MICROBIAL PROTEIN EXTRACTION FROM PALM OIL MILL EFFLUENT

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ABSTRACT

*Palm oil mill effluent (POME) is abundantly produced during palm oil milling process and is treated for pollutant reduction without any revenue or profit. The main objective of this research was to evaluate POME as a substrate for microbial protein production and analyse the removal rate of pollutant. This study used hydrolysed POME containing fermentable sugars as carbon source for the cultivation of microbes. Raw POME was initially pre-treated with Celluclast® enzyme using the following conditions: Concentration (1.5% to 5.0% v/v); incubation temperature (45°C-55°C), rotation speed (100-200 rpm) and pH (4.0-5.5). After pre-treatment, the hydrolysate contained 41.63 g L^{-1} reducing sugars with 56 000 mg L^{-1} chemical oxygen demand (COD). Baker's Yeast (*Saccharomyces cerevisiae*) was then cultivated onto the raw or hydrolysed POME at 30°C for seven days. The amount of yeast biomass produced was 28.92 g L^{-1} with 24.79% protein content. Adding to this, the COD value was reduced by 79.12%. The yeast fermentation in hydrolysed POME recorded the highest increase in biomass and protein contents of 3.44 and 7.74 folds, respectively. The findings revealed that POME is a promising raw material for microbial biomass protein production and simultaneously remove the pollutant from POME.*

INTRODUCTION

Malaysia processed more than 97.8 million tonnes of fresh fruit bunches (FFB) in 2018 (Kushairi *et al.*, 2019). Apart from the production of crude palm oil, the mills also generated enormous amount of palm oil mill effluent (POME) every year. In general, 1 t FFB would generate 0.67 m³ POME (Ma, 1999). As a result, more than 65 million m³ POME was discharged from the mills. Without treatment, raw POME is a polluting material with high chemical oxygen demand (COD) (51 000 mg L^{-1}), biological oxygen demand (BOD) (25 000 mg L^{-1}) and total suspended solids (TSS) (18 000 mg L^{-1}) (Nahrul Hayawin *et al.*, 2017).

Raw POME is basically a dark brown oily waste which consists of carbohydrate, protein, nitrogen (N), lipids, minerals and other nutrients. Dried POME contains 39.3% cellulose and 24.6% hemicellulose (Khaw *et al.*, 2008). Conventionally, POME is treated using the open pond system consisting of a cooling pond, an acidification pond, and the anaerobic and aerobic ponds. However, this system has its limitation where the BOD value is merely managed to reduce from 25 000 mg L^{-1} to 50-100 mg L^{-1}. Many advanced technologies on POME treatment are being introduced including membrane ultrafiltration (Numan *et al.*, 2019), membrane nanofiltration (Ali Amat *et al.*, 2015), electrocoagulation (Nasrullah *et al.*, 2018), and a hybrid system that combines the activated carbon adsorption and ultrasound cavitation (Parthasarathy *et al.*, 2016). However, these advanced systems require very high capital and operating costs without returning any profits to the palm oil millers although they are very effective in reducing BOD, COD, TSS and colour.

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Alternatively, these waste streams can be converted from an economic liability into a source of revenue. POME could be converted via bioconversion into value-added products such as bioenergy and biochemical. The high compositions and concentrations of carbohydrate, protein, N, lipids, minerals and other nutrients in POME make it possible to reuse the effluent for biotechnological processing (Nurul Adela *et al.*, 2020). Therefore, researchers are starting to propose on recovering high value products from POME in order to increase the revenue of the palm oil millers and at the same time will promote sustainable oil palm industries (Chia *et al.*, 2020). The recovery processes mostly practised in mills include biogas capture and biomass co-composting. Numerous attempts have been made to utilise POME in composting such as through vermicomposting and co-composting with empty fruit bunch (EFB) and decanter cake (Nahrul Hayawin *et al.*, 2017). Another sustainable approach on POME utilisation is as a substrate for microbial fermentation and cultivation. For instance, microalgae can be cultivated in POME for bioremediation as well as a feedstock for biodiesel production (Kamyab *et al.*, 2015). Furthermore, hydrolysed POME could be used to produce bioflocculant via POME-isolated *Bacillus marisflavi* cultivation. The resulted bioflocculant is potentially be used for wastewater treatment and algae recovery (Nurul Adela *et al.*, 2016; 2020).

Another potential approach for resource recovery from POME is for production of single cell protein (SCP). SCP refers to sources of mixed proteins extracted from pure or mixed cultures of microorganisms. It is one of high quality dietary products for animals feed such as ruminants, pig, chicken and aquaculture application that enable to replace soymeal and fishmeal due to its high protein and nutrient content (Patelski *et al.*, 2020; Sharif *et al.*, 2021). Crude SCP which is also termed as bioprotein, microbial protein or biomass are widely produced from agricultural waste sources such as molasses, dairy waste, fruit waste, starch rich waste, bran, poultry waste *etc.* (Spalvins *et al.*, 2018). SCP produced from different microbes has high protein content (30%-70%) as compared to different green plants and animal sources. Previously, several types of microorganisms have been studied for the production of SCP such as yeast, bacteria, fungi and algae (Anupama and Ravindra, 2000). Microorganisms that are widely used in SCP production include *Candida utilis* and *Saccharomyces cerevisiae* since both microorganisms are safe for consumption. For instance, *S. cerevisiae* that is produced in undiluted POME is able to produce 4.42 g L^{-1} protein biomass with 27% protein content. Another study by Izah (2018) highlights the cultivation of *S. cerevisiae* in palm oil and cassava mill effluent. Based on proximate composition the

results are also promising especially with protein content which is higher than 17% but with low yield (approximately 4 g L^{-1}).

