

Decolorization of Biogas Effluent from Palm Oil Mill Using Combined Biological and Physical Methods

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ABSTRACT

Two mixed cultures (Super LDD.1 and Super LDD.2) were used as inocula (10%) for the decolorization and treatment of biogas effluent from palm oil mill (BEPOM) under aerobic (2.5 volume of air per volume of medium per minute, vvm) and microaerobic conditions, respectively. With the supplementation of 10% palm oil mill effluent (POME) as nutrients, Super LDD.1 and Super LDD.2 showed higher decolorization than the Super EM culture under microaerobic conditions after 4 d cultivation. The optimum inoculum size for both sources was 20% (volume per volume) and Super LDD.1 produced higher decolorization (27.0%) and phenol removal (20.4%) than Super LDD.2 (16.7% and 12.1%, respectively) after 2 d cultivation. However, Super LDD.1 exhibited slightly lower chemical oxygen demand (COD) removal than Super LDD.2 (27.8% and 29.2%, respectively) after 6 d cultivation. The effluent after treatment by Super LDD.1 was subsequently tested for the effect of pH and palm ash concentration (1–15%, weight per volume) under mixing conditions (on 125 rpm shaker) at room temperature (30 ± 3 °C) for 1 h. The highest decolorization (84.7%), phenol removal (90.8%) and COD removal (88.9%) were achieved at 15% (w/v) palm ash. The results indicated the potential treatment of BEPOM using a biological method (Super LDD.1 culture) in combination with a physical method (palm ash) which was better than using each treatment alone.

Keywords: decolorization, biogas effluent, biological methods, physical method

INTRODUCTION

The palm oil industry is an important agro-industry in Southern Thailand producing 1.35×10^6 t palm oil in 2010 (Office of Agricultural Economics, 2010). The palm oil mill extraction process generates three major sources of the effluent: separator sludge, sterilizer condensate and hydrocyclone effluent with a total quantity per fresh fruit bunch (FFB) of $0.87 \text{ m}^3 \cdot \text{t}^{-1}$ (H-Kittikul

et al., 1994). Nowadays, palm oil mill effluent (POME) is commonly used for biogas production as a replacement for treatment using an anaerobic pond. The biogas system employed in most Thai palm oil mills is a completely stirred tank reactor and an anaerobic covered lagoon. Biogas is now a source of renewable energy that could be used directly or converted to heat and electricity. Therefore, electricity can become another major product in some palm oil mills in Thailand.

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After anaerobic treatment, the biogas effluent has a dark brown color with high organic matter (COD) and some phenol; hence, further treatment is needed. The brown color in palm oil mill effluent is the result of the decomposition of plants cells such as phenolic compounds (tannic and humic acid) from lignin and melanoidins from the Maillard reaction of carbohydrate and protein, as these cause the color in the sugar processing (Zahrim *et al.*, 2009). The bioremediation of color from industrial wastes has been studied by using a variety of microorganisms such as *Lactobacillus plantarum* (Limkhuanuwan and Chaiprasert, 2010), *Streptomyces* sp. (Zhou and Zimmermann, 1993), *Saccharomyces cerevisiae* (Aksu and Donmez, 2003) and *Bacillus* sp. (Nakajima-Kambe *et al.*, 1999; Dawkar *et al.*, 2009). Since these microorganisms are present in the mixed cultures of Super LDD.1 and Super LDD.2, they would be tested for decolorization and treatment of biogas effluent.

Besides biological methods, physical methods are also applied for decolorization using palm ash, a solid waste generated after the combustion of palm fibers and palm shells as boiler fuel for steam generation in the palm oil mill. Palm ash was reported to successfully remove dyes from textile industry effluent (Ahmad *et al.*, 2007; Isa *et al.*, 2007). The current study aimed to decolorize and treat biogas effluent from a palm oil mill by using biological and physical methods.

