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POME TREATMENT USING AB-101 MICROBIAL CONSORTIUM: PERFORMANCE AND PROPOSED MECHANISM

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ABSTRACT

Due to the high organic load content, palm oil mill effluent (POME) has undergone various treatment systems. Most palm oil millers prefer to add a new approach to improve the existing treatment system but the investment and operation costs are too high to be incurred. Therefore, this study emphasizes the AB-101 microbial consortium performance in treating POME under the original operating parameters (0.2% AB-101 volume percentage, 7.5% molasses volume percentage and 100 ppm bio-activator dosage). The percentage reductions of POME characteristics under the original operating factor were 67.6% biochemical oxygen demand (BOD), 59.2% chemical oxygen demand (COD), 82.8% total suspended solids (TSS) and 66.7% oil and degrease (O&G). Meanwhile, POME treated with AB-101 under the optimal operating parameters (0.01% AB-101 volume percentage, 9.85% molasses volume percentage and 43.8 ppm bio-activator dosage) showed better characteristics of 92.9% BOD, 65.3% COD, 93.4% TSS and 95.5% O&G. Based on the proposed mechanism, lignocellulose degradation was greater when AB-101 was added into POME which improved the primary treatment of POME through enhanced anaerobic digestion.

Keywords: AB-101 microbial consortium, palm oil mill effluent, performance, POME characteristics, treatment mechanism.

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INTRODUCTION

Palm oil mill effluent (POME) has very high organic load content, where the chemical oxygen demand (COD) is about 50 000 mg / L and biochemical oxygen demand (BOD) is about 25 000 mg / L (Lokman *et al.*, 2021). It also has high concentrations of phenolic compounds and intense colour (Mohammed and Chong, 2014). As part of the organic pollutants, the fibre residues in POME stemming from the oil extraction process also increase the turbidity of the wastewater, which complicates its treatment (Ng *et al.*, 2019). In the long run, it has the potential to cause water pollution, food source depletion and aquatic species extinction (Tan and Lim, 2019). Therefore, POME must be treated prior to discharge into the watercourse. Conventionally, most palm oil mills in Malaysia adopt ponding systems comprising sequential anaerobic (primary treatment), facultative and aerobic ponds (secondary treatment) to treat POME (Iskandar *et al.*, 2018). Among the reasons is that the open ponding system incurs relatively low capital and

operating costs, with a simple design and minimum energy consumption (Tan and Lim, 2019). In addition, the ponding system distinctly represents a biological treatment (Cheng *et al.,* 2021), which involves anaerobic digestion occurring in anaerobic ponds. Predominant microorganisms that grow in the absence of dissolved oxygen in the anaerobic pond degrade high organic load contents in POME (Zainal *et al.,* 2017).

Nevertheless, anaerobic process is known to have disadvantages in terms of the large area required, low treatment efficiency and high hydraulic retention time of 30-90 days (Iskandar *et al.,* 2018). It is considered time-consuming as the indigenous bacterial population responsible for degradation requires a relatively long time to adapt to the environment before starting to consume organic matter. In addition, the average biochemical oxygen demand (BOD) that can be reduced using the ponding systems is only around 100-200 mg/L and still does not comply with the standard discharge limit of below 100 mg/L (Zainal *et al.*, 2017), which is currently the prominent issue in palm oil industry nowadays. The incompetent performance of ponding system has prompted the mill owners to install tertiary treatment before discharging the treated effluent. Among the tertiary treatments that have been considered in recent years are chemical coagulation, flocculation, flotation (Asis *et al.*, 2016; Johan and Zahari, 2017; Poh *et al.,* 2014), photocatalytic (Ng and Cheng, 2016; Saputera *et al.,* 2021), membrane technology (Bello and Abdul Raman, 2017; Chan and Chong, 2018), integrated photocatalytic-membrane (Sidik *et al.,* 2019), adsorption (Ibrahim *et al.,* 2017; Mohammed and Chong, 2014), and advanced oxidation process (AOP) (Bello and Abdul Raman, 2017). Although satisfactory results have been demonstrated by these alternative technologies, the high costs of capital investment, operation, processing and maintenance constrain the actual-scale application of a small fraction of these technologies in the long term (Foo, 2019; Saputera *et al.*, 2021). In addition, some of these technologies generate acidic secondary effluent that entails further treatment before discharge (Abdulsalam *et al.*, 2020).

