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ORIGINAL PAPER

Production of bioethanol by direct bioconversion of oil-palm industrial effluent in a stirred-tank bioreactor

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Abstract The purpose of this study was to evaluate the feasibility of producing bioethanol from palm-oil mill effluent generated by the oil-palm industries through direct bioconversion process. 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 (4% 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 (pO_2%), 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 4.6% (v/v) or 36.3 g/l was achieved at a temperature of 32°C, pH of 6, and pO_2 of 30%. The results of the model validation test under the developed optimum process conditions indicated that the maximum production was increased from 4.6% (v/v) to 6.5% (v/v) or 51.3 g/l with 89.1% chemical-oxygen-demand removal.

Keywords Palm-oil mill effluent (POME) · Bioethanol · Direct bioconversion · *T. harzianum* · *S. cerevisiae*

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Introduction

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. 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 [1, 2]. In addition, POME has little inhibiting effect on microbial growth due to a certain content of lignin and phenolic compounds [3]. It is estimated that 0.5–0.75 t of POME can be discharged from every tonne of oil palm fresh fruit [4]. Several processes are currently being used to treat this effluent. An anaerobic ponding system is one of the major treatment processes practiced by most of the oil-palm industries. The major drawbacks of this process are that it requires large land areas and emits biogas, which pollutes the environment [5]. Some other applications, such as the production of citric acid [6], biohydrogen [7], oil palm-based activated carbon [8], and stone mastic asphalt with oil palm fiber [9] have been attempted. Due to low yield and lack of information for scale-up, most of the processes are restricted from further development. Direct bioconversion of POME for bioethanol production would be economic, practical, and useful, and while by itself it might not solve the pollution problem, it might be a step in the right direction for satisfying the global demand for bioethanol. In addition, the development and implementation of such technologies would provide employment, reduce oil imports, and provide a partial solution to the disposal of wastes [10].

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 [11–14]. 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 [15–20].

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 (pO_2%), 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

Palm-oil mill effluent as substrate

POME was collected from Seri Ulu Langat Palm Oil Industry, Dengkil, Malaysia. 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.

Microbial cultures for fermentation

A total of four strains, three fungal and one yeast, were used. The fungal strains were *T. harzianum*, *P. chrysosporium*, and *M. hiemalis*, and the yeast was *S. cerevisiae.* The strains were selected from the laboratory stock based on their potential for biodegradation and biocatalytic activity (lignocellulolytic enzymes) [21–23]. The strains were subcultured on potato dextrose agar (PDA) plates once a month.

Inoculum preparation for yeast and fungi

One successful individual colony of *S. cerevisiae* was taken from a PDA plate and inoculated into a 100-ml Erlenmeyer flask containing 50 ml of yeast-malt extract medium (YM) with a composition of 20 g/l of yeast and 10 g/l of malt extract. The inoculated sample was incubated at 30°C for 24 h at 150 rpm. The concentration of the cells was measured for further use in fermentation (10^8 cells/ml).

For the preparation of fungal inoculums, 7-day PDA 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 get the suspension inoculum. The suspension inoculums were collected after filtration through Whatman #1 filter paper. 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 to be 2×10^7 spores/ml using a hemacytometer.

Development of direct (single-step) bioconversion for bioethanol production

A 1,000-ml Erlenmeyer flask containing 400 ml of POME 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 [24]. Based on the inoculation strategy, four experiments designated as runs were carried out to evaluate the direct bioconversion process for bioethanol production (Table 1).

The optimum medium and process compositions used in this study were obtained from the previous study and were as follows: 1% POME (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°C temperature, 200 rpm agitation, and pH 5 [25]. Samples were autoclaved at 121°C for 15 min and inoculated with different combinations of mixed cultures as shown in Table 1. Sampling was done everyday and analyzed for pH and total sugar and ethanol contents. Experiments were done with three replications.

Optimization of process conditions in a 2-l stirred-tank bioreactor

To optimize the process conditions in a 2-l stirred-tank bioreactor for the production of bioethanol, a fractional

Table 1 Experimental design for the development of direct bioconversion of POME into bioethanol production

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

factorial design with one center 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 (pO_2), 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 [10, 12, 25]: pO_2 10% (–), 20% (0), and 30% (+); temperature 25°C (–), 32.5°C (0), and 40°C (+); and pH 3 (–), 6 (0), and 9 (+).

