VOL. 5, NO. 4, JULY 2010 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

STATISTICAL OPTIMIZATION OF OPERATING CONDITIONS FOR THE BIOCONVERSION OF PALM OIL MILL EFFLUENT INTO BIOETHANOL IN A 3 LITRE COMPUTER CONTROLLED STIRRED TANK BIOREACTOR

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ABSTRACT

The purpose of this study was the statistical optimization of operating conditions for the bioconversion of oil-palm industrial effluent into bioethanol in a three litre computer controlled stirred tank bioreactor and to evaluate the feasibility of producing bioethanol from palm-oil mill effluent generated by the oil-palm industries. The bioethanol production was carried out through the treatment of compatible mixed cultures such as Thrichoderma harzianum, Phanerochaete chrysosporium, Mucor hiemalis, and yeast, Saccharomyces cerevisiae. Simultaneous inoculation of T. harzianum and S. cerevisiae was found to be the mixed culture that yielded the highest ethanol production as 4.5% v/v or 31.6 g/l. Statistical optimization was carried out to determine the operating conditions of the stirred-tank bioreactor for maximum bioethanol production by a two-level fractional factorial design with a single central point. The factors involved were oxygen saturation level (pO2%), temperature, and pH. A polynomial regression model was developed using the experimental data including the linear, quadratic, and interaction effects. Statistical analysis showed that the maximum ethanol production of 4.8% (v/v) or 38.6 g/l was achieved at a temperature of 32^0C, pH of 6, and pO2 of 30%. The results of the model validation test under the developed optimum process conditions indicated that the maximum production was increased from 4.8% (v/v) to 6.6% (v/v) or 52.4 g/l with 80% chemical oxygen demand removal.

Keywords: model, palm-oil mill effluent, operating conditions, bioconversion process, bioethanol production, statistical optimization.

INTRODUCTION

Palm oil is said to be nature's gift to the world. Consumed for more than 5,000 years, its nutritional value, health benefits and value as a natural resource continue to be discovered even today. Palm-oil mill effluent (POME) is an abundant organic residue that is generated by palm-oil mills during the process of extracting palm oil from fresh fruit bunches of oil palms. Oil palm is the only fruit that can give two types of oil, extracted from the fruit of the oil palm which are chemically different. Palm oil comes from the mesocarp (flesh of the fruit) and the lauric palm kernel oil from the kernel at the fruit's core. The two oils are separated in the oil palm bunches by the thick shell of palm kernels. Semi-solid at room temperature, these oils or fats can be fractionated into solid and liquid fractions known as stearins and oleins, respectively. They can also be processed through physical or chemical refining to yield either refined, bleached and deodorized (RBD) or neutralized, bleached and deodorized (NBD) palm oil and palm kernel oil. Combinations of these processes lead to various types of palm oil and palm kernel oil products. The high content of carbohydrates (29.55%), proteins (12.75%), nitrogenous compounds, and lipids with a considerable amount of cellulose and nontoxic minerals provides a good source of microbial fermentation. In addition, POME has little inhibiting effect on microbial growth due to certain content of lignin and phenolic compounds (Wattanapenpaiboon N, Wahlqvist ML 2003). It is estimated that 0.5-0.75 t of POME can be discharged from every tonne of oil palm fresh fruit. Several processes are currently being used to treat this effluent.

Anaerobic digestion system

Generally, palm oil mill effluent treatment plants (ETPs) are operated on two-phase anaerobic digestion process followed by extended aeration process. This two-phase anaerobic process gives excellent pollutant destruction efficiency of above 95% while extended aeration ensures that the final pollutant levels in the effluent are within the stipulated limits set by the Department of Environment (DOE). In the anaerobic digestion process, the raw POME is first converted into volatile fatty acids by acid forming bacteria. The volatile acids are then converted into methane and carbon dioxide. The advantages of anaerobic digestion system are:

a) The two phase system allows greater control of digester environmental conditions.
b) Long solid retention times allow better biodegradation efficiencies.
c) Additional settling of liquor ensures minimum loading to the aerobic process.
d) There is capability to cope with full effluent load, regardless of fluctuation.