Therefore, the opportunity to explore the potential of improving the existing processes is wide open for researchers to obtain a more cost-effective approach for POME treatment compared to tertiary treatment through the use of microorganisms. Anaerobic digestion is the main part of biological treatment processes that rely heavily on the mutual and syntrophic interactions of microorganism consortium to breakdown complex organic matter into soluble monomers, such as amino acids, fatty acids, simple sugars and glycerol (Anukam *et al.,* 2019). Remediation of organic contaminants in POME using microorganisms, such as fungi and bacteria, has demonstrated promising performance (Abdulsalam *et al.,* 2020). In recent years, several studies have utilised microbes in treating POME. Hazman *et al.* (2018) utilised *Chlamydomonas* sp. UKM 6 and *Chlorella* spp. UKM 8 for POME treatment in 21 days and discovered that *Chlamydomonas* sp. UKM 6 could remove chemical oxygen demand (COD) by 56%, nitrogen by 65% and phosphorous by 34%. Bala *et al.* (2018a) investigated indigenous mixed microbial consortium isolated from POME to reduce organic load and POME biodegradation. The consortium was able to reduce BOD by 90.23%, COD by 91.06% and total suspended solids (TSS) by 92.23%. On the other hand, Suseela and Muralidhar (2018) isolated five fungal species from POME dump sites and evaluated their efficacy in the bioremediation of POME. The reductions in COD, BOD and oil and grease content were 80.28%, 88.23% and 87.34%, respectively. According to Ganapathy *et al.* (2019), bacteria, moulds, yeasts, and fungus can perform complete degradation of oil-based wastewater such as POME. Nevertheless, recent studies on microorganisms for POME treatment have only been carried out on a laboratory scale and not on a pilot scale.

Thus, the application of AB-101 microbial consortium may exhibit its potential to enhance the existing anaerobic degradation and improve the quality of the final POME discharged. This approach may also incur low capital and operating costs as the microorganisms are dosed into existing treatment process. According to Abidi *et al.* (2021), AB-101 is a thick dark-brown liquid manufactured from different types of fruits and plants through customised fermentation. It is non-toxic and biodegradable as no chemicals are added throughout the manufacturing process. AB-101 is used by industries by inoculating the acclimatised AB-101 microbial consortium (bio-activator) into the industrial effluent treatment system (IETS) to improve the quality of the final effluent discharged. It could remediate the microbial community in the effluent by intensifying the growth of the microorganisms that are specifically needed with respect to the causative pollutants present in the effluent.

According to Abidi *et al.* (2021), AB-101 is a microbial consortium consisting of various types of beneficial microorganisms, of which an increase in volume percentage of AB101 with respect to the total volume of bio-activator prepared would directly increase the activated microorganisms in the bio-activator. For each amount of bio-activator dosed into POME, the number of microorganisms would continue to increase to degrade the pollutants. In addition, higher volume percentage of molasses also contributed to greater POME

characteristics reduction. Molasses is rich in micro- and macronutrients that are food for microbes so it can grow and perform optimally in reducing organic pollutants (Abidi *et al.*, 2021). Moreover, POME generally has low C/N ratio which inhibits anaerobic digestion due to insufficient carbon as energy source to microorganisms to compensate nitrogen into synthesising enzymes responsible for anaerobic digestion (Wang *et al.*, 2016). Therefore, the addition of molasses as supplementary carbon source may significantly re-adjust the C/N ratio to the optimum value that favours effective anaerobic digestion (Farizzuan *et al.*, 2019).

Furthermore, Bala *et al.* (2017) postulated that the synergistic effect of mixed microbial consortium for POME treatment has induced effective biodegradation. The ability of microbial consortium in degrading POME characteristics has been reported by several previous studies. For instance, Bala *et al.* (2018a) discovered in their study that the mixed microbial consortium containing bacterial and fungi strains isolated from POME process has succeed in reducing organic load in POME over 90%. Said *et al.* (2019) also reported bacteria in consortium had greater synergy as they metabolise the cellulose, protein, oil and fat in POME, and reduced BOD, COD and TSS from 73%-91%. A consortium culture has exhibited outstanding performance as each consortium member strain acted in synergy to breakdown different organic pollutants in wastewaters.

Regardless of the potential AB-101 to improve the anaerobic treatment of POME, further research into the effect of operating parameters on the performance of AB-101 is needed to expand its use in palm oil mills. Therefore, the collaboration with the AB-101 manufacturing company is an effort to initiate an analytical study to evaluate and optimise the application of this product for POME treatment. In addition, the lack of research and data documentation for the application of AB-101 microbial consortium for POME treatment in palm oil mills became an opportunity to embark this study. Thus, the AB-101 performance in treating POME was evaluated in this study under the original operating parameters in reducing BOD, COD, TSS and oil and grease (O&G). Comparison was also made for the characteristics of POME treated by AB-101 under original and optimum operating parameters. For better understanding, the mechanism of AB-101 in increasing the enzymatic activity of lignocellulose degradation of POME, and the overall anaerobic degradation rate are also presented. This study is expected to add significant values to the palm oil industry to resolve the POME issues that have contributed to several pollution cases in Malaysia.

