Review Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

SACCHARIFICATION OF LIGNOCELLULOSIC BIOMASS FROM *PHOMA EXIGUA* AND ETHANOL PRODUCTION FROM *SACCHAROMYCES CEREVISIAE* USING COST EFFECTIVE FABRICATED LAB SCALE FERMENTER

Madhu H N*[1], Manasa V Anand[1], Gautham Subramaniam[1], Pradeep R Jain[1]

[1] Department of Biotechnology, Dayananda Sagar College of Engineering, Bengaluru, India

ABSTRACT

The continued use of petroleum products is now widely considered unsustainable owing to the depletion of fuel reserves and their contribution to global warming. Renewable, efficient and eco-friendly fuels are necessary for environmental and economic sustainability. Our work aims at ethanol production from lignocellulosic materials using the fungus *Phoma exigua (P. exigua)* and the brewers' yeast *Saccharomyces cerevisiae (S. cerevisiae)* for hydrolysis of cellulose to glucose and fermentation of glucose to ethanol respectively. In this project, we have designed and fabricated a fermenter using cost effective materials which facilitates maintenance of the required fermentation conditions including pH, temperature, anaerobic conditions and proper agitation of the media which indeed increases the ethanol yield. From laboratory flask studies we have determined the effect of temperature and pH on the growth of *S. cerevisiae*, thus finding optimum conditions for its growth. The same conditions were maintained during the fermentation process. Finally, we have carried out a full-fledged fermentation process while maintaining all the optimum conditions using our fabricated fermenter, and spanning a time of 5 days. We have successfully produced ethanol with an encouraging yield of 29.56 mg/ml from wood shavings and 32.01 mg/ml from tissue paper respectively.

KEYWORDS: **Biofuels, Ethanol production, *P. exigua*, *S. cerevisiae*, fermentation, Fermenter design and fabrication**

*** Madhu H N**

Department of Biotechnology, Dayananda Sagar College of Engineering, Bengaluru, India

Received on : 20-09-2016
Revised and Accepted on : 28-12-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b484-493

INTRODUCTION

The continued use of petroleum products is now widely considered unsustainable owing to the depletion of fuel reserves and the contribution of these fuels to global warming.[1] The price fluctuations on the international energy markets with the increasing emissions of greenhouse gases and rising energy dependency on fossil fuels has led to move towards the production of alternative, renewable, efficient and cost-effective energy sources with lesser emissions.[3] Biofuels are of most interest and play a crucial role in the global energy infrastructure among the various potential sources of renewable energy. It is recognized as possible primary energy source and as an ideal recyclable energy carrier. In comparison with using hydrogen or solar energy as transportation fuel, Bioethanol is considered the most favourable choice as biofuel since biofuels are renewable, biodegradable and cost-effective.[10] Biofuels can be solids, liquids or gases so long as they are derived directly from biological sources. There are various biofuel sources and the most common solid biofuel is lignified cellulose (wood) that can be burned for energy.[15] Lignocellulose is the most abundant and renewable carbohydrate which offers significant opportunity to attain sustainability in energy supplies through its use, as approximately 100 billion tons bio-mass is produced in the biosphere every year.[6] Microalgae and macroalgae has been of interest that can be used as a possible biofuel resource for biodiesel production.[16] Several eukaryotic microalgae have the ability to store significant amounts of energy rich compounds which can be utilized for the production of various distinct biofuels.[14] The most abundant and renewable carbohydrate which offers significant opportunity to attain sustainability in energy supplies through its use is the Lignocellulose.[6] Bioethanol can be produced by the saccharification of renewable resources using microbial cellulose complex to supplement the depleting fossil fuels. The major bottle neck in the economical production of biofuel is the efficient conversion of cellulose and hemicelluloses into their respective fermentable sugars.[9] Lignocelluloses undergoes hydrolysis by a mixture of hydrolytic enzymes during bioconversion.[5] Plant pathogens can produce a variety of hydrolytic enzymes to break down cellulosic cell wall and to fulfill their nutrient requirements.[4] Plant pathogens serve as a novel enzyme source for improved hydrolysis of lignocellulosic biomass for biofuel production.[8] The prerequisite in the utilization of lignocellulose for ethanol production is to efficiently yield a fermentable hydrolyzate rich in glucose from the cellulose content present in the feedstock. The employment of enzymes for the hydrolysis of lignocellulose is considered as the most viable strategy to offer advantages over other chemical conversion routes.[13] *S. cerevisiae* yeast is used to convert glucose into ethyl alcohol. The yeast cell contains enzyme catalysts that provide an energetically favourable pathway for the reaction. The environmental conditions that affect yeast cell growth are the availability of major and minor nutrients, the temperature, pH, and dissolved oxygen concentration. Cell metabolism and growth are maintained by a large coupled set of simultaneous chemical reactions wherein essential cell nutrients are necessary for most reactions to occur.[11] Yeast cells have evolved so that they can thrive in more acidic environments than many competing organisms. As yeast cells consume their nitrogen source, hydrogen ions are released decreasing the pH of the solution. The amount of dissolved oxygen in the fermentation broth has major implications for the reactions that occur in yeast. Ethanol may be produced from glucose in the presence of oxygen by aerobic fermentation, under some conditions.[12] Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for the limited crude oil.[7] Pretreatment of lignocellulosic materials to remove lignin and hemicellulose can significantly enhance the hydrolysis of cellulose. Optimization of the cellulase enzymes and the enzyme loading can also improve the hydrolysis.[2] This work aims at ethanol production from lignocellulosic materials using the fungus *P. exigua* and the brewers' yeast *S. cerevisiae* for hydrolysis of cellulose to glucose and fermentation of glucose to ethanol respectively. A fementer was designed and fabricated using cost effective materials which facilitates maintenance of the required fermentation conditions including pH, temperature, anaerobic conditions and proper agitation of the media which indeed increases the ethanol yield. From laboratory studies, the effect of temperature and pH on the growth of *S. cerevisiae* was determined, thus finding optimum conditions for its growth. The same conditions were maintained during the fermentation process.