MATERIALS AND METHODS

Microorganisms and raw materials

The biological method used mixed cultures (Super LDD.1 and Super LDD.2) provided by the Land Development Department, Ministry of Agriculture and Cooperatives of Thailand, while consortium Super EM culture was commercially available.

The raw biogas effluent from a palm oil mill (BEPOM) and palm ash were collected from Green Glory Co., Ltd and The Southern

Palm (1978) Co., Ltd., respectively, Surat Thani province, Thailand. The required volume of BEPOM was thawed to room temperature (27–28 °C) before use. Palm oil mill effluent (POME) was collected from Laptawee Co., Ltd, Satun province, Thailand. BEPOM and POME were determined for chemical oxygen demand (COD), total suspended solids (TSS), total solids (TS), color, total nitrogen (TN) and total phosphorus.

Analytical methods

COD, TSS, TS, pH and total phosphorus were analyzed in accordance with the Standard Methods for the Examination of Water and Wastewater (American Public Health Association, 1998). Color was determined using the platinum-cobalt standard method (American Public Health Association, 1998) compared to the American Dye Manufacturers Institute (ADMI) method using a spectrophotometer (Spectroquant® UV/VIS spectrophotometer Pharo 300; Merck Darmstadt, Germany) and color measurements were reported in units of platinum-cobalt (PtCo). Phenol was analyzed following the method described by Garcia Garcia *et al.* (2000). TN was analyzed using commercial test kits from Spectroquant (Merck KGaA; Darmstadt, Germany).

Selection of the inoculum source and cultivation conditions on decolorization of biogas effluent from palm oil mill

Preparation of inocula

Prior to use as inocula, the two mixed cultures needed to be activated as specified by the manufacturer (the Land Development Department, Thailand). Super LDD.1 of 2.5 g was added into 500 mL water and stirred for 10 min, whereas Super LDD.2 of 5.0 g was added into 500 mL water and stirred for 5 min. Super EM was activated by mixing EM with molasses and water in the ratio of 1:1:100, respectively, and then incubating in a closed container for 7 d before use.

Microbial count in the inoculum

Microbial counting in the three inoculum

sources was conducted using the dilution plate count method. The samples of Super LDD.1, Super LDD.2 and Super EM were diluted in the range 1×10^{-1} – 1×10^{-9} by 0.85% NaCl. Then, 0.1 mL of 1×10^{-1} – 1×10^{-9} dilution was dropped on plate count agar plates, then incubated at room temperature (30 ± 3 °C) for 7 d before counting.

Selection of the inoculum source

The two sources of mixed cultures (Super LDD.1, Super LDD.2) and the consortium (Super EM) were tested for their ability to decolorize BEPOM. The inoculum size of 10% (volume per volume; v/v) was added into 150 mL BEPOM (nonsterilized) with 10% POME as supplemented nutrients. Each sample was cultivated at room temperature (30 ± 3 °C) for 7 d under microaerobic conditions. Samples were taken every 24 h for 7 d and determined for color. The inoculum source that demonstrated higher decolorization than the control (without any inoculum) was chosen for further studies.

Selection of cultivation conditions

The selected inoculum source was inoculated into 150 mL biogas effluent (sterilized) with 10% POME as supplemented nutrients. Incubation was conducted under aerobic conditions (an aquarium pump was used to supply air through a porous stone diffuser that was located at the base of the reactor with an air flow rate of 2.5 volume of air per volume of medium per min, vvm) and microaerobic conditions (no supply air) at room temperature (30 ± 3 °C) for 7 d. The optimum conditions were chosen for further studies.

Effect of inoculum size on decolorization of biogas effluent from palm oil mill

The selected inoculum source was used to study the effect of inoculum size (5, 10, 15 and 20%, v/v) on decolorization, COD removal and phenol removal in the 400 mL BEPOM (nonsterilized) without nutrients addition. It should be noted that a different batch of the BEPOM was used which had higher values of color, COD and phenol than that used above in the first experiment. The experiment

was incubated at room temperature (30 ± 3 °C) for 7 d under aerobic conditions (2.5 vvm).