Since POME contains insoluble carbohydrates with high molecular weight compounds such as cellulose, hemicellulose and starch, it requires pre-treatments to hydrolyse the complex carbohydrates in POME prior to fermentation to aid or speed up the microbial process (Nurul Adela *et al.*, 2016). Khaw *et al.* (2008) has determined that POME solid contains about 39.3% cellulose and 24.6% of hemicellulose and these can be converted to fermentable sugars through hydrolysis process. Pre-treatments could disrupt the structure of lignocellulosic materials, hence releasing more reducing sugars which enhance the production of biochemicals. For instance, the sugar content in POME treated with cellulase enzyme increased from 12.45 to 27.13 g L^{-1} (Nurul Adela *et al.*, 2016). Xylanase enzyme enables the release of sugars from hemicellulose in POME prior to biogas production and improves the hydrolytic reaction that increases the methane production (Prasertsan *et al.*, 2017). Cellulolytic fungi such as *Trichoderma harzianum* and *Mucor hiemalis* are added in order to convert carbohydrate polymers in POME into fermentable sugar prior to yeast fermentation for bioethanol production (Alam *et al.*, 2009). It was also reported that alkaline pre-treatment by using 2.58% sodium hydroxide (NaOH) could increase the fermentable sugar content by 9.35% relative to untreated POME (Izzi *et al.*, 2020). This showed that alkaline pre-treatment is also one of viable pre-treatment methods in addition to enzymatic hydrolysis method.

However, exploration of POME as a substrate for microbial protein production is still lacking compared to bioenergy and microalgae. To date, very limited studies are available on the utilisation of POME for SCP production (Iwuagwu and Ugwuanyi, 2014). Therefore, the aim of this article was to evaluate POME, either in raw or hydrolysed form, as potential material for obtaining the yeast biomass and producing microbial protein as a crude SCP. The pollutant removal will also be quantified in terms of COD reduction. This approach may recover and utilise value-added products from the palm oil mills, particularly from POME before being discharged into water courses. Apart from generating profits, this approach will indirectly help to safeguard our environment.

MATERIALS AND METHODS

Raw Materials

Samples of mixed raw effluent (MRE) containing steriliser condensate and clarification underflow sludge were collected at the sludge pit

from Palm Oil Mill Effluent Technology Centre (POMTEC), located in Negeri Sembilan, Malaysia. These samples were transported and kept under 4°C.

Cellulase (Celluclast® 1.5 L FG) used in MRE hydrolysis was obtained from Novozyme (M) Sdn. Bhd. The enzyme is commercially produced from *Trichoderma reesei* with the activity of 700 endo-glucanase units (EGU) g^{-1}.

Dry yeast (Mauripan) was inoculated onto a basal medium consisting of glucose (50 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.025 g L^{-1}), monopotassium phosphate (KH_2PO_4) (0.25 g L^{-1}), peptone (1.15 g L^{-1}) and yeast extract (2.5 g L^{-1}). All chemicals were purchased from local companies with 100% purity and for molecular biology grade. The pH of the prepared medium was adjusted to 5.0 and sterilised in an autoclave (model 50HV Hirayama) at 121°C for 15 min (Keturah *et al.*, 2014).

Methods

In this study, raw POME was firstly enzymatically hydrolysed using cellulase enzyme in order to release fermentable sugars as a carbon source for the microbe's growth. One-factor-at-time (OFAT) method was used to determine the optimal conditions in producing fermentable sugars via POME hydrolysis. NaOH was also added prior to POME hydrolysis to condition the substrate's pH as well as to enhance the fermentable sugar production. Then, the cultivation of *S. cerevisiae* was performed in various POME to basal medium ratios that are 0:100, 50:50 and 100:0. Moreover, the biomass yield, protein content and COD reduction were determined to identify the most suitable POME medium for maximum microbial protein production and pollutant removal. Analytical measurements were performed based on DOE standard method and APHA method. The overall process flow for MRE utilisation for microbial biomass protein production is illustrated in *Figure 1*.

Compositional Analysis of Mixed Raw Effluent (MRE)

A total of 200 mL sludge POME was filtered using cellulose filter (Whatman Filter No. 1) and put in a thimble and dried overnight in an oven. The dried solid MRE was then subjected to Soxhlet extraction with 130 mL petroleum ether for 4 hr to remove residual oil and extracts. The solid residue was then dried overnight at 60°C to remove traces of the solvent. The dried solid residue was then analysed for hemicellulose, cellulose and lignin as described by Ayeni *et al.* (2015). The cellulose content (% w/w) was calculated, assuming that oil and extractives, hemicellulose, lignin and cellulose were the only solid components in the entire mixed raw POME. The calculation is shown in Equation (1).