MATERIALS & METHODS

IDENTIFICATION OF SUITABLE MICROBIAL STRAINS

P. exigua was identified as a capable cellulose hydrolyzing fungus and *S. cerevisiae* was identified to be the ideal candidate to perform the fermentation of glucose to ethanol.

CULTIVATION OF P. EXIGUA AND S. CEREVISIAE

Pure cultures of *P. exigua* and *S. cerevisiae* were obtained from National Collection of Industrial Microorganisms, Pune. The pure cultures were received in agar slants. *P. exigua* cultures were grown and maintained in Potato Dextrose Agar medium (Potato infusion – 200 g, Dextrose – 20 g, Agar – 15 g, Distilled water – 1000 ml, pH 5.6 $\pm$ 0.2, Temperature - 25^{o}C) and *S. cerevisiae* was grown and maintained in Malt Glucose Yeast Peptone medium (Malt extract – 0.3 g, Glucose – 1.0 g, Yeast extract – 0.3 g, Peptone – 0.5 g, Agar – 2.0 g, Distilled water – 100 ml, pH 6.4 $\pm$ 0.4, Temperature - 28^{o}C) .

SUB-CULTURING AND CULTURE MAINTENANCE

Sub- culturing was done by inoculating the culture onto the Malt Glucose Yeast Peptone media (MGYP) and Potato Dextrose Agar gels for future culture requirements. To perform the sub-culturing, the above

mentioned media were prepared. The media were autoclaved and then cooled to room temperature. Then under aseptic conditions, the media were inoculated with their respective cultures. Laminar Air Flow cabinet was not used for fungi, as it posed a risk of the spreading of spores. Once inoculation was complete, the cultures were maintained at 28°C.

STUDYING THE GROWTH PATTERN OF S. CEREVISIAE AT DIFFERENT TEMPERATURES

Isolated organisms were grown under temperature conditions to determine the optimum growth conditions. The varying temperature conditions were 30°C, 35°C, and 40°C. The condition where best growth was observed was considered as optimum. Malt Glucose Yeast Peptone medium, MGYP (Malt extract – 0.3 g, Glucose – 1.0 g, Yeast extract – 0.3 g, Peptone – 0.5 g, Agar – 2.0 g, Distilled water – 100 ml, pH 6.4 ± 0.4, Temperature - 28°C a was prepared at pH 5.0 ±0.5in three 250 ml conical flasks and autoclaved at 121°C, 15 lb/in^2 pressure for 15 minutes. *S. cerevisiae* was inoculated into the media using an inoculation loop. The absorbance value immediately after inoculation was recorded at 600nm and this value was used as a blank for further tests of absorbance. The conical flasks were incubated in a shaker incubator at the varying temperature conditions. The flasks were incubated for a period of 18 hours and were regularly monitored. Absorbance values were calculated for the cultures grown in each flask after every 2 hours. Once all the absorbance values of the cultures, at their respective temperatures, were determined (at regular 2 hour intervals) for a total period of 18 hours, graphs of Absorbance vs. Incubation Time were plotted. Using the growth curves, the optimum pH conditions were determined.[4]

STUDYING THE GROWTH PATTERN OF S. CEREVISIAE AT VARYING PH CONDITIONS

Isolated organisms were grown in media with a varying range of pH to determine the optimum growth conditions. The varying pH conditions were 4, 5.5 and 7. The condition where best growth was observed was considered as optimum. Malt Glucose Yeast Peptone medium, MGVP (Malt extract – 0.3 g, Glucose – 1.0 g, Yeast extract – 0.3 g, Peptone – 0.5 g, Agar – 2.0 g, Distilled water – 100 ml, pH 6.4 ± 0.4, Temperature - 28°C was prepared at pH 4, 5.5 and 7 in three 250 ml conical flasks and autoclaved at 121°C, 15 lb/in^2 pressure for 15 minutes. *S. cerevisiae* was inoculated into the media using an inoculation loop. The absorbance value immediately after inoculation was recorded at 600nm and this value was used as a blank for further tests of absorbance. The conical flasks were incubated in a shaker incubator at a temperature of 30°C. The flasks were incubated for a period of 18 hours and were regularly monitored. Absorbance values were calculated for the cultures grown in each flask after every 2 hours. Once all the absorbance values of the cultures having pH 4, 5.5 and 7 were determined (at regular 2 hour intervals) for a total period of 18 hours, graphs of Absorbance vs. Incubation Time were plotted. From observation of the growth curves, the optimum pH conditions were determined.[4]