Extended aerobic process

In the extended aerobic system, the anaerobic liquor is aerated to further reduce the BOD content. In addition to providing oxygen, the floating aerators also

ensure complete mixing is achieved and the pod contents are always in suspension. In this process, levels of beneficial micro-organisms are increased which in turn hasten the conversion of pollutants into carbon dioxide, water and energy (Korrapati Narasimhulu, Parcha Sreenivasa Rao. 2009). The aerobic suspension is allowed to settle in a settling tank to ensure production of a fairly clean supernatant. The main advantages of extended aerobics systems are its high BOD removal efficiency and low solid yield.

Ponding system

The raw effluent is treated using a ponding system comprising of three phases, i.e. anaerobic, facultative, and algae processes. Although the system takes a longer retention time of 90 days, it is less sensitive to environment changes, stable, efficient and could guarantee excellent pollutant biodegradation efficiency of above 95%.

Bioreactor system

This is a simple and innovative bioreactor process that is capable of treating POME efficiently. The system is superior to the conventional system as it operates with very short hydraulic retention times, takes high organic loading, requires less space and is more environmentally friendly.

Composting system

The composting system offers an effective solution to the oil palm industry's perennial problem of waste disposal. The composting system utilizes 100% POME and EFB and uses a technically advanced method to convert these waste matters into compost. EFB are firstly shredded using a high speed hammer mill and then stacked into windrows of 1.5 meter high by 45 meter length in an open field. POME with BOD levels less than 10,000 ppm is then pumped from the pond and sprayed onto these windrows at a specified rate at 3 days intervals. The windrows are turned regularly using a windrow-turner for better mixing and aeration. Composting accelerants are sprayed once at the start of the process to accelerate the composting process. Throughout the composting process, the windrows are covered by an air-permeable covering to avoid drenching by heavy rain and to prevent leaching of nutrients. The covering is crucial for the control of temperature and moisture content, two key factors that affect the speed of composting and quality if the end product. The compost is mature after 70 days and is ready for use. The compost, when used in sufficient quantity, is capable of replacing 66% of chemical fertilizers. Due to the high demand for biofuels, bioethanol production from starch, sugar, crops, and agricultural residues is expected to increase. Biofuel crops include corn, corn cobs, corn stover, starch, rice, wheat, sorghum, and sugar cane. Most of these resources compete with human food production, as well as having high production prices that restrict their industrial production. Lignocellulosic materials include agricultural residues (e.g., crop residues and sugar cane bagasse), herbaceous crops (e.g., alfalfa, switchgrass), forestry wastes, wastepaper, and other wastes that could serve as alternative resources for bioethanol production, due to their lower prices and local abundance (Kim S, Dale BE 2004) Limited research has been done on bioethanol production by direct bioconversion of lignocellulosic and carbohydrate-based materials, especially POME, which is a new substrate to be reported. The present study proposes the statistical optimization of processing conditions such as oxygen saturation level (pO2%), pH, and temperature in the utilization of POME for direct bioethanol production in a stirred-tank bioreactor with the co-culture of lignocellulolytic fungi and Saccharomyces cerevisiae. In this process, the direct bioconversion of POME into ethanol occurs in three steps. The first step is the delignification of lignocellulosic materials from their complex structure by lignocellulolytic fungus (Trichoderma harzianum and/or Phanerocheate chrysosporium). The second step is the depolymerization of the carbohydrate polymers (cellulose and hemicellulose) into reducing sugars (glucose, fructose, xylose etc.) using cellulolytic enzymes produced by the cellulolytic fungi (T. harzianum/Mucor hiemalis), followed by the third step, fermentation of sugars by yeast (S. cerevisiae) for bioethanol production.

