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Research Article

Treatment and Valorization of Palm Oil Mill Effluent through Production of Food Grade Yeast Biomass

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Palm oil mill effluent (POME) is high strength wastewater derived from processing of palm fruit. It is generated in large quantities in all oil palm producing nations where it is a strong pollutant amenable to microbial degradation being rich in organic carbon, nitrogen, and minerals. Valorization and treatment of POME with seven yeast isolates was studied under scalable conditions by using POME to produce value-added yeast biomass. POME was used as sole source of carbon and nitrogen and the fermentation was carried out at 150 rpm, 28 ± 2°C using an inoculum size of 1 mL of 10^6 cells. Yeasts were isolated from POME, dump site, and palm wine. The POME had chemical oxygen demand (COD) 114.8 gL^{-1}, total solid 76 gL^{-1}, total suspended solid (TSS) 44 gL^{-1} and total lipid 35.80 gL^{-1}. Raw POME supported accumulation of 4.42 gL^{-1} dry yeast with amino acid content comparable or superior to the FAO/WHO standard for feed use SCP. Peak COD reduction (83%) was achieved with highest biomass accumulation in 96 h using *Saccharomyces* sp $L_3{}^1$. POME can be used as carbon source with little or no supplementation to achieve waste-to-value by producing feed grade yeast with reduction in pollution potential.

1. Introduction

Palm oil is the most widely consumed vegetable oil and accounts for about 33% of total vegetable oil production in the world [1]. Global palm oil production has been dominated by Indonesia and Malaysia and to a lesser extent by Colombia, Thailand, and Nigeria. Combined, these countries produce over 93% of global palm oil output [2, 3]. Nigeria is currently the fifth leading producer with over 930,000 metric tons annually [4]. The palm oil industry in Nigeria is a major agroenterprise especially in the southern parts where palm trees grow in the wild and in plantations [5]. About 80% of the palm oil industry in Nigeria is dominated by smallholders who typically use manual equipment and, to a lesser extent, semimechanized processors for processing palm fruit [2, 3]. Processing of palm fruit in both methods employs large volumes of water. This results in the production of copious volumes of the liquid waste known as palm oil mill effluent (POME) [6, 7]. Estimates of the volume of POME produced per litre of palm oil extracted from palm fruits are few and variable. This is occasioned by several variables including differences in the efficiencies of the different processes and nature of the fruit. Manual processes appear to be the least efficient in terms of volume of POME generated, with excess of 10 litres of POME being generated for each litre of oil produced in some instances. Ohimain and Izah [8] reported that 72–80 liters of water are required to process one ton of fresh fruit bunches in the semimechanized process. Of these, 72–75% ends up as POME.

POME is a high strength pollutant with a low pH (due to the organic and free fatty acids arising from partial degradation of palm fruits before processing). The characteristics of POME depend on the quality of the raw material and the production processes [9], but it typically contains large amounts of total solids (40,500–75000 mgL^{-1}) and oil and grease (2000–8300 mgL^{-1}). Its suspended solids content is in the range of 18,000–47,000 mgL^{-1}, total nitrogen in the range of 400–800 mgL^{-1}, while the ash content is between 3000–42000 mgL^{-1} [10]. POME has very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in the range of 25,000–54,000 mgL^{-1} and 50,000 to >

100,000 mgL^{-1}, respectively. These values are 100 times more than that of municipal sewage [11–14].

No matter how effective the method of processing, POME discharged from a mill is objectionable and could pollute streams, rivers, or surrounding land [15]. When discharged into water bodies it turns the water brown, smelly, and slimy and causes anoxygenation [16], and may kill fishes and other aquatic organisms thereby denying humans access to good water for domestic use [17]. Also, application of untreated POME on soil alters its physic-chemical properties, causing undesirable decreases in pH and increases in salinity. Unfortunately, most oil palm processors still discharge raw effluent directly into nearby streams and rivers and on land [18].

