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CORN COB FERMENTATION TO BIOETHANOL BY CO-CULTURE OF *SACCHAROMYCES CEREVISIAE* AND *ZYMOMONAS MOBILIS*

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ABSTRACT

The intent of this work was to study the optimization co culturing Saccharomyces Cerevisiae and Zymomonas Mobilis (2:3 ratio) on dried corn cob as substrate for the production of bio-ethanol. Substantial cost reductions may be possible if starch based agro waste such as dried corn cobs are used instead of corn. In this study, corn cobs which are in abundance and do not interfere with food security was subjected to saccharification and fermentation process by co-culture of Saccharomyces Cerevisiae and Zymomonas mobilis for 5 days. Considering parameters like cell density, reducing sugars and ethanol concentration were determined. The results of the study revealed that the combination of yeast and bacteria undergoing EMP and ED pathway converts sugar into alcohol. The substrate was hydrolyzed to produce 154 ug/ml reducing sugar concentrations. Optimal ethanol yield of 11.46 % (v/v) was obtained after 5th day of fermentation. ANOVA shows this test is significant and purity of ethanol was confirmed by GC analysis. The results of this study suggest that agro waste contain fermentable sugars can no longer be discarded into our environment but should be converted to useful products like bioethanol.

KEY WORDS

Co-culture fermentation, Saccharomyces Cerevisiae, Zymomonas Mobilis and Bioethanol.

INTRODUCTION:

A new biotechnological approach for the production of ethanol by fermentation from the renewable carbohydrate materials for use as an alternative liquid fuel has been attracting worldwide interest. The main contributive parameter of bioethanol is the cost of raw material and in order to reduce the overall cost of production corn cob which is abundant and do not interfere with food security was used for this experiment. (Ward and Singh, 2002). Corn cobs represents an important biomass resource for fuel alcohol production, because of its chemical composition and high density of starch, compared to other forms of biomass, and thus premise as an alternative bioresource for the production of ethanol through fermentation (Hang, et. al. 1981, 1986; Roukas, 1994). The starch can be hydrolyzed to monomer units of carbohydrates and can be used by the microorganisms in fermentation process. The production of industrial and fuel ethanol commonly involves three steps: 1) liquefaction of starch by α- amylase, 2) enzymatic saccharification of liquefied product by using amyloglucosidase to produce glucose, and 3) fermentation of glucose to ethanol (Sree et. al., 2004).

Co-culture processes present the opportunity to establish stable and profitable biotechnological

bioprocesses and produce value added products from economical raw materials such as agricultural residues (Manas Ranjan et.al.,2013). Mixed culture systems have demonstrated promise in hydrogen, methane, ethanol and polyhydroxy alkanoates productions from renewable resource, increasing potential revenue and reducing environmental impacts. The synergistic interaction between different yeasts provides a tool for the implementation of new fermentation technologies. In biofuel research, the co-culture process of *Saccharomyces cerevisiae* and *Zymomonas mobilis* has been extensively studied with attempts to achieve a one-batch, simple process to ferment glucose for the production of bioethanol using corn starch substrate (Rodriguez et. al.,2006). The model treats the mixed culture as a single virtual microorganism catalyzing the most common fermentative pathways, producing ethanol, acetate, propionate, butyrate, lactate, hydrogen, carbon dioxide, and biomass. The product spectrum is obtained by maximizing the biomass growth yield which is limited by catabolic energy production (Eric L. Huang et. al., 2014). The objective of this study is therefore to produce ethanol from a cheap commonly available agricultural waste such as corn cob with aid of a co-culture of *Saccharomycescerevisiae and Zymomonas mobilis.*

MATERIALS AND METHODS:

Preparation of Corn cobs as in Substrate: -

Thoroughly dried corn cobs were milled in a flour to obtain a coarsely milled powder. The initial step in ethanol production using dry-grind technology is to reduce the particle size of corn by grinding it (Kelsall and Lyons, 1999) . In a 250 ml conical flask, 20 g of corn flour is added along with 100 ml of distilled water. This slurry is cooked, at 121˚ C and 15 psi for 30 minutes in an autoclave. The heat and mechanical shear of the cooking process break apart the starch granules. It is followed by the treatment with enzymes amylase (saccharification) and glucoamylase (liquefaction) to make slurry for the use of starch as in substrate for ethanol production.

