Production of Bioethanol from Spontaneous Fermentation of Palm Oil Mill Effluent (POME).

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Abstract: The feasibility of bioethanol production from spontaneous fermentation of palm oil mill effluent was carried out. Cooled POME (1.5L) was allowed to ferment spontaneously for 21 days for analysis of microbial quality, free fatty acid, lipase activity and ethanol contents. Effect of pH and supplementation on ethanol and other parameters were also determined every 3 days for 21 days. Ethanol content increased as the concentration of glucose increased and highest ethanol content (0.9%) was recorded at 25g/L and 30g/L of glucose in POME. Sugar cane bagasse supplementation had highest ethanol content (0.9%) at 20g/L and 30g/L thereafter decreased as its supplementation increased. Corn steep liquor supplementation did not have any effect on ethanol production from POME. Fermented POME supplemented with corn steep liquor had the highest lipase activity (0.00756µg/ml/day) and free fatty acid (2.561%) at 150ml/L. POME supplemented with 30g/L glucose and 30g/L sugar cane bagasse had the highest record of ethanol content (2.3%) at pH of 8.5 with highest free fatty acid (5.029%) at pH 6.5 on the 12th day of fermentation and highest lipase activity (0.03200µg/ml/day) was recorded at pH 6.5 on the 3rd day of fermentation. A relative high bioethanol can be produced by spontaneously fermenting POME with addition of 30g/L of glucose and 30g/L of sugar cane bagasse for 12 days at pH of 8.5.
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1. Introduction

The oil palm is a perennial crop that originated in the tropical rain forest of West Africa. It spread to South America in the 16th century and to Asia in the 19th century. During the 1970s, Asia overtook Africa as the principal oil palm producing region in the world (Olagunju, 2008). In Africa, no part of the oil palm is considered waste. The residue after oil has been extracted is called palm kernel cake, which is useful in feeding livestock. The leaves of oil palm are used for making brooms, roofing and thatching, basket and mats. The thicker leaf stalks are used for walls of village huts. The bark of the palm frond is peeled and woven into baskets (Komolafe and Joy, 1990).

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 (Habib *et al*., 1979; Wu *et al*., 2009).

In many parts of the world, demand for ethanol as an alternative fuel source has steadily increased (Sheoran *et al*., 1998) due to dwindling fossil fuel resources and increased gasoline prices. Biofuel crops include corn, corncobs, corn stover, starch, rice, wheat, sorghum, and sugar cane (Suresh *et al*., 1999; Verma *et al*., 2000; Latif and Rajoka, 2001; Kadam and McMillan, 2002). Most of these resources compete with human food production, as well as having high production prices that restrict their industrial production. Lignocellulosic materials which include agricultural residues (e.g., crop residues and sugar cane bagasse), herbaceous crops (e.g., alfalfa, switchgrass), forestry wastes, wastepaper, and other wastes could serve as alternative resources for bioethanol production, due to their lower prices and local abundance (Sun and Cheng, 2002; Kim and Dale, 2004).

Palm oil mill effluent has great potential as a substrate for acetone, butanol and ethanol fermentation because it contains a mixture of carbohydrates including starch, hemicellulose, sucrose and other carbohydrates that can be utilized by microorganisms (Lee *et al*., 1995) Some applications, such as the production of citric acid (Jamal *et al*., 2005), biohydrogen (Cheong *et al*., 2004), oil palm-based activated carbon (Alam *et al*., 2006), and stone mastic asphalt with oil palm fiber (Muniandy, 2000) from POME have been attempted. Due to low yield and lack of information for scale-up, most of the processes are restricted from further development. This research work is aimed at determining the possibility of producing bioethanol from spontaneous fermentation of palm oil mill effluent.

2. Materials and Methods

Collection of Sample

Palm Oil Mill Effluent (POME) was collected aseptically from Palm Oil Mill Industry in Oyedeji's Village, Igbo-Elerin Area of Lagelu Local Government, Ibadan, Oyo State. The sample was collected during production of palm oil at a temperature between 80ºC and 90ºC. It was brought to the laboratory, allowed to cool to room temperature and kept at 4ºC until use.

Fermentation and Sampling

One and half litres (1.5L) of cooled sample was aseptically measured into sterile 2L Erlenmeyer flask, covered with sterile aluminium foil and allowed to ferment spontaneously for 21 days. One hundred and fifty millilitre (150ml) sample was taken every 3 days for analysis of microbial quality, ethanol content, lipase activity and free fatty acid content.