A 2-L BIOSTATB laboratory-scale fermenter (Sartorius BBI Systems) with Rushton turbine with a total working volume of 1.5 l 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 pO_2 probe and acid, base, and antifoam pumps were calibrated before the inoculation. The pO_2 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 (pO_2) was maintained by agitation of the impeller, which was cascaded to the stirrer only. Temperature, agitation, foaming, level, pO_2, 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 experiment (run) was 4 days, and a 30-ml sample was withdrawn form the reactor vessel every day. The sample was filtered with Whatman No. 1 filter paper and centrifuged (Eppendorf AG 22331, Hamburg, Germany) 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 14. The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). This analysis included the Fisher's F test (for overall model significance), 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). A 2D contour plot was presented to evaluate the parameters tested within the surface of the response.

Bioethanol production under optimum process conditions: validation of the model

A final experiment to validate the model under optimum process conditions (pO_2, 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.

Analytical analysis

Bioethanol was measured by using ethanol determination kits (QuantiChrom Ethanol Assay Kit DIET-500, Gentaur Molecular Products, Brussels). The COD was measured using the HACH method [26], and total suspended solids (TSS) of treated samples were observed using the standard methods of the American Public Health Association (APHA) [27]. The total sugar was determined by the phenol sulfuric acid method [28] with spectrophotometer at 490 nm, and pH was measured using pH meters. Data are the average of three replicates.

Results and discussion

Development of direct (single-step) bioconversion for bioethanol production

Four experimental runs were carried out to evaluate the direct (single-step) bioconversion of POME 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 was *Saccharomyces cerevisiae* (SC). Most of the fungi and yeast were previously proven to be compatible with each other [24]. 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 Fig. 1. The concentration of bioethanol increased with increased fermentation time; however, all runs showed that the ethanol concentration decreased at the end of the fermentation time. 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 compared to the other runs. The maximum ethanol produced, 4% (v/v) or 31.6 g/l, was recorded on the third day of fermentation for run 2, and there was a sharp decline in ethanol concentration on days 4 and 5. For 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 [21, 22]. In another study, Nakamura et al. [29] reported that an ethanol concentration of 15% (v/v) was obtained during 72-h fermentation of Jerusalem artichoke tubers in mixed culture of *S. cerevisiae* and *A. niger*.

As shown in Fig. 2, the concentration of total sugar was found to fluctuate throughout the fermentation period. This is due to the hydrolysis of carbohydrate/lignocellulosic materials by fungal strains and simultaneous conversion of sugars to ethanol by *S. cerevisiae*. For run 2, the total sugar concentration fell tremendously from day 1 to day 2, indicating rapid consumption of sugar by the microorganisms. The highest total sugar concentration (1.48 g/l) was recorded on day 1 of fermentation for run 2, while it was lowest on day 2 at 0.40 g/l.

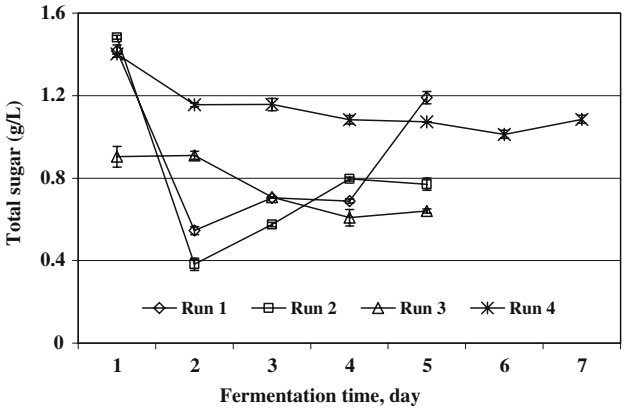

Fig. 2 Total sugar concentration (g/l) of different experimental runs over the course of fermentation. *Run 1* TH-SC (simultaneous inoculation), *Run 2* TH-SC, *Run 3* MH-SC, *Run 4* PC-TH-SC (for abbreviations, see Table 1)

The initial pH of the broth was set at 5. The pH results shown in Fig. 3 indicate that the pH of each run decreased throughout the fermentation time. 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 (Fig. 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.

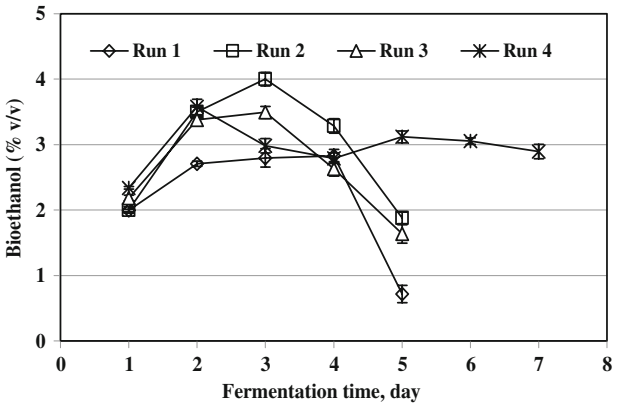

Fig. 1 Production of bioethanol under different treatment strategies as part of the development of a direct bioconversion process. *Run 1* TH-SC (simultaneous inoculation), *Run 2* TH-SC, *Run 3* MH-SC, *Run 4* PC-TH-SC (for abbreviations, see Table 1)