STUDYING THE GROWTH PATTERN OF P. EXIGUA AT DIFFERENT TEMPERATURE CONDITIONS

The culture media, Potato Dextrose Agar (Potato infusion – 200 g, Dextrose – 20 g, Agar – 15 g, Distilled water – 1000 ml, pH 5.6 ± 0.2, Temperature - 25°C) was prepared and poured into three different Petri dishes. The Petri dishes were inoculated with spores of the fungus *P. exigua* by streaking the plate with an inoculation loop containing the spores. After inoculation, the plates were sealed with paraffin wrap and incubated at different temperatures i.e. 35°C, 40°C and 28°C (Room Temperature).The plates were incubated for 5 days at their respective temperatures and on the 5th day, the morphological features of the culture were observed and recorded. The temperature at which there was profuse growth was considered as the optimum temperature.

PRETREATMENT OF LIGNOCELLULOSIC MATERIALS BY CHEMICAL METHODS

3 g of tissue paper was pretreated by soaking in acetone for 24 hours. 5 g of wood shavings was retreated by heating at 80°C in 5N NaOH. The pre-treated substrates were neutralized and dried under sun for 2 to 3 days.

HYDROLYSIS OF PRETREATED LIGNOCELLULOSIC MATERIALS BY P. EXIGUA

100 ml of submerged fermentation media (KH_2PO_4 - 0.2g, $MgSO_4$ - 0.1g, $(NH_4)_2SO_4$ - 0.4g, $FeSO_4$ - 0.08g, $MnSO_4$ - 0.008g, $ZnSO_4$ - 0.006g, Wood Shavings - 5g, Tissue Paper - 3g, distilled water – 1000 ml, Either Wood shavings or Tissue paper is to be used as the sole carbon source) was prepared in 2 separate 250ml conical flasks – one with pretreated tissue paper as the sole carbon source and the other with pretreated wood shavings as the sole carbon source. The pH was set to 5.5 ± 0.2. The media was sterilized by autoclaving at 121°C, 15 lb/in^2 for 15 min. After sterilization, the medium was inoculated aseptically with a disc of *P. exigua* culture. The inoculated medium was kept for incubation at 28°C in a shaker incubator. The hydrolysis was carried out for a period of one week. After one week, a DNS test was performed to check the glucose content of the hydrolysate. A graph of Absorbance vs. Glucose Concentration was plotted and the glucose content was assayed.[2]

ETHANOL PRODUCTION IN LAB SCALE USING FERMENTATION SHAKE FLASK STUDIES

200ml of fermentation medium (Glucose – 30 g, yeast extract – 5g, NH_4Cl – 2 g, Peptone – 1 g, KH_2PO_4 – 0.1 g, NaCl – 0.08 g, $MgCl_{2\cdot6}H_2O$ – 0.07 g, $CaCl_{2\cdot2}H_2O$ – 0.01 g, Distilled Water – 1000 ml) was prepared and split into two conical flasks. The pH was adjusted to 5.5.

The supplements and sugar components of the medium were separately autoclaved. Autoclaving was done at 121°C, 15 lb/in^2 for 15 min. Each conical flask was filled with 100ml of fermentation medium, which was prepared as per the above composition. The pH of the medium was adjusted to 5.5 and it was heated in the microwave oven to ensure dissolution of salts and substrates. The conical flasks containing the medium were autoclaved at 121°C, 15 lb/in^2 for 15 min. Glucose was added in the form of hydrolysate from the hydrolysis by *P. exigua*. The amount of glucose added was according to the ratio mentioned above. The hydrolysate was autoclaved separately. Sterile Glucose solution was added to the medium aseptically once the autoclave was complete. Next, an inoculation loop full of *S. cerevisiae* culture was inoculated into the medium. The conical flasks were placed in a shaker incubator maintained at 30^{o}C and the shaker was run at an agitation of 190 rpm. After 3 days of fermentation, a Potassium Dichromate test was performed to check the ethanol content. A graph of Absorbance vs. Ethanol Concentration was plotted and the ethanol content was assayed .This was done for both the conical flasks.[2]

DESIGN AND FABRICATION OF A 3 LITER FERMENTER USING COST-EFFECTIVE MATERIALS

Our fermenter uses an array of devices and sensors to monitor the reaction that comprised of a high speed motor taken from a drilling/screwing to facilitate uninterrupted agitation of the fermentation broth, a regulator to regulate the speed of the motor, a heating coil with a maximum heating capacity of 400^{o}C, a pH sensor to constantly monitor the pH of the fermentation broth, a thermostat to maintain the temperature in the reaction vessel to a stipulated value, a digital tachometer to measure the speed of the motor in rpm and a digital thermometer to read the temperature in the reactor vessel. The reaction conditions were considered as major variables in designing our fermenter. Parameters like reaction temperature, pH, agitation and maintenance of an anaerobic condition were considered. Once these factors were determined and appropriate ranges for these parameters were assigned, the design of the fermenter was finalized. Following the designing of the fermenter, fabrication was outsourced to Surya Steel Works, S.P Road, Bengaluru.