Isolation Method

POME was serially diluted, and appropriate dilutions were pour-plated on different growth media in duplicate. Culture on Nutrient Agar (NA) and de Mann Rogosa Sharpe (MRS) agar were inverted and incubated at 37ºC for 48 hours and incubated aerobically and anaerobically respectively. Cultures on Yeast Extract Agar (YEA) and Potato Dextrose Agar (PDA) were incubated at room temperature (30±2ºC) for 3 and 5 days respectively. All plates were counted after incubation period.

Identification of Isolates

All the bacteria isolates were identified using microscopic, biochemical and physiological characteristics (Sneath *et al*., 1986). Yeasts were identified according to Kreger-van Rij (1984) and Barnett *et al*. (1990). Mould isolates were identified according to their micro-morphology as well as the colour and nature of their sporulating structures and conidia (Barnett and Hunter, 1972; Onions *et al*., 1981).

Determination of Acidity (pH)

The pH of the fermenting sample was determined using the electrode probe of pH meter throughout the sampling period. The pH meter was calibrated with buffers 4, 7 and 9.

Determination of Ethanol Content of the Samples

Ethanol content of the fermented samples was determined using gravimetric method. The specific gravity was determined using density bottle. One hundred millilitres of the samples was distilled using electric heating mantle. The weight of empty density bottle was taken. The distillate was poured into a dry density bottle which had a volume of 25ml and weighed. Equal volume of distilled water was also poured into a dry density bottle and weighed. The weight of the empty dry density bottle was subtracted from both the weight of density bottle containing fermenting Palm Oil Mill Effluent distillate and that of distilled water. The specific gravity was determined by dividing the weight of the distillate of Palm Oil Mill Effluent by the weight of the distilled water. This is according to AOAC (1990).

$$\text{Specific Gravity} = \frac{W_1 - W}{W_2 - W}$$

Where W1= weight of the density bottle plus weight of distillate of Palm Oil Mill Effluent

W2= weight of the density bottle plus weight of distilled water and

W= weight of empty density bottle

The specific gravity was used to determine the concentration of ethanol using Ethyl Alcohol Conversion Table

Determination of Lipase Activity

Fifteen millilitres of fermented Palm Oil Mill Effluent was measured and 0.1N sodium hydroxide (NaOH) was titrated against it to pH 9.5 using pH meter in order to quantify the volume of sodium hydroxide that was used (Onilude *et al*., 2010). A unit of lipase activity is defined as the amount of NaOH used in the titration to bring the reaction mixture to pH of 9.5 under the defined assay condition (Young and Wood, 1977). Lipase activity was calculated using modified method of Kanimozhi *et al*. (2011).

$$\text{Lipase Activity } (\mu g/ml/day) = \frac{\text{Volume of alkali consumed x Normality of NaOH}}{\text{Time of Incubation x Volume of the POME}}$$

Determination of Free Fatty Acid

Sodium hydroxide (0.1N) was titrated against fifteen millilitres of fermented Palm Oil Mill Effluent to pH of 9.5 and the volume of NaOH used was quantified (Onilude *et al*., 2010). The Free Fatty Acid was calculated using modified method of Kanimozhi *et al*. (2011).

$$\text{Free Fatty Acid } (\%) = \frac{\text{Volume of alkali consumed x Constant Value } (0.02825) \text{ x } 100}{\text{Volume of POME}}$$

Effect of Different Supplements on Production of Bioethanol

POME was supplemented with 3 different supplements at different concentrations. The supplements (concentrations) are: Sugar Cane Bagasse (10g/L, 20g/L, 30g/L, 40g/L and 50g/L), Corn Steep Liquor (50ml/L, 100ml/L, 150ml/L, 200ml/L and 250ml/L) and Glucose (10g/L, 15g/L, 20g/L, 25g/L and 30g/L). Ethanol concentration,

lipase activity, free fatty acid and pH of the samples were determined at 0 day and 12 day.

Effect of pH on Bioethanol Production from supplemented POME

Effect of different pH on POME supplemented with 30g/L glucose and 30g/L sugar cane bagasse was carried out to determine the best pH for higher ethanol yield. The initial pH of samples were adjusted to 4.5, 5.5, 6.5, 7.5 and 8.5 in order to know the effect of the pH on the combination of sugar cane bagasse and glucose for ethanol production. Sample was fermented for 15 days. Ethanol concentration, lipase activity, free fatty acid and pH of the sample were determined every 3 days for 15 days.