Cellulose (% w/w): 100% - hemicellulose - lignin - (oil + extractives)   Equation (1)

Enzymatic Hydrolysis for MRE Pre-treatments

A volume of 100 mL MRE samples were adjusted to pH 4.7 (using 0.1 M citrate buffer or 0.1 M NaOH), followed by the addition of 1 mL cellulase enzyme. The samples were then incubated at 50°C with 150 rpm rotation for 24 hr (Noorshamsiana *et al.*, 2013). After the incubation process, the POME hydrolysate mixture was then sterilised at 121°C for 15 min. Parameters for the incubation were varied as follows: Enzyme dosages (1% to 5% v/v), temperatures (45°C-55°C), agitation rate (100-200 rpm) and pH (4.0-6.0).
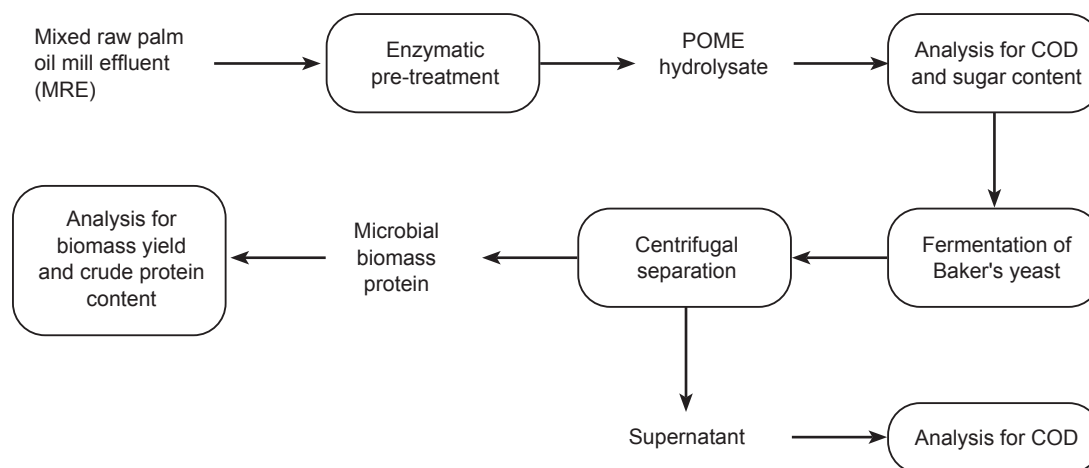

Figure 1. Process flow for mixed raw palm oil mill effluent (POME) utilisation for microbial biomass protein production.

Isolation of Baker's Yeast into Pure Culture

An amount of 1 g dry yeast was stirred in 100 mL distilled water in sterile condition inside laminar flow cabinet to prevent contamination. After 10-fold serial dilutions, 0.1 mL of the suspension was spread onto the surface of malt extract agar plates. Visible individual yeast colony was transferred onto new agar plates for seed culturing. All yeast cultures were incubated at 30°C for 48 hr.

Yeast Cultivation in MRE

One full loop of pure single colony from the seed culture was inoculated into 10 mL growth medium (GM) in a 50 mL Falcon tube overnight to promote yeast growth. The production of crude SCP was performed in a 250 mL flask using 100 mL POME medium or GM. The flask was inoculated with 10% (v/v) of the overnight seed culture and incubated at 30°C with agitation at 150 rpm for seven days using a rotary incubator shaker (MaxQ Mini 4450, Thermo Fisher Scientific, USA). After seven days, the fermentation was halted and the biomass was harvested. The resulting yeast biomass was subjected to centrifugal separation prior to the protein content analysis. The residual supernatant was collected for COD measurement.

The effect of medium composition for fermentation was studied by varying the POME to GM ratio. Two types of POME medium, *i.e.,* POME hydrolysate and untreated MRE were used in the fermentation medium preparation. The compositions of fermentation medium are shown in *Table 1*.

Chemical Oxygen Demand (COD) Analysis

Samples were diluted 10 times with distilled water for COD analysis. A volume of 0.2 mL diluted samples were transferred into the High Range Plus COD reagent vial (HACH, COD range 1500-15 000 ppm). The mixtures of samples and reagents were heated in the reactor at 150°C for 2 hr and then cooled to room temperature. The COD was then measured using a HACH DR890 colorimeter (APHA, 2018).

Reducing Sugar and Glucose Content

The reducing sugar content was measured by dinitrosalicylic acid (DNS) method (Miller, 1959). Approximately 1.5 mL sample was centrifuged at 5000 rpm for 20 min and 1 mL supernatant was decanted into a boiling tube. After adding 2 mL DNS reagent, the solution was then boiled in a water bath for 10 min. Then, the solution was made up to 10 mL with distilled water. The absorbance of the sample solution was measured at 540 nm using a UV-Visible spectrophotometer. The DNS calibration curve was developed using glucose standard concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 g L^{-1}.

Biomass Concentration

The biomass was collected by centrifuging the culture medium at 5000 rpm for 20 min. The supernatant was discarded and the pellet was washed several times with distilled water and then dried in an oven at 80°C for 24 hr (Keturah *et al.,* 2014). The biomass concentration or biomass yield was quantified in g L^{-1}.