ETHANOL PRODUCTION AT OPTIMUM CONDITIONS USING THE FABRICATED FERMENTER

2 liters of fermentation medium (Glucose – 30 g, yeast extract – 5g, NH_4Cl - 2 g, Peptone – 1 g, KH_2PO_4 – 0.1 g, NaCl – 0.08 g, $MgCl_2.6H_2O$ – 0.07 g, $CaCl_2.2H_2O$ – 0.01 g, Distilled Water – 1000 ml) was prepared and split into two conical flasks. The pH was adjusted to 5.5. The supplements and sugar components of the medium were separately autoclaved. Autoclaving was done at 121°C, 15 lb/in^2 for 15 min. The fermenter was disinfected by washing thoroughly with ethanol (70%).Once disinfection was done, the sterile media at pH 5.5, was added aseptically into the fermenter vessel. Glucose was added in the form of hydrolysate from the hydrolysis by *P. exigua*. The amount of glucose added was according to the ratio mentioned above. The hydrolysate was autoclaved separately. Sterile Glucose solution was added to the fermenter aseptically once the autoclave was complete. After addition of media and all components required for fermentation, all the valves except the ones on the lid were sealed. Nitrogen air (98%) was flushed into the fermenter to flush out oxygen and maintain an anaerobic condition. Once this was done, the valves were sealed. The agitator was set to spin at 160 rpm. The temperature was maintained between 30^{o}C – 35^{o}C.The power was turned on and the fermenter was left to perform the process for 3 days. After 3 days of fermentation, a Potassium Dichromate test was performed to check the ethanol content. A graph of Absorbance vs. Ethanol Concentration was plotted and the ethanol content was assayed.[12]

RESULTS & DISCUSSIONS

CULTIVATION OF PHOMA EXIGUA AND SACCHAROMYCES CEREVISIAE

The microorganisms were successfully cultured in their respective media, as shown in Figures 1 (a) and Figures 1 (b).

STUDYING THE GROWTH PATTERN OF SACCHAROMYCES CEREVISIAE AT DIFFERENT TEMPERATURES

The optimum temperature for the growth of *Saccharomyces cerevisiae* was observed to be 30°C. By close observation of the results (Graph 1), it is evident that the growth is best at a temperature of 30^{o}C. Hence, this temperature was considered as optimum and was used for cultivation of *Saccharomyces cerevisiae* and also during the fermentation process.

STUDYING THE GROWTH PATTERN OF SACCHAROMYCES CEREVISIAE AT VARYING PH CONDITIONS

pH of 5.5 was the optimum for growth and cultivation of *Saccharomyces cerevisiae*. By close observation of the graphs in Figure 5.4, it is evident that the growth is best at a pH of 5.5. Hence, this pH was considered as optimum and was used for cultivation of *Saccharomyces cerevisiae* and also during the fermentation process (Graph 2**)**.

STUDYING THE GROWTH PATTERN OF PHOMA EXIGUA AT DIFFERENT TEMPERATURE CONDITIONS

Phoma exigua morphological development was best seen at 28^{o}C (RT). Since *Phoma exigua* is a mold, the methods to detect growth such as colorimetric estimation (which is used for yeasts) will not work. Hence, the agar plates of *P. exigua* cultures were placed at different temperatures and their morphological developments were observed (Figure 2). The temperature at which better morphological development was observed was considered as optimum.

PRETREATMENT OF LIGNOCELLULOSIC MATERIALS BY CHEMICAL METHODS

The wood shavings were successfully pretreated using 5N NaOH and the tissue paper was pretreated using acetone (Figure 3). Chemical pretreatment is mainly done to delignify the lignocellulosic materials. Delignification ensures that the materials are free of the recalcitrant lignin and are left with almost pure cellulosic mass (consisting of celluloses and hemicelluloses). Chemical pretreatment is an essential step in the hydrolysis of lignocellulosic materials.

HYDROLYSIS OF PRETREATED LIGNO - CELLULOSIC MATERIALS BY PHOMA EXIGUA

Phoma exigua hydrolysed the cellulose present in the 2 samples to glucose successfully test (Figure 4(a) and Figure 4(b)). The glucose content after hydrolysis was assessed with a DNS. Amount of glucose present was found to be 5.2 mg/ml (avg. of 2 trials)for wood shavings sample (Table 1). Amount of glucose present was found to be 6.3mg/ml (avg. of 2 trials**)** for tissue paper sample (Table 1). Hydrolysis of cellulose to glucose can be assessed by DNS test. The aldehyde group of glucose converts 3, 5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid, which is the reduced form of DNS. Water is used up as a reactant and oxygen gas is released during the reaction. The formation of 3-amino-5-nitrosalicylic acid results in a change in the amount of light absorbed, at wavelength 540 nm. The absorbance measured using a colorimeter is directly proportional to the amount of reducing sugar. Here, we have assessed the quality of the hydrolytic activity of *Phoma exigua* by using the DNS test to assay how much of glucose is present in unit volume of the hydrolysate.