Statistical Analysis

The experimental data was analysed using Analysis of Variance to determine the means. The level of significance was set at $P \leq 0.05$. The data were analysed using SPSS version 19.

3. Results

The microorganisms isolated and characterized were identified as *Bacillus carotarum*, *Bacillus lentus*, *Bacillus stearothermophilus*, *Bacillus pumilis*, *Micrococcus luteus*, *Yarrowia lipolytica*, *Candida intermedia*, *Candida tropicalis*, *Clavispora lusitaneae*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceous* and *Penicillium* species.

Table 1 shows the Ethanol concentration, free fatty acid, lipase activity and pH of fermenting Palm Oil Mill Effluent. The pH decreases as the fermentation day increases from starting time (6.17) till the 18th day (4.88) of fermentation with a slight increase on the 21st day of fermentation. The lipase activity decreases with time from onset (0.0112µg/ml/day) till day 15 (0.00256µg/ml/day) with a slight increase in day 18 (0.002889µg/ml/day) and thereafter decreases. Free fatty acid increases with time from 0.3164% to 1.1526% by 12th day and decreases thereafter. Ethanol concentration was the same throughout the fermentation period.

Table 1. Ethanol concentration, free fatty acid, lipase activity and pH of fermenting palm oil mill effluent

Sample	Fermentation time (day)	pH	Ethanol concentration (%)	Lipase activity (µg/ml/day)	Free Fatty Acid (%)
1	0	6.17	0.5	0.0112	0.3164
2	3	5.39	0.5	0.006933	0.5876
3	6	5.19	0.5	0.004467	0.7571
4	9	5.11	0.5	0.003867	0.9831
5	12	5.03	0.5	0.0034	1.1526
6	15	4.94	0.5	0.00256	1.0848
7	18	4.88	0.5	0.002889	1.469
8	21	4.91	0.5	0.001924	1.1413

From Table 2, there was no ethanol production at the onset of fermentation irrespective of the quantity of added glucose. By 12th day of fermentation, ethanol concentration ranges from 0.5% to 0.9% by addition of 25g/L and 30g/L of glucose. These ranges are significantly different ($P \leq 0.05$) from each other. Lipase activity at all the concentration of glucose is higher at onset of fermentation than at 12th day. The lipase activity of unfermented POME is not significantly different from each other while that of 12-day fermented POME increases with increase in concentration of glucose and highest lipase activity of 0.01389µg/ml/day recorded at 25g/L glucose. Statistical analysis revealed that lipase activities of the 12-day fermented POME are significantly different ($P \leq 0.05$) with the difference in concentration of added glucose.

The free fatty acid of unfermented POME increases as the concentration of glucose increases and the highest free fatty acid of 2.825% was recorded at 30g/L of glucose while the lowest free fatty acid of 2.034% was observed in unsupplemented POME. The 12-day fermented POME has the lowest free fatty acid (2.354%) in unsupplemented sample of 0g/L and highest (4.708%) at 25g/L supplementation. The free fatty acid of both unfermented and 12-day fermented POME were significantly different ($P \leq 0.05$) from each other at different concentration of glucose within each sampling period.

A relatively steady pH irrespective of glucose concentration was recorded at the onset while a drastic increase in acidity/decrease in pH as a result of glucose supplementation was observed in fermented POME. Highest acidity (3.3) was observed at 20g/L and 30g/L glucose supplementation on 12-day fermented POME. Statistically, there was little or no difference in pH values except for unsupplemented sample.

Table 3 shows the effect of supplementation of sugar cane bagasse in POME on pH, lipase activity, ethanol and free fatty acid at 0 day and 12 day. Ethanol was not produced at the onset (0 day) of fermentation irrespective of the quantity of sugar

cane bagasse supplementation. Ethanol content of 12-day fermented POME increases as sugar cane bagasse concentration increases and later decreases with increased supplementation. Statistical analysis revealed a significant difference ($P \leq 0.05$) between the unsupplemented and sugar cane bagasse supplemented POME.

