Journal of Geoscience and Environment Protection, 2019, 7, 1-12
https://www.scirp.org/journal/gep
ISSN Online: 2327-4344
ISSN Print: 2327-4336

Efficacy of Purple Non Sulphur Bacterium *Rhodobacter sphaeroides* Strain UMSFW1 in the Utilization of Palm Oil Mill Effluent

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How to cite this paper: Al Azad, S., Chin, F. S., & Lal, M. T. B. M. (2019). Efficacy of Purple Non Sulphur Bacterium *Rhodobacter sphaeroides* Strain UMSFW1 in the Utilization of Palm Oil Mill Effluent. *Journal of Geoscience and Environment Protection, 7,* 1-12.
https://doi.org/10.4236/gep.2019.710001

Received: July 19, 2019
Accepted: October 8, 2019
Published: October 11, 2019

Abstract

Sustainable use of palm oil mill effluent (POME) has been the major focus in the recent development in palm oil industry due to the fact that environmental issue brought by POME. The purpose of this study was to determine the optimum incubation period of purple non-sulphur bacterium (PNSB) in reduction of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) in settled POME and to determine the dry cell weight, TN, TP and cell yield of PNSB. Pure isolate of *Rhodobacter sphaeroides* strain UMSFW1 was cultured in settled POME under anaerobic condition at 2500 lux illumination on light intensity at a temperature of 30˚C ± 2˚C for 144-h. Parameters such as COD (mg/L), dry cell biomass (g/L), TP (mg/L) and TN (mg/L) in settled POME and bacterial cells were analyzed. A total reduction of TN (43.9%) in settled POME and a total increase of TN (43.2%) in bacterial cell were recorded at the end of experiment. At the same time the reduction of 51.5% chemical oxygen demand was determined from the POME. The highest dry cell weight of 2.44 g/L with cell yield 0.39 (mg/cell/mg COD) was achieved at the end of experiment. A total 24.7% of TP reduction in settled POME was achieved in 144-h culture, but while a maximum 10% of TP in bacterial cell was achieved in 48-h culture. This study shows that PNSB *Rhodobacter sphaeroides* strain UMSFW1 grows well by using settled POME as substrate and is capable to remove TN in the settled POME and assimilate into bacterial biomass. This study could provide us a further insight in the nutrient removal and COD removal in the bioremediation process by bacterium *Rhodobacter sphaeroides* strain UMSFW1.

Keywords

Purple Non-Sulphur Bacteria, Palm Mill Effluent, Chemical Oxygen Demand, Nutrients and Growth