ETHANOL PRODUCTION IN LAB SCALE USING FERMENTATION SHAKE FLASK STUDIES

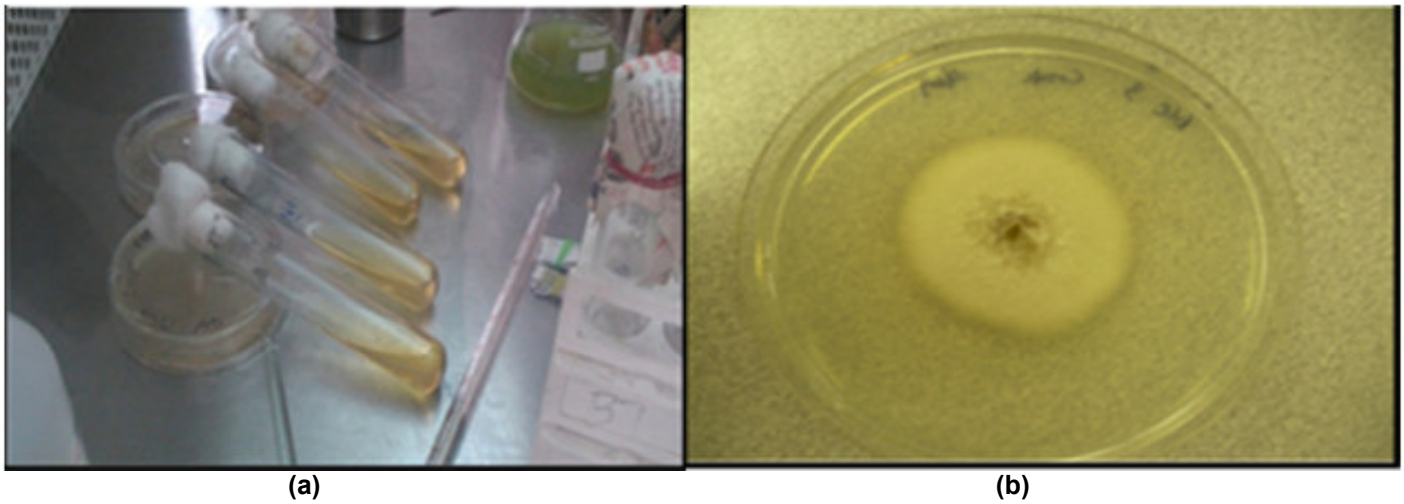
Colorimetric quantification is based on the formation of green color resulting from the treatment of standard or sample with potassium dichromate in presence of sulfuric acid. Absorbance maximum for ethanol was found to be 578 nm. The linearity of absorbance versus concentration of ethanol was observed by plotting calibration curve. Here, we observed the amount of ethanol present in the fermented broth from both wood shavings as well as tissue paper. Ethanol content was proportional to the glucose content of the medium before fermentation (Table 2 and 3)) It is clear that tissue paper is a better raw material than wood shavings in terms of ethanol yield. The ethanol concentration for the wood shavings sample was found to be 5.54 mg/ml for the absorbance of 0.158 at 600 nm. The ethanol concentration for the tissue paper sample was found to be 7.50 mg/ml for the absorbance of 0.19 at 600 nm.

FABRICATED FERMENTER

The fermenter was fabricated using 304 Stainless Steel (Figure 5 & Figure 6). Having a capacity of 5 litres, the fermenter comes equipped with a removable top, an agitator attached to a high torque motor, a heating coil, a cooling jacket, digital pH and temperature sensors and inlet/outlet pipes with sealed with ball valves. The fermentation conditions were maintained to carry out the process for long periods without problems.

ETHANOL PRODUCTION AT OPTIMUM CONDITIONS USING THE FABRICATED FERMENTER

Here, we observed the amount of ethanol present in the fermented broth from both wood shavings as well as tissue paper. Ethanol content was proportional to the glucose content of the medium before fermentation. The maximum ethanol concentration of 29.56 mg/ml for Saw Dust sample was obtained for the absorbance of 0.64 at 600 nm and the maximum ethanol concentration of 32.01 mg/ml for tissue paper sample was obtained for the absorbance of 0.69 at 600 nm. From Table 5.4 and Figure 5.14, it is clear that tissue paper is a better raw material than wood shavings in terms of ethanol yield.