Effect of pH and palm ash concentration in combined treatment

The BEPOM after treatment with the selected inoculum source with the pH adjusted (to pH 4) and without the pH adjusted (pH 9.15; the control) were used in this study. Palm ash was added at concentrations of 1, 3, 6, 9, 12 and 15%, weight per volume (w/v). The experiment was carried out on a shaker (125 rpm) at room temperature (30 ± 3 °C). Samples were taken every 15 min for 60 min.

RESULTS AND DISCUSSION

Characteristics of the biogas effluent from palm oil mill, palm oil mill effluent and palm ash

The characteristics of BEPOM and POME were determined and found to have values for COD of 1,832 and 55,000 mg.L⁻¹, phenol of 29.58 and 965 mg.L⁻¹, color of 1,633 and 24,000 PtCo and pH at 7.5 and 4.7, respectively (Table 1). Palm ash was oven dried at 105 °C for 24 hr, and kept at room temperature till used (without size selection). The chemical composition of palm ash collected from the same lot used in this study were reported by Noparat (2008) as: silicon dioxide (44.84%), aluminum oxide (1.12%), calcium oxide (12.01%), magnesium oxide (3.22%), potassium oxide (4.99%) and sulfur trioxide (1.66%).

Selection of inoculum source and cultivation condition on decolorization of biogas effluent from palm oil mill

The viable count of the mixed cultures measured in colony forming units (CFU) of Super LDD.1, Super LDD.2 and Super EM were 9.6×10^8 , 3.6×10^8 and 1.1×10^7 CFU.mL⁻¹, respectively. These inocula were employed to treat and decolorize the raw BEPOM under microaerobic conditions. Super LDD.1 and Super LDD.2 showed substantially higher

decolorization (52.6% and 53.1%) than the control (no inoculum added) (45.8%) and Super EM (42.8%), respectively, after 4 d cultivation (Table 2). Therefore, the mixed cultures were selected. The high efficiency of Super LDD.1 was due to the presence of aerobic cellulose decomposing fungi (*Corynascus* sp., *Scytalidium* sp., *Chaetomium* sp. and *Scopulariopsis* sp.) and the aerobic bacteria (*Bacillus* sp.) as well as actinomycete (*Streptomyces* sp.) in the inoculum used (Leaungvutiviroj *et al.*, 2007). In addition, the high efficiency of Super LDD.2 resulted from the activity of facultative alcoholic yeast (*Saccharomyces* sp.), lactic acid bacteria (*Lactobacillus* sp.) and protein catabolism bacteria (*Bacillus* sp.) (Pasda *et al.*, 2007). Decolorization by Super LDD.2 was higher than that from *Lactobacillus plantarum* SF5.6 for palm oil mill effluent (15.9%) (Limkhuansuwan and

Chaiprasert, 2010) and *Bacillus* sp. for molasses wastewater (35.5%) under thermophilic and anaerobic conditions (Nakajima-Kambe *et al.*, 1999). In addition, it was also higher than that from *Bacillus megaterium* and *Bacillus cereus* for predigested distillery effluent under aerobic conditions (33.0%) (Jain *et al.*, 2002).

The selected mixed cultures (Super LDD.1 and Super LDD.2) were cultivated under microaerobic conditions and aerobic conditions. Super LDD.1 exhibited higher COD removal under aerobic conditions (33.3%) than under microaerobic conditions (14.3%) after 4 d cultivation (Figure 1a). For Super LDD.2, the COD removal under microaerobic conditions (32.0%) was substantially higher than that under aerobic conditions (28.6%) after 6 d cultivation (Figure 1b).

Table 1 Characteristics of palm oil mill effluent (POME) and biogas effluent (BE) from palm oil mill.