MATERIALS AND METHODS

Collection of POME Sample

The raw POME sample was collected about 20 L from the first (anaerobic) pond of Tai Tak Palm Oil Mill Sdn. Bhd., Kota Tinggi, Johor, Malaysia using a 30 L high-density polyethylene (HDPE) container. To obtain POME sample, the container was half-filled with the sample and inverted several times to rinse any impurities from the inner walls of the container. The sample was then re-discharged to the same pond but at different points. This step was repeated before the final sampling. The container was properly labelled (company name, type, and collection date) and sent to the UTHM downstream laboratory. The sample was stored in a cold room at 4°C to prevent the occurrence of any microbiological activities naturally.

Collection of AB-101 and Molasses Samples

Based on information provided by the manufacturer, AB-101 contained a number of beneficial bacteria and fungi. Approximately 650 mL of AB-101 sample was obtained from the manufacturer in Johor, Malaysia (*Figure 1*), in an aseptic 1 L amber bottle to prevent the penetration of any light or heat from the surroundings so as not to induce possible microbiological reactions. Exposure of light to the microbes may lead to inactivation of some microbes and lower abundance of viable bacteria communities (Fahimipour *et al.*, 2018). The bottle was tightly sealed using a stopper and parafilm to prevent oxidation due to the presence of air. While the AB-101 sample was transported from the manufacturer to the laboratory, it was stored in a portable isolated icebox (5 L) containing 3 kg of dry ice to ensure that the remaining microorganisms in the AB-101 were in an inactive state. Thus, no biological reaction would occur during the logistical step. Similarly, 1 kg of molasses was also collected from the same manufacturer and packed tightly in the carboy. The molasses was delivered to the laboratory and stored at room temperature before use.

Figure 1. AB-101 sample retrieved from the manufacturer.

Preparation and Application of AB-101 for POME Treatment

For AB-101 application, the product was prepared or cultured in separate containers as illustrated in *Figure 2*. Initially, about 0.2% v/v (2 L) of AB-101 microbial consortium and 7.5% v/v (75 L) of molasses were premixed with 1000 L of POME. Molasses is used as carbon source to improve carbon to nitrogen (C:N) ratio. Raw POME is the media containing a considerable amount of nutrient and beneficial indigenous microorganisms (Udaiyappan *et al.*, 2020) as well as targeted pollutants. Next, the mixture was aerated continuously using an air pump for at least 72 hr continuously for acclimatisation purposes. During the preparation of the bio-activator, it took several minutes for the operating mode to change to aerobic conditions, which was intended to hibernate AB-101. Changing the conditions for a short time did not have a significant effect on bacterial growth. Nitrogen was purged to the reactor to ensure the sustainability of the bio-activator while it was growing under different mode conditions. Then, the ready product, known as bio-activator AB-101, was dosed into the anaerobic pond as advised by the product manufacturers' team from Indonesia that designed this method of application. The details of ingredients and manufacturing process are kept as proprietary information by the manufacturer due to on-going patent application.

In this study, experiments were conducted at the palm oil mill using a pilot scale bioreactor via intermediate bulk container (IBC) or tote tank as the main treatment tank. *Figure 3* depicts the schematic diagram of a pilot-scale bioreactor and the actual pilot scale of the POME treatment. The dosage of bio-activator AB-101 inoculated into raw POME in the tote tank of 1000 L capacity was 100 ppm or 0.1 L per day. For five consecutive days, the bio-activator was dosed into POME daily and left under anaerobic condition without external aeration or mixing. To ensure proper dispersion of AB-101, the mixture was stirred gently after each daily dosing.

Performance Evaluation of AB-101 under Original Operating Parameters

The performance of POME treatment based on original operating parameters was evaluated on-site using the pilot-scale. The original parameters were obtained based on the personal communication with the manufacturer. The collected raw POME samples were tested in terms of BOD, COD, TSS, and O&G. Then, 0.2% v/v (2 L) of the AB-101 microbial consortium and 7.5% v/v (75 L) of molasses were premixed with 1000 L of POME as summarised in *Table 1*.

Then, the treated POME was also tested for BOD, COD, TSS and O&G. The percentage reduction was calculated by comparing between treated POME (after treatment) with untreated POME (before treatment) as indicated by Equation (1).

$$\text{Reduction percentage (\%)} = \left(\frac{\text{(value before-value after)}}{\text{(value before)}} \right) \times 100\% \quad (1)$$

Performance Evaluation of AB-101 under Optimum Operating Parameters

In addition, the performance of the AB-101 was evaluated under optimum operating parameters for comparison with the original operating parameters. Optimisation was conducted using Response Surface Methodology via Central Composite Design. Three operating variables were analysed during optimisation, namely the percentage of AB-101 used in bio-activator (range: 0.1%-1.0%), percentage of molasses added in bio-activator (range: 0.0%-10.0%), and dosage volume of bio-activator into the