1. Introduction

Palm oil industry not only contributed revenue to the country, but also generated severe impacts to the environment. By-product such as empty fruit bunches (EFB), palm kernel, fibre, shell and palm oil mill effluents (POME) are produced during the extraction process of crude palm oil (DOE, 1999). Among these by-product, POME management has being the most concerned issue in the industry because of its huge quantities in production. It was estimated that for every tonne of crude palm oil, 5 to 7.5 tonnes of water is used and more than 50% of this amount of water will be ended up as POME (Ahmad et al., 2003). POME is an acidic thick brownish nutrient-rich liquid that includes high organic loading materials which also supported the growth of single cell organisms, like phytoplankton and beneficial microbes. However, excessive quantities of untreated POME if released to environment will rapidly reduce dissolved oxygen of river especially rivers with low waste assimilative capability. The waste may float to the surface preventing oxygen from dissolving into the water. Anaerobic condition in the water body activates anaerobic microbes leading to the production of poisonous gas such as hydrogen sulphide. To avoid such situation, POME must have to undergo treatment processes. Treatment of POME usually involves two processes which are physical and biological process. Pre-treatment steps are usually categorized in physical treatment including screening, sedimentation and oil removal in oil traps (DOE, 1999). While, in the secondary treatment, biological treatment systems are employed to treat POME. Biological treatment can further be categorized into anaerobic and aerobic processes in which most microbes such as bacteria, fungi and algae are being used to treat POME. For example, microbes that are commonly being found in the biological treatment of POME are fermentative bacteria *Thermoanaerobacterium aotearoense*, denitrifying bacteria *Bacillus subtilis*, nitrifying bacteria *Nitrobacter sp.* and *Nitrosomonas sp.*, phosphorus accumulating bacteria *Flavobacterium sp.*, fungi *Aspergillus niger* and microalgae *Chlorella pyrenoidosa* (Tan et al., 2015). These microbes are usually native to POME and each of them has its own role in the degradation of POME. Biological treatment often results in the production of excess biomass in the form of sludge. Purple non-sulphur bacteria (PNSB) is one of the potential candidate in the bioremediation of POME because of its high adaptability, ability of switching various mode of energy-generating systems according to the available environmental conditions (Wei, 2016) and the ability of PNSB to grow directly in high organic load wastewater (Sasaki et al., 1991). Furthermore, PNSB is economically attractive because several reports showed the ability of PNSB to utilize wastewater as substrate to produce various useful products such as polyhydroxybutyrate (PHB) (Khatipov et al., 1998) and single cell protein production (Kobayashi & Kobayashi, 1995), while simultaneously reducing chemical oxygen demand in wastewater and ability to reduce nitrogen and phosphorus level in the domestic waste water (Hulsen et al., 2014). PNSB is also used in the bioremediation of POME to reduce the chemical oxygen demand (COD) with maximize the production of bacterial biomass (Azad & Shaleh, 2015) and characterize the nutritional values of POME grown biomass to support the growth rotifers (Loo et al., 2013). The species of *Rhodopesudomonas* possess characteristics which readily breakdown lipids. The bacterium *R. palustris* can utilize POME with the formation of hydrogen gas (Suwansaard et al., 2009). However, research on the efficiency of PNSB to reduce total nitrogen and total phosphorus in POME are limited. Hence, this study was aimed to determine the dry cell weight and uptake of total nitrogen and total phosphorus by purple non-sulphur bacterium with the reduction of chemical oxygen when culture in settled palm oil mill effluent (POME).

2. Materials and Methods

2.1. Collection of Palm Oil Mill Effluent (POME)

POME sample was collected from Lumadan Mill, Beaufort located at 5˚18'29"N 115˚42'16"E in Sabah. Malaysia. The sample was collected directly from the POME drainage point by using plastic bottles. The sample was brought to the Chemical Oceanography Lab of Borneo Marine Research Institute (BMRI), University Malaysia Sabah for characterization.

2.2. Purple Non-Sulfur Bacterium and Media Used

Pure freshwater isolate *Rhodobacter sphaeroides* strain UMSFW1 was obtained from Borneo Marine Research Institute culture collection. Media 112 was used as bacterial growth media and also for inoculums development. Media 112 was prepared by mixing 10.0 g of yeast extract, 0.5 g of magnesium sulphate and 1.0 g of di-potassium hydrogen phosphate and dissolving them in one litre of distilled water. Inoculums of bacterium also developed in 112 synthetic media. A 48-h of *Rhodobacter sphaeroides* was then used as inoculum in the utilization of POME.

2.3. Utilisation of Palm Oil Mill Effluent (POME)

Supernatant of settled POME was collected and the pH was adjusted to 7.0 by adding in 1.0 N sodium hydroxide (NaOH) solution. In one litre Schott's bottle 700 ml of settled POME was dispensed and 30% (v/v) of 48-h previously grown inoculum of *Rhodobacter sphaeroides* strain UMSFW1 was added. Mixture was topped up with Media 112 and the bottles cap was hand-tightened to create anaerobic condition inside the bottles. The bottles were then incubated for 144-h with 2.500 lux illumination light intensity at temperature of 30˚C ± 2˚C. The bottles were manually inverted two times/day to allow even mixing of bacteria and settled POME inside the bottles. Schott's bottle which contained only 1000 mL of settled POME was also incubated in same condition and used as control.

2.4. Samplings and Analytical Parameters

During the six days (144-h) experimental period, destructive sampling was carried out every day with a random removable of three bottles and used up for analysis.