Preparation of Growth Medium: -

The growth medium for *Saccharomyces Cerevisiae*is Yeast Extract Peptone Dextrose (YEPD) comprising of Yeast extract (10 g), Peptone (20 g), Glucose (20 g), dH2O (1000 mL) and adjusting pH 5.4. The bacterial culture *Zymomonas mobilis* is grown on Nutrient medium containing Beef extract (10 g), Peptone (10 g), Glucose (2.0 %), Nacl (5.0 g), dH2O (1000mL) pH is adjusted to 7.0-7.5. The whole mixture for both of the fermentation microbes is sterilized by autoclaving at 121˚ C at 15 psi for 15 minutes. (A.M. Deshmukh-Handbook of Media, Stains and Media Preparation).

Preparation of Inocula and Fermentation Medium: -

The inoculum for *Saccharomyces cerevisiae and Zymomonas mobilis* was prepared in their above stated respective medium and was inoculated into liquid medium and was incubated at their optimum growth conditions. This inoculum was used as 10 % in sterilized corn mash. The fermentation broth (100ml) comprising of (% w/v), Peptone, 8g; yeast extract, 8g; and the product of the saccharification of corn starch as the fermentating sugar (Orji Jerry et. al.,2016).

Determination of Cell Density: -

Cell density is denoted as viable cell density which is the number of living cells per unit volume. The cells obtained after centrifugation was filtered and washed with 0.95% saline to remove the residual substrates and cell debris and it is dried in hot air oven at 70˚ C. and 10 % v/v (5 % *saccharomyces cerevisiae* + 5 % *Zymomonas mobilis*) inoculum size is used to check the appropriate ratio used for the ethanol production by Co-culturing *S.cerevisiae and Z.mobilis.*

Co-culture Fermentation: -

In this fermentation media above stated- 10% of inoculum (2:3) of *S.cerevisiae and Z.mobilis* was inoculated aseptically into production medium (starch hydrolysate) and fermentation was carried out at 30˚ C at 150 rpm for 24 hrs. and then in static mode till the end of fermentation i.e. 5 days. Samples were collected at regular intervals and were checked for residual sugar and ethanol production.

Quantitative Analysis of Reducing Sugar: -

It is carried out using 3.5 –DNSA, the concentration of reducing sugar present in the flask is determined by the absorbance at 540 nm using spectrophotometer. Thus, the concentration values were extrapolated from the glucose standard curve. (Sadashivam and Manicham).

Quantitative Analysis of Ethanol Present in distillate: -

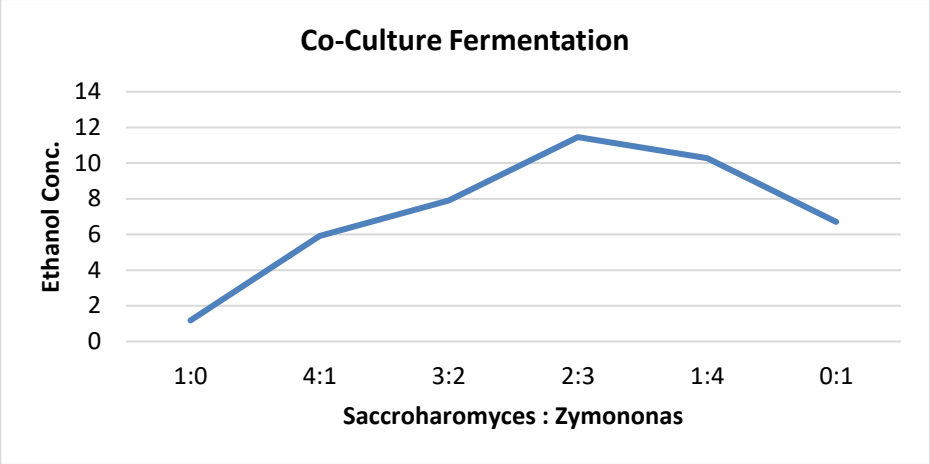
The concentrations of the distillates were extrapolated from the density standard curve. Furthermore the pH, refractive index, and specific gravity values were extrapolated from the standard curves prepared from each parameter since the concentration of the distillate were known (J.Itelima et. al.,2013). The Ethanol

concentration was measured by gas chromatography equipped with UV detector, after distillation of the culture media and separation of Ethanol.

