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Palm Oil Mill Effluent as Alternate Carbon Source for Ammonia Removal in Wastewater Treatment

(Efluen Kilang Kelapa Sawit sebagai Punca Karbon Silih Ganti untuk Penyingkiran Ammonia dalam Rawatan Air Sisa)

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ABSTRACT

To address high demand in searching for carbon sources alternatives in ammonia wastewater treatment, comparison among various carbon sources in term of pollutants reduction efficiency was essential to determine the most cost-effective carbon source selection for industry scale in bulk amount. This study focuses on investigating palm oil mill effluent (POME) as the alternate carbon source for supporting ammonia oxidizing bacteria (AOB) in ammonia removal of glove industrial wastewater treatment. Ammonia reduction efficiency was compared between POME with molasses, one of the most commonly used carbon sources. POME as carbon source in ammonia wastewater treatment had shown significant comparable reduction efficiency as compared to molasses. Furthermore, the study on various mixture ratios of POME-molasses had also shown further improvement in ammonia reduction efficiency. At the optimum ratio of 50:50 (v/v) POME-molasses as carbon source mixture, the ammonia reduction in the treatment system had achieved 53.11% reduction, which reduced ammonia content down to 10.49 mg/L NH_3. In this study, the results suggested that POME showing great potential to be the new cost-effective carbon source alternative in industry scale treatment.

Keywords: Ammonia removal; carbon source; molasses; palm oil mill effluent; wastewater treatment

ABSTRAK

Untuk memenuhi permintaan yang tinggi dalam mencari punca karbon alternatif dalam rawatan air sisa berammonia, perbandingan antara punca karbon daripada sudut kecekapan mengurangkan pencemaran penting untuk menentukan pemilihan punca karbon yang paling menjimatkan kos bagi skala industri dalam jumlah pukal. Kajian ini memfokus kepada penggunaan efluen kilang kelapa sawit (POME) sebagai punca karbon alternatif untuk sokongan pengoksidaan ammonia bakteria (AOB) dalam rawatan pembuangan sisa ammonia bagi industri sarung tangan. Kecekapan pengurangan ammonia dibandingkan antara POME dengan molases, salah satu punca karbon yang sering digunakan. POME sebagai punca karbon dalam rawatan air sisa ammonia telah menunjukkan kecekapan pengurangan yang ketara berbanding molases. Selain itu, kajian menggunakan pelbagai nisbah campuran molases-POME juga menunjukkan peningkatan kecekapan pengurangan ammonia. Pada nisbah optimum, 50: 50 (v/v) POME-Molases sebagai campuran punca karbon, pengurangan ammonia dalam sistem rawatan telah mencapai pengurangan 53.11%, yang mengurangkan kandungan ammonia ke 10.49 mg/L NH_3. Dalam kajian ini, keputusan menunjukkan POME menunjukkan potensi yang tinggi untuk menjadi punca karbon alternatif dengan kos efektif dalam rawatan berskala industri.

Kata kunci: Efluen kilang kelapa sawit; molases; penyingkiran ammonia; punca karbon; rawatan air sisa

INTRODUCTION

Over decades, researchers had been investigating for better approaches in industrial wastewater treatment. Different kinds of industries had also invested enormous amount of grants and effort for the improvement on their wastewater treatment process. Among the types of wastewater treatment methods available, biological wastewater treatment has been always favored among industries due to its cost effectiveness and ease in maintenance (Mohammadi et al. 2010). Malaysia as one of the main rubber-latex producing country, ammonia contents in wastewater effluents had always been a challenge for the respective industries to comply with the industrial effluent discharge standards regulated under Department of Environmental (DOE) Malaysia. Whereas, ammonia content in effluent discharge are limited at 10 mg/L NH_3-N for Standard A, and 20 mg/L NH_3-N for Standard B (DOE 2009).

Ammonia oxidizing bacteria (AOB) had been well studied for the capability of reducing ammonia effectively in wastewater treatment (Yapsakli et al. 2011). The ammonia removal mechanism of bacteria namely nitrification and denitrification, are processes where several groups of bacteria had come together to utilize and digest ammonia (Keluskar et al. 2013). During the removal process, carbon sources are essential for supporting the bacteria growth and survival within the treatment systems (Yang et al. 2012). Among the choices of carbon sources available, molasses had been commonly chosen by industry for sustaining bacteria density in the sequential batch reactor (SBR) tanks. Molasses was referred to the syrup left over after the crystallization of sugars when juice extracted from sugarcane or sugar beets is boiled down (Duraisam et al. 2017). It contains a high concentrated level of vitamins and minerals, while sugars in molasses are sucrose, glucose, and fructose. Despite the availability of molasses, heavy bulk usage of molasses could still be a huge cost towards industry expenses in wastewater treatment.