Total nitrogen (mg/L) and total phosphorus (mg/L) uptakes by bacterium and reduction of chemical oxygen demand (mg/L) were determined according to standard methods (APHA, 2005). The characterized parameters such as, pH, oil and grease (mg/L), total solids (mg/L) total suspended solids (mg/L), volatile suspended solids (mg/L) and total nitrogen (mg/L) and total phosphorus (mg/L) of settled and unsettled POME were determine according to the standard methods (APHA, 2005). The growth of the bacterium *Rhodobacter sphaeroides* (X_{max}) was determined by using dry cell weight (g/L) according to standard method (Sawada et al., 1977).

$$\text{Dry cell weight}(\text{mg/L}) = \frac{W_2 - W_1}{V} \times 1000$$

where

W_2 = Final weight of the test tube;

W_1 = Initial weigh of the test tube;

V = volume of sample.

The specific growth rate (μ_{max} per h) and cell yields ($Y_{x/s}$) were determined and using following formulas (Sasaki & Nagai, 1979):

$$\mu_{max} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100$$

(μ_{max}: specific growth rate, e: base of natural logarithms, W_2: cell dry biomass at time T_2 and W_1: initial cell dry biomass at time T_1)

$$\text{Cell yield}(Y_{x/s}) = \frac{\text{Dry biomass produced}(\text{mg/L})}{\text{COD reduction}(\text{mg/L})}$$

Kruskal-Wallis Test in SPSS was used to determine significance difference in dry cell biomass production, total nitrogen reduction and reduction of total phosphorus at different intervals of time.

3. Results

3.1. Characterisation of POME

Upon settled the physico-chemical characteristics of POME was observed to decrease, but in certain parameters (**Table 1**). Although, there observed in the reduction of total nitrogen (TN) and total phosphorus (TP) of 70.3% and 35.8% respectively, but the available concentrations of TN and TP are more than enough to support the growth of bacterium *Rhodobacter sphaeroides* while cultured in settled POME.

The reduction of 43.9% of total nitrogen (from 239 mg/L to 105 mg/L) and total phosphorus of 24.7% were observed in 144-h of culture. On the other hand, a reduction of 51.5% of chemical oxygen demand was observed while *Rhodobacter sphaeroides* cultured in settled POME (**Table 2**).

Table 1. Physico-chemical characteristic of raw and settled palm oil mill effluent (POME).

Parameter	Raw POME	Settled POME	Reduction (%)
pH	3.68	3.78	-
Chemical Oxygen Demand (mg/L)	39,900	21,450	46.2
Total Solid (mg/L)	50,782 ± 1215	12,885 ± 40.86	74.6
Total Volatile Solid (mg/L)	43,099 ± 988	9510 ± 46.78	77.9
Total Suspended Solid (mg/L)	12,318 ± 265	1624 ± 146	86.8
Oil and Grease (mg/L)	4132 ± 70.68	151 ± 26.03	96.3
Total Nitrogen (mg/L)	804 ± 53.49	239 ± 100.75	70.3
Total Phosphorus (mg/L)	120 ± 5.07	77 ± 3.96	35.8

Table 2. Summary of chemical oxygen demand, total nitrogen and total phosphorus in settled palm oil mill effluent (POME) when culture with bacterium *Rhodobacter sphaeroides* strain UMSFW1 from 0-h to 144-h of culture anaerobic condition with 2500 lux light intensity at 30˚C ± 2˚C.

POME	Initial mean concentration, mg/L (0-h)	Final mean concentration, mg/L (144-h)	Total reduction after 144-h of culturing period (%)
Chemical oxygen demand	21460 ± 204.00	11050 ± 108.00	51.5
Total nitrogen	239 ± 12.31	105 ± 9.14	43.9
Total phosphorus	77 ± 1.33	19 ± 1.18	24.7

Values express as mean ± SD.