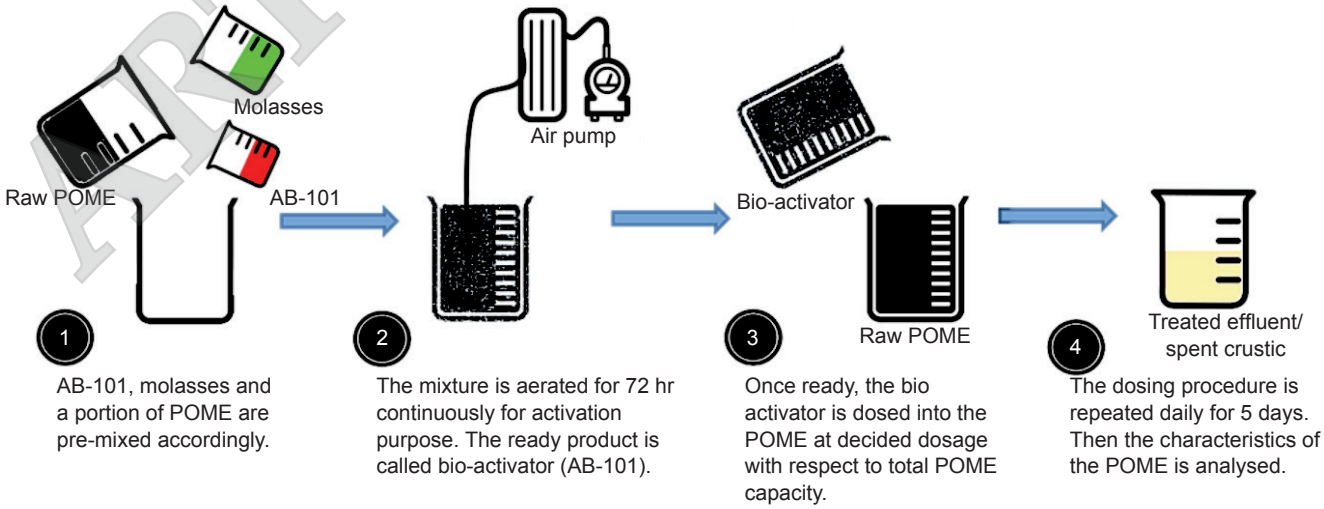

Figure 2. Method of AB-101 application for POME treatment.

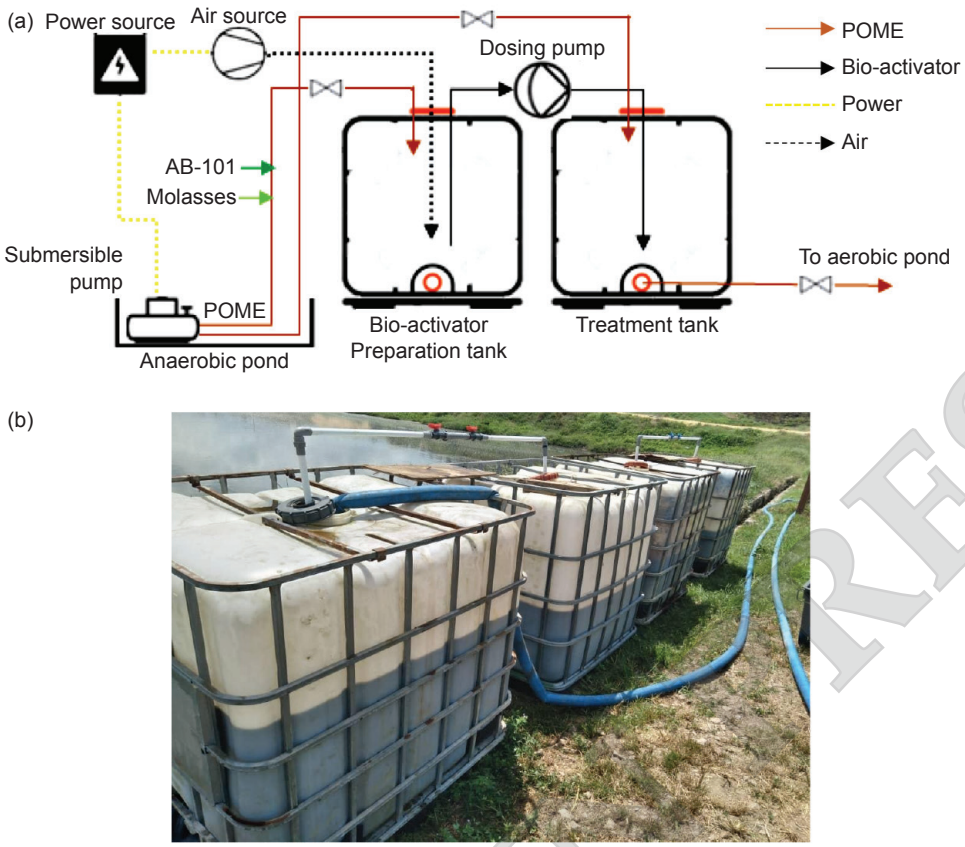

Figure 3. (a) Schematic diagram of pilot-scaled experiment; (b) actual pilot-scale of POME treatment using AB-101.