Lipase activity of fermented POME (12 day) at all concentration is higher than lipase activity of unfermented POME (0 day). The addition of different concentration of sugar cane bagasse to fermenting POME shows an increase in lipase activity as the concentration of supplement increases till 40g/L and decreases by addition of 50g/L sugar cane bagasse. Statistical analysis shows that addition of sugar cane bagasse to both unfermented and 12-day fermented POME significantly affect the lipase activity ($P \leq 0.05$) except unsupplemented and 10g/L supplemented POME fermented for 12 days.

Table 2. Effect of glucose supplementation of fermented POME on pH, lipase activity, ethanol and free fatty acid

Glucose concentration (g/L)	Fermentation period (day)							
	pH		LA(µg/ml/day)		FFA (%)		Ethanol (%)	
	0	12	0	12	0	12	0	12
0	4.7^{a}	4.8^{c}	0.07200^{a}	0.00694^{a}	2.034^{a}	2.354^{a}	ND	ND
10	4.9^{c}	3.4^{b}	0.09600^{a}	0.01267^{b}	2.712^{b}	4.294^{b}	ND	0.5^{a}
15	4.9^{c}	3.4^{b}	0.09330^{a}	0.01317^{c}	2.750^{c}	4.463^{c}	ND	0.5^{a}
20	4.8^{b}	3.3^{a}	0.09867^{a}	0.01383^{e}	2.789^{d}	4.689^{e}	ND	0.5^{a}
25	4.9^{c}	3.4^{b}	0.09933^{a}	0.01389^{f}	2.806^{e}	4.708^{f}	ND	0.9^{b}
30	4.9^{c}	3.3^{a}	0.10000^{a}	0.01367^{d}	2.825^{f}	4.633^{d}	ND	0.9^{b}

Mean values with similar superscript along the column are not significantly different ($P \leq 0.05$)
Key: LA = Lipase activity, FFA = Free fatty acid, ND = Not detected

Free fatty acid increases as the concentration of sugar cane bagasse increases. Supplementation of POME with sugar cane bagasse has significant effect on free fatty acid of both unfermented (0 day) and 12-day fermented POME. Increase in sugar cane bagasse concentration resulted in increase in free fatty content of 12 days fermented POME till 40g/L concentration while further increase resulted in a decrease in free fatty acid produced. As the concentration of sugar cane bagasse supplementation increases, the pH of the 12 days fermented POME steadily decreases till 40g/L sugar cane bagasse supplementation and increases later at 50g/L addition. The pH of supplemented and unfermented (0 day) POME is higher than that of supplemented and 12-day fermented POME. Statistically, supplementation of POME with sugar cane bagasse shows significant difference ($P \leq 0.05$) in the pH within the different fermentation days.

Table 4 shows the effect of supplementation of corn steep liquor in POME on ethanol, pH, lipase activity and free fatty acid at 0 day and 12 day. The lipase activity of all concentration of corn steep liquor fermented POME is lower than lipase activity of unfermented POME (0 day). Ethanol was not detected in all concentration of corn steep liquor. The highest lipase activity (0.00756µg/ml/day) of fermented POME was recorded at concentration of 150ml/L while the lowest (0.00628µg/ml/day) was recorded at 50ml/L whereas the highest lipase activity (0.1267µg/ml/day) for unfermented POME was at 150ml/L POME and the lowest (0.0720µg/ml/day) was in unsupplemented POME. Statistical analysis reveals that there is significant difference ($P \leq 0.05$) in lipase activity of all concentration of corn steep liquor for both unfermented (0 day) and fermented POME except for 50g/L and 250ml/L of fermented (12-day) POME where there was no significant difference.

At day 12, free fatty acid increases from corn steep liquor concentration of 50ml/L to 150ml/L and thereafter decreases. The highest free fatty acid (2.561%) was recorded at 150ml/L and the lowest (2.090%) at 250ml/L at day 12. The free fatty acid for supplemented and unfermented is higher than the free fatty acid of supplemented and fermented POME. In the unfermented sample, the highest free fatty acid (3.578%) was recorded at 150ml/L while the lowest (2.034%) was at unsupplemented POME. The free fatty acid increases as the concentration of corn steep liquor increases till 150ml/L and thereafter decreases at day 0. There is significant difference ($P \leq 0.05$) in free fatty acid at all the concentrations of corn steep liquor for both fermented and unfermented POME.