3.2. Growth Characteristic of Bacterium *Rhodobacter sphaeroides* in Settled POME

Growth characteristics in term of dry cell weight, total nitrogen and total phosphorus in bacterium *Rhodobacter sphaeroides* stain UMSFW1 during 144-h of culturing in settled palm oil mill effluent is shown in Table 3. The highest dry cell weight (X_{max}) of 2.4 g/L was achieved by bacterium cultured under 2.5 klux at 144-h of culture (Table 3). On the other hand the uptakes of total nitrogen and total phosphorus in bacterium cell observed to be increased by 43.2% (on 120-h) and 10% (on 48-h) respectively. Statistical analysis using one-way ANOVA shows significance differences (p = 0.02) among the days with the highest on 144-h of culture.

The uptakes of TN and TP during the experimental time followed the increase in trends among the days, but with little fluctuation (Figure 1). Statistical analysis showed that there were no significant differences (p = 0.78) in the uptake of TN among 72-h and 120-h culture (Figure 1). Similarly no significant differences (p = 0.92) were observer in the uptakes of TP in bacterium cell after 24-h culture at the end of experiment. On the other hand, the specific growth rate (μ_{max}

Table 3. Growth characteristic of purple non-sulphur bacterium *Rhodobacter sphaeroides* strain UMSFW1 when culture in settled palm oil mill effluent from 0-h to 144-h under anaerobic condition with 2500 lux light intensity at 30˚C ± 2˚C temperature.

Parameters	Initial mean (mean ± sd.)	Final (mean ± sd.)	Remarks
Dry Cell Weight (mg/L)	10.5 ± 0.009	2435 ± 25.35	X_{max} (2.4 g/L) on 144-h of culture
Total Nitrogen (%)	118 ± 1.25	169 ± 3.20	Uptakes of 43.2% on 120-h of culture
Total Phosphorus (%)	12.9 ± 0.015	14.1 ± 0.129	Uptakes of 10% on 48-h of culture
Cell Yield (mg/cell/mg of COD)	-	-	$Y_{x/y}$ of 0.39 ± 0.12 (g/cell/g COD) on 144-h of culture
Specific Growth Rate (per hour)	-	-	μ_{max} (0.25 ± 0.002/h) on 120-h of culture

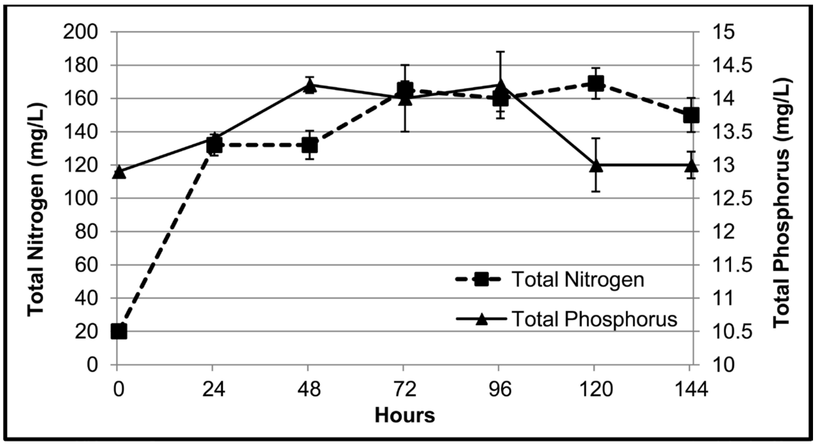

Figure 1. Total nitrogen (mg/L) and total phosphorus (mg/L) in cells of bacterium *Rhodobacter sphaeroides* strain UMSFW1 while culture in settled POME under anaerobic condition with 2500 lux light intensity at 30˚C ± 2˚C temperature.

per hour) was the highest of μ_{max} (0.25 ± 0.002/h) on 120-h of culture. Significant different values ($p = 0.03$) were found in specific growth among the 48-h.