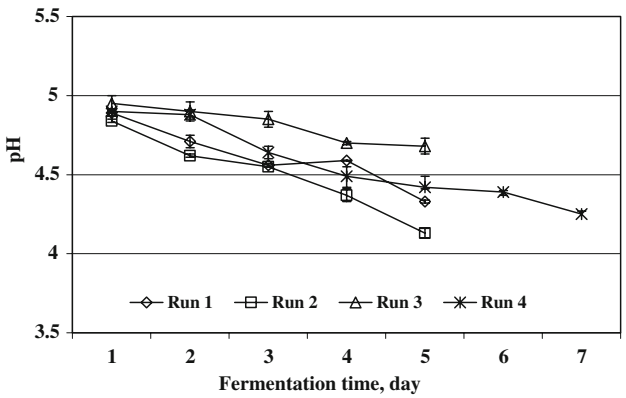

Fig. 3 pH observed during direct bioconversion of POME for bioethanol production. *Run 1* TH-SC (simultaneous inoculation), *Run 2* TH-SC, *Run 3* MH-SC, *Run 4* PC-TH-SC (for abbreviations, see Table 1)

Table 2 Experimental and predicted results for bioethanol production obtained by the experimental design

Run no.	Factors			Ethanol concentration[a] (%, v/v)	
	pO_2 (%)	Temperature (°C)	pH	Measured	Expected
1	10 (−)	25 (−)	3 (−)	1.6	1.7
2	10 (−)	32.5 (0)	9 (+)	3.4	3.5
3	20 (0)	32.5 (0)	6 (0)	1.5	1.6
4	30 (+)	32.5 (0)	3 (−)	4.5	4.4
5	30 (+)	40 (+)	9 (+)	1.7	1.6

The *minus sign* indicates the minimum value for a given factor, the *plus sign* the maximum value, and *0* the central point

[a] The ethanol concentration is based on volume % in which 1% (v/v) ethanol is equivalent to the concentration of 7.9 g/l

Optimization of process conditions in a 2-l stirred-tank bioreactor

The most effective experimental conditions (run 2) for the direct bioconversion of POME for bioethanol production were examined for further optimization. Three process conditions, pO_2 (X_1), temperature (X_2), and pH (X_3), were observed to determine their effects on the single-step bioconversion of POME into bioethanol production in a 2-l 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 pO_2 was 30%, temperature 32.5°C, and pH 3 (Table 2).

By using the statistical software Minitab Release 14, 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 pO_2 (X_1), temperature (X_2), and pH (X_3) is as follows:

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

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. Therefore, 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 ANOVA is presented in Table 3. The ANOVA of the quadratic regression model demonstrated that the model was highly significant. The computed F value (422.56) 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. 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 3 Analysis of variance (ANOVA) for the quadratic model

Source	Degrees of freedom	Sum of squares	Mean squares	F value	P value
Regression	5	21.91	4.38	422.56	0.000
Residual error	9	0.093	0.01		
Total	14	22.00			

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

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

* Significant at $P < 0.01$

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 pO_2 (X_1^2), and linear term of pO_2 (X_1).

The 2D contour plot is an interpretation of the regression equation used to evaluate the optimum values within the ranges of the variables considered [30]. The main target of the response surface is to obtain the optimum values of the variables efficiently so that the response is maximized. The maximum predicted value is represented by the surface confined in the smallest ellipse in the contour diagram. A perfect interaction between the independent variables occurs when elliptical contours are obtained. The response surface (2D contour plot) described by the model equation to estimate ethanol production based on the independent variables pO_2 and temperature is shown in Fig. 4. 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 center point (32.5°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°C. Nigam [10] reported that the pH of a continuous stirred-tank reactor (CSTR) was maintained at 4.5 for ethanol production from pineapple-cannery waste. Stevenson and Weimer [31] noted that a pH range of 3.8–5.5 was feasible in the fermentation of cellulose to ethanol by *Trichoderma* strain. According to Galbe and Zacchi [32], a temperature of 35°C for simultaneous saccharification and fermentation (SSF) is a compromise, but the development of a thermotolerant yeast strain is expected to improve the performance of SSF. The SSF of corn cobs into ethanol by yeast was carried out at 37°C in a shaker at 150 rpm [11]. Saha and Ueda [33] reported that maximum ethanol was produced at 38°C by *S. cerevisiae* in the fermentation of glucoamylase-treated starch. Verma et al. [12] reported that the optimum temperature for maximum ethanol production using starch in co-cultures of amylolytic yeasts and *S.cerevisiae* 21 was 30°C, and there was no remarkable loss in ethanol yield up to 40°C.