MATERIALS AND METHODS

Collection of POME, Microbial culture inoculums preparation

Palm oil mill effluent was collected from A.P. Oil Industry, Kammam, India. The sample effluent was obtained at the point of discharge to the aerobic ponding system. The sample collected was stored at 4°C for further use. The fungal strains T. harzianum, P. chrysosporium, and M. hiemalis, and the yeast was S. cerevisiae were used in which three fungal and one yeast. The strains were sub-cultured on potato dextrose agar plates once a month after selecting from the laboratory stock based on their potential for biodegradation and biocatalytic activity. One successful individual colony of S. cerevisiae was taken from a potato dextrose agar plate and inoculated into a 100-ml Erlenmeyer flask containing 50 ml of yeast-malt extract medium with a composition of 10 g/l of malt extract and 20 g/l of yeast. The inoculated sample was incubated at 30°C for 24 h at 150 rpm. The concentration of the cells was measured as 110 cells/ml for further use in fermentation. For the preparation of fungal inoculums, seven-day potato dextrose agar plate cultures of each strain were collected. A total of 30 ml of sterilized distilled water was used to wash a culture plate with an L-shaped glass rod to obtain the suspension inoculum. The suspension inoculums were filtered through Whatman #1 filter paper and collected. The inoculum was poured into a 250-ml shake flask and stored at 4°C in a chiller for future use. The concentration of spore suspensions was determined as 2 9 112 spores/ml using a hemacytometer.

Development of single step bioconversion for bioethanol production and optimization of process conditions in a 3 litre stirred-tank bioreactor

A one litre Erlenmeyer flask containing 400 ml of Palm oil mill effluent was used to develop the direct bioconversion process with several compatible mixed cultures. The compatible mixed cultures were designed based on combinations of fungus with yeast as a common factor. Three combinations were used: T. harzianum (TH) and S. cerevisiea (SC) as TH-SC; M. hiemalis (MH) and S. cerevisiea (SC) as MH-SC; and T. harzianum (TH), M. hiemalis (MH), and S. cerevisiea (SC) as TH-MH-SC. The combination of P. chrysosporium (PC) and S. cerevisiea (SC) as PC-SC was not considered in this study as it was not found to be compatible in a previous study. According to the inoculation strategy, four experiments designated as runs were carried out to evaluate the direct bioconversion process for bioethanol production (Table-1).

Table-1. Microbes combination and inoculation time for the single step bioconversion of palm oil mill effluent into bioethanol.

Run	Microbes	Inoculation time
1.	T. harzianum and S. Cereviasiae (TH-SC)	TH was inoculated at the beginning and SC on the third day for 5-day fermentation
2.	T. harzianum and S. Cereviasiae (TH-SC)	Broth strains were inoculated at the beginning for 5-day fermentation
3.	M. hiemalis and S. Cereviasiae (MH-SC)	MH was inoculated at the beginning and SC on the third day for 5-day fermentation
4.	P. chrysosporium, T. harzianum and S. Cereviasiae (PC-TH-SC)	PC was inoculated at the beginning, TH on the second day and SC on the fifth day for 7-day fermentation

The optimum medium and process compositions used in this study were as follows: 1% palm oil mill effluent (w/w, total suspended solids, TSS), 2% (w/w) wheat flour (easily biodegradable nutrients), 800 mg/l KH_2PO_4, 3% (v/w) inoculum, 30^0C temperature, 200 rpm agitation, and pH 5. Samples were autoclaved at 121^0C for 15 min and inoculated with different combinations of mixed cultures as shown in Table-1. Sampling was done every day and analyzed for pH and total sugar and ethanol contents. Experiments were done with three replications.

