials	1	52,470	52,470.00
Electrical layout/wirings	1	3250	3250.00
1” Stainless steel cap	1	173/pc	173.00
¾” Stainless steel cap	1	129.75/pc	129.75
½” brass cap	2	42/pc	84.00
¼” x 3” Stainless steel nipple	2	129.75/pc.	259.75

Table 3 (Cont.) Bill of Materials

ITEM	QTY	UNIT PRICE	COST (Php)
¼” x ½” nipple reducer	1	72.75/pc	72.75
Rubber Gasket	1x1 ft	120/ft	120.00
19 x 38 x 7 7/8 oil seal	1 pc	85/pc	85.00
1” G.I union	2 pc	49/pc	98.00
1” brass ball valve	3 pc	379.75/pc	1,139.25
1” G.I Tee	1 pc	43.5/pc	43.50
1” x 1 ½ “ G.I nipple	1 pc	54/pc	54.00
90° ¼ “ G.I Stainless steel elbow	1 pc	109.75/pc	109.75
TOTAL		119,888.75	

Table 4. Energy Consumption and Cost of the SSF-D Apparatus

INSTRUMENT	ENERGY CONSUMPTION	COST / kWh
Motor	13.8943 kWh	78.22
Heating Coil of SSF	11.25 kWh	63.34
Heating Coil of Distillation	2.25 kWh	12.67
Total Energy Consumption	27.3943 kWh	
TOTAL ENERGY COST		154.23

Materials of Construction

The apparatus was designed to provide a controlled environment to achieve an optimal product formation using biological catalysts. Microorganisms were frequently sensitive to strong shear stress and to thermal and chemical influences. Since these microorganisms greatly affect the success of the reaction, this was used as the basis for the selection of materials. The materials of construction considered should not adversely affect, nor be adversely affected by, the desired microbial activity, either by interaction with the fermentation medium or by harbouring unwanted organisms. Also, it must be resistant to corrosion by the nutrient medium and products and resistant to rust which might contaminate the broth, and to the effects of sterilization temperatures. Stainless steel has met the requirements of the prototype and it was used as the major material for construction of the apparatus. The SSD and distillation vessel, agitator, pipes and elbows were all made of stainless steel. Another factor considered was the cost of the materials. Other parts which would not come in contact with the microorganisms used more affordable materials. Carbon steel was chosen as the material for the frame of the prototype.

Fabrication and Assembly

A deal was made with Mr. Rommel Camacho, owner of Sampaloc Mufflers located at Sta. Rita, Batangas City, for the fabrication of the prototype. Most of the raw materials were readily available there. Auxiliary devices, temperature controller and pH controller, wirings and other supplementary materials were gathered. The assembly and adjustments were also done in the said shop.

Main problem in the prototype fabrication was the availability and affordability of the controllers and materials. Initial pH controller proposed was automated which automatically add acid or base to maintain the values set in the controller but since the said controller was not available locally and it has high cost in international market, the researchers opted for pH controller with manual pH adjustments . A single temperature controller was used for both the SSF vessel heating system and distillation heating system to minimize the cost of fabrication of the temperature controller. A second hand motor with a rating of ¼ hp was also used due to financial limitations. The fabrication of a condenser was removed due to its cost and long time required for fabrication.

Other modifications of the design arose after preliminary testing of the prototype.

To accelerate the cooling process of the prototype, a copper tube cooling system was installed. Two types of impeller were used, the upper impeller was paddle type while the lower impeller was pitch-blade and larger dimension was also used for even mixing of the reactor.

For the agitation controller an inverter was used to control the speed of a ¼ hp motor with a maximum speed of 600rpm. For the heating system, heating coils was used and control panel with thermocouples to regulate the desired temperature. For the cooling system, copper tubing was used as a heat exchanger. Acid and bases could be fed through a hose attached to the top plate and a pH indicator was used to monitor the pH. There was a limewater container connected to the top plate for the $CO_2$by-productproduced during the process. The SSF vessel was connected to the distillation vessel through a stainless pipe with a globe valve. A condenser could be attached to the top plate of the distillation vessel. Each vessel had a drain valve for cleaning.