(a) **(b)**

FIGURE 1
Cultivation of P. exigua and S. cerevisiae
(a) S. cerevisiae grown in MGYP Agar Slants
(b) P. exigua grown in Potato Dextrose Agar

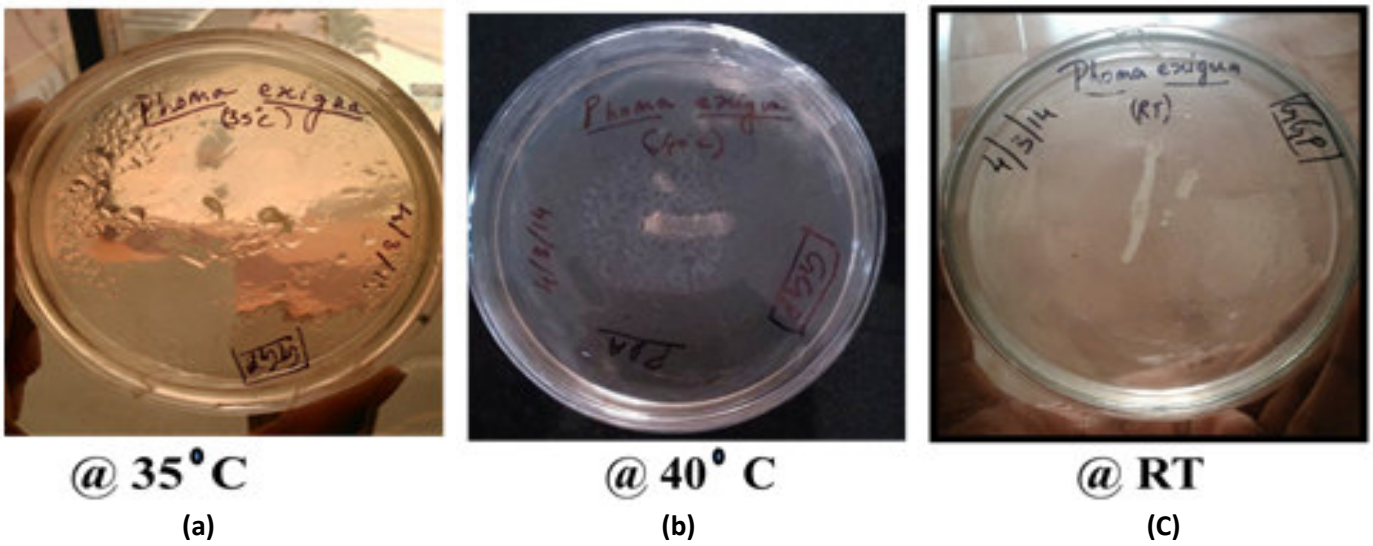


FIGURE 2
Growth Pattern of P.exigua at Different Temperature Conditions: (a) Growth Pattern of P. exigua at 35°C (b) Growth Pattern of 40°C (c) Growth Pattern of Room temperature

(a) Pretreatment

(b) Dried wood shavings

(c) Dried tissue papers

FIGURE 3
Chemical Pretreatment of Ligncellulosic Materials:
(a)Pretreatment (b) Dried wood shavings
(c) Dried tissue papers

(*a) Wood Shavings and Tissue Paper before Hydrolysis*

(*b) Wood Shavings and Tissue Paper after Hydrolysis*

FIGURE 4

FIGURE 5
Fabricated fermenter

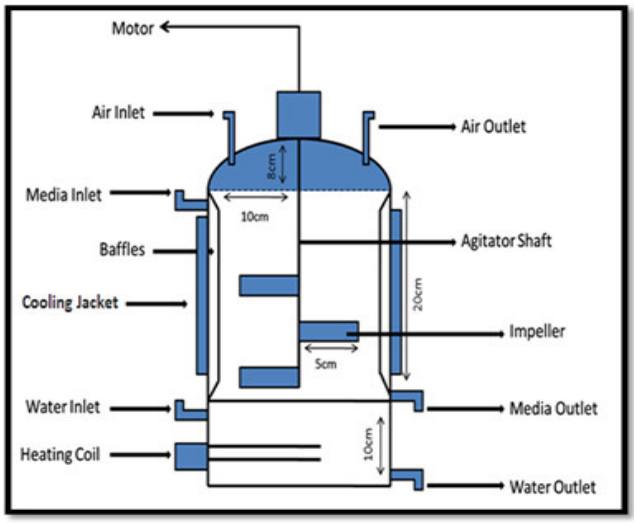
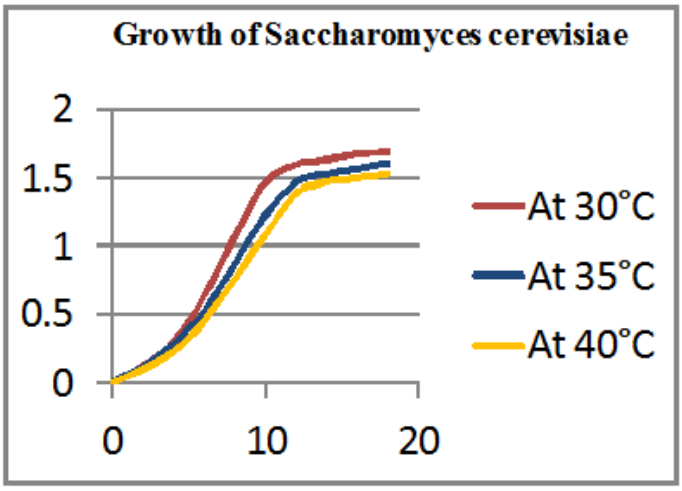
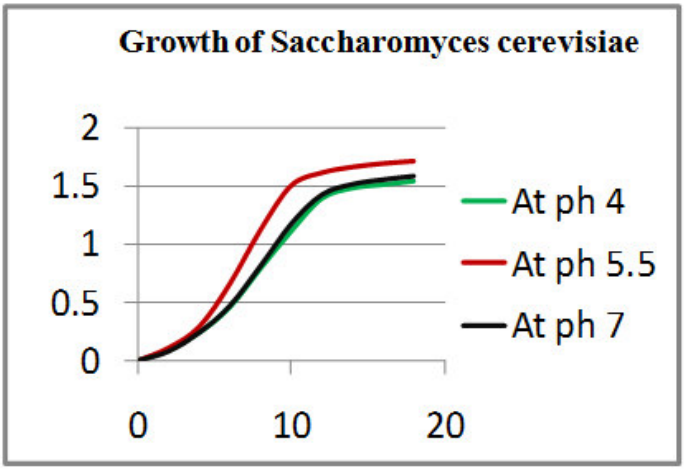