4. Discussion

4.1. Characteristic of Settled Palm Oil Mill Effluent (POME)

During settlement total suspended solid (TSS) in the palm oil mill effluent (POME) was able to reduce up to 86.8%. High suspended solid could reduce the light penetration through water. Settled POME increase the penetration of light that is required by purple non-sulphur bacteria (PNSB) under anaerobic-light condition to grow (Loo et al., 2013). Thus, settled POME is a more suitable media used to grow purple non-sulphur bacteria than raw POME. Light is an important factor to promote the growth and sustain life of purple non-sulfur bacteria (Prasertsan et al., 1997). Chemical oxygen demand of settled POME has been reduced almost half of the raw POME. This may probably due to the removal of organic suspended particle solid in the POME and the available nutrients settled POME were enough to support the growth and survival of PNSB under anaerobic light culture system (Azad & Shaleh, 2015). PNSB is able to use low-molecular organic components to produce their own energy source under anaerobic light condition (Wei, 2016). The total nitrogen in settled the POME may be limiting factor for the growth of some species of microorganisms, but the organism can survive. In addition POME the carbon based rich organic substrate can support the growth of Purple non sulfur bacteria (Azad & Shaleh, 2015). PNSB has unique properties as it can grow well diverse environmental and cultural conditions. In fact, purple non-sulfur bacteria prefer highly polluted areas than unpolluted areas in the nature (Middleburg & Nieuwenhuize, 2000). The optimum pH values for the growth of PNSB are within the range of 6.5 - 7.5. Although the pH value of POME was highly acidic, but adjusted to 7.0 with 1 N HCL to get optimum growth of *Rhodobacter sphaeroides* strain UMSFW1 during the experimental period.

4.2. Effectiveness of Bacterium *Rhodobacter sphaeroides* in the Utilisation of POME as Substrate

Maximum reduction of 51.5% Chemical oxygen demand (COD) was determined in present study. The reduction of COD decreases with the increase in dry cell weight. Nutrient recovery from domestic waste water by purple non-sulphur bacteria (PNSB), *Rhodobacter sphaeroides* showed that COD increased significantly over time, indicating that not only organic matter was assimilated, but substantial amount was generated from phototrophic activity due to some of the soluble COD was accumulated as polyhydroxybutyrate (PHA) (Hulsen et al., 2014). Bacterium *Rhodovulum sulfidophilum* W-1S utilized intra-celluar PHA as electron donors for hydrogen production instead of using external organic sources (Suwansaard et al., 2009). However, PHA of the bacterium was not analyzed in this experiment. The COD reduction for current study (55.5%) in 144-h culture was relatively less compared to reduction of 82% COD in 96-h culture (Azad & Shaleh, 2015). This might be due to the strain of PNSB that was isolated from different source used in process and also gives different growth rate in the substrates. In this study the performance bacterium *Rhodobacter sphaeroides* stain UMSFW1, an isolate from freshwater pond mud was observed less than the *Rhodobacter sphaeroides* strain UMSPSB3, an isolate from POME. The highest bacterial biomass (X_{max}) of 6.5 g/L (dry weight) was obtained after 96-h culture with 20% (v/v) inoculum level, but 82% reduction of COD was noticed in 30% inoculums level (Azad & Shaleh, 2015). On the hand, the highest biomass (X_{max}) of 2.44 g/L was observed after 144-h culture in the bacterium *Rhodobacter sphaeroides* stain UMSFW1 in this experiment. Consequently the cell yield ($Y_{x/y}$, g cell/g COD) of 0.98 and 0.39 in the settled POME were determined with the strain *Rhodobacter sphaeroides* strain UMSPSB3 and *Rhodobacter sphaeroides* stain UMSFW1 respectively. Further under same conditions in microaerobic-light, only one strains of bacteria out of 92 isolates of the PNSB performed the best growth in raw supplemented wastewater in competition with the indigenous organisms (Kantachote et al., 2005). The performance of various species of purple non-sulfur bacterium for high cell yield to achieve single cell protein (SCP) using industrial wastewater as substrate with simultaneous reduction of chemical oxygen demand (COD) are well documented (Kobayashi & Kobayashi, 1995). Purple non-sulfur bacterium, *Rhodocyclus gelatinosus* was prepared in synthetic G5 media from whom 10% (v/v) inoculum was used in cultures of diluted tune condensate and dry cell biomass of 5.7 g/L with 86% reduction in COD were obtained (Prasertsan et al., 1997). Bacterium *Rhodovulum sulfidophilum*, an isolate from mangrove mud while cultured in settled and undiluted sardine processing wastewater with a 15% (v/v) inoculum had shown a reduction of 76% and 68% of COD under aerobic dark and anaerobic light conditions respectively (Azad et al., 2003). The performance of PNSB in treating wastewater was affected by several factors such as types of bacterial strains, preference conditions of the bacteria when treating wastewater and the preference nutrients in the wastewater. This may lead to an inference that current species of *Rhodobacter sphaeroides* stain UMSFW1 had a low efficiency and performance in POME for the COD removal with the simultaneous production of bacterial cell under the provided conditions.