Parameter	BE	POME	BE+10% POME	
			Before treatment	After treatment
pH	7.5	4.7	7.1	8.4
COD (mg.L ⁻¹)	1,832	55,000	7,330	3,995
TS (mg.L ⁻¹)	4,340	38,500	8,190	-
TSS (mg.L ⁻¹)	2,370	-	-	-
Phenol (mg.L ⁻¹)	29.58	965	126	60.9
Colour (PtCo)	1,633	24,000	4,033	1,900
Total nitrogen (mg.L ⁻¹)	250	1,130	363	-
Total phosphorus (mg.L ⁻¹)	71.5	280	99.5	-
Apparent Color	Dark brown	Brown	Dark brown	Dark brown

COD = Chemical oxygen demand, Ts = Total solids, TSS = Total suspended solids, PtCo = Platinum-cobalt unit of measurement from the Standard Methods for the Examination of Water and Wastewater (American Public Health Association (1998).

Table 2 Decolorization of raw biogas effluent from palm oil mill with supplementation of 10% palm oil mill effluent by two types of mixed cultures and a consortium under microaerobic conditions after 7 d cultivation.

Experiment	Decolorization (%) after time period						
	1 day	2 day	3 day	4 day	5 day	6 day	7 day
Control	28.7±1.3	35.7±1.5	46.8±1.5	45.8±1.7	48.9±1.4	48.0±1.8	51.4±2.0
10% Super LDD.1	32.9±1.5	44.0±1.3	47.7±1.2	52.6±1.4	53.4±1.3	52.9±1.5	53.7±1.4
10% Super LDD.2	30.1±2.0	44.7±1.8	49.7±1.5	53.1±1.8	55.1±1.8	55.6±1.7	57.9±1.9
10% Super EM	26.2±2.3	43.9±2.0	43.6±2.0	42.8±1.6	45.4±2.1	42.7±2.0	43.6±2.1

However, nutrients supplementation by adding POME (10%) caused an increase in the color, COD and phenol values both at the beginning and after the experiment compared to the control (Table 1). Therefore, Super LDD.1 and Super LDD.2 were chosen for further studies without POME addition.

Effect of inoculum size on decolorization of biogas effluent from palm oil mill

The effect of various inoculum sizes (5, 10, 15 and 20% v/v) of Super LDD.1 and Super LDD.2 on the decolorization of the BEPOM was studied. For Super LDD.1, the inoculum size at

20% v/v showed the highest decolorization (27.0%) under aerobic conditions (2.5 vvm) after 2 d cultivation (Figure 2a). The inoculum size of Super LDD.2 at 20% v/v also gave higher decolorization (17.0%) than the other sizes (1.0, 6.0 and 7.0%, respectively) under microaerobic conditions after 6 d cultivation (Figure 2b). Although higher in decolorization, the Super LDD.1 showed slightly lower COD removal (27.8%) after 2 d cultivation under aerobic conditions (Figure 3a) than did Super LDD.2 (29.2%) after 6 d cultivation under