FIGURE 6
Schematic representation of the fermenter



Graph 1
Observation of S. cerevisiae's growth pattern under different temperature



Graph 2
Growth Pattern of S. cerevisiae at Varying pH Conditions

Table 1
Glucose assay using Di-Nitrosalicylic(DNS)Colorimetric method

Sl.No:	Sample	Amount of Glucose (mg/ml)
1	Tissue Paper	6.25
2	Wood Shavings	5.2

Table 2
Ethanol Production at Optimum Conditions using the Fabricated Fermenter

Sample	Weight of Ethanol in Sample (mg/ml)
Saw Dust	29.56
Tissue paper	32.01

Table 3
Ethanol Production in Lab Scale using Fermentation Shake Flask Studies

Sample	Weight of Ethanol in Sample (mg/ml)
Saw dust	5.54
Tissue Paper	7.50

DISCUSSION

There are plenty of chemical treatment methods reported in literature to treat lignocellulosic materials for ethanol production. In this study, we have integrated biological hydrolysis of lignocellulosic materials using *P. exigua* and the ethanol production using *S. cervesiae*. Using a mixture of the different sugarcane parts (bagasse-straw-tops, 1:1:1, in a dry-weight basis), it was possible to achieve a 55% higher enzymatic conversion and a 25% higher ethanol yield, compared to use of the bagasse alone.[21] The sugars released from waste paper were subsequently converted into ethanol with the yield of ethanol of 0.38 g ethanol g(-1) sugar with Saccharomyces cerevisiae CTM-30101.[19] Simultaneous saccharification and fermentation (SSF) of furfural residues (FR) pretreated with green liquor and hydrogen peroxide (GL-H2O2) with Cassava residues (CR) saccharification liquid was investigated. Using 0.06 g/g-H_2O_2-GL-pretreated FR with CR at a ratio of 2:1 resulted in 51.9 g/L ethanol concentration.[18] Cynara cardunculus (cardoon) pretreated by steam explosion (SE) was involved in second-generation bioethanol production using separate hydrolysis and fermentation (SHF) or simultaneous saccharification and fermentation (SSF) processes. The ethanol fermentation in SSF mode was found to be faster than SHF process providing the ethanol concentration of 18.7 g L(-1) and a yield of 26.6g ethanol/100 g CSEOH or 10.1 g ethanol/100 g untreated cardoon.[17] In our study using wood shavings and tissue paper we observed ethanol production of 29.56 mg/ml and 32.01 mg/ml respectively.

CONCLUSION

In this work we have successfully demonstrated ethanol production in a lab scale low cost fabricated fermenter through biological hydrolysis of lignocellulosic materials using *P. exigua* and *S. cervesiae*. This is a promising feature for biological hydrolysis of lignocellulosic material using *P. exigua* and ethanol production using *S. cervesiae*. The amount of glucose present for wood shavings sample by DNS test was found to be 5.2 mg/ml and Amount of glucose present for tissue paper sample by DNS test was found to be 6.3 mg/ml. The amount of ethanol present in the fermented broth from both wood shavings as well as tissue paper was observed from which it can be concluded that tissue paper is a better raw material than wood shavings in

terms of ethanol yield. In this work we have designed and fabricated a small scale cost effective semi automated fermenter to maintain the anaerobic conditions and optimum controlled growth for hydrolysis and ethanol production. We have optimized the fermenter for the temperature controlling using PID – temperature controller. The stirring was controlled at 50 – 1200 RPM using tachometer. The anaerobic conditions were maintained by flushing inert gas Nitorgen in the head space of the fermenter. The pH was controlled manually through acid base vertical reservoirs for every 2 hours.