4.3. Growth Characteristic of Bacterium *Rhodobacter sphaeroides* in Settled POME

Dry cell biomass of 2.44 g/L was recorded in 144-h culture, which indicated that bacterium *Rhodobacter sphaeroides* stain UMSFW1 grew well in settled palm oil mill effluent (POME). Total nitrogen in settled POME was decreased gradually with 43.9% in 144-h culture. The PNSB strain *Rhodobacter sphaeroides* strain UMSPSB3 has the capability to reduce the total nitrogen in the settled POME under anaerobic light condition and did not act as limiting factor (Azad & Shaleh, 2015). However, result shows that growth of bacterium *Rhodobacter sphaeroides* strain UMSPSB3 was not limited by amount of total nitrogen in settled POME. The decrease of total nitrogen in substrate is believed to be assimilated into metabolites and used up by bacteria to carry out daily cell activities. PNSB may produce proteinase to convert protein in the wastewaters from fish processing industry into simple and readily absorbed molecules such as peptides, amino acids and ammonia (De Lima et al., 2011). This activity represent important tool for the reduction of organic nitrogen content in industrial effluents and thus, avoiding eutrophication processes in nature aquatic environment had also been stated by them. On the other hand, a total of 43.2% increase of total nitrogen in bacteria cell at 120-h culture indicated that nitrogen contained in settled POME was assimilated by *Rhodobacter sphaeroides* strain UMSFW1 biomass rather than destructive assimilation of nitrogen. Nitrogen in the sewage was assimilated by PNSN into biomass instead of removing through destructive accumulation (Hulsen et al., 2014). The conceptual design was developed based on the utilization of POME *Rhodobacter sphaeroides* strain UMSPSB3 to achieved dual benefits: bioremediation of wastewater and production of beneficial bacterium biomass (Azad & Shaleh, 2015). Total nitrogen in bacterial cell was closely related to biomass production of bacteria. In addition total nitrogen in bacterial cell was observed the highest on 120-h culture (**Figure 1**), but the maximum dry cell weight was recorded in 144-h culture. This may probably because bacteria cell had utilized the nitrogen for the production of useful metabolites instead of assimilation for biomass production. Phototrophic bacteria have high protein content with good amount of essential amino acids, vitamins, biological co-factors and fewer amounts of nucleic acids (Merugu et al., 2012). The PNSB can grow in POME although their growth rates are slower than cultured synthetic 112 media, but the bacterial biomass generated from POME has better nutritional quality in term of essential fatty acid composition (Suwansaard et al., 2009). In addition extracellular polymeric substances, like enzyme produced by purple non-sulfur indicative in the assimilation of nitrogen being documented by researchers. *Afifella marina* strain ME (KC205142) produces protease under anaerobic light conditions at temperature of 30˚C ± 2˚C. The proteolytic activity was positively correlated with the dry cell weight in *A. marina* (Azad et al., 2013). The production of these value added products may probably lead to the high total nitrogen in the bacteria cell.