Highest pH for unfermented (4.92) and 12-day fermented (4.82) POME was recorded at 50ml/L corn steep liquor and the least was recorded at 200ml (4.57) for unfermented (0 day) and 250ml/L (4.72) for fermented (12-day). A relatively steady pH was observed at 100ml and 150ml at both periods. Statistical analysis shows that the pH of fermented and unfermented POME are significantly different ($P \leq 0.05$) with different concentration of corn steep liquor except at 150ml and 100ml that are not significantly different for fermented POME.

Table 3. The Effect of supplementation of sugar cane bagasse in POME on pH, lipase activity, ethanol and free fatty acid

Sugar Cane Bagasse Concentration (g/L)	Fermentation Period (day)							
	pH		LA (µg/ml/day)		FFA (%)		Ethanol (%)	
	0	12	0	12	0	12	0	**12**
0	4.73^{d}	4.80^{f}	0.0720^{a}	0.0069^{a}	2.034^{a}	2.354^{a}	ND	ND
10	4.79^{e}	4.07^{e}	0.1000^{c}	0.0072^{a}	2.825^{c}	2.448^{b}	ND	0.5^{a}
20	4.73^{d}	3.92^{d}	0.0960^{b}	0.0098^{b}	2.712^{b}	3.315^{c}	ND	0.9^{b}
30	4.72^{c}	3.70^{b}	0.1080^{e}	0.0102^{c}	3.051^{e}	3.465^{d}	ND	0.9^{b}
40	4.71^{b}	3.61^{a}	0.1027^{d}	0.0115^{e}	2.900^{d}	3.898^{f}	ND	0.5^{a}
50	4.70^{a}	3.77^{c}	0.1093^{f}	0.0111^{d}	3.389^{f}	3.769^{e}	ND	0.5^{a}

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: LA = Lipase Activity, FFA= Free Fatty Acid, ND = Not Detected

Table 4. The Effect of Concentration of Corn Steep Liquor in POME on pH, Lipase Activity and Free fatty acid at 0 day and 12 day

Concentration of corn steep liquor (ml/L)	Fermentation period (day)							
	pH		LA (µg/ml/day)		FFA (%)		Ethanol	
	0	12	0	12	0	12	0	12
0	4.73^{c}	4.80^{c}	0.0720^{a}	0.00694^{d}	2.034^{a}	2.354^{e}	ND	ND
50	4.92^{f}	4.82^{d}	0.1067^{e}	0.00622^{a}	3.013^{e}	2.109^{b}	ND	ND
100	4.79^{d}	4.78^{b}	0.1027^{c}	0.00628^{b}	2.900^{c}	2.128^{c}	ND	ND
150	4.80^{e}	4.77^{b}	0.1267^{f}	0.00756^{e}	3.578^{f}	2.561^{f}	ND	ND
200	4.57^{a}	4.72^{a}	0.1053^{d}	0.00644^{c}	2.976^{d}	2.185^{d}	ND	ND
250	4.62^{b}	4.72^{a}	0.0987^{b}	0.00617^{a}	2.787^{b}	2.090^{a}	ND	ND

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: LA = Lipase activity, FFA= Free fatty acid, ND = Not detected

Table 5 shows the effect of pH on Lipase Activity, Free Fatty Acid and ethanol on fermented POME at 0 day and 12 day. Free fatty acid of unfermented (0-day) POME decreases from pH of 3.5 to 7.5 and then increases at 8.5 while that of fermented (12-day) POME increases from pH of 3.5 to 5.5 and later decreases as the pH is increasing till pH 7.5 thereafter increases. Lowest free fatty acid was recorded for both fermented (1.563%) and unfermented (1.431%) POME at pH of 7.5 while the highest free fatty acid was recorded for fermented (3.861%) at 8.5 and unfermented (4.030%) at 3.5. Statistical analysis reveals that there is significant difference in free fatty acid at all pH for both fermented and unfermented POME. Ethanol was not detected irrespective of the pH.

The lipase activity of unfermented POME (0-day) is higher than lipase activity of fermented POME (12-day) at all the pH. Lipase activity of unfermented POME decreases from pH 3.5 to pH 7.5 and then increases at pH 8.5. The highest lipase activity (0.1427µg/ml/day) of unfermented POME was recorded at pH of 3.5 and the lowest (0.0506µg/ml/day) was at pH of 7.5 while fermented POME has the highest lipase activity (0.01139µg/ml/day) at 8.5 and the lowest (0.00461µg/ml/day) at 7.5. Statistical analysis shows that different pH of both fermented and unfermented POME has significant effect on lipase activity.