Several treatment technologies have been applied to POME with varying levels of success. These include ponding [19], aerobic digestion [20], anaerobic digestion [21], and physicochemical treatments [22, 23]. These methods seek to dispose of POME without any consideration to the current trend in the management of high strength agro-food wastes that seeks to reprocess them through value addition [24]. The management of agro-food wastes has evolved from treatment for disposal to beneficial utilization of resources through valorization [24–26]. Valorization is a concept that seeks the recovery of value added products from wastes and effluents through the application of cost effective technologies. Since POME has currently little or no recycling value, it constitutes an environmental hazard, undergoing slow (acidic) degradation in pits from which it emits strong, foul odour causing air pollution and contamination of ground and surface waters and agricultural land besides vector attraction. Attempts have been made to achieve valorization of POME through production of microbial biomass, enzymes, energy, and biochemicals [27–38].

Cost effective disposal of POME hinges on a sustainable and economic method for treatment. Where this can be coupled to some value addition it will become an incentive resulting in a no-loss waste treatment, or a reduction in the overall cost, with the achievement of clean environment being a bonus. The possibility of using POME for biomass production has been suggested and, in this regard, room for innovation exists in both the choice of microbe and fermentation environment. It is envisaged that development of a process for production of yeast biomass from POME will reduce the time and cost in the cycle-to-value and thereby create opportunities for the valorization of a waste stream the accumulation and environmental concern for which can only increase with increasing global production of palm oil. This work was carried out to study the treatment and valorization of POME through production of yeast biomass and to assess the quality of the biomass in terms of amino acid content.

2. Materials and Methods

2.1. Collection of POME. Raw POME was obtained from a local palm oil processor employing manual process in Ejuona Obukpa Community in Nsukka Local Government Area of Enugu State, Nigeria. Samples were collected in clean containers and transported to the laboratory. The fresh POME was dispensed in 500 mL containers and stored frozen in a deep freezer when not used immediately.

2.2. Isolation and Identification of POME Utilising Yeasts. Yeasts were isolated from stale POME and soil taken from POME dump site and from palm wine (all collected from the same community as above). Stale POME, soil, and palm wine samples were collected in sterile sample bottles and taken immediately to the laboratory for analysis. Samples were processed by 1 : 10 serial dilution in sterile half strength peptone water and plated by pour plate method on Saboraud Dextrose Agar (SDA) medium (Oxoid, England) supplemented with chloramphenicol (50 μgmL^{-1} final concentration). Plates were incubated at room temperature (30°C ± 2°C) for up to 72 hours or until yeast colonies appeared if earlier. Yeast colonies that appeared on the media were purified on fresh plates of SDA. Pure colonies of representative isolates were stored on slants of SDA at 4°C until needed.

2.2.1. Identification of Yeast Isolates. All the yeasts isolates were identified using conventional microbiological methods based on their cultural, morphological, and physiological/biochemical characteristics as described by Kurtzman and Fell [39]. Isolates were also tested for their ability to growth at 37°C and 40°C. Microscopy was carried out with a drop of lacto-phenol cotton blue stain at ×40 objective.

2.2.2. Carbon Fermentation Test. Each isolate was tested for ability to ferment different sugars with the production of acid and or gas. Fermentation basal medium containing bromothymol blue indicator was prepared with 2% sugars (except for raffinose which was used at 4%). Basal medium was prepared by dissolving 4.5 g of powdered yeast extract, 7.5 g of peptone, and 26.7 mg of bromothymol blue indicator in 1 litre of distilled water. A 6 mL volume of the medium was dispensed in fermentation tube containing inverted Durham tube for detection of gas production. The tubes were capped and sterilized at 121°C for 10 minutes. Suspensions of the isolated yeast cells were made from 24 h cultures of isolates using sterile distilled water. Each tube containing the test medium was inoculated with 0.1 mL of the yeast suspension ($5 \times 10^6\ mL^{-1}$) and incubated at 25°C for two weeks. The tubes were shaken and inspected at frequent intervals for accumulation of gas in the Durham tube and change of colour of indicator. A positive result was indicated by a colour change from deep green to yellow for acid production and accumulation of air in the Durham tube for gas production. The results were scored according to the scheme of Kurtzman and Fell [39].