TABLE 1. COMPOSITIONS OF FERMENTATION MEDIUM FOR SINGLE CELL PROTEIN PRODUCTION

Composition	The ratio of POME: Growth medium			Composition of nutrient in fermentation medium (g in 100 mL)				
	Untreated MRE (mL)	POME hydrolysate (mL)	Growth medium (mL)	Glucose	Yeast extract	Peptone	KH$_2$PO$_4$	MgSO$_4$
Reference Control 1 (CHP)		100	100	5	0.3	0.5	0.1	0.05
Control 2 (CP)	100							
1		50	50	2	0.3	0.5	0.1	0.05
2		100						
3		100		2	0.3	0.5	0.1	0.05
4	50		50	2	0.3	0.5	0.1	0.05
5	100							
6	100			2	0.3	0.5	0.1	0.05

Note: POME - palm oil mill effluent; MRE - mixed raw effluent; CP - control sample for untreated MRE; CHP - control sample for POME hydrolysate.

Crude Protein

Protein content (%) was determined by total nitrogen (TN) method with traditional conversion factor of 6.25 to convert N into protein content (Maehre *et al.*, 2018). The TN analysis was conducted using Dumas combustion method (Durmatherm, Gerhardt) with reactor temperature at 980°C. During the determination of TN, oxygen was used for combustion while helium was used as the carrier gas. This method was selected due to shorter analysis times, ease of operation and improved safety compared to the Kjeldahl method but with similar precision (Müller, 2017). The protein yield (g L^{-1}) was calculated by multiplying the biomass yield (g L^{-1}) and protein content (%) of the biomass.

RESULTS AND DISCUSSION

Compositional Analysis of MRE

The cellulose, hemicelluloses, lignin, oil content and moisture content were analysed prior to POME pre-treatment. Results obtained from the triplicate analysis revealed that the MRE contained water (90%-95%), solids (4%-7% dry weight), as well as oil and extractives (2%-3% dry weight). The lignocellulosic content of solid fraction in MRE was quantified. The solid MRE contained 44%-58% cellulose, 7%-11% hemicellulose, and 27%-43% lignin (*Table 2*). It is believed that high lignin content in POME contributed to the dark brownish POME colour together with tannin, humic acid and fulvic acid-like substance as well as phenolic compounds (Zahrim *et al.*, 2014). The cellulose amount was

higher than the value reported by Khaw *et al.* (2008) whereas the hemicellulose content was comparable with Prasertsan *et al.* (2017). These findings may be due to the differences in sampling points, mills processes, and other factors (oil palm age and locality). Moreover, *Table 2* shows the organic content (measured as COD), total solids, crude protein, TN, reducing sugars, and glucose in MRE. The measured values obtained in this study were within the range when compared to previous studies. Apart from organic constituents, raw POME contains minerals and other nutrients such as phosphorus, potassium, calcium, and magnesium (Nahrul Hayawin *et al.*, 2017). The available cellulose and hemicellulose in POME can be converted into simple sugars and further utilised as fermentation media, while the available minerals and nutrients may be useful for microbial growth.

Pre-treatment of MRE for Fermentable Sugar Production

Polysaccharides in lignocellulosic materials are made from long homopolymer chain of glucose units connected by beta acetyl linkages. The low digestibility of lignocellulosic biomass for conversion into fermentable sugars is usually due to its lignin content, acetyl groups, and crystallinity. Therefore, physical and chemical pre-treatments followed by enzymatic hydrolysis are essential in order to improve the accessibility of the sugar components (Silvamany *et al.*, 2015). Enzymatic MRE pre-treatment using commercial cellulase enzymes were carried out. Optimisation of enzymatic hydrolysis was carried out using

TABLE 2. COMPOSITIONS OF MIXED RAW PALM OIL MILL EFFLUENT

Parameters	Unit	This study	Other studies
COD	mg L^{-1}	48 000-91 300	52 000-114 800
Moisture	%	90-95	95-96
Total solid	g L^{-1}	29-70	38-73
Total carbohydrate	%	NA	22.27-29.55
Crude protein	%	8.40-17.63	12.31-12.75
Total nitrogen	mg L^{-1}	536-1 295	420-8 540
Reducing sugar	g L^{-1}	5.29-14.79	1.53-12.45
Glucose	g L^{-1}	0-2.32	0.87-1.01
Cellulose	% dry weight	44-58	38-39
Hemicellulose	% dry weight	7-11	23-24
Lignin	% dry weight	27-43	22-26

Note: NA - not analysed.

Source: Kamal *et al.*, 2012; Khaw *et al.*, 2008; Nahrul Hayawin *et al.*, 2017; Nurul Adela *et al.*, 2016; Prasertsan *et al.*, 2017; Saifuddin and Refal, 2014.

OFAT method by varying one factor while keeping other parameters fixed in each experiment. In the first experiment, temperatures were varied at 45°C, 50°C and 55°C while pH, agitation, and enzyme dosage were fixed at 5.0, 150 rpm, and 1% (v/v), respectively. Fermentable sugar production measured as reducing sugar content was optimum at 50°C incubation temperature (*Figure 2*). The optimum temperature for the reaction and stability of three components, namely, endoglucanase, cellobiohydrolase, and beta-glucosidase in *Trichoderma* sp. is between 50°C and 60°C (Mun *et al.*, 2008). However, enzymatic hydrolysis activity becomes slower at 55°C and thus, producing less fermentable sugars.