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Prototype Modifications

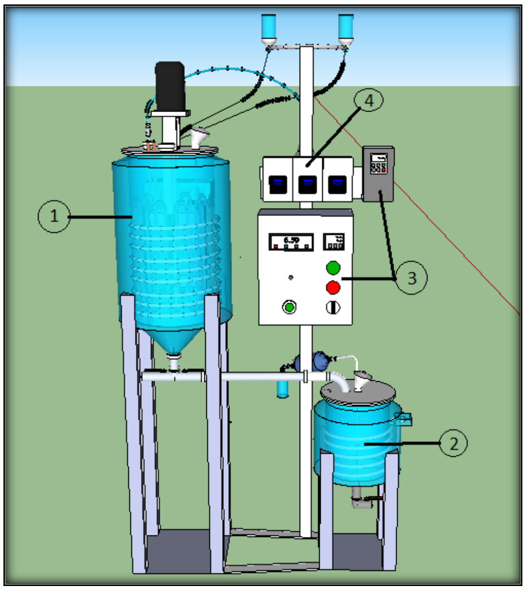

Figure 1. Parts of the SSF-D Apparatus

1. Simultaneous Saccharification Fermentation Vessel

1.1 SSF Inner Vessel

All the reactions in the SSF process will occur in this vessel from pretreatment of the biomass to fermentation process.

1.2 SSF Outer Vessel

This vessel served as insulation for the heating coil from the outside. It was filled with fiberglass to avoid too much heat loss while in the process of heating the broth.

1.3 SSF Heating Coil

It was fabricated in a way that its contact with the SSF inner vessel will be maximized and the supply of heat would suffice the demand of the vessel. The heating coil was divided into 3 part which has 3 loops.

1.4 SSF Cooling Tube

This was used to lessen the time of cooling the SSF vessel. Its material of construction was copper tubing with a diameter of 0.01m.

1.5 SSF Top Plate

This serves as cover and ports for all the auxiliary devices in the SSF vessel.

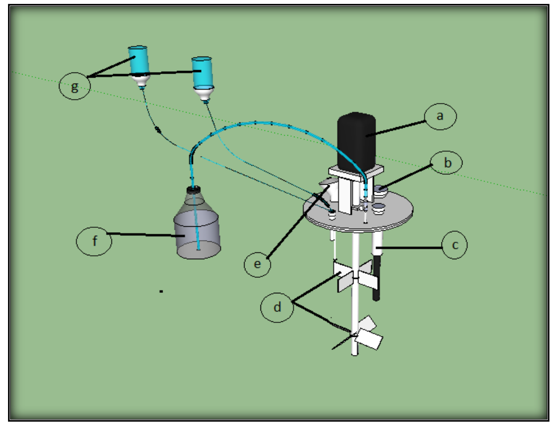

Figure 2. SSF Top Plate & Ports

a. ¼ hp electric motor which provides power for agitation.
b. Feed port which serves as the means of passage of the feed to the vessel.
c. pH probe port supports the pH probe to stabilize its position inside the vessel.
d. Impeller was designed to have two types of blades (paddle and pitch blade) for the even mixing of the feed
e. A type K thermocouple was used to measure the temperature of the broth.
f. Lime water vessel is where the CO2 is directed
g. Acid/base feeder is connected through a host to the vessel and aids the adjustment of pH.

2. Distillation Vessel

2.1 Distillation Inner Vessel

The distillation process will occur in this vessel.

2.2 Distillation Outer Vessel

This vessel serves as insulation for the heating coil from the outside. It is filled with fiberglass to avoid too much heat loss while in the process of heating the broth.

2.3 Distillation Heating Coil

It was fabricated in a way that its contact with the distillation inner vessel be maximized and the supply of heat would suffice the demand of the vessel.

2.4 Distillation Top Plate

This serves as cover and ports for all the auxiliary devices in the SSF vessel. The material of construction of this vessel was stainless steel with a thickness of 5mm and has a diameter of 0.25m.

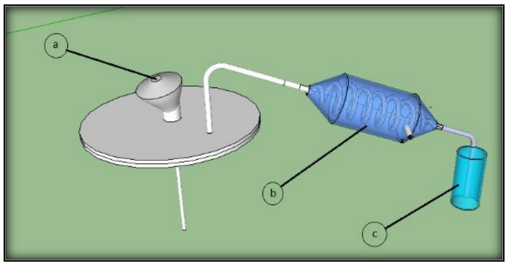

Figure 3. Distillation Top Plate & Ports

a. A type K thermocouple was used to measure the temperature of the broth on the distillation process.
b. The condenser that was use was connected at the top plate using a rubber tubing.
c. A flask was used to collect the bioethanol distillate.