2.2.3. Carbon Assimilation Test. Each isolate was tested for ability to assimilate different sugars using Yeast Nitrogen Base (YNB) agar slants containing 2% sugar (except for raffinose which was 4%). The media were prepared as described by Kurtzman and Fell [39]. Slant of the test medium was inoculated with 0.1 mL suspension of test isolate. The tests were incubated at 25°C in a cool incubator and inspected after 1, 2, and 3 weeks. A positive result is indicated by the growth of

the yeasts after some days and heavy growth signifies strong assimilation [39].

2.2.4. Nitrogen Assimilation Test. This test was carried out as for carbon assimilation test but using Yeast Carbon Base (YCB) agar containing different nitrogen sources. Each isolate was tested for ability to utilize different nitrogen compounds as sole source of nitrogen. The two nitrogen sources used were potassium nitrate (test nitrogen source) and ammonium sulphate (positive control). YCB was prepared as described by Kurtzman and Fell [39] and inoculated as above. The tests were incubated at 25°C in a cool incubator and inspected for growth after 1, 2, and 3 weeks. A positive result is indicated by the growth of the yeasts after some days and heavy growth signifies strong assimilation.

2.2.5. Growth at Different Temperatures. This test was carried out using glucose-peptone-yeast extract (GPYE) broth. The broth was prepared by dissolving 20 g of glucose, 10 g of peptone, and 5 g of yeast extract in 1 litre of distilled water. A 6 mL volume of this medium was dispensed in test tubes, plugged with cotton wool, and sterilized at 121°C for 15 minutes. Each of the tubes was inoculated with 0.1 mL of test yeast suspension incubated at 25°C, 30°C, 37°C, and 40°C for one week. The tubes were inspected each day for growth.

2.3. Preparation of Yeast Inoculum for Growth in POME. Inoculum was prepared by adding 5 mL of sterile 0.85% normal saline onto 2-day-old SDA slant cultures in universal bottles and gently rubbing with sterile wire loop to dislodge yeast growth. The yeast concentration was adjusted to approximately 5.0×10^6 cells mL^{-1} using a haemocytometer and fresh inoculum was prepared from 24 hour culture for each parameter [40]. Prior to fermentation, POME was allowed to completely thaw at room temperature, boiled, and filtered while still hot by simple surface filtration using a double layered muslin cloth to remove coarse solids followed by fine filtration through Whatman Number 41 filter paper.

2.4. Determination of Physicochemical Parameters of POME. The total solids (TS) and total suspended solids (TSS) were determined as described in standard methods [41]. The chemical oxygen demand (COD) was determined by using modified titrimetric/dichromate oxidation method [41]. The total dissolved solid (TDS) was determined using Hanna portable TDS metre. Total ash was determined by ignition of the total solids in a muffle furnace at 550°C (Gallenkamp, size 3, England). Total lipid was determined by extraction with chloroform/methanol (2 : 1), according to the method of Folch et al. [42]. Total nitrogen was determined using Kjeldahl method [43]. The pH of POME as well as change in pH of the fermenting medium was measured using Hanna portable pH meter (HANNA HI 198107, USA). The organic carbon was determined by dichromate oxidation method [44].

2.5. Screening of Isolates for Biomass Production and COD Reduction. Isolates were screened to determine their ability to degrade COD of POME and to produce biomass under culture conditions. Erlenmeyer flasks of 250 mL capacity containing 25 mL of POME were set up in duplicates and sterilized at 121°C for 15 min. When cooled, each flask was inoculated with 1 mL suspension of the test isolates and incubated at 28 ± 2°C on a rotary shaker incubator (model KS 4000 I Control (IKA-Werke GmbH Germany)) at 150 rpm for 7 days. Sterile POME medium was included as control. Changes in the COD and physicochemical parameters of POME were monitored throughout the fermentation period as above.