In general, instead of using a buffer solution, MRE mixed with NaOH gave higher reducing sugar content. For instance, based on *Figure 2*, samples that were added with 100 mL 0.1 M of citrate buffer solution produced 13.14 g L^{-1} of fermentable sugars at incubation temperature of 50°C. In contrast, samples that were added with 100 mL 0.1 M NaOH produced about 34.78 g L^{-1} sugars. NaOH acts as a medium to adjust the pH which increases the amount of reducing sugars produced after the enzymatic hydrolysis. This finding is in agreement with Khaw *et al.* (2008) where lignin degradation by alkali will enhance the conversion of MRE into simple sugars. Alkali helps to promote hydrolysis and splitting of polymers into smaller molecules. During alkaline hydrolysis, the lignocellulosic compound swelled, leading to alteration of lignin structure and breaking of the ester and glycosidic chains (Noorshamsiana *et al.*, 2017).

Further experiments were carried out by treating the samples with 100 mL 0.1 M NaOH for pH adjustment. It was observed that the highest amount of fermentable sugars was produced when the pH of the substrate was at 4.7 (*Figure 3*). However, sugar production was not directly affected by the increment of enzyme concentration

and agitation rate (*Figures 4* and *5*). Much lower enzyme loading was preferred in order to reduce the processing costs. Thus, the enzyme concentration of 1.0% (v/v) which gave comparable sugar production was used. Although the COD values were not affected by all parameters, however, COD values increased with the production of fermentable sugars. Based on OFAT method, the optimum parameters obtained for enzymatic hydrolysis of MRE using cellulase enzyme were pH 4.7, temperature 50°C, agitation rate 150 rpm, and enzyme dosage of 1 mL/100 mL MRE. MRE hydrolysate with reducing sugar content of about 41.63 g L^{-1} was produced whereas the COD value was 56 800 mg L^{-1}, compared to control that produced only 12.44 g L^{-1} of fermentable sugars (*Figure 4*). The fermentable sugars are utilised to complement glucose that is usually used in the preparation of conventional fermentation medium, due to the relatively high MRE hydrolysate reducing sugar content.

Yeast Growth Profile in Hydrolysed POME

The viable cell counts of the yeast isolate in the POME hydrolysate added with glucose, N source, and minerals are presented in *Table 3*. The cell count can only be visible after 72 hr cultivation. There was an intense growth of yeast after day 5 and day 6 of cultivation. However, after day 7, a reduction in viable cell count was recorded (4.00 × 10^8 CFU mL^{-1}). The mean viable cell counts of *S. cerevisiae* in POME hydrolysate were found to decrease after day 7. The reduction in growth can be attributed to limiting nutrients and oxygen, arising from the depletion of nutrients and oxygen in the media. Autolysis is enhanced by the exhaustion of nutrients and oxygen (Ojokoh and Uzeh, 2005). Thus, yeast cultivation will stop at day 7 in order to obtain maximum biomass yield from the fermentation.
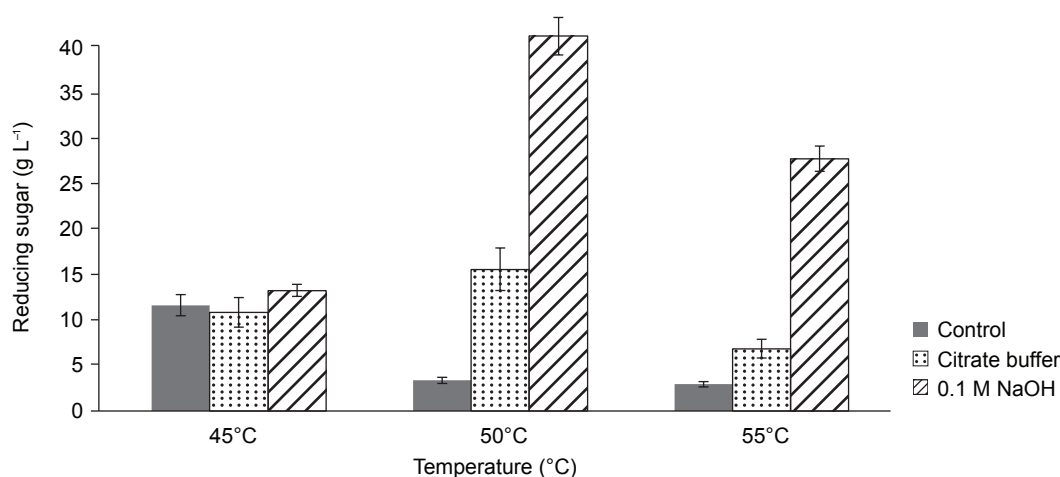