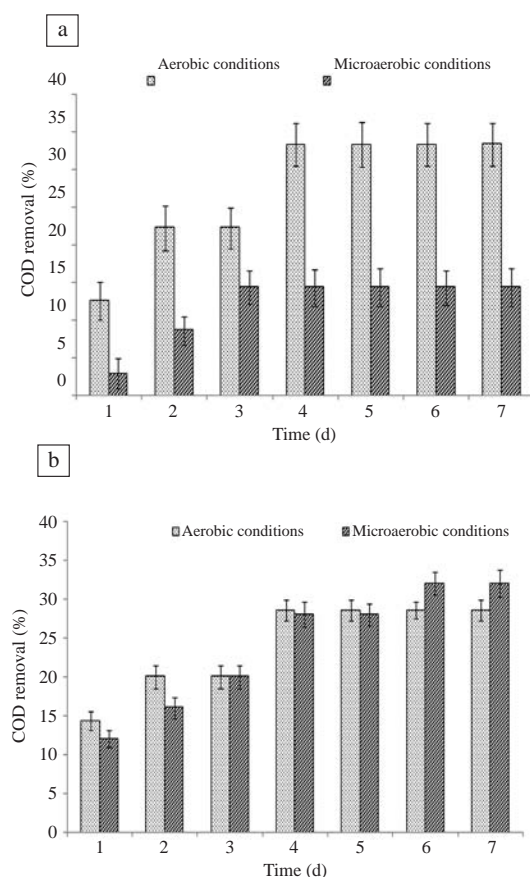


Figure 1 Comparison of chemical oxygen demand (COD) removal between aerobic conditions and microaerobic conditions of: (a) Super LDD.1 and (b) Super LDD.2. (Error bars show \pm SD).

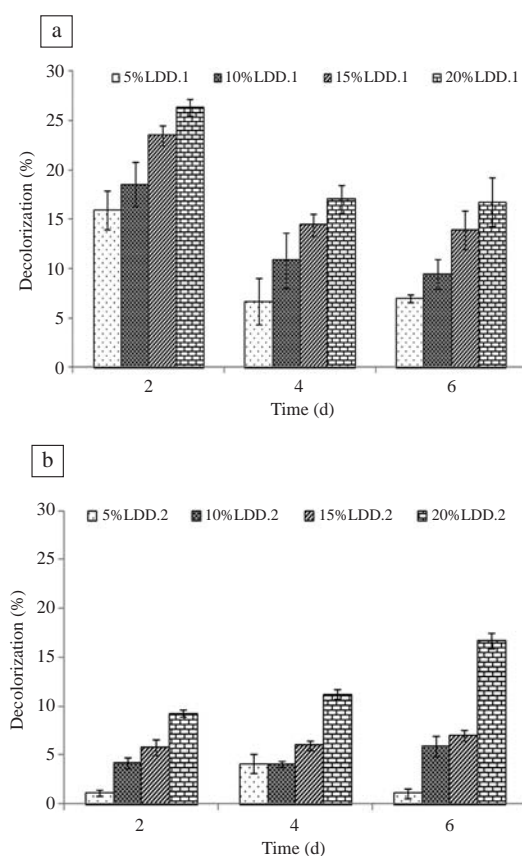


Figure 2 Effect of inoculum size (without palm oil mill effluent supplementation) on decolorization of biogas effluent from palm oil mill using: (a) Super LDD.1 under aerobic conditions (2.5 volume of air per volume of medium per minute) and (b) Super LDD.2 under microaerobic conditions. (Error bars show \pm SD).

microaerobic conditions (Figure 3b). However, in terms of removal efficiency, the Super LDD.1 was more efficient in the treatment of BEPOM than Super LDD.2 (13.9% per day compared to 4.9% per day, respectively). Many microorganisms present in Super LDD.1 and Super LDD.2 were reported to possess decolorization ability. *Streptomyces* BW130 decolorized Anthraquinone Blue (27.0%) and Azo-copper Red (73.0%) after 14 d cultivation by biosorption mechanisms (Zhou and Zimmermann, 1993). *Lactobacillus*

plantarum showed 55.0% decolorization of olive mill wastewater (Lamia and Moktar, 2003). The color removal performed by microorganisms occurred via two methods: bioaccumulation and biodegradation. In bioaccumulation, the microorganism can absorb dyes into the cell wall and thus color removal is dependent on biomass and mass transfer effects in the medium, while in biodegradation, the dyes are oxidized by the enzymatic system (Silveira *et al.*, 2009).