At the end of culture the biomass was harvested by centrifuging the culture medium at 5000 ×g for 20 minutes, using Hettich II centrifuge. The pellets were washed several times with cold distilled water and then dried in an oven at 80°C, using preweighed filter paper to constant weight (Sartorius model AGBS 323S Sartorius, AG Germany). This was followed by the filtration of the supernatant through a preweighed membrane filter of 0.45 μm pore size. The filters were washed with distilled water severally and then dried at 80°C for at least 16 h to constant weight. The biomass concentration was estimated from the membrane and filter paper weight difference with and without the dried sample. The determinations were performed in duplicate. The supernatant was used for determination of residual COD.

2.6. Amino Acid Analysis of Yeast Biomass. Amino acid analysis was done in accordance with the Technical method of AOAC [41]. Exactly 1 g of the dried yeast biomass was placed in the conventional hydrolysis tubes. To each tube 100 μL of 6 $molL^{-1}$ HCl containing 30 mL phenol and 10 mL 2-mercaptoethanol (6 $molL^{-1}$ HPME) were added and the tubes were evacuated, sealed, and hydrolyzed at 110°C for 22 hours. After hydrolysis, HCl was evaporated in a vacuum bottle heated to 60°C. The residues were dissolved in ultrapure water (HPLC) grade, containing ethylene diamine tetra acetic acid (EDTA). The hydrolyzed samples were derivatised (45 minutes per sample) on Waters 616/626 HPLC by reacting free amino acids, under basic condition, with phenylisothiocyanate (PITC) to obtain phenyl-thiocarbamyl (PTC) amino acid derivatives and analyzed by using reverse phase HPLC (Waters 616/626 LC USA). A set of standard solutions of the amino acids were prepared from Pierce Reference standards H (1000 μmol) into autosampler crops and also derivatised. These standards (0.0, 0.5, 1.0, 1.5, 2.0 μmol) were used to generate a calibration file that was used to determine the amino acid contents of the samples. After the derivatisation methanol solution (1.5 N) containing the PTC-amino acids were transferred to a narrow bore waters 616/626 HPLC system for separation.

The separation and quantization of the PTC-amino acids were done on a reverse phase 18 silica column and the PTC chromophores were detected at 254 nm. The column temperature was 60°C and elution took 30 minutes. The buffer system used for separation was 140 mm sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B. The program was run using a gradient of buffer A and buffer B concentration and ended with a 55% buffer B concentration at the end of the gradient. The chromatographic peaks areas

Table 1: Morphological and microscopic characteristics of the isolated yeasts.

Characteristics	L_3^1	SP^5	TMCC	TWC	TWDC	V_4^2	V_4^5
Colour on SDA	Creamy white	Tannish white	Glistening Cream	Cream	White	Creamy white	Cream
Colour on Chromagar	Purple	Pale pink	White	Blue	Dark blue	Purple	Pale pink
Colony surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Texture	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous	Butyrous
Margin	Irregular	Irregular	Entire	Irregular	Irregular	Circular	Irregular
Elevation	Sl. raised	Raised	Raised	Raised	Raised	Sl. Raised	Flat
Cell shape	Spherical	Ellipsoidal	Globose	Ellipsoidal	Spherical	Globose	Spherical
Ascospore	P	P	P	P	P	P	P
Pseudomycelium	P	P	A	P	P	P	P
True mycelium	A	A	A	A	P	A	A
Blastoconidia	P	P	P	P	P	P	P
Pellicle	A	P	A	P	A	A	P

Key: P = present; A = absent; sl. = slightly.

Table 2: Physiological characteristics of the isolated yeasts.

Physiological test	L_3^1	SP^5	TMCC	TWC	TWDC	V_4^2	V_4^5
Glucose	FA	FA	SA	FA	FA	FA	FA
Galactose	FA	FA	SA	SA	FA	FA	FA
Sucrose	FA	FA	SA	FA	FA	FA	FA
Raffinose	FA	WA	SA	FA	–	FA	FA
Maltose	–A	–A	–A	–	FA	–	–A
Lactose	–	–	–	–	–	–	–
Soluble starch	–	–	–	–	–A	–	–
Xylose	–	–A	–	S	–A	–	–A
Nitrate	–	+	+	–	–	–	–
Growth at 25°C	+	+	+	+	+	+	+
Growth at 30°C	+	+	+	+	+	+	+
Growth at 37°C	+	–	+	+	+	+	+
Growth at 40°C	+	+	+	+	+	+	+
Probable identity	*Saccharomyces* sp	*Pichia* sp	*Candida* sp	*Candida* sp	*Candida* sp	*Saccharomyces* sp	*Pichia*