Table 6 shows the effect of pH on ethanol concentration of POME supplemented with 30g/L of sugar cane bagasse and 30g/L of glucose. Ethanol production increases with increase in pH and fermentation day. The ethanol concentration ranges from 0% in control POME to 2.3% at pH 8.5 on 12th day of fermentation. Generally, pH 8.5 had the highest ethanol production at all days of fermentation. Higher ethanol production was recorded at pH 7.5 and 8.5. Statistical analysis revealed that an increase in pH has significant effect on the quantity of ethanol produced.

Table 7 shows the effect of pH on free fatty acid of POME supplemented with 30g/L sugar cane bagasse and 30g/L of glucose. The least free fatty acid was observed generally in unsupplemented (control) samples at all fermentation days except in unfermented and 3-day fermented POME. The free fatty acid production increases with increase in fermentation days within the 9th and 12th fermentation period except in control POME at 15 day and 6th day in pH 8.5 adjusted POME. Generally at all period of fermentation, free fatty production increases with increase in pH till pH 6.5 and later decreases with further increase in pH. The highest free fatty acid (5.029%) in supplemented POME was recorded at pH 6.5 on the 12th day fermentation period. Statistical analysis shows that the free fatty acids are significantly different in all the pH at all the days of fermentation except for pH 5.5 and 6.5 of 15th day of fermentation.

Table 5. The effect of pH on lipase activity, free fatty acid on fermented POME

pH	Fermentation period (day)					
	Lipase activity (µg/ml/day)		FFA (%)		Ethanol (%)	
	0	12	0	12	0	12
3.5	0.1427^{f}	0.00633^{c}	4.030^{f}	2.147^{c}	ND	ND
4.5	0.1333^{e}	0.00761^{d}	3.767^{e}	2.580^{d}	ND	ND
5.5	0.0933^{d}	0.00833^{e}	2.637^{d}	2.825^{e}	ND	ND
6.5	0.6530^{c}	0.00561^{b}	1.846^{c}	1.902^{b}	ND	ND
7.5	0.0506^{a}	0.00461^{a}	1.431^{a}	1.563^{a}	ND	ND
8.5	0.0533^{b}	0.01139^{f}	1.507^{b}	3.861^{f}	ND	ND

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: FFA = Free fatty acid, ND = Not detected

Table 6. Effect of pH on ethanol concentration (%) of POME supplemented with 30g/L of sugar cane bagasse and 30g/L of glucose.

pH	Fermentation period (day)					
	0	3	6	9	12	15
Control	0.5^{a}	0.5^{a}	ND	ND	0.5^{a}	ND
4.5	0.5^{a}	0.5^{a}	0.5^{a}	0.9^{b}	1.4^{c}	1.4^{c}
5.5	0.5^{a}	0.5^{a}	ND	0.5^{a}	0.9^{b}	0.5^{a}
6.5	0.5^{a}	0.5^{a}	0.5^{a}	0.5^{a}	0.9^{b}	0.9^{b}
7.5	0.5^{a}	0.5^{a}	1.4^{b}	1.4^{c}	1.4^{c}	1.4^{c}
8.5	0.5^{a}	0.9^{b}	1.4^{b}	1.8^{d}	2.3^{d}	1.4^{c}

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: Control= POME without any supplement, ND = Not detected

Table 8 shows the effect of pH on lipase activity of POME supplemented with 30g/L sugar cane bagasse and 30g/L of glucose. Generally, the lipase activity decreases as the fermentation day increases for all the pH. At all period of fermentation, lipase activity increases with increase in pH till pH 6.5 and later decreases with further increase in pH. The least lipase activity (0.00684µg/ml/day) for supplemented POME was observed at pH 8.5 of 15-day fermented POME. Statistical analysis revealed that there was significant difference (P≤0.05) in lipase activity at all the pH for all the fermentation days except at pH 5.5 and 6.5 of 15-day fermented POME that is not significantly different from each other.