3. Control Panel
The control panel is an electrical device consisting of a flat insulated surface that contains switches and dials and meters for controlling and monitoring the auxiliary devices. It is an ease of access in controlling the parameters being observed by the apparatus such as temperature and pH. The agitator speed controller has a frequency inverter which allows the user to set the desired speed up to 600rpm.

4. Circuit Breakers
The circuit breakers provides simplified installation - fewer components result in higher overall reliability .It also made the apparatus safer when ground fault protection was needed.

Preparation of User's Manual

A user's manual was provided for better understanding of the prototype. It includes the features of the equipment, description of the prototype, specifications (parts and its functions), layout of the system (layouts and pictures), operation procedure, and maintenance and safety.

Experimental Procedure

A laboratory experiment was conducted as basis of comparison for the percent ethanol of the prototype. The experiment was conducted at Batangas State University, Main Campus II Laboratory 1.The parameters that were considered were the temperature and pH

Bioethanol production from cornstarch using laboratory procedure was done under the same parameters used in prototype procedure. The amount of biomass used were 10% of the amount of biomass used in the prototype operation.

Media composition

The growth medium used for preparing S. cerevisiae contained in grams per 50 ml: Dextrose, 1; peptone, 1; yeast extract, 0.5; Bacterial culture, 0.75. After weighing all the components, it was put in sterilized beaker and constantly stirred in a hot water bath. The medium was autoclaved and transferred on a sterilized test tube. It was rested until the medium solidify and the mother culture was streaked. The new culture was incubated for 3 days before inoculation [7].

Pretreatment

A 25% (w/v) solution of cornstarch was cooked until gelatinized. The solution was cooled and pretreated using α-amylase enzyme with enzyme activity of 115 IU/mL obtained from Enzyme Research Laboratory of *UPLB* National Institute of Molecular Biology and *Biotechnology (BIOTECH)*. The pretreatment was carried out in beaker at controlled conditions of pH 6.0 and temperature 70°C for two hours using 23 mL of α-amylase enzyme to liquefy the starch present [8].

Simultaneous Saccharification and Fermentation (SSF)

Bioethanol production by co-culture of mold and yeast was carried out using enzyme activity of 260 IU of glucoamylase enzyme and 24 h old Saccharomyces cerevisiae simultaneously. Fifty mL of 24-h old S. cerevisiae cell culture containing 72×10^5 cells was inoculated to 1000 ml of pretreated corn starch solution (The same S. cerevisiae inoculum was utilized for the prototype operation.). The glucoamylase enzyme was added to fermentation medium. Fermentation was carried out at 30° and pH of 5.0[8].

Distillation

Distillation of the fermented broth was carried out at a controlled temperature of 78.4^0C – boiling point of ethanol [9].

Evaluation of the efficiency of the SSF apparatus

The testing of the prototype's efficiency was conducted at Tierra Verde Subdivision, Batangas City. After the fabrication and assembly of the prototype, the researchers conducted preliminary procedures to evaluate the performance of the developed SSF apparatus before the actual testing to ensure that the apparatus is in good working condition. The researchers used a spare thermocouple to ensure that the temperature reading is accurate.

Cornstarch was utilized for the production of bioethanol and fed into the SSF apparatus with the same controlled parameters of the laboratory procedure.

Evaluation of the Bioethanol

Bioethanol was collected after the distillation through a condenser and placed at a translucent flask completely sealed with a rubber cork. Proportion of the prototype yield and the laboratory yield were directly collected through vials and were taken to BIOTECH for the % ethanol analysis.

Statistical Treatment of Data

For performance evaluation, the standard deviation of the laboratory samples and the prototype samples will be evaluated and will be compared to check its performance and the laboratory samples will serve as the control. As for the% ethanol, it will also be compared, performance will be based on the consistency on the percentage ethanol produced and the % ethanol itself based on what setup can be achieved the highest performance on maximum yield. The comparison of the yields t-test was used for evaluation.

RESULTS AND DISCUSSION

One of the problems encountered during the laboratory experiment was maintaining the working area and the fermentation broth sterilized. The sterility was an important part of the experiment so that unwanted microorganisms may not hinder the enzymes and the yeast from their optimum activity.

Also, the pH and the temperature of the broth were not properly maintained due to technical problems of the laboratory equipment. There was an inconsistency in the reading of the pH meter. The temperature was not sustained during the process because the electric stove was the heating source that was used and a thermometer was used to monitor the temperature. The researchers had to manually adjust the temperature by turning on/off the electric stove to control the temperature.