A decrease of 24.7% of total phosphorus (TP) in settled palm oil mill effluent (POME) was achieved after 144-h culturing period with purple non-sulphur bacterium *Rhodobacter sphaeroides* strain UMSFW1 (**Table 2**). The result from this experiment shows the inefficiency of *Rhodobacter sphaeroides* strain UMSFW1 for the uptake of total phosphorus (10%) in settled POME. Total phosphorus in bacterial cell experienced an increase in 48-h culture and reached maximum value and gradually decreased from 48-h to 144-h culture (**Figure 1**). The initial increase of total phosphorus in bacterial cells was probably due to the accumulation of polyphosphate as intracellular storage although polyphosphate was not analyzed in current experiment. However, the decrease after 48-h culture may probably due to the polyphosphate being degraded (Liang et al., 2010). Overall, performance total phosphorus in the settled POME and PNSB were relatively unstable but the growth of the cell continued. A net decrease in the bacterium cell the end of the experiment was probably due to the mobilization of the organic phosphorus compound in the settled POME (Hulsen et al., 2014). However, a maximum percentage total phosphorus reduction (10.2%) in settled palm oil mill effluent (POME) in the current study was comparatively lower than the uptake of 26.8% total phosphorus in domestic waste water (Hulsen et al., 2014). The better uptake of TP obtained in 2.7 klux anaerobic light illumination at 30˚C ± 1˚C across 144-h culturing period provided with 100 rpm agitation speed in the bioremediation of domestic wastewater (Hulsen et al., 2014). The comparatively lower reduction percentage of total phosphorus in current study may probably due to the low light intensity (2.5 klux) and lack of agitation in the current studies. In the study of bioremediation of sardine processing wastewater, agitation had the significant effects on the growth characteristic of PNSB under aerobic dark condition (Azad et al., 2001). There was a positive correlation between agitation speed and the growth characteristic of bacteria in the bioremediation of under anaerobic light condition (Azad et al., 2003). Under anaerobic light condition (4.0 klux and 150 rpm), 58% of phosphorus could be removed from swine wastewater without supplementation after 144-h (Kim et al., 2004). The production of extracellular nucleic acids (phosphorus based compound) in purple non-sulfur marine bacterium *Afifella marina* strain ME (KC205142) observed better with high light intensity as The highest extracellular nucleic acid yield of 7.48 mg/g dry cell weight was recorded at continuous light condition with 5000 lux illumination intensity in 72-h culture (Soon et al., 2013). This experiment indicated that light intensity and agitation could affect the phosphorus removal from wastewater. Furthermore, different bacterial strains such as *Rhodopseudomonas palustris* (Kim et al., 2004) and *Rhodobacter sphaeroides* (Hulsen et al., 2014) selected for bioremediation was probably one of the factors resulting in low efficiency phosphorus removal in this study. The light, environmental pH, bacteria species and composition of nutrients in the wastewater are the factors affect phosphorus removal by bacteria (Liang et al., 2010). Bacterium *Rhodopseudomonas palustris* exhibited higher internal phosphorus content compared to other isolated strains such as *Rhodobacter blasticus. R. palustris* could accumulate internal phosphorus up to 13% to 15% of its dry cell weight under anaerobic illuminated incubation conditions (Liang et al., 2010). In addition *Rhodobacter sphaeroides* was prevented from polyphosphate accumulation under nitrogen limitation. When nitrogen was limited, nitrogenase-dependent H_2 photosynthetic photo-evolution would compete for ATP available, which this situation could prevent polyphosphate accumulation (Liang et al., 2010). The details of how enzymatic activities affect total phosphorus removal by *Rhodobacter sphaeroides* strain UMSFW1 was not studied in current study, but requires future investigation.

5. Conclusion

So, results of current study suggested that PNSB *Rhodobacter sphaeroides* strain UMSFW1 was able to grow in settled palm oil mill effluent (POME). However, the performance of nutrients removal and assimilation into biomass of this strain were remained unsatisfactory and poorly understood. Thus, more studies should be carried out to improve the performance of *Rhodobacter sphaeroides* strain UMSFW1 in the uptakes of nutrients in different light intensities and agitation speed using POME as substrate with an aim to achieve dual benefit.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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