To optimize the process conditions in a 3 litre stirred tank bioreactor for the production of bioethanol, a fractional factorial design with one centre point was applied with the best experimental run obtained from the study of the development of direct bioconversion (Table-1). Three factors (parameters)-oxygen saturation level (pO2), temperature, and pH-were selected for process optimization considering their linear, quadratic, and interaction effects. Using the compatible mixed culture in one system, the maximum (+), minimum (-), and central (0) levels for the factors were selected based on the previous study and literature review as follows: pO2 10% (-), 20% (0), and 30% (+); temperature 25^0C (-), 32.5^0C (0), and 40^0C (+); and pH 3 (-), 6 (0), and 9 (+). A 3-L BIOTRON laboratory-scale fermenter with a six blade Rushton turbine with a total working volume of 2.5 litre was used. The initial pH of the substrate was adjusted according to the FFD and automatically controlled throughout the fermentation time by the addition of 2 M NaOH and 2 M HCl into the fermenter. The pH probe was calibrated before the sterilization of the media, and the pO2 probe and acid, base, and antifoam pumps were calibrated before the inoculation. The pO2 probe was calibrated by sparging nitrogen gas and air into the broth; however, no antifoam agent was used since no foaming occurred. The dissolved oxygen (pO2) was maintained by agitation of the impeller, which was cascaded to the stirrer only. Temperature, agitation, foaming, level, pO2, and pH were maintained automatically by microprocessor control of the bioreactor. No additional air was supplied by sparging for bioethanol production. The total time of the fermentation process for each run was 4 days, and a 25-ml sample was withdrawn from the reactor vessel every day. The sample was filtered with Whatman No. 1 filter paper and centrifuged at 13,000 rpm for 20 min prior to analysis. Each sample was analyzed for pH, concentration of bioethanol, and total sugar. A regression model was developed from the experimental design with the response of bioethanol as the dependent variable using the statistical software Minitab Release 15. The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). This analysis included the Fisher's F test, its associated probability P (F), and coefficient of determination R^2, which measures the fit of the regression model. It also includes the t value for the estimated coefficients and the associated probabilities P (t).

Bio-ethanol production under optimum process conditions- Analytical analysis

Validation of the model a final experiment to validate the model under optimum process conditions (pO2, temperature, and pH) was carried out for 5 days of fermentation. A sample was analyzed everyday for the analysis of bioethanol, total sugar, and chemical oxygen demand (COD) as part of the bioconversion. Bioethanol was measured by using ethanol determination. The COD was measured using the HACH method, and total suspended solids (TSS) of treated samples were observed using the standard methods. The total sugar was determined by the phenol sulphuric acid method with

spectrophotometer at 490 nm, and pH was measured using pH meters. Data are the average of three replicates.

RESULTS AND DISCUSSIONS

Development of single-step bioconversion for bioethanol production

Four experimental runs were carried out to evaluate the single step bioconversion of palm oil mill effluent into ethanol utilizing lignocellulolytic fungi and yeast. Three types of fungi were used: Trichoderma harzianum (TH), Mucor hiemalis (MH), and Phanerochaete chrysosporium (PC), and the yeast used were Saccharomyces cerevisiae (SC). Most of the fungi and yeast were previously proven to be compatible with each other. The runs were designed based on the times of microbial inoculation, which were either simultaneous or one at a time (Table-1). Several analyses were conducted to investigate the production of ethanol, concentration of total sugar, and pH. From these analyses, the best experiment, run 2, was selected for the development of direct bioconversion towards the bioethanol production. The production of bioethanol under different experimental conditions (Table-1) is shown in Figure-1.

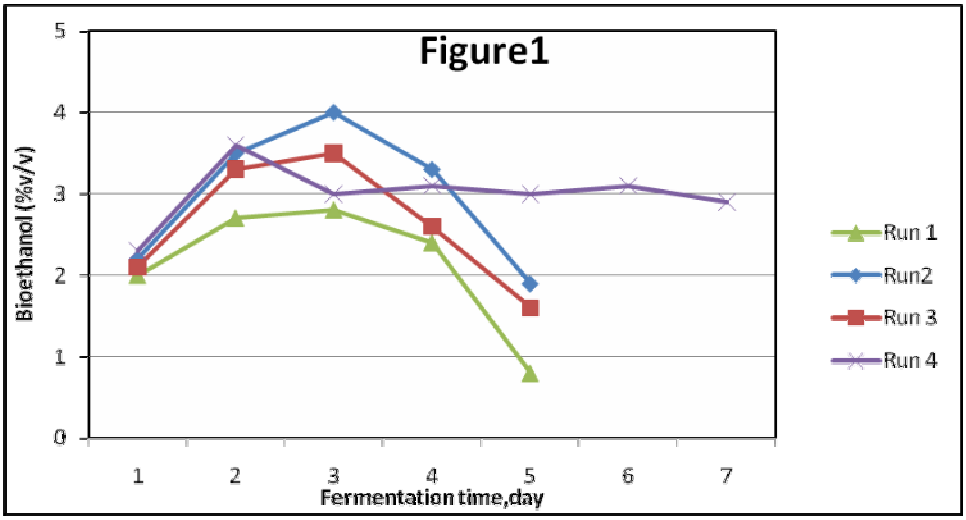

Figure-1. Production of bioethanol under different treatment strategies as part of the development of a direct bioconversion process. Run 1 TH-SC (simultaneous inoculation); Run 2 TH-SC; Run 3 MH-SC; Run 4 PC-TH-SC.