Therefore, there is a need to find an alternate cost-effective carbon source substitute in industry scale treatment, where bulk amount is utilized. Previous study had shown that palm oil mill effluent (POME) consists of relatively high total organic carbon (TOC) (Aljuboori et al. 2014). Study also showed that POME consists of huge communities of bacteria composing even nitrifying and denitrifying bacteria (Verla et al. 2014). These findings suggested that POME could be a great carbon source alternative for AOB in ammonia removal treatment. POME is referred to the liquid waste that combined with the wastes from sterilizer condensate and cooling water from palm oil processing, usually oily and consisted of various suspended components (Heuzé et al. 2015). Thus, in this research, the utilization of POME as alternate carbon source in ammonia wastewater treatment is studied. Ammonia removal efficiency of AOB with POME and molasses as carbon source were compared, and different mixture ratios of molasses-POME were also investigated for further improvement in term of ammonia reduction efficiency.

Materials and Methods

Preparation of Treatment Materials

Fresh ammonia wastewater sample from Sequential Batch Reactor (SBR) tank influent were collected from local glove manufacturing plant. Wastewater sample was stored in carboy drum packaging (approximately 30 L each) and delivered to laboratory for further treatment experiment procedures. Initial ammonia content in collected wastewater sample was measured as 17-23 mg/L NH_3, with pH value around 6.5-7.5. Fresh POME sample were requested from local palm oil mill industry and stored in plastic containers (approximately 10 L each). Fresh molasses was purchased from local supplier. All materials were stored and kept under 4°C fridge until further usage in research experiments.

Bacteria Inoculum Preparation

Selected pure indigenous bacteria isolates previously isolated and identified from local glove industry SBR tanks, *Ochrobactrum intermedium* (Isolate H) & *Bacillus subtilis* (Isolate J), were inoculated on Tryptic Soy Agar (TSA) plate and incubated at 35°C for at least 24 h until significant bacteria colonies size had been observed. Single pure bacteria colonies were then transferred from agar plates to Tryptic Soy Broth (TSB) solution and incubated in shaking incubator at 35°C with 160 rpm for 24 h. Fully grown bacteria cultures with OD_{600} minimum at 1.0 were then subjected to subsequent wastewater treatments.

Treatment Comparison Between Molasses and POME

Wastewater treatment tanks (10 L) were all prepared and setup with aeration and heating bars. Fresh wastewater samples were poured into treatment tanks, with molasses and POME implemented at C/N ratio at 20:1, respectively. Bacteria culture (H & J) were inoculated in all treatment tanks at 5% (v/v) inoculums size. Negative control was setup with bacteria cultures, but no carbon sources provided. All treatment tanks were aerated and heated at 35°C. Carbon sources (C/N at 20:1) were replenished at 3rd hour of treatment. Treatments were conducted for 6 h, with ammonia readings measured hourly with a colorimeter (Orion AQUAfast AQ3700, Thermo Scientific).

Treatment Comparison Among Molasses-POME Ratios

Wastewater treatment tanks (10 L) were prepared and setup identically as stated. POME samples were spread onto sterile petri dishes and exposed under UV light for 30 min. CFU counts for UV-treated and untreated POME was conducted and collected on TSA plates after 24 h of incubation at 35°C. Fresh wastewater samples were then aliquoted into treatment tanks, with molasses-POME (C/N at 20:1) mixture as carbon sources implemented at different mixture ratio of 25%, 50%, and 75% (v/v), respectively. Bacteria culture (H & J) were inoculated in each treatment tank at 5% (v/v) inoculums size. Negative control was setup with bacteria cultures, but no carbon sources provided. All treatment tanks were aerated and heated at 35°C. Carbon sources (C/N at 20:1) were replenished at 3rd hour of treatment. Experiments were conducted for 6 h, with ammonia readings measured hourly with colorimeter (Orion AQUAfast AQ3700, Thermo Scientific).