TABLE 1. ORIGINAL OPERATING PARAMETERS FOR AB-101 APPLICATION

Operating factor	Unit	Value
AB-101 volume percentage (v/v)	%	0.2
Molasses volume percentage (v/v)	%	7.5
Bio-activator dosage	ppm	100

POME (range: 20-80 ppm). A detailed description of the optimisation procedure is reported by Abidi *et al.* (2021).

Analysis of POME Sample Characteristics

The BOD, COD, TSS and O&G of the POME were analysed before and after treatment with AB-101 according to American Public Health Association (2015). The instruments used for the characterisation of the sample were DO meter (Hach) for BOD, and DR6000™ UV-VIS Spectrophotometer (Hach) for COD. POME samples were taken out a few hours before testing and left at room temperature (~25°C). Temperature and pH of the POME were recorded using thermometer and pH probe, respectively. The sample container was inverted several times to ensure the consistency of the content taken from the sampling points. About 4 L of samples were poured into the transparent HDPE pan and placed in the fume hood for 24 hr to prevent undesired odours from the samples infiltrating the room atmosphere. Initial colour, odour and height of sludge and scums were recorded. The treatment efficiency can be calculated according to Equation (2).

$$\text{Efficiency} = \frac{(P_{raw} - P_{final})}{P_{raw}} \times 100\% \quad (2)$$

where, P_{raw} is parameter of raw POME before treatment (initial), and P_{final} is parameter of POME after treatment (final).

RESULTS AND DISCUSSION

Performance of AB-101 in Treating POME under Original Operating Parameters

Under the original operating parameters, the performance of POME treatment using AB-101 via pilot scale was evaluated. The characteristics of raw POME were analysed on-site before conducting the experiments to ensure the accuracy of the results obtained. The BOD_3, COD, TSS and O&G of POME before treatment are summarised in *Table 2*. It was found that BOD_3 was 8 500 mg/L, COD was

TABLE 2. CHARACTERISTICS OF POME AFTER TREATMENT WITH AB-101 UNDER ORIGINAL OPERATING PARAMETERS

Parameter	Unit	POME characteristic		Reduction percentage (%)
		Before	After	
BOD_3 at 30°C	mg/L	8 500	2 750	67.6
COD	mg/L	24 500	10 000	59.2
TSS	mg/L	14 500	2 500	82.8
O&G	mg/L	1 110	370	66.7

Note: BOD - biochemical oxygen demand; COD - chemical oxygen demand; TSS - total suspended solid; O&G - oil and grease.

24 500 mg/L, TSS was 14 500 mg/L and O&G was 1110 mg/L. After treatment with AB-101 under the original operating parameters, the characteristics of the treated POME were as follows: BOD_3 = 2750 mg/L, COD = 10 000 mg/L, TSS = 2500 mg/L and O&G = 370 mg/L. The reduction percentages of each parameter were calculated and shown in *Table 2*. Accordingly, the reduction percentages of BOD_3, COD, TSS and O&G were 67.6%, 59.2%, 82.8% and 66.7%, respectively.

Comparison of POME Parameter Reduction Percentages after Treated with AB-101 under Optimum and Original Operating Conditions

The differences in the performance of AB-101 in treating POME under the original and optimum operating parameters are illustrated in *Figure 4*. The performance was evaluated based on the ability of AB-101 microbial consortium in reducing BOD, COD, TSS and O&G in POME samples. Optimum operating parameters were obtained from our previous study by Abidi *et al.* (2021). Under the optimum operating parameters, the BOD reduction percentage was 92.9%, while 67.6% was under the original operating parameters. For COD, the reduction percentage under the optimum operating parameters was 65.3%, and the percentage was 59.2% under the original operating parameters. Furthermore, the reduction percentage of TSS was 82.8% under the original operating parameters and then increased drastically to 93.4% under the optimum operating parameters. Last but not least, the reduction percentage of O&G increased from 66.7% to 95.5% for POME treated by AB-101 under original and optimum operating parameters, respectively, as depicted in *Figure 4*. Thus, it can be inferred that the reduction in BOD, COD, TSS and O&G of POME under optimum conditions was greater than the original parameters.