Although the ethanol concentration decreased at the end of the fermentation time for all runs, the concentration of bioethanol increased with increased fermentation time. When compared to the other runs, in run 2, the inoculation of T. harzianum co-culture with S. cerevisiae at the beginning was shown to be the best experimental run, yielding a higher amount of ethanol production. On the third day of fermentation the maximum ethanol produced, 4.5% (v/v) or 31.6 g/l was recorded for run 2, and there was a sharp decline in ethanol concentration on days 4 and 5. For the run 4, a longer fermentation time (7 days) led to fluctuations in ethanol production with lignocellulolytic fungi (P. chrysosporium and T. harzianum) and S. cerevisiae. As the enzymatic system of these fungi is delayed by secretion, the ethanol production increases through 5 days of fermentation while it decreases at day 6.

As shown in Figure-2, due to the hydrolysis of lignocellulosic materials by fungal strains and simultaneous conversion of sugars to ethanol by S. cerevisiae the concentration of total sugar was found to fluctuate throughout the fermentation period. For run 2, from day 1 to day 2, the total sugar concentration fell tremendously, indicating rapid consumption of sugar by the microorganisms. The highest total sugar concentration (1.50 g/l) was recorded on day 1 of fermentation for run 2, while it was the lowest on day 2 at 0.40 g/l. The initial pH of the broth was set at 5. The pH results shown in Figure- 3 indicate that the pH of each run decreased throughout the fermentation time.

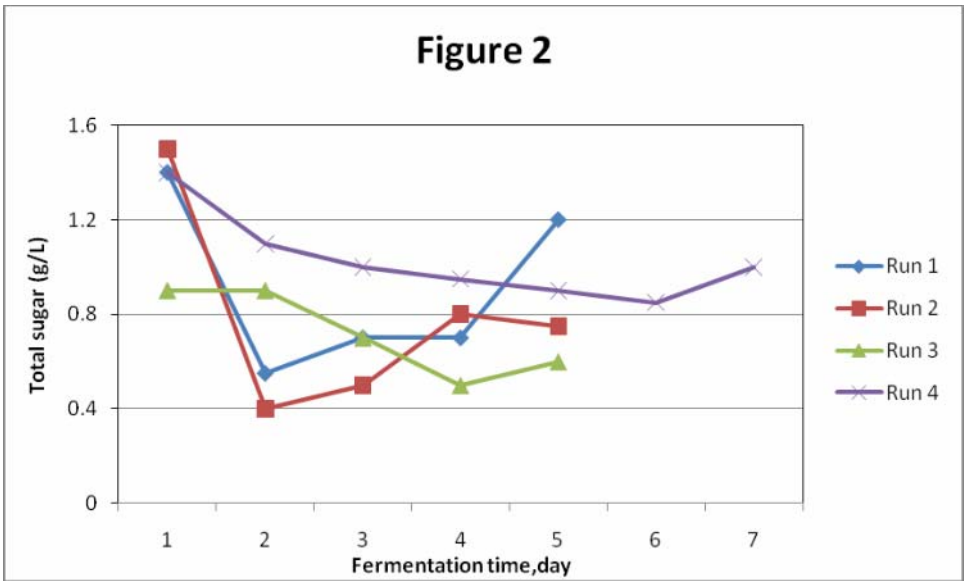

Figure-2. Total sugar concentration (g/l) of different experimental runs over the course of fermentation. Run 1 TH-SC (simultaneous inoculation); Run 2 TH-SC; Run 3 MH-SC; Run 4 PC-TH-SC.