Results and Discussion

AN Removal Efficiency Between Molasses and POME

Ammonia removal efficiency of selected bacteria isolates (H and J) was compared under two different carbon sources, POME and molasses. Negative control conducted with no carbon source implemented had shown minimal reduction in ammonia content, from 18.87 mg/L NH_3 to 17.24 mg/L NH_3, about 8.64% reduction (Table 1), and does not achieved DOE Standard A. This showed that the presence of carbon source is crucial in wastewater treatment, where it supports bacteria growth and performance in ammonia reduction. Carbon source is needed as major energy source for the growth of bacteria in the treatment system (Yang et al. 2010). Whereas, wastewater treatments with carbon sources presented showed significant reduction in ammonia content (Figure

TABLE 1. Ammonia removal efficiency comparison between molasses and POME

Hour (h)	AN value (mg/L NH_3)		
	Carbon sources		
	Negative control	Molasses	POME
0	18.87 ± 0.07 [a]	17.49 ± 0.04 [b]	17.24 ± 0.04 [c]
1	18.30 ± 0.05 [a]	16.75 ± 0.06 [b]	15.62 ± 0.09 [c]
2	18.71 ± 0.04 [a]	15.62 ± 0.05 [b]	14.48 ± 0.10 [c]
3	18.63 ± 0.05 [a]	14.72 ± 0.04 [b]	12.93 ± 0.08 [c]
4	18.14 ± 0.06 [a]	12.44 ± 0.03 [b]	10.65 ± 0.03 [c]
5	17.89 ± 0.08 [a]	10.82 ± 0.03 [b]	9.68 ± 0.04 [c]
6	17.24 ± 0.07 [a]	9.76 ± 0.02 [b]	8.70 ± 0.04 [c]

* Superscript indicated the significant shifts in relation to the assessed value (p<0.05): (a,b,c) for carbon sources towards ammonia values

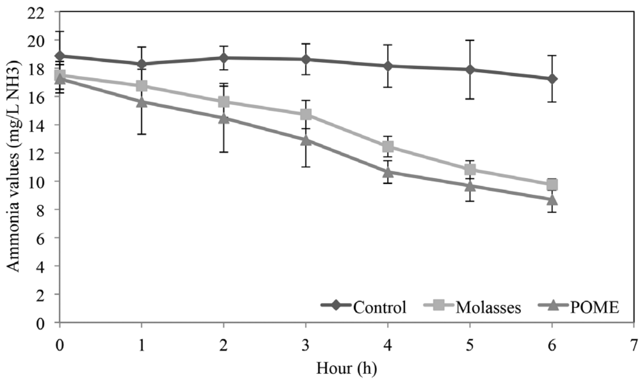

FIGURE 1. Ammonia wastewater treatment comparison between molasses and POME

1). Treatment with molasses had reduced ammonia content from 17.49 mg/L NH_3 to 9.76 mg/L NH_3, which represented 44.20% reduction (Table 1). While, treatment with POME reduced ammonia content from 17.24 mg/L NH_3 to 8.70 mg/L NH_3, represented 49.54% reduction (Table 1). In this experiment, treatment with POME had shown comparable ammonia reduction efficiency in term of percentage reduction as compared with molasses. POME was found to consist of rich total organic carbon as well as contain high nutrients to be utilized for bioremediation process (Kanu & Achi 2011). This suggested that POME as a promising carbon source alternative at a lower cost for various industrial wastewater treatments. In both treatments with carbon sources implemented, ammonia contents had also successfully reduced below 10 mg/L NH_3 within 6 h of treatment, respectively, (Figure 1), to be complied with DOE Standard A regulations for industrial effluents discharge. Treatment with POME as carbon source had even shown slightly better performance than treatment with molasses whereas the lowest ammonia value achieved by using POME as carbon source is at 8.70 mg/L NH_3.