Key: FA = fermentation and gas production; –A = assimilation only; + = positive; – = negative.

were identified and quantified using a Dionex chromeleon data analysis system attached to the HPLC System. The calibration curve or file prepared from the average values of the retention times (minutes) and areas (in Au) of the amino acids in 5 standard runs was used. Amino acid was expressed as g100 g^{-1} of proteins and compared with FAO/WHO [45] reference.

2.7. Statistical Analysis of the Experimental Data. All results were expressed based on duplicate determinations. Data collected were subjected to analysis of variance (ANOVA) using GenStat discovery edition 4 and means were compared using least significant difference at 95% confidence and separated using Duncan new Multiple Range Test.

3. Results

3.1. Identification of POME Utilizing and Associated Yeasts. Seven representative isolates of POME utilizing yeasts were selected from a total of more than 100 initial isolates based on the amount of biomass accumulated and identified. Two of the representative isolates identified as *Candida* sp TMCC and *Pichia* sp SP^5 were obtained from POME dump site, another two identified as *Candida* spp. TWDC and TWC were from stale POME, while the remaining three isolates identified as *Saccharomyces* spp L_3^1 and V_4^2 and *Pichia* sp V_4^5 were from palm wine. Table 1 shows the morphological characteristics of the isolates yeasts, while Table 2 shows their physiological characteristics.

3.2. Physicochemical Parameters of POME. The proximate characteristics of the raw POME used in this study are presented in Table 3. The POME contained high amount of total solids (76,000 mgL^{-1}), total suspended solids (44,000 mgL^{-1}), total dissolved solids (32,000 mgL^{-1}), organic carbon (15128 mgL^{-1}), lipid (3,580 mgL^{-1}) ash (4,000 mgL^{-1}), and COD (114,800 mgL^{-1}) but a low amount of total nitrogen (420 mgL^{-1}) and had an acidic pH (3.9). On the bases of these, the C : N ratio of 36 : 1 was calculated.

Table 3: Proximate composition of raw POME (pH 3.9).

Parameter	Concentration (mg/L)
COD	114,800
Total solid	76,000
Total dissolved solid	32,000
Total suspended solid	44,000
Total lipid	3,580
Total nitrogen	420
Ash	4,000
Total organic carbon	87,140

3.3. Biomass Accumulation and COD Degradation by Isolates. As shown in Figure 1, the isolates differed from one another in their ability to reduce COD content of POME in the course of fermentation for biomass production and reduction in pollution potential. The isolates achieved peak reduction of POME COD content after 72, 96, 120, or 144 hours. Isolate L_3^1 achieved the highest reduction in COD (83% reduction after 96 hours), while SP^5 achieved the least COD reduction (73% reduction after 72 hours). During the fermentation, there was a rise in pH of the POME medium for all the isolates from the initial acid value to alkaline levels (Figure 2). The yield of biomass by the isolates is shown in Figure 3. Biomass accumulation mirrored the trend of COD removal and increased with duration of fermentation to a peak point corresponding to time of maximum COD removal after which it decreased. A slight exception was in the case of isolate TWDC, which showed increase in biomass production throughout the fermentation. Isolate L_3^1 showed increase in biomass production up to 96 hours when a maximum of 4.42 gL^{-1} (the highest in the process) was obtained after which it declined while isolate SP^5 achieved maximum production of 3.14 gL^{-1} at the 72 hours. Isolates TMCC and TWC yielded their maximum biomass of 1.94 gL^{-1} (the least in the process) and 2.22 gL^{-1}, respectively, after 120 hours, while TWDC gave maximum biomass yield of 3.06 gL^{-1} at 168 hour. Isolate V_4^2 gave maximum biomass yield of 3.02 gL^{-1} after 72 hours, while isolate V_4^5 yielded its maximum biomass of 3.46 gL^{-1} after 144 hours.