Yeasts, under anaerobic conditions, metabolize glucose to ethanol primarily by the Embden-Meyerhof pathway [34]. However, Kosaric [34] showed that a small concentration of oxygen must be provided to the fermenting yeast, as it is a necessary component in the biosynthesis of polyunsaturated fats and lipids. Typical amounts of O_2 to be maintained in the broth are 0.05–0.10 mm Hg oxygen tension. Other researchers have noted the effects of oxygen-limited conditions on ethanol production from xylose by *P. stipitis* and *C. shehatae* [35, 36].

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 favorable microbial growth, preferably pH 6. The value of optimized pO_2 was maintained at 30%. Therefore, the only factor varied in order to determine the developed process condition was temperature. At 32°C, the maximum ethanol concentration was found to be 4.6% (v/v) or 36.3 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 Fig. 5. The results indicated that the maximum ethanol production of 6.5% (v/v) or 51.3 g/l was achieved on day 4 of fermentation. The ethanol concentration decreased after 4 days of fermentation. For the production of ethanol

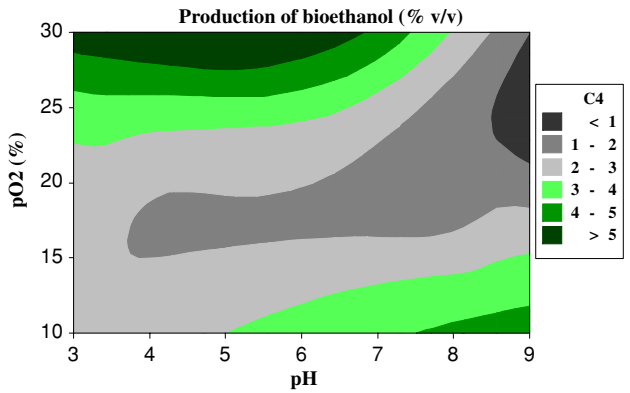

Fig. 4 2D contour plot obtained by the interaction of pO_2 (%) and pH for the maximum response (bioethanol) (temperature 32.5°C)

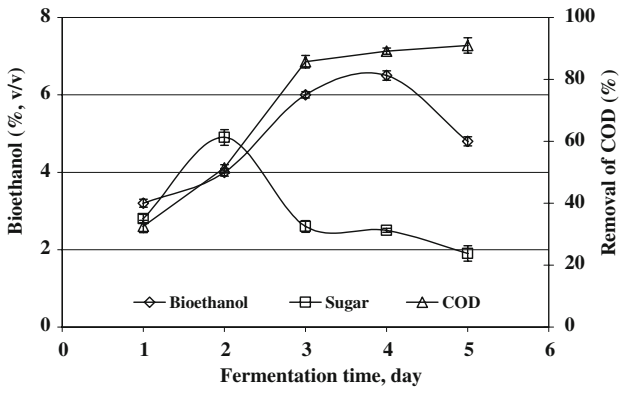

Fig. 5 Production of bioethanol and biodegradation of POME by direct bioconversion under the developed process conditions in the stirred-tank bioreactor (temperature 32°C, pO_2 30%, pH 6)

from cellulose by *T. reesei* and *S. cerevisiae*, Srinivas et al. [37] reported that they obtained the highest ethanol concentration (0.17 g/l) at 30 h of fermentation, while Verma et al. [12] reported that maximum ethanol (24.8 g/l) was produced in 48 h of fermentation by *S. cerevisiae* 21 (distiller's yeast) and *S. cerevisiae* (baker's yeast).

The result shown in Fig. 5 is that the total sugar concentration under optimum conditions was recorded to be highest at day 2 (4.9 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*. Lezinou et al. [38] 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 [18].

The COD in the treated POME at day zero was found to be 111 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 (as food and energy). In Fig. 5, 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

Development of direct (single-step) bioconversion by the compatible mixed culture of *T. harzianum* and *S. cerevisiae* was achieved with maximum bioethanol production of 4% (v/v) on the third day of fermentation. The optimization study showed that a concentration of ethanol of 4.6% v/v or 36.3 g/l was observed under the optimum conditions of 30% pO_2, temperature 32°C, and pH 6. The direct bioconversion process with optimum conditions enhanced the bioethanol production to 6.5% (v/v) or 51.3 g/l. The removal of COD as part of the biodegradation of POME was found to be 89.1% after 4 days of treatment. This study shows a potential solution for POME management through the production of bioethanol, which would be an alternative for ultimate disposal in future research.

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