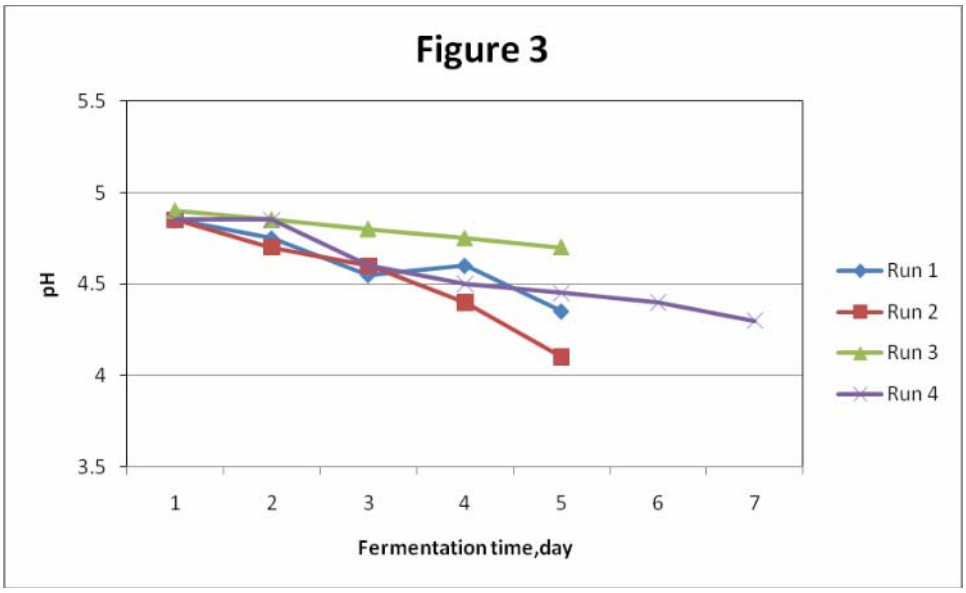

Figure-3. pH observed during direct conversion of POME for bioethanol production. Run 1 TH-SC (simultaneous inoculation); Run 2 TH-SC; Run 3 MH-SC; Run 4 PC-TH-SC.

The decrease in pH indicated that fermentation reaction was occurring in the broth. The pH of run 2 (inoculation of TH and SC at the beginning) dropped significantly throughout the process while the pH of run 3 (inoculation of MH at the beginning and SC on the third day) showed a slower rate of pH decrease (Figure-3). The lowest pH achieved was on day 5 of run 2 when the pH reached 4.1. An unusual observation can be seen in run 1 where there was a slight increase in pH at day 4. Optimization of process conditions in a 3 litre stirred-tank bioreactor. The most effective experimental conditions (run 2) for the single step bioconversion of palm oil mill effluent for bioethanol production were examined for further optimization. Three process conditions, pO2 (X_1), temperature (X_2), and pH (X_3), were observed to determine their effects on the single-step bioconversion of palm oil mill effluent into bioethanol production in a 3 litre stirred-tank bioreactor. In order to determine the optimum conditions for direct bioconversion, five runs were designed using two-level fractional factorial design with a single central point. The total fermentation time for each experiment was set to 4 days. From the optimization experiment, the highest concentration of ethanol, 4.4% v/v or 34.7 g/l was achieved in run 4 where the pO2 was 30%, temperature 32.5^{0}C, and pH 3 (Table-2).

Table-2. Predicted and experimental results for bioethanol production by the experimental design.

Run No.	pO_2 (%)	Temperature (^0C)	pH	Ethanol concentration* in % (v/v expected)	Ethanol concentration in % (v/v measured)
1.	10(-)	25(-)	3(-)	1.7	1.6
2.	10(-)	32.5(0)	9(+)	3.5	3.4
3.	20(0)	32.5(0)	6(0)	1.6	1.5
4.	30(+)	32.5(0)	3(-)	4.4	4.5
5.	30(+)	40(+)	9(+)	1.6	1.7

The minus sign indicates the minimum value for a given factor and the plus sign the maximum value and 0 the central point.
* The ethanol concentration is based on volume% in which 1 % (v/v) ethanol is equivalent to the concentration of 7.9 g/l.