3.4. Proximate Composition and Amino Acid Profile of Microbial Biomass. Following the biomass yield screen, isolate L_3^1 was selected for production of biomass for proximate and amino acid analysis. This isolate was selected for biomass production and amino acid analysis because it produced the most biomass of all the isolates and was also very effective in the reduction of waste pollution potential (COD). The biomass produced in POME had a moisture content of 8.95% and a dry matter content of 91.05%. The biomass crude protein content was approximately 27% while the fat, carbohydrate, crude fibre, and ash contents were 0.83%, 35.45%, 4.70%, and 6.12%, respectively, for biomass harvested at peak biomass content (96 hours culture). Table 4 shows the amino acid profile of the biomass protein. A comparison of the amino acid composition of the biomass with FAO standard for SCP protein intended for use in animal feeding indicates that the biomass was comparable to or superior to the recommended standard with respect to a number of amino acids, while being inferior with respect to only a few others.

4. Discussion

In this study, seven yeasts isolated from palm wine (L_3^1, V_4^2 and V_4^2), stale POME (TWDC and TWC), and POME dump site (TMCC and SP^5) were screened for their efficiency in reducing the COD content (pollution potential) of POME while producing biomass. Based on their colonial and physiological characteristics [39], the isolates were identified as species of *Saccharomyces* (L_3^1 and V_4^2), *Pichia* (V_4^5 *and* SP^5), and *Candida* (TMCC, TWC, and TWDC).

The proximate composition of POME used in this study indicates high total solid but was considerably different from those used in some previous studies [7, 11–13]. This is probably due to differences in the nature of the mill and process operation. The POME used in this study was obtained from local palm oil extractors who use manual method of oil extraction. This method is inefficient in recapturing all the oil in the palm fruits. The manual method employs smaller volume of water (relative to the mechanized process) to achieve oil extraction and allows for a considerable reduction in the volume of wastewater generated in the process. However, the reduction in effluent volume results in a more concentrated effluent with higher content of organic matter. The use of limited volume of water for oil extraction particularly in the upland area of this study is understandable since water is not available in unlimited quantities outside the wet season. It is also possible that the fruits may have undergone some deterioration prior to processing thereby increasing the polluting potential of the effluent. Delay in processing of produce results in degradation of oil/lipids into low molecular weight organic acids that remains in solution and so are not extracted with oil. This leads to low pH and high content of soluble solids and pollution potential of the ensuring POME, and is common during the fruit season when many manual small holder processors are regularly overwhelmed.

The POME used in this study had a pH of 3.9. This value falls in the range of 3.5 to 5.0 reported by other authors [46–48]. It is however lower than the guideline value (pH 6–9) for effluent from vegetable oil processing [49]. The total lipid content (3580 mgL^{-1}) was comparable to but lower than the range of 4000–6000 mgL^{-1} reported by Ma [14] and much higher than the regulatory discharge limit of oil and grease in POME (50 mgL^{-1} [7]), a value that is low and considerably challenging to achieve in most POME management procedures. COD values reported for POME vary considerably from less than 42,000 mgL^{-1} to over 112,000 mgL^{-1} [7, 11–13]. The value of COD (114, 800 mgL^{-1}) recorded in this study was high but compares closely to figures that have been reported. It is, however, several orders of magnitude higher than the IFC [49] guideline value of 250 mgL^{-1} for effluent from vegetable oil processing and so requires significant treatment prior to disposal. It is also