Moreover, *Figure 5* displays differences in the appearance of untreated POME, POME treated with AB-101 under the original operating parameters and POME treated with AB-101 under the optimum operating parameters. Prior to treatment, raw POME was highly viscous, had colloids and appeared solid opaque black. A large amount of total suspended solids was clearly visible with a pungent odour. After five days of treatment under the original operating parameters, POME sample turned brownish black with slightly higher turbidity and less pungent odour compared to untreated POME. Similar phenomenon has been reported by Okwute *et al.* (2015) when three different bacteria were used to treat POME. In their study, the turbidity of the samples inoculated by the bacteria increased after four days onwards. The causative factor is more likely due to the presence of nitrogen and phosphorus in the mineral salt media which are vital for biodegradation activity (Adesodun and Mbagwu, 2008).

Meanwhile, POME treated under the optimum operating parameters exhibited slight discolouration where its colour changed to slightly brownish, unlike POME treated under the original operating parameters. The dark brownish colour of the raw effluent is due to excessive concentration of tannins, melanoidin and lignin compounds (Tamrin and Zahrim, 2017; Tan *et al.*, 2017). The change in the POME colour from dark brownish to slightly brownish could be attributed to the adaptation of microbes which then secrete appropriate enzymes to degrade the colour (Abdulsalam *et al.*, 2020). In other words, microbes entail a longer retention period for them to gradually adapt to the conditions of the medium prior to degrading the colour compounds. In terms of odour, POME treated under the original and optimum operating parameters had a less unpleasant odour than raw POME (untreated).

The Proposed Mechanism of AB-101 Microbial Consortium for POME Treatment

To better understand the working principle of AB-101, its mechanism is elaborated in this section. The AB-101 increases the enzymatic activity of lignocellulose degradation of POME, thus improving the overall anaerobic degradation treatment. Conventional anaerobic digestion of POME is often reported to be ineffective possibly due to several parameters, including hydrolysis. Owing to high organic contents in POME, hydrolysis occurs at a very low rate (Akyol *et al.*, 2019; Rosa *et al.*, 2020). It is

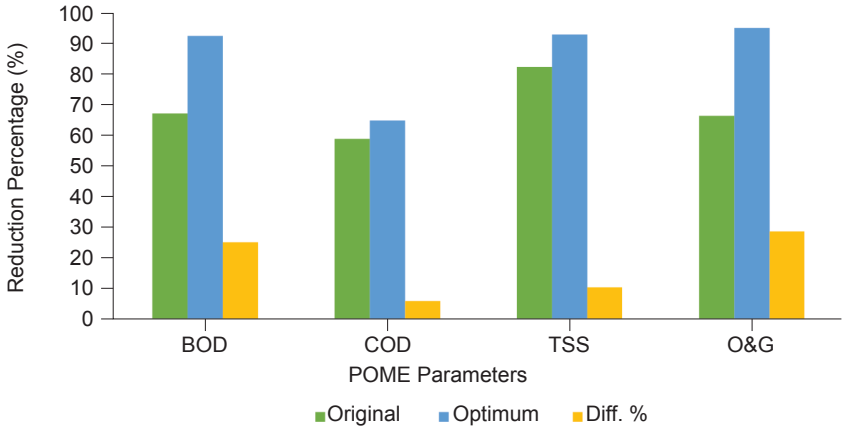

Figure 4. Comparison of POME treatment performance using AB-101 under original and optimum operating parameters.

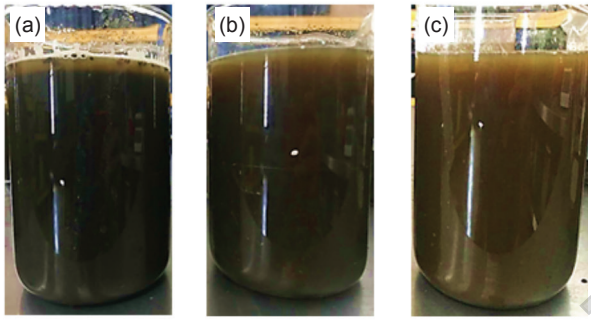

Figure 5. Appearance of POME in different conditions: (a) before treatment, (b) after treatment (original operating factor) and (c) after treatment (optimum operating parameters).

known that POME is rich in lignocellulose materials, namely cellulose, hemicellulose and lignin. Based on compositional analysis, 39.0% cellulose and 24.6% hemicellulose have been reported to be present in dried POME (Khaw *et al.*, 2008). On another note, 44.0%-58.0% cellulose, 7.0%-11.0% hemicellulose, and 27.0%-43.0% lignin are contained in the mixed raw effluent obtained from steriliser condensate and clarification underflow sludge (Nor Faizah *et al.*, 2022).