RESULTS:

The ability of the amylase and glucoamylase is to breakdown the starch of the corn cobs into reducing sugar and convert it into ethanol. The results are represented in the Figure 1 below. It shows at the ratio 2:3 *(Saccharomyces: Zymomonas)* reducing sugar was found 158 ug/ml where it was found to produce maximum ethanol of 11.46% at 5th day of fermentation.

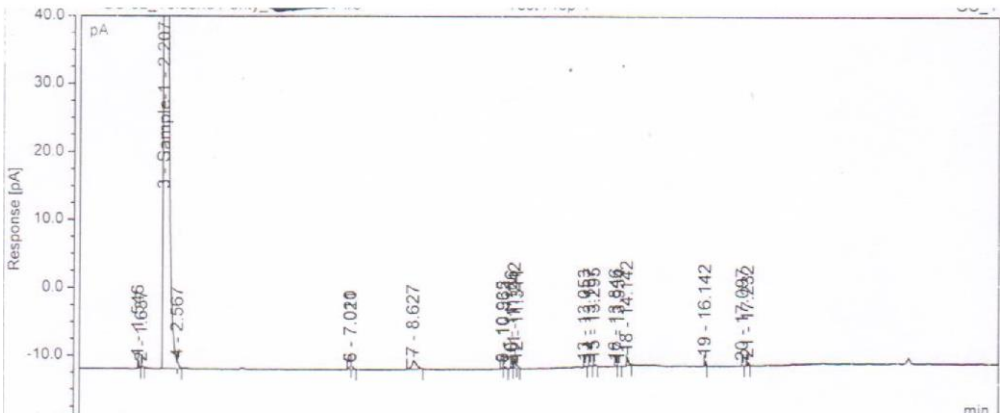
The fermented medium when treated with only *S. cerevisiae* and *Z.mobilis* without co-culturing it was found to produce less amount of ethanol with 1.18 % and 6.71 % respectively. The ratio 4:1 (*Saccharomyces: Zymomonas)* also produced lower amount of ethanol with 5.92 %. *S. cerevisiae* undergoes EMP pathway and *Z.mobilis* follows ED pathway for breakdown of starch into glucose releasing an end product Ethanol. Hence, below graph represents ratio 2:3 (*Saccharomyces: Zymomonas)* with highest ethanol yield having concentration 11.46 v/v. Distillate when subjected to GC analysis the purity for ethanol was at 97% representing highest peak in GC chromatogram attached below.



ANOVA Analysis

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.087293	2	0.043647	6.887954	0.010181	3.885294
Within Groups	0.07604	12	0.006337			
Total	0.163333	14				

Above ANOVA table shows this test is significant with p < 0.05.



GC Chromatogram

DISCUSSION:

Co-culturing microbes for Ethanol production from substrate starch has proven to be one of the efficient way to increase the yield of the product. Many investigations have been done on co-culturing two or more microbes for bioethanol production using different substrates. Nisha Sharma et.al., in their studies of bioethanol production showed maximum 11.26 g/l ethanol yield achieved with co-culture of *S.cerevisiae II+ P.stipitis* with fermentation efficiency of 44.16 % under SSF. Tri Widjaja et. al.,optimized Palmyra Palmsap fermentation using co-culture of *S.cerevisiae* and *P.stipitis* and obtained highest yield 0.32 g/g of ethanol. Manas Rajan et. al. used co-culture of *Trichoderma* spp. and *S. Cerevisiae* in SSF and produced bioethanol and found 65 % higher than monoculture. Eric L Huang et. al., co-culture process with *S.cerevisiae* and *Scheffersomyces stipites* using shotgun proteomics. D.V Adegunloye et. al. showed the effect of fermentation on production of bioethanol from peels of cocoyam using *Aspergillus niger* and S*accharomyces cerevisiae.* Mohammed Abouzied et. al. investigated direct fermentation of potato starch to ethanol by co-culture of *A. niger* and *S. cerevisiae.*

REFERENCES:

Biochemical methods – S.Sadasivam and A.Manickam.