By using the statistical software Minitab 15, the regression equation was generated based on the experimental results obtained. The generated second-order quadratic model showing the production of bioethanol (Y, volume %) with independent variables of pO2 (X_1), temperature (X_2), and pH (X_3) is as follows:

$$Y = - 30.6 - 0.909X_1 + 2.59X_2 + 0.0527X_3 + 0.0242X_1^2 - 0.0415X_2^2 \quad \text{---- (1)}$$

The terms X_3^2, X_1X_2, X_1X_3, and X_2X_3 have been removed from the equation since they are highly related to other X variables. So, the model indicated that no interactions were found to be significant among the variables. The regression equation and coefficient of determination R^2 were evaluated in order to test the fitness of the design of the experiment or model. The model showed a high R^2 (0.996) and a high adjusted R^2 (adj) (0.994), which indicates that the model is highly significant. The corresponding analysis of variance (ANOVA) is presented in Table-3.

Table-3. Analysis of variance for the quadratic model.

Source	Degree of freedom	Sum of squares	Mean squares	P value	F value
Regression	5	21.91	4.38	0.000	424.60
Residual error	9	0.093	0.01		
Total	14	22.00			

The analysis of variance of the quadratic regression model demonstrated that the model was highly significant. The computed F value (424.60) indicated that overall, the model was highly significant with a high confidence level. This is also supported by very low probability value (P = 0.000). The t and P values for the linear and quadratic elements are summarized in Table-4.

Table-4. Statistical analysis showing coefficients, P values and t values.

Predictor	Coefficient	Standard error coefficient	P value	T value
Constant	-30.6	1.28	0.000*	-23.74
X_1, pO_2	-0.909	0.035	0.000*	-26.86
X_2, Temperature	2.59	0.085	0.000*	30.45
X_3, pH	0.0527	0.04	0.189	1.42
X_1^2	0.0242	0.0007	0.000*	32.24
X_2^2	-0.0415	0.0011	0.000*	-37.42

* Significant at P<0.01

The significance of each coefficient or factor was determined by the Student t distribution and P values. The variables with low probability levels contribute to the model, whereas others with high probability levels can be neglected and eliminated from the model. The low values of P of <0.05 and the larger magnitude of t indicate a more significant correlation of coefficients. Table-4 shows that all P values were <0.01 except for the pH (P>0.05), which indicated that the model terms X_1, X_2, X_1^2, and X_2^2 have a significant effect on ethanol production. The computed t value represents the level of significance of the effect of the variables on ethanol production. Thus, it could be concluded that the variable with the largest effect was the squared term of temperature (X_2^2) followed by the linear term of temperature (X_2), squared term of pO2 (X_1^2), and linear term of pO_2 (X_1). The results indicate that the pH range of 5-6 and a high range of pO_2 (25-30%) results in maximum ethanol production (5-6%, v/v) when the temperature is at the centre point (32.5^{0}C). In general, yeast is able to grow and efficiently ferment substrates into ethanol at pH values of 3.5-6.0 and temperatures of 28-35^{0}C. Bioethanol production with developed process conditions: validation of the model a final experiment for the validation of the model was carried out under the optimum process conditions obtained from the statistical approach. Since the pH factor (X_3) was not shown to be significant in the model, it was set at a reasonable level for favourable microbial growth, preferably pH 6. The value of optimized pO2 was maintained at 30%. Therefore, the only factor varied in order to determine the developed process condition was temperature. At 32^{0}C, the maximum ethanol concentration was found to be 4.8% (v/v) or 38.6 g/l, which was calculated using Eq. 1. Any temperature below or above this point resulted in lower ethanol production. The ethanol concentration was measured starting from the first day of fermentation. Ideally it was assumed that there was no ethanol at the beginning of the reaction. The production of bioethanol with developed bioconversion is shown in Figure-4.