In general, POME is characterized as highly polluting wastewater if it is directly discharged to environment due to its high chemical oxygen demand (COD) and biological oxygen demand (BOD) (Adela et al. 2014). In the study by Poh et al. (2010) on characterizing POME in high crop season showed overall characteristic of POME with 49000 mg/L BOD, 79000 mg/L COD, 42600 mg/L total volatile solids (TVS), and 250:2.9:3.2 of COD:N:P ratio, indicating high biodegradability of POME. On average, each ton of fresh fruit bunches (FFB) processed could generate about one ton of liquid waste with 27 kg BOD, 62 kg COD, 35 kg suspended solid (SS) and 6 kg oil and grease (Zafar 2018). POME usually showed 100 times more BOD and COD than common municipal sewage. Studies found that POME itself carried a high community of own indigenous bacteria such as hydrolytic bacteria, fermentative or acidogenic bacteria, and acetogenic bacteria from palm oil industry (Bala et al. 2014). To sustain ammonia reduction efficiency with dedicated bacteria isolates, these undesired indigenous bacteria community from POME needs to be resolved as it could serve as competitors for the nutrient and carbon source in wastewater treatment (Ohimain et al. 2017). To optimize the ammonia reduction efficiency, dedicated bacteria isolates need to be ensured as the dominant group and survived in the wastewater treatment systems (Zhao et al. 2013). Despite consisting rich organic carbon and nutrient minerals, POME also found to have lots of chemical residues from palm oil mill process, which was harmful, and potentially inhibiting the performance of ammonia oxidizing bacteria (Shavandi et al. 2012). For instance, in study by Verla et al. (2014) showed the presence of heavy metals in POME such as Cd, Fe, Cu, Cr, and Ni at both fresh and stale effluents. These undesired chemical residues could severely suppress the efficiency of ammonia reduction or perhaps kill the applied bacteria inside the treatment system (Igbinosa & Igiehon 2015). As compared to POME, characteristic of glove industry wastewater is usually safer and showing slightly lower content in BOD, COD, suspended solids, and chemical residues (Mohammadi et al. 2010). However, glove wastewater normally consisted of high amount of total nitrogen as major concern and presence of chlorine due to the upstream processing of natural rubber. The high ammoniacal nitrogen content is due to the use of substantial quantity of ammonia in preservation of latex (Gamaralalage et al. 2016).

To reduce the suppressing effect from POME on ammonia reduction efficiency in glove wastewater treatment, different ratios of molasses-POME mixture had thus been investigated in subsequent study for the optimum ammonia reduction efficiency in treatments. The idea of molasses-POME mixture is to dilute the potential chemical residues from POME while providing required nutrients to support bacteria growth. POME samples were also exposed to UV light radiation to reduce the undesired indigenous bacteria competitor prior ammonia wastewater treatment application (Rizzo et al. 2014).

THE REMOVAL EFFICIENCY COMPARISON AMONG MOLASSES-POME MIXTURE RATIOS

Ammonia removal efficiency of selected bacteria isolates under three different mixture ratios of POME with molasses were studied. Negative control with absence of carbon source showed minimal reduction in ammonia content from 21.80 mg/L NH_3 to 19.60 mg/L NH_3 at only 10.09% reduction (Table 2), and barely achieved below 20 mg/L NH_3 for complying DOE Standard B regulations on industrial effluent. This result again showed the importance of carbon source as bacteria's energy source to grow and survive inside the treatment systems (Pant et al. 2010). Without the presence of any carbon sources, dedicated bacteria isolates could hardly reduce ammonia content in the treatment tanks. On the other hands, treatment with 25% molasses:75% POME (v/v) had reduced ammonia from 22.29 mg/L NH_3 to 15.78 mg/L NH_3 as lowest value, represented 29.21% reduction (Table 2). Secondly, treatment with 50% molasses:50% POME (v/v) reduced ammonia from 22.37 mg/L NH_3 to 10.49 mg/L NH_3, as 53.11% reduction (Table 2). Lastly, treatment with 75% molasses:25% POME (v/v) reduced ammonia from 22.69 mg/L NH_3 to 15.45 mg/L NH_3, shown 31.91% reduction (Table 2).

The results supported that carbon source is essential in bioremediation whereas applied bacteria utilized the

TABLE 2. AN removal efficiency comparison among molasses-POME mixture ratios

Hour (h)	AN value (mg/L NH_3)			
	Molasses-POME mixture ratio (M:P)			
	Negative control	25M:75P	50M:50P	75M:25P
0	21.80 ± 0.81 [a]	22.29 ± 0.12 [b]	22.37 ± 0.61 [c]	22.69 ± 0.40 [d]
1	22.20 ± 1.05 [a]	18.87 ± 2.68 [b]	21.15 ± 0.12 [c]	19.60 ± 2.00 [d]
2	21.96 ± 0.53 [a]	17.73 ± 2.27 [b]	20.58 ± 0.23 [c]	19.19 ± 2.70 [d]
3	21.15 ± 0.90 [a]	17.32 ± 0.72 [b]	19.19 ± 0.12 [c]	18.95 ± 1.51 [d]
4	20.90 ± 0.92 [a]	17.00 ± 1.40 [b]	17.65 ± 0.61 [c]	19.03 ± 0.40 [d]
5	19.93 ± 0.61 [a]	15.78 ± 1.33 [b]	13.99 ± 1.13 [c]	16.84 ± 0.53 [d]
6	19.60 ± 0.75 [a]	16.43 ± 1.44 [b]	10.49 ± 1.83 [c]	15.45 ± 0.12 [d]