Generally, cellulose comprised long homopolymer chain of glucose units linked by a beta acetyl linkage, while hemicellulose consists of a branched heteropolymer of pentoses (xylose and arabinose), hexoses (glucose, galactose, mannose) and sugar acids (acetic) (Silvamany *et al.*, 2015). Moreover, cellulose and hemicellulose are strongly bound to lignin via covalent and hydrogen bonds, which contribute to their robust structure and resistance to treatment (such as hydrolysis) (Ufodike *et al.*, 2020). Unlike cellulose and hemicellulose, enzymatic hydrolysis is unable to hydrolyse lignin without the aid of pre-treatment such as alkaline pre-treatment. According to Balat (2011), the hydrolysis of cellulose generates glucose and cellobiose, while the degradation of hemicellulose generates arabinose, xylose, mannose, rhamnose, galactose and glucose. Cellulose is naturally resistant to enzymatic attack as it is protected by the surrounding matrix of hemicellulose, lignin and pectin (Wong *et al.*, 2008). To hydrolyse cellulose and hemicellulose in POME, specific enzymes are applicable. These specific enzymes attributed to the degradation of different biomass waste can be typically secreted by bacteria (Rupani *et al.*, 2022). Under appropriate conditions and enzymes, the sugar content in POME can be released by enzymatic hydrolysis method due to disruption of the lignocellulose material structure (Nurul Adela *et al.*, 2016).

Through this study, the application of AB-101 microbial consortium has pioneered the potential to improve the primary treatment of POME via anaerobic digestion. This could be explained by understanding the mechanism involved during the process. *Figure 6* illustrates the possible mechanisms between the consortium and POME samples which helped enhance the characteristics of the treated POME. In *Figure 6(a)*, raw POME has deficiencies in microbial community diversity, particularly enzyme-secreting microorganisms capable of degrading lignin (laccase, lignin peroxidase and manganese peroxidase), such as white, brown and soft-rot fungi (Ganapathy *et al.*, 2019). These fungi have poor adaptability to pH and temperature, oxygen limitations and high contamination risk, which may infer those indigenous fungi in POME are either denatured or working at minimum capacity (Azman *et al.*, 2019; Bala *et al.*, 2018b). Thus, the deficiency of these particular microorganisms significantly inhibits the hydrolysis rate of lignocellulosic biomass in POME (Vu *et al.*, 2020).

Figure 6(b) illustrates that the application of AB-101 may improve the diversity in microbial communities in the respective POME systems. As a microbial consortium-based product, AB-101 contains several different microorganisms, particularly beneficial bacteria and fungi. These