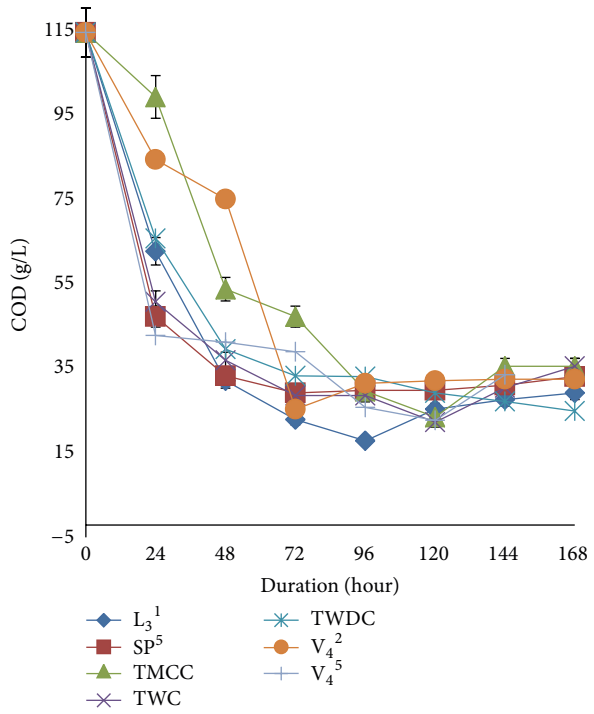

Figure 1: Changes in COD of POME with fermentation time.

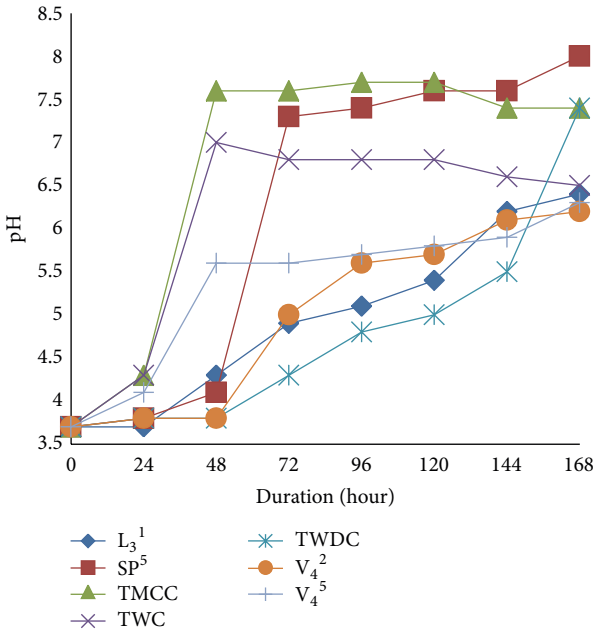

Figure 2: Changes in pH of POME with fermentation time.

consistent with the high values of TS obtained and may be the result of incomplete extraction of lipid as has been reported by Oswal et al. [50].

The yeast isolates obtained in this study varied considerably in their ability to grow in POME and accumulate biomass. They also showed variations in their ability to reduce the COD load of the POME. Of the seven isolates

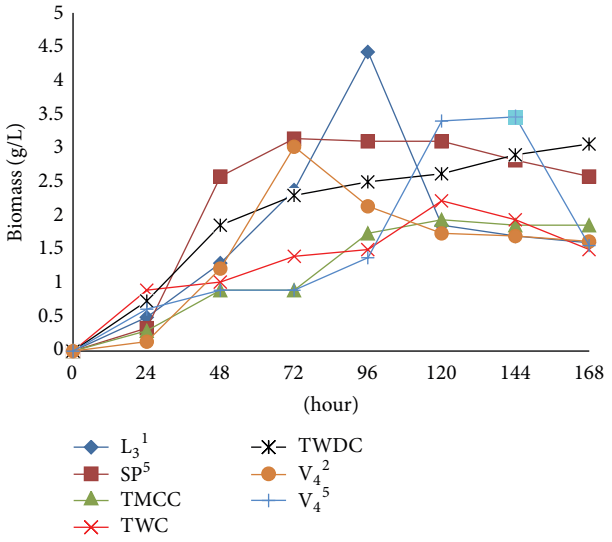

Figure 3: Biomass yield of the different isolates in POME.