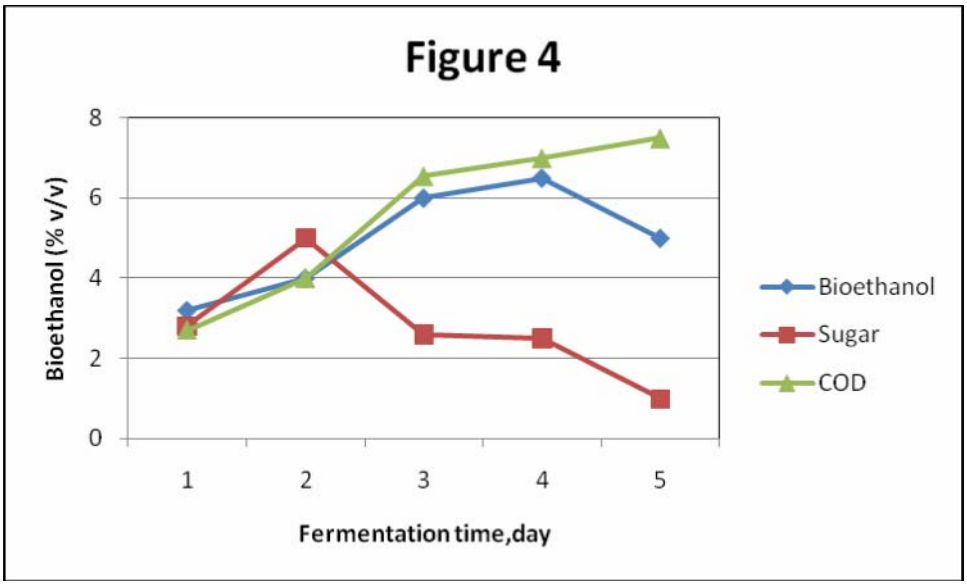

Figure-4. Production of bioethanol and biodegradation of palm oil mill effluent by single step bioconversion under the developed process conditions in a 3 litre computer controlled bioreactor available in the Department of Biotechnology, National Institute of Technology, Warangal. (Temperature 32^{0}C, pO_2 30%, pH 6).

The results indicated that the maximum ethanol production of 6.6% (v/v) or 52.4 g/l was achieved on day 4 of fermentation. The ethanol concentration decreased after 4 days of fermentation. The result shown in Figure-4 is that the total sugar concentration under optimum conditions was recorded to be highest at day 2 (4.8 g/l) and lowest at day 5 (1.9 g/l). The concentration of total sugar rapidly declined from the maximum level to the lowest level just before it rose again until the end of fermentation time. The fluctuation of the sugar released may be due to the secretion of cellulolytic enzymes by T. harzianum and the simultaneous conversion of sugar to ethanol by S. cerevisiae (Srinivas D, Rao KJ, Theodore K, Panda T. 1995). Lezinou *et al*., reported in their study that bioconversion of cellulose to ethanol involves acid or enzymatic hydrolysis of the biopolymer followed by the fermentation of resulting soluble oligosaccharides to ethanol. The direct conversion of modified wheat straw to ethanol can be also conducted by utilizing a co-culture of C. thermocellum strain and anaerobic-bacteria-fermenting pentoses, namely C. thermosaccharolyticum and C. thermohydrosulphuricum, respectively. The COD in the treated POME at day zero was found to be 114 g/l. The COD decreased with the fermentation time due to the consumption of soluble and insoluble organic substances in POME by the microorganisms for ethanol production as

well as cell growth and maintenance. In Figure-4, the removal of COD showed a pattern of increasing as more nutrients and organic matter were removed throughout the fermentation time. The COD removal obtained by the microbial treatment of POME was 89.1% after 4 days of direct bioconversion process and slightly increased to 91% in the final days of fermentation (5 days).

CONCLUSIONS

Single-step bioconversion developed by the compatible mixed culture of T. harzianum and S. cerevisiae was achieved with maximum bioethanol production of 4.5% (v/v) on the third day of fermentation. The optimization study showed that a concentration of ethanol of 4.8% (v/v) or 38.6 g/l was observed under the optimum conditions of 30% pO_2, temperature 32^0C, and pH 6. The single step bioconversion process with optimum conditions enhanced the bioethanol production to 6.6% (v/v) or 52.4 g/l. The removal of chemical oxygen demand as part of the biodegradation of palm oil mill effluent was found to be 80% after 4 days of treatment. This study shows a potential solution for palm oil mill effluent management through the production of bioethanol, which would be an alternative for ultimate disposal in future research.

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