FUTURE ASPECTS OF RESEARCH

In this work we have successfully demonstrated ethanol production in a lab scale low cost fabricated fermenter through biological hydrolysis of lignocellulosic materials which can be further enhanced for large scale production. This is a promising feature for biological hydrolysis of lignocellulosic material using *P. exigua* and ethanol production using *S. cervesiae*. The ethanol production can be enhanced using other fungal cultures.

CONFLICT OF INTEREST

Conflict of interest declared.

REFERENCES

1. Chisti Y. (2007) Biodiesel from Microalgae, Biotechnology advances; 25(3): 294-306.
2. Deswal D., Khasa Y.P., Kuhad R.C. (2011). Optimization of cellulase production by a brown rot fungus *Fomitopsis sp. RCK2010* under solid state fermentation,Bioresour. Technol., 102 (10): 6065–6072.
3. Dwivedi P., Alavalapati J.R.R., Lal P. (2009). Cellulosic ethanol production in the United States: Conversion technologies, current production status, economics and emerging developments, Energy for Sustainable Development, 13(5): 174–182.
4. Gibson D. M., King B. C., Hayes M. L., Bergstrom G. C.(2011). Plant pathogens as a source of diverse enzymes for lignocellulose digestion, Curr. Opin. Microbiol., 14 (3): 264–270.
5. Henrissat B., Teeri T.T., Warren R.A. (1998). A scheme for designating enzymes that hydrolyse the polysaccharides in the cell walls of plants, FEBS Lett., 425(2): 352–354.
6. Jarvis M. (2003). Cellulose stacks up., Nature, 426 (6967): 611–612
7. Jun H., Bing Y., Keying Z., Xuemei D., Daiwen C.(2009). Strain improvement of Trichodermareesei Rut C-30 for increased cellulase production, Indian J. Microbiol., 49 (2): 188–195.
8. King B.C., Waxman K.D., Nenni N.V., Walker L.P., Bergstrom G.C., Gibson D.M. (2011). Arsenal of plant cell wall degrading enzymes reflects host preference among plant pathogenic fungi,Biotechnol. Biofuels, 16(4): 4.
9. Klein-Marcuschamer D., Oleskowicz-Popiel P., Simmons B.A., Blanch H.W. (2012). The challenge of enzyme cost in the production of lignocellulosic biofuels, Biotechnol. Bioeng, 109(4): 1083–1087.
10. Nigam P.S., Singh A. (2011). Production of liquid biofuels from renewable resources, Progress in Energy and Combustion Science, BioresourTechnol, 37(5): 52–68.
11. Saunders R. M. and Walker J. R. (1999). The sugars of wheat bran, American Association of Cereal Chemists, 47: 85-92.
12. Senthilguru K., George T. S., Vasanthi N. G., Kannan K. P. (2011). Ethanol Production from Lignocellulosic Waste, World Journal of Science and Technology, 1(11): 12-16.
13. Zheng Y., Pan Z., Zhang R. (2009). Overview of biomass pretreatment for cellulosic ethanol production,Metabolic engineering, 2(3): 51-68.
14. Radakovits R., Jinkerson R. E., Darzins Al, Posewitz M. C. (2010). Genetic Engineering of Algae for Enhanced Biofuel Production, Eukaryotic Cell, 9(4): 486-501.
15. Sumbhate S., Nayak S., Goupale D., Tiwari A., Rajesh S. (2012). Colorimetric Method for the Estimation of Ethanol in Alcoholic-Drinks, Journal of Analytical Techniques 1(20): 1-6.
16. Liang M. H., Jiang J. G. (2013). Advancing oleaginous microorganisms to produce lipid via metabolic engineering technology, Progress in lipid research, 52(4):395-408.
17. Fernandes M. C, Ferro M. D, Paulino A. F, Mendes J. A, Gravitis J, Evtuguin D. V, Xavier A. M (2015), Enzymatic saccharification and bioethanol production from Cynara cardunculus pretreated by steam explosion, Bioresource Technology, 186:309-15.
18. Ji L, Zheng T, Zhao P, Zhang W, Jiang J (2016), Ethanol production from a biomass mixture of furfural residues with green liquor-peroxide saccarified cassava liquid, BioMed Central biotechnology, 16(1):48.
19. Guerfali M, Saidi A, Gargouri A, Belghith H (2015), Enhanced enzymatic hydrolysis of waste paper for ethanol production using separate saccharification and fermentation, Applied Biochemistry and Biotechnology, 175(1):25-42
20. Li H, Wu M, Xu L, Hou J, Guo T, Bao X, Shen Y (2015), Evaluation of industrial Saccharomyces cerevisiae strains as the chassis cell for second-generation bioethanol production, Microbial Biotechnology, 8(2):266-74
21. Pereira S. C, Maehara L, Machado C. M, Farinas C. S (2015). 2G ethanol from the whole sugarcane lignocellulosic biomass, Biotechnology for biofuels, 8:44.