*Superscript indicated the significant shifts in relation to the assessed value (p<0.05): (a,b,c,d) for molasses-POME mixture ratios towards AN values

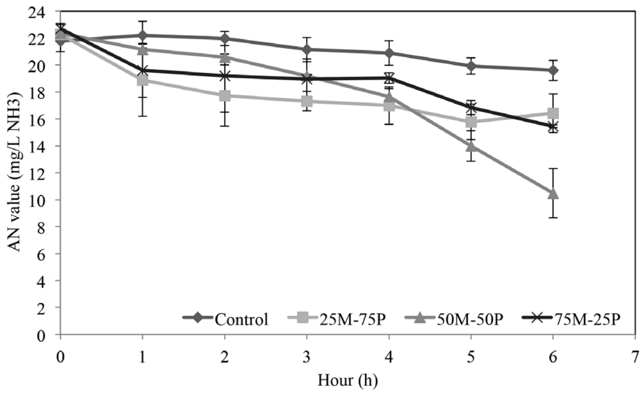

FIGURE 2. AN wastewater treatment comparison among molasses-POME mixture ratios

organic carbon as energy source for survival and growth while consuming ammonia as nitrogen source (Vital et al. 2010). Among the three different mixture ratios been investigated, both treatments with mixture ratio at 25M:75P and 75M:25P had shown similar ammonia percentage reduction by achieving lowest ammonia content at 15.78 mg/L and 15.45 mg/L NH_3, respectively, achieving DOE Standard B. Meanwhile, treatment with mixture ratio at 50M:50P had shown improved ammonia percentage reduction, and nearly achieving DOE Standard A at 10.49 mg/L NH_3. It suggested that mixture ratio of 50M:50P has the best balance in term of organic carbon and nutrients supply for the bacteria inoculated. In mixture of molasses and POME, molasses serves as the main organic carbon supply, while POME provides various micro- and macro-nutrients for further enhancement of bacteria growth (Kavitha et al. 2013). With sufficient support amount of energy source for bacteria growth in the treatments, bacteria showed better capability in consuming ammonia as nitrogen source which reducing ammonia content in the wastewater indirectly.

Through the exposure of POME samples towards UV light, potential undesired bacteria competitors from POME samples were significantly suppressed (Nelson et al. 2013). CFU counts for UV-treated POME showed 4.33×10^4 cfu/mL, while untreated POME showed 8.67×10^6 cfu/mL. UV-treated POME had nearly 200 times less viable cells in content as compared to untreated POME. This pre-treatment of POME is to ensure that applied bacteria will be the active dominant bacteria in the treatment system for ammonia removal. As for the chemical residues in the POME, the mixing of carbon sources diluted and reduced the content of the potential harmful substances, thus lowering the suppressing effect towards dedicated bacteria isolates in the treatment systems (Qureshi et al. 2010). In this study, it suggested that carbon source mixture ratio of 50% (v/v) molasses with 50% (v/v) POME is the optimal ratio for ammonia removal treatment. With the replacement of 50% (v/v) carbon sources in wastewater treatment with POME, it could lower the overall cost significantly in a larger scale treatment, especially on industrial scale where tons of carbon source are purchased.

Conclusion

In this study, POME had been utilized as high potential substitute for replacement of other carbon source, especially molasses in term of industry scale wastewater treatment on ammonia removal. In treatment comparisons between using POME and molasses, treatment with POME had shown significant comparable ammonia reduction efficiency. Despite having the chemical residues and indigenous bacteria competitors, diluting POME with molasses into various ratios further improved ammonia removal in the treatment systems. With the optimal ratio of 50:50 (v/v) POME-molasses, reduction on ammonia is the highest among other ratios which nearly achieving the DOE Standard A (10 mg/L NH_3) for ammonia content in industrial effluent discharge. Result in this study had proven that the potential of POME to be treated as alternate replacement substitute for other carbon sources. Although this study had mainly focused on reducing ammonia as contaminant in wastewater, more works and studies could be focused on utilizing POME in order to further improve and reveal its promising applications onto other industry wastewater treatments.

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