The phenol removal efficiency values (26.5–28.7%) at all inoculum sizes tested (5–20%, v/v) of Super LDD.1 were not substantially different (Figure 4a). These values were higher than those of Super LDD. 2 (11.9–13.5%) after 6 d cultivation (Figure 4b). The ability to degrade phenol depended on the strain as well as the

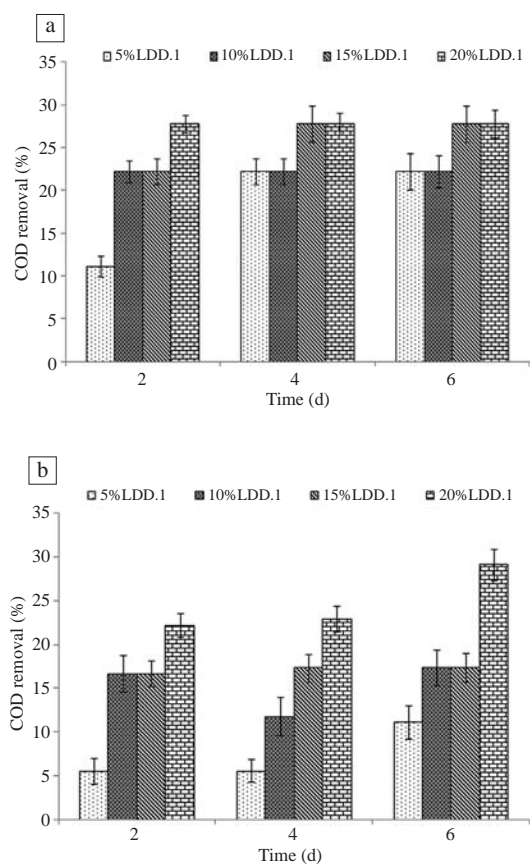


Figure 3 Effect of inoculum size (without palm oil mill effluent supplementation) on chemical oxygen demand (COD) removal of biogas effluent from palm oil mill: (a) Super LDD.1 under aerobic conditions (2.5 volume of air per volume of medium per minute) and (b) Super LDD.2 under microaerobic conditions. (Error bars show \pm SD).

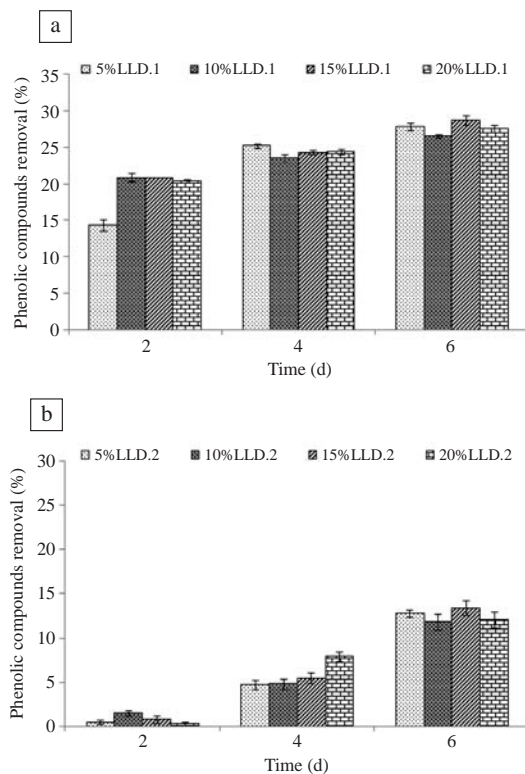


Figure 4 Removal of phenol in biogas effluent from palm oil mill using: (a) Super LDD.1 (aerobic conditions) and (b) Super LDD.2 (microaerobic conditions). (Error bars show \pm SD).

phenol concentration. For example, *Bacillus cereus* degraded 99.1%, 100%, 94.8%, 44.3% and 10.4% phenol from its initial concentrations of 100, 500, 1,000, 1,500, and 2,000 mg.L⁻¹, respectively (Banerjee and Ghoshal, 2010). In addition, the immobilized *Bacillus insolitus* could remove more than 50% of the 2,4-dichlorophenol using suspended *Bacillus insolitus* (Wang *et al.*, 2000). Many types of aerobic bacteria are capable of using aromatic compounds as the sole source of carbon and energy (Melo *et al.*, 2005). The typical pathway for metabolizing an aromatic compound is to dehydroxylate the benzene ring to form a catechol derivative and then to open the ring through ortho or meta oxidation (Melo *et al.*, 2005). Based on the above results, Super LDD.1 was chosen for further study as it exhibited higher decolorization, COD and phenol removal.