Figure 2. Effect of incubation temperature on reducing sugar concentration (enzyme dosage: 1 mL/100 mL substrate, pH: 4.7, agitation rate: 150 rpm).
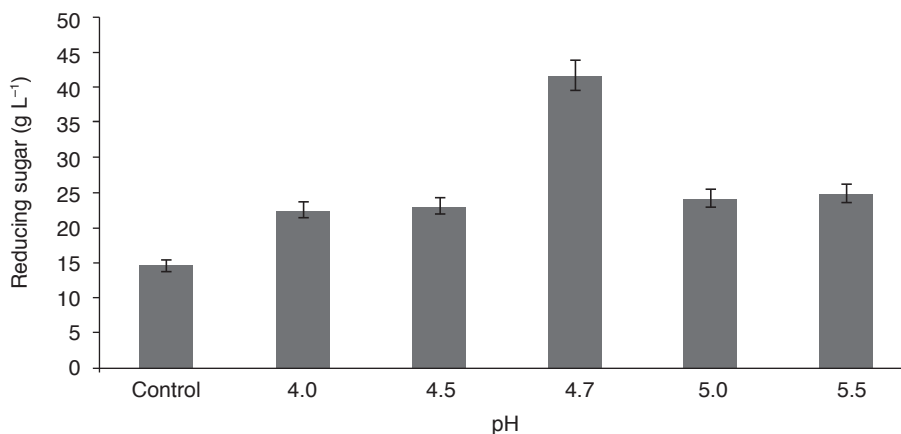
Figure 3. Effect of pH on fermentable reducing sugar production (enzyme dosage: 1 mL 100 mL substrate, agitation rate: 150 rpm, incubation temperature: 50°C).
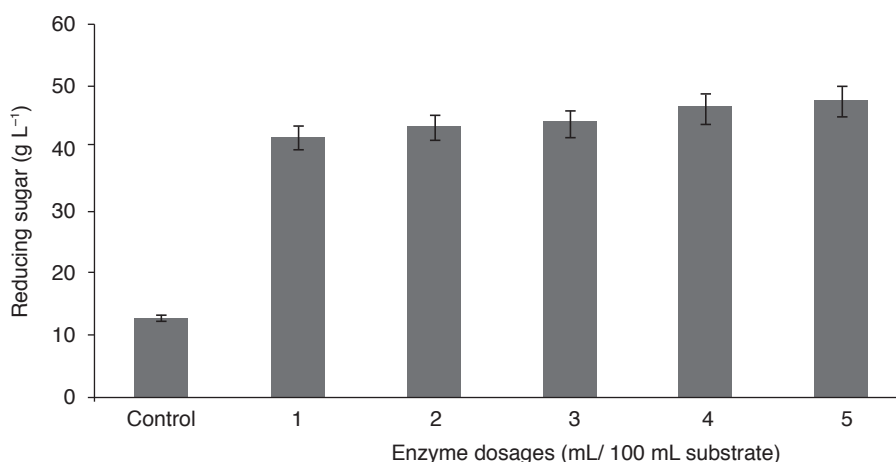

Figure 4. Effect of enzyme dosages on fermentable sugar production (pH: 4.7, agitation rate: 150 rpm, incubation temperature: 50°C).
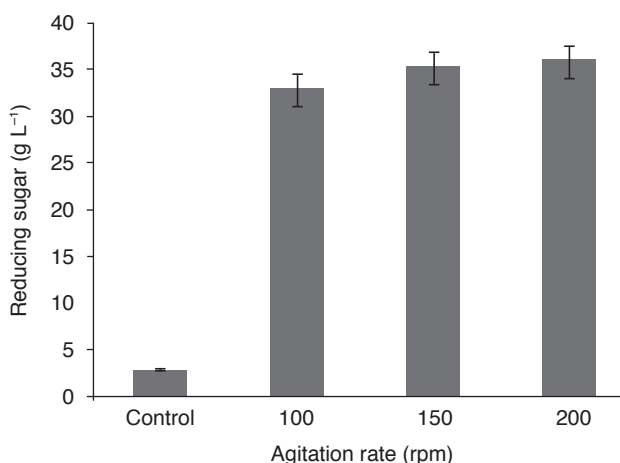

Figure 5. Effect of agitation rate on fermentable sugar production (enzyme dosage: 1 mL/ 100 mL substrate, pH: 4.7, incubation temperature: 50°C).

Production of Microbial Biomass Protein with Different POME Compositions

After 7 days of fermentation, the growth of *S. cerevisiae* in control and experimental media

TABLE 3. VIABLE CELL COUNT OF *S. cerevisiae* IN POME HYDROLYSATE MEDIUM DURING 7-DAYS INCUBATION

Day	CFU mL^{-1}
1	Not visible
2	Not visible
3	5.00×10^7
4	8.00×10^7
5	2.04×10^8
6	1.03×10^9
7	4.00×10^8

were determined by quantifying the biomass yield. The biomass yield, COD reduction, and protein content obtained after yeast fermentation in different medium containing hydrolysed MRE (POME hydrolysate) or untreated POME as well as supplemented fermentation medium are tabulated in *Table 4*. Compositions 1 to 3 consisted of POME hydrolysate as fermentation medium supplemented with nutrients as described in the