D. V. Adegunloye1 and D. O. Udenze (2017)," Effect of Fermentation on Production of Bioethanol from Peels of Cocoyam Using *Aspergillus niger* and *Saccharomyces cerevisiae"*.Journal of Advances in Microbiology 4(2): 1-8.

Eric. L. Huang and Mark. G. Lefsrud, (2014)," Fermentation monitoring of a Co-Culture Process with Saccharomyces cerevisiae and Scheffersomyces stipitis Using Shotgun Proteomics".Journal of Bioprocessing &Biotechniques, 4(1).

Eric. L. Huang, and Mark. G. Lefsrud, (2014) Fermentation Monitoring of a Co-Culture Process with *Saccharomyces cerevisiae and Scheffersomyces stipitis* Using Shotgun Proteomics, Journal of Bioprocessing and biotech.

Handbook of Media, Stains and Reagents used in Microbiology (2007) –A.M.Deshmukh.

Hang YD, Lee CY, Woodams EE. (1981) A solid-state fermentation system for production of ethanol from apple pomace. J Food Sci.; 47: 1851–1852.

J.Itelima, A.Ogbonna, S.Pandukur, J.Egbere and A.Salami (2013),"SimaltaneousSaharification and fermentation of corn cobs to bioethanol by coculture of *Aspergillus niger* and *Saccharomyces cervisiae*. International Journal Of environmental science and Development, 4(2), 239-242.

Kelsall, D.R., and T.P. Lyons. 1999. Grain dry milling and cooking for alcohol production: designing for 23% ethanol and maximum yield. In: The alcohol textbook. 3rd ed. K.A. Jacques, T.P. Lyons and D.R. Kelsall Ed. Nottingham University Press. Nottingham, UK.

Manas Ranjan Swain, Jyoti Mishra and Hrudayanath Thatoi,(2013)" Bioethanol Production from Sweet Potato (Ipomoea batatas L.) Flour using Co-Culture of *Trichoderma sp. And Saccharomyces cerevisiae* in Solid-State Fermentation, Brazillian Archieves of bioloy and technology.,56(2): 171-179,

Nisha Sharma and Nivedita Sharma (2016)," Bioethanol Production under Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and Co-Fermentation (SSCF) as well As It's Scale up in a Stirred Tank Bioreactor. International Journal of Innovative Research in Science, Engineering and Technology, 5(2): 1572-1576.

Orji Jerry, Kelechi Aleke, Ejikeugwu Chika, NwachiAnthonia, Moses Ikechukwu, Eluu Stanleyand Ugbo Emmanuel," Bioethanol Production from Corncob Hydrolysed by Cellulase of*Aspergillus niger*Using *Zymomonas mobilis* and *Saccharomyces cerevisiae* Isolated from Palm Wine".

Rodrguez J, Kleerebezem R, Lema JM, Van Loosdrecht M (2006) "Modeling Product Formation in Anaerobic Mixed Culture Fermentations". BiotechnolBioeng 93: 592-606.

Sree NK, Sridhar M, Suresh K, Bannat IM, Rao LV. (2004),'High alcohol production by repeated batch fermentation using an immobilized osmotolerant *Saccharomyces cerevisiae". J. Ind. Microbiol.* 24: 222-226.

Tri Widjaja, Toto Iswanto, Elly Agustiani, Ali Altway, Belli Martha JudikaSilaban and Li Felix Yuwono (2017)," Optimization of palmyrapalmsap fermentation using co-culture of *Saccharomyces cerevisiae* and *Pichia stipites*. ARPN Journal of Engineering and Applied Sciences, 12, (23):6817-6824.

Ward OP, Singh A. (2002)" Bioethanol technology: developments and perspectives." *Adv. Appl. Microbiol*; 51: 53–80.

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