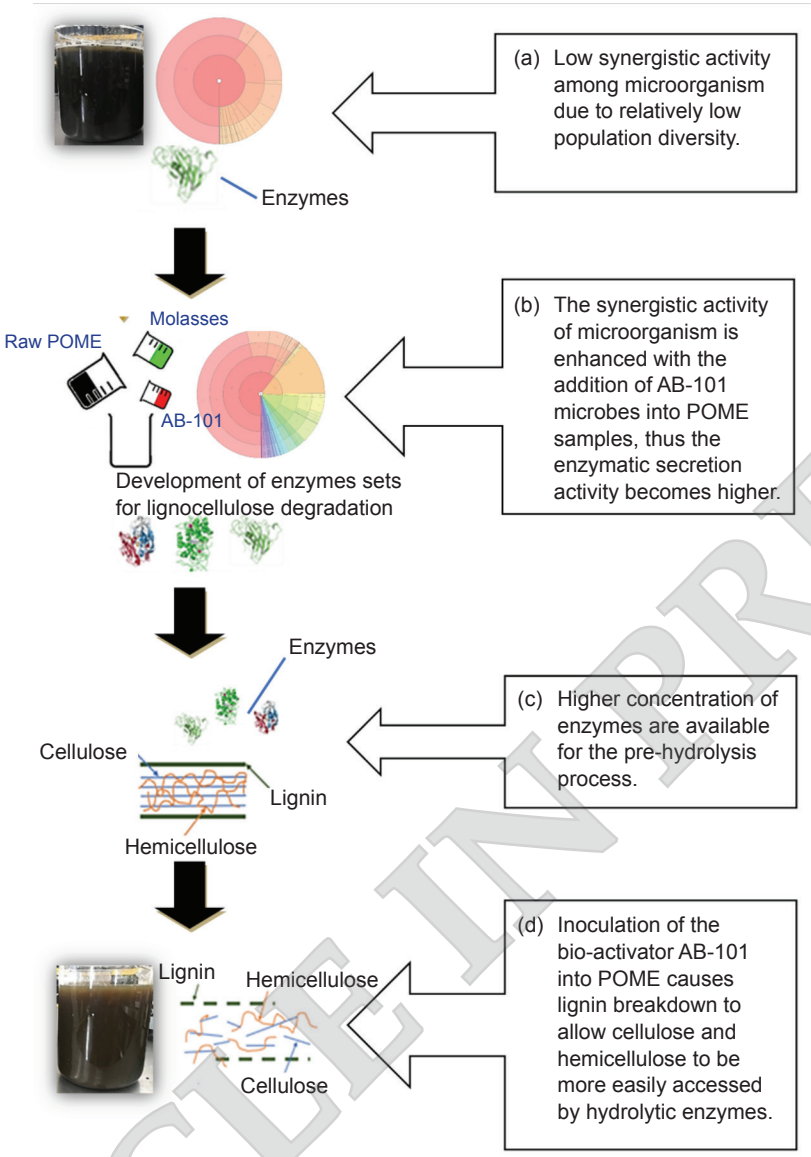

Figure 6. The proposed mechanism of AB-101 application for POME treatment.

microorganisms synergistically improved the metabolic activity among them and the indigenous microorganisms in POME, thereby inducing greater enzymatic activity. Higher variation of microorganisms was further activated in isolated system at improved nutritional levels (C/N ratio) with the addition of molasses. In AB-101 application, sugarcane molasses was selected as supplementary carbon source. It contains about 50.0% (w/v) total sugar (sucrose, glucose and fructose), 0.5%-0.9% (w/v) nitrogen and 10.0% (w/v) inorganic salts, which are the main features associating with good enhancement in nutritional value within micro fauna in acclimatisation process by providing additional source of proteins to enhance enzymatic activity and improve nutritional efficiency of culture system (Becerril-Cortés *et al.*, 2018). Carbon sources such as molasses or dextrose dissolve more easily in culture mediums compared to rice bran, thus liberating more quickly the carbon for microbial protein generation for enzymatic activity (Serra *et al.*, 2015).

After activation, the mixture of microorganisms demonstrated potential metabolic activity at the exponential growth phase and has higher enzymatic secretion level. Furthermore, POME is globally considered a lignocellulose agricultural liquid waste due to the presence of highly insoluble suspended solids and large particle-sized organic content derived from empty fruit bunch (EFB), comprising mainly cellulose, hemicellulose and lignin (Mahmod *et al.*, 2017; Rosa *et al.*, 2020). This limits the lignocellulose hydrolysis and consequently leads to low degradation efficiency in the overall process of anaerobic digestion due to the ineffective preliminary degradation of lignin as an external component (Hii *et al.*, 2012; Liang *et al.*, 2018).

In *Figure 6(c)*, the concentrations of lignin and hemicellulose-degrading enzymes may be higher as POME was dosed with the bio-activator AB-101. Meanwhile, *Figure 6(d)* illustrates how microorganisms were activated in POME via the application of AB-101 which effectively degraded organic and inorganic pollutants including lignin. Inoculation of the bio-activator AB-101 into POME was presumed to alter the lignocellulose structure of POME, leading to the breakdown of polysaccharides and lignin chains. As a result, cellulose and hemicellulose are more easily accessible by microorganisms and hydrolytic enzymes (Vu *et al.*, 2020), as evidenced in *Figure 5* through discolouration. By activating AB-101 along with a small portion of POME, it is able to activate the dormant indigenous bacteria or fungi that may be present in AB-101 and/or the respective POME (Ambarsari and Harahap, 2017).

In summary, when bio-activator was dosed into POME, the microorganisms activated in AB-101 and indigenous microorganisms in POME formed synergistic enhancements through syntropic communication among microorganisms resulting in greater lignocellulose degradation. This increased the enzymatic activity of lignocellulose degradation as well as increased the hydrolysis and overall anaerobic degradation processes. Thus, it can be inferred that the application of AB-101 at better operating parameters indeed improved the performance of POME treatment. This in turn provides an improvement in the quality of POME before entering the secondary treatment system and may eliminate the need for tertiary treatment.

CONCLUSION

In conclusion, the performance of AB-101 for POME treatment under the original operating parameters in the pilot scale was successfully evaluated. The application of AB-101 under original operating parameters, *i.e.*, AB-101 volume percentage of 0.2%, molasses volume percentage of 7.5% and bio-activator dosage of 100 ppm, resulted in reduction percentages of POME characteristics as follows: BOD = 67.6%, COD = 59.2%, TSS = 82.8% and O&G = 66.7%. In comparison, the optimum operating parameters (0.1% AB-101 volume percentage, 9.96% molasses volume percentage and 43.7 ppm bio-activator dosage) successfully improved the reduction of BOD, COD, TSS and O&G by 92.9%, 65.3%, 93.4% and 95.5%, respectively, better than the original operating parameters. Through the treatment mechanism, it was clear that the hydrolysis rate and overall anaerobic digestion process was enhanced as a result of greater lignocellulose degradation with the addition of AB-101.

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