TABLE 4. EFFECT OF DIFFERENT FERMENTATION MEDIUM ON BIOMASS YIELD, COD REDUCTION AND PROTEIN CONTENT

Composition No.	Media	Biomass yield (g L⁻¹)	COD reduction (%)	Protein content (%)	Protein yield (g L⁻¹)
Reference	100% GM	29.51 ± 0.35	22.63 ± 0.05	58.63 ± 0.95	17.30 ± 0.25
CHP	Control hydrolysed POME	8.34 ± 0.32	62.22 ± 2.50	11.05 ± 0.04	0.92 ± 0.03
CP	Control untreated MRE	29.93 ± 1.89	59.87 ± 1.47	17.23 ± 0.41	5.45 ± 0.44
1	50% HP + 50% GM	18.35 ± 1.21	55.39 ± 1.00	24.42 ± 0.11	4.52 ± 0.30
2	100% HP	17.66 ± 1.20	68.32 ± 2.50	18.72 ± 0.42	3.21 ± 0.20
3	HP + 2% glucose + YE + peptone + minerals	28.92 ± 1.37	79.12 ± 1.87	24.79 ± 0.33	7.34 ± 0.43
4	50% UP + 50% GM	26.64 ± 0.99	56.98 ± 2.30	18.92 ± 0.66	5.28 ± 0.34
5	100% UP	31.13 ± 3.00	62.85 ± 2.50	24.00 ± 1.96	6.78 ± 0.51
6	UP + 2% glucose + YE + peptone + minerals	51.66 ± 3.47	56.07 ± 2.70	18.09 ± 0.70	9.83 ± 0.79

Note: POME - palm oil mill effluent; COD - chemical oxygen demand; CP - control sample for untreated MRE; CHP - control sample for POME hydrolysate; HP - hydrolysed POME; UP - untreated MRE; YE - yeast extract; GM - growth medium.

method section. Composition 3 which contained 100 mL POME hydrolysate supplemented with 2 g glucose, 0.5 g peptone, 0.3 g yeast extract, 0.1 g KH_2PO_4, and 0.05 g magnesium sulphate ($MgSO_4$) produced the highest biomass yield and COD reduction at 26.74 g L⁻¹ and 79.12%, respectively. The recorded density of yeast growth for this composition was 1.03×10^9 CFU mL⁻¹. This shows that the yeast cells have utilised more sugars and produced more biomass (Keturah *et al.*, 2014). In contrast, the biomass yield, COD reduction, and protein content in control POME hydrolysate were 8.33 g L⁻¹, 62.22% and 11.05%, respectively. No colony was formed in aliquot spotted agar plate indicating that the biomass and protein determined in the POME hydrolysate control was not affected by the yeast growth. For composition 2 (100% hydrolysed POME), the growth of yeast was observed in the medium but with lower biomass yield, since no glucose and nutrients were added into this medium. This indicated that the yeast growth is highly dependent on limited fermentable sugars available in POME hydrolysate with reducing sugar content of approximately 4 g/100 mL. Previous study showed that lower yeast growth was observed when the fermentation media (papaya extract juice) were diluted with 400 mL and 600 mL sterile water as compared by using undiluted juice as the fermentation medium. The poor cell growth in diluted fermentation substrate may be attributed to reduction of available sugars and in the concentration of growth factors (Ojokoh and Uzeh, 2005).

Composition 6 which contained 100 mL untreated POME supplemented with 2 g glucose, 0.3 g yeast extract, 0.5 g peptone, 0.1 g KH_2PO_4, and 0.05 g $MgSO_4$ produced the highest biomass yield (51.66 g L⁻¹). However, the high biomass yield recorded may be contributed by the initial suspended solids that existed in the untreated POME samples. This can be seen from the raw POME control samples (29.93 g L⁻¹ solid biomass). COD reduction during fermentation was relatively lower at 56.07%. The growth of yeast was observed in this medium with the addition of N source (yeast extract and peptone) together with carbon source (existing COD) in the POME samples. COD reduction of 62.85% in medium 5 (100% untreated POME) was highest among untreated POME samples. Since no additional carbon sources and nutrients were added into this medium, the yeast utilised any carbon, N and micronutrient sources that were readily available in the untreated POME samples. The yeast (*S. cerevisiae*) may also be able to hydrolyse complex carbohydrate in untreated POME into sugars that serve as carbon source in synthesising microbial biomass during the fermentation process (Aruna *et al.*, 2017).

The biomass produced after fermentation was analysed for protein content. The initial protein content in untreated POME and POME hydrolysate were 17.26% and 11.05%, respectively. The highest protein content was obtained in composition 3 (24.79%). With this amount, the resulting biomass is quite suitable for animal feed use (Iwuagwu and Ugwuanyi, 2014). The protein yield in composition 3 was the highest (7.34 g L⁻¹) among POME hydrolysate medium due to its highest biomass yield. Protein yield in untreated POME was higher (11.8 g L⁻¹) than POME hydrolysate, but with lower protein increment (5.45 g L⁻¹) compared to initial protein content prior to yeast fermentation. However, the biomass yield was comparable to GM although the protein content was much lower. This is due to 5% glucose was used as carbon source in GM whereas

only 2% glucose was added with 4% reducing sugar (mixed of glucose and pentose) in medium 3. Earlier study stated that *S. cerevisiae* preferentially utilises glucose as the source of carbon. However, following its depletion, it can utilise a wide variety of other carbons including non-fermentable compounds (Turcotte *et al.*, 2009).