Effect of palm ash concentration and pH in combined treatment

The biological treated effluent (pH 9.15) was further decolorized by the physical method. An increase in the palm ash concentration by 1, 3, 6, 9, 12 and 15% (w/v) resulted in higher decolorization (9.5, 31.7, 60.3, 61.9, 65.9 and 76.2%, respectively) at the initial pH of 4 (and final pH values of 8.4, 8.5, 8.7, 8.8, 8.8 and 8.8, respectively) (Figure 5a) than with no pH adjustment (the control) (3.2, 28.6, 50.8, 54.0, 61.1, and 69.1%, respectively) after 1 h reaction time (Figure 5b). The results indicated that acidic conditions enhanced the adsorption of color by the palm ash as the positive charge dominates on the surface of the adsorbent. Thus, a sufficiently high electrostatic attraction exists between the positively charged surface of the adsorbent and the negatively charged dye species (Isa *et al.*, 2007). On the other hand, the lower adsorption rate at higher pH may have been due to the release of OH⁻ ions and caused ionic repulsion between the negatively charged active sites of the adsorbent (Ahmad and Hameed, 2009).

The final co-treated effluent samples with

palm ash (9–15% w/v and pH adjusted to pH 4) were colorless with an ADMI color value below 300 units which passed the discharged effluent standard. In the case of no initial pH adjustment, a higher palm ash concentration (12–15% w/v) was required to produce a value below 300 color units. Moreover, an increase in the palm ash concentration resulted in an increase in the phenol removal (0.9, 27.9, 45.9, 45.9, 54.9 and 73.0%, respectively) as well as the COD removal (14.3, 21.4, 35.7, 50.0, 64.3 and 64.3%, respectively) with the adjusted initial pH at 4 after 1 h reaction time (Figure 6a). Without adjustment of the initial pH (9.15), lower values were recorded for the phenol and COD removal than with an initial pH 4 (Figure 6b).

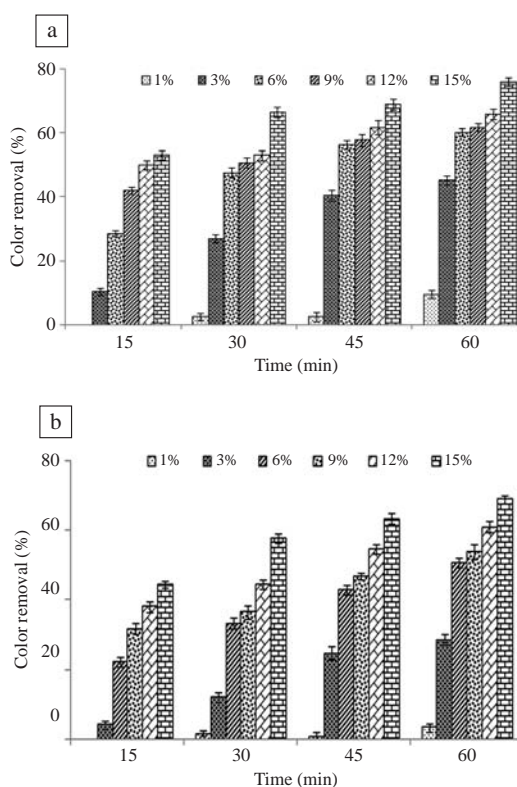


Figure 5 Effect of palm ash concentration and initial pH used for decolorization of biotreated biogas effluent from palm oil mill: (a) Initial pH adjustment (pH = 4) and (b) No initial pH adjustment (pH = 9.15). (Error bars show \pm SD).