The same procedure and parameters were considered in the prototype testing. The operation of the prototype ran smoothly during the whole SSF process. The temperature and the pH were easily maintained and monitored properly using the controller. Adjustments in the temperature and the pH were done easier. It was also easier to heat the vessel since it was jacketed with a heating coil and there was also a cooling tube inside the vessel so that it was easy to lower the temperature. The broth was also agitated using two types of blades (paddle and pitch blade) for the even mixing of the feed. The sterility of the broth was also maintained since the whole process was done in a closed vessel and there was a port for the feed.

One of the problems encountered during the operation of the prototype was its maintenance. It was difficult to clean because it was heavy to open the top plate and hard to detach its piping. The induction motor also tends to overheat so it was turned off after 8 hours of operation and rest for an hour. The pH was also manually adjusted.

During the distillation process, the main problem that was encountered was leakage of steam but was solved by adding silicone gasket.

Quantitative Analysis of Bioethanol

The bioethanol produced from four trials, two of which from the laboratory experiment and the other two from the prototype experiment, were analyzed for its %ethanol.

Table 5. Results of Bioethanol Analysis

Samples	Components
	%ethanol (%v/v)
Laboratory Experiment	
Trial 1	6.48 ± 0.20
Trial 2	6.76 ± 0.03
Prototype Testing	
Trial 1	7.43 ± 0.09
Trial 2	9.53 ± 0.15

Statistical Treatment of Data

The standard deviation for the prototype has a greater deviation compared to the laboratory. This indicated that the laboratory runs had lesser external interferences over that of the prototype sample runs. But considering the percentage ethanol produced, the one from the prototype had a greater percent ethanol compared to that from the laboratory run with a percentage difference of 28.09668. It is probable that the optimum condition for fermentation was achieved during the prototype run but due to external interferences the runs became varied. While in the laboratory run, the optimum condition was almost achieved with consistent operation run.

Table 6. %Ethanol t-test Results

	n-1	Mean	SD	Variance
Laboratory	5	6.62	0.1997	0.0399
Prototype	5	8.48	1.1555	1.3352
%Difference	28.0966767			

***95% CI (2.92662, 0.79338); level of significance = 0.05*
*** t-values (3.885244, 2.228)*
***f-values (15.09512, 5.05)*

Based on the t-test results, t_0 equal to 3.8852 is greater than $t_{critical}$ equal to 2.2280, an indication of rejection of the researchers' null hypothesis. In addition, based on f-test, f_0 equal to 15.0951 is greater than $f_{critical}$equal to 5.05, an indication also that the researchers' null hypothesis is rejected.

Moreover, based on the confidence interval results, the CI did not pass the 0 value since the upper had a value of 2.92662 and the lower with a value of 0.79338. This result showed that it did not support the null hypothesis at 0.05 level of significance.

Therefore, there is a significant difference between the %ethanol obtained from the SSF apparatus with that from the laboratory process. The mean percent ethanol of the prototype exceeded the mean percent ethanol of the laboratory sample.

CONCLUSION AND RECOMMENDATION

The developed simultaneous saccharification fermentation-distillation apparatus was carefully designed and improved to meet the proponents' aim to enhance its performance.The SSF vessel of the prototype has a maximum capacity of 20L while 10L for the distillation vessel. The prototype had some minor leaks due to fitting considerations and fabrication. This issue was addressed by using gaskets readily available in the market. Temperature ranges during saccharification and fermentation process was resolved thus improving both the yield and process efficiency.

There is a significant difference between the %ethanol obtained from the SSF Apparatus with that from the laboratory process.

The prototype was able to produce bioethanol with a %ethanol higher than the ones obtained from the laboratory process, thus its efficiency had been proven. On the other hand, based from the computed standard deviation, the laboratory results were more precise thus proving its advantage from the prototype in terms of consistency of performance.

The prototype fabrication including all auxiliary parts cost PhP 119,888.75. The prototype was reasonably expensive due to its materials of construction, sensitivity and operating conditions requirement.

The instruction manual of the apparatus was provided for operation, trouble shooting and maintenance procedures.

The following recommendations were made: replacement of the insulation material for both the SSF and Distillation vessels; programmable logic controller was recommended for automated pH adjustments.

1. The use of submersible pump for the cooling system was highly recommended.
2. Filtering of the fermentation broth before distillation was also recommended.

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