Table 5 shows that medium compositions affect the increment of biomass and protein yield after fermentation. The use of 100% POME hydrolysate supplemented with inorganic carbon and N source as well as micronutrient in fermentation medium (composition 3) gave substantial increase in biomass and protein yield of more than 3.44 and 7.74 folds, respectively. The crude protein content in that particular sample was 24.79%. Nevertheless, composition 6 recorded the highest biomass and protein yield of 51.66 and 9.83 g L^{-1}, respectively. The biomass and protein yield increment after yeast fermentation were only 1.71 and 1.89 folds,

respectively. These findings demonstrated similar trend with a study by Aruna *et al.* (2017) in which fermented yam peels with *S. cerevisae* supplemented with N source produced 15.54% crude protein as compared to unfermented yam peels and non-supplemented *S. cerevisae* fermented yam peels that produced 6.60% and 11.08% crude protein, respectively. In addition, COD reduction was not influenced by composition of the fermentation medium.

Compared to other studies presented in *Table 6*, this study managed to produce higher biomass but with lower protein content. This is because most of the biomass was in the form of suspended solid (readily present in the raw material). However, yeast fermentation seemed to significantly increase the protein content in POME hydrolysate from 11% to 29%. For untreated POME substrate, the protein increment was from 17% to 24%. The COD reduction of 79.12% was comparable to Barker and

TABLE 5. INCREMENT OF BIOMASS AND PROTEIN YIELD AFTER YEAST FERMENTATION IN HYDROLYSED POME

Samples	Media	Biomass yield increment (fold)	Protein yield increment (fold)
1	50% HP + 50% GM	2.20 ± 0.72	4.90 ± 1.62
2	100% HP	2.02 ± 0.34	3.34 ± 0.44
3	HP + 2% glucose + YE + peptone + minerals	3.44 ± 0.41	7.74 ± 1.07
4	50% UP + 50% GM	0.97 ± 0.16	1.05 ± 0.19
5	100% UP	0.94 ± 0.16	1.28 ± 0.17
6	UP + 2% glucose + YE + peptone + minerals	1.71 ± 0.13	1.89 ± 0.51

Note: POME - palm oil mill effluent; COD - chemical oxygen demand; CP - control sample for untreated mixed raw POME (MRE); CHP - control sample for POME hydrolysate; UP - untreated MRE; HP - hydrolysed POME; YE - yeast extract; GM - growth medium.

TABLE 6. COMPARISONS ON YEAST AND FUNGAL BIOMASS PRODUCTIONS FROM DIFFERENT SUBSTRATES

Substrate/ Microorganism	Biomass yield (g L^{-1})	COD reduction (%)	Protein content (%)	Protein yield (g L^{-1})	References
Untreated POME/ *S. cerevisiae*	63.84 ± 6.01	59.02 ± 5.34	18.09 ± 2.81	11.80 ± 1.55	This study
POME hydrolysate/ *S. cerevisiae*	28.92 ± 3.43	79.12 ± 3.73	24.79 ± 1.31	7.34 ± 1.06	This study
POME/ *Saccharomyces* sp. L3	4.42	83.00	27.00	1.19	Iwuagwu and Ugwuanyi (2014)
Pineapple waste/ *S. cerevisiae*	5.71	n/a	n/a	2.76	Dhanasekaran *et al.* (2011)
Cabbage juice/ *S. cerevisiae*	8.00	n/a	35.00	2.00	Choi and Park (2003)
Vegetable waste hydrolysate/ *S. cerevisiae*	8.10	n/a	41.30	3.35	Stabnikova *et al.* (2005)
Potato wastewater/ *Candida utilis*	33.25	n/a	36.70	12.20	Kurcz *et al.* (2018)
Steriliser condensate/ Isolated cellulolytic fungi	8.30	n/a	30.30	2.48	Cheah and Leslie (1987)

Note: n/a - not available; POME - palm oil mill effluent; COD - chemical oxygen demand.

Worgan (1981) with a reduction of 77% COD during mycoprotein production from POME using *Yarrowia lipolytica* NCIM 3589 culture. On the other hand, COD reduction was slightly lower when compared to the use of POME for bioethanol and biogas production at 91% and 82%, respectively (Alam *et al.*, 2009; Prasertsan *et al.*, 2017).

CONCLUSION

POME hydrolysate was utilised as medium for the production of microbial biomass protein, that is also known as crude SCP. Fermentation of mixed raw POME either in raw or hydrolysed form, using *S. cerevisiae* with and without the addition of carbon and N sources resulted in microbial protein production. However, higher yield was obtained when fermentation of the *S. cerevisiae* was carried out using undiluted POME hydrolysate as the substrate. The fermentation produced 28.92 g L^{-1} biomass with 24.79% protein content and consequently reduced organic pollutant up to 79.12% COD reduction. The yeast fermentation in undiluted hydrolysed POME supplemented with additional carbon and N sources recorded the highest increase in biomass and protein contents that of 3.44 and 7.74 folds, respectively. Considering these significant enhanced protein contents in the POME, it is a potential protein supplement in animal diet. Besides this, scale-up studies should be conducted in order to develop the present process, which would offer a low-cost and abundant substrate for SCP production.

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