Table 7. Effect of pH on free fatty acid (%) of POME supplemented with 30g/L of sugar cane bagasse and 30g/L of glucose

pH	Fermentation Period (day)					
	0	3	6	9	12	15
Control	0.866^{e}	2.260^{c}	2.561^{a}	2.599^{a}	2.787^{a}	2.825^{a}
4.5	1.036^{f}	1.996^{a}	2.957^{b}	4.181^{d}	3.917^{d}	4.087^{d}
5.5	0.848^{d}	2.279^{d}	3.390^{c}	4.445^{e}	4.953^{e}	4.708^{e}
6.5	0.697^{c}	2.712^{f}	3.729^{e}	4.595^{f}	5.029^{f}	4.708^{e}
7.5	0.414^{b}	2.449^{e}	3.654^{d}	3.654^{c}	3.465^{c}	3.390^{c}
8.5	0.301^{a}	2.128^{b}	3.804^{f}	3.202^{b}	3.239^{b}	2.900^{b}

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: Control= POME without any supplement

Table 8. Effect of pH on lipase activity (µg/ml/day) of POME supplemented with 30g/L of sugar cane bagasse and 30g/L of glucose

pH	Fermentation period (day)					
	0	3	6	9	12	15
Control	0.03067^{e}	0.02667^{c}	0.01511^{a}	0.01022^{a}	0.00822^{a}	0.00667^{a}
4.5	0.03667^{f}	0.02356^{a}	0.01744^{b}	0.01644^{d}	0.01156^{d}	0.00964^{d}
5.5	0.03000^{d}	0.02689^{d}	0.02000^{c}	0.01748^{e}	0.01461^{e}	0.01111^{e}
6.5	0.02467^{c}	0.03200^{f}	0.02200^{e}	0.01807^{f}	0.01483^{f}	0.01111^{e}
7.5	0.01467^{b}	0.02889^{e}	0.02156^{d}	0.01437^{c}	0.01022^{c}	0.00800^{c}
8.5	0.01067^{a}	0.02511^{b}	0.02244^{f}	0.01259^{b}	0.00956^{b}	0.00684^{b}

Mean values with similar superscript along the column are not significantly different (P≤0.05)
Key: Control= POME without any supplement

Discussion and Conclusion

The highest ethanol content recorded in this work without any supplement was 0.5% (3.95g/L) while the highest ethanol content with supplementation was 2.3% (18.17g/L). Kalil *et al*. (2003) recorded ethanol concentration of 4g/L by fermenting POME with *Clostridium acetobutylicum* which is closed to the ethanol concentration that was recorded in this research work without supplementation but lower than that of supplemented POME. On the contrary, Alam *et al*. (2009) recorded 6.5% (51.3 g/L) and Narasimhulu and Nanganuru (2010) recorded 6.6% (52.4g/L) of ethanol from POME which are higher than the ethanol concentration recorded in this research work although Alam *et al*. (2009) and Narasimhulu and Nanganuru (2010) used starter cultures which was not used in this research work.

The best pH for spontaneous production of ethanol in this research using supplements was 8.5 which is in contrast to the work of Kalil *et al*. (2003) and Alam *et al*. (2009). Kalil *et al*. (2003) had the best production of ethanol at pH of 5.8, however Kalil *et al*. (2003) used a starter culture which was not used in this research work and did not use supplement. Alam *et al*. (2009) had the maximum production of ethanol from palm oil mill effluent at pH range of 5-6. In this research work, POME was spontaneously fermented with different supplements at different concentrations.

The pH of POME decreases as the fermentation day increases. This is in accordance with the work of Alam *et al*. (2009) and Narasimhulu and Nanganuru (2010). There were situations where there were sudden increase in pH during fermentation; this was also reported by Parihar (2012). The increase in pH could be correlated with proteolytic activity which yields ammonia into the medium that causes increase in pH (Parihar, 2012).

It was observed in this research that lipase activity of POME supplemented with sugarcane bagasse is higher than that of POME supplemented with corn steep liquor after 12 days of fermentation a report similar to that of Vaseghi *et al*. (2012). Different supplements have different effects on lipase activity, this is in accordance with the work of Kanimozhi *et al*. (2011) who used different substrate and found out that different substrates have different effect on lipase activity. Report has also shown that the amount of substrate used has great influence on extracellular lipase production (Iftikhar *et al*., 2011).

There is possibility of producing bioethanol from palm oil mill effluents which can be with and without addition of supplement(s). Supplementation of POME with glucose and Sugar cane bagasse can increase the concentration of bioethanol that is produced in spontaneous fermentation. The best supplementation for bioethanol production can therefore be achieved at 30g/L glucose and 30g/L sugarcane bagasse at pH 8.5 at a fermentation period of 12 days.

The authors thus recommended further work on other possible optimization parameters that can lead to higher yield.

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