screened, *Saccharomyces* sp L_3^1 showed the highest COD reduction of 83% in 96 h. This is comparable to the COD reduction value (82%) reported by Alam et al. [51] when *Aspergillus niger* (A103) was used to produce citric acid in POME. If the reduction of COD were the sole end of the process then the used *Saccharomyces* sp L_3^1 will be considered more economical, even though the product of this process retained higher COD than the regulatory requirement for final disposal. Barker and Worgan [52] reported 77% COD reduction in POME used for mycoprotein production after 2 days, while Oswal et al. [50] obtained higher COD reduction (96%) in culture of *Yarrowia lipolytica* NCIM 3589. Using *Rhodotorula glutinis* Saenge et al. [53] on the other hand obtained only 66% COD removal in POME during lipid and carotenoid production. Our process also compares with but is slightly better in COD reduction than 80% reported during anaerobic digestion of POME [21]. Growth of *Saccharomyces* sp L_3^1 in POME was accompanied by a rise in pH to 8.0. This is consistent with the report of Wu et al. [35], who explained the rise due to utilization of organic acid and would make adjustment of pH during mass propagation of yeasts in this medium unnecessary.

The peak biomass accumulation of 4.42 gL^{-1} obtained by *Saccharomyces* L_3^1 shows the isolate as the best for biomass production in POME (Figure 1). This result is consistent with data reported by Nwuche et al. [54], who obtained maximum biomass of 4.0 gL^{-1} when *A. niger* ATCC 9642 was cultured in POME. Saenge et al. [53] on the other hand, obtained 7.5 gL^{-1} biomass when the oleaginous *Rhodotorula glutinis* TISTR was used to produce biomass, lipid, and carotenoid in POME. The total protein content of *Saccharomyces* L_3^1 at peak biomass production was 27% while the fat, carbohydrate, crude fibre, and ash contents were 0.83%, 35.45%, 4.70%, and 6.12% of dry biomass, respectively, making the resulting biomass quite suitable for feed use.

A comparison of the amino acid composition of the biomass with FAO standard for SCP protein intended for

Table 4: Amino acid profile of isolate L_3[1] cultured in POME in g/100 g of protein.

Amino acids	Amino acid content g/100 g protein	FAO/WHO Standard
*Phe + Tyr	8.68	6.3
Isoleucine	3.09	2.8
Leucine	10.25	6.6
Lysine	6.01	5.8
Methionine	5.05	2.5
Tryptophan	6.76	1.1
Valine	7.13	3.5
Threonine	3.57	3.4
Histidine	1.91	1.9
Total essential	52.45	33.9
Proline	1.87	10.7
Alanine	2.97	6.1
Glutamine	2.95	
Glutamic acid	11.1	14.7
Glycine	3.71	2.2
Serine	3.57	7.7
Arginine	3.85	5.2
Aspartic acid	2.60	7.7
Asparagine	8.54	
Trimethylserine	3.53	
Total nonessential	34.70	54.3
Total amino acids	87.15	88.2

FAO/WHO (1991) [45].
*Phe and Tyr were taken together.

use in animal feeding (Table 4) indicates that the biomass was comparable to or superior to the recommended standard with respect to a number of amino acids, while being inferior with respect to only a few others, mostly nonessential amino acids. The amino acid profile of this isolate shows that it is also superior to the that of thermophilic *Bacillus stearothermophilus* isolated from thermophilic digestion of agricultural residue [24]. The superior content of essential amino acid in this biomass relative to FAO standards for feed is interesting considering the status of the substrate as waste and the possibility of using this process to produce valuable biomass while achieving economic waste disposal. As culture conditions affect the amino acid profiles of microbial biomass [24] these may be manipulated in optimization processes to improve the content of desired amino acids. It is remarkable that biomass produced in this waste is nutritionally qualitative. In many rural Nigeria villages stale POME with visible yeast and fungal growth is usually traditionally fed to swine either directly or used in compounding feed.

5. Conclusion

This work has shown that POME can be used to produce yeast biomass by fermentation without costly pretreatment or nutrient supplementation. The biomass production process worked as a cost effective biological treatment for the reduction of pollution in the industrial wastewater, requiring simple and feasible methods that can be operated in the industry, so minimizing by products. In small scale process the cultured POME may also be fed as enriched feed directly to animals or used in compounding feed. This will remove the cost that may be associated with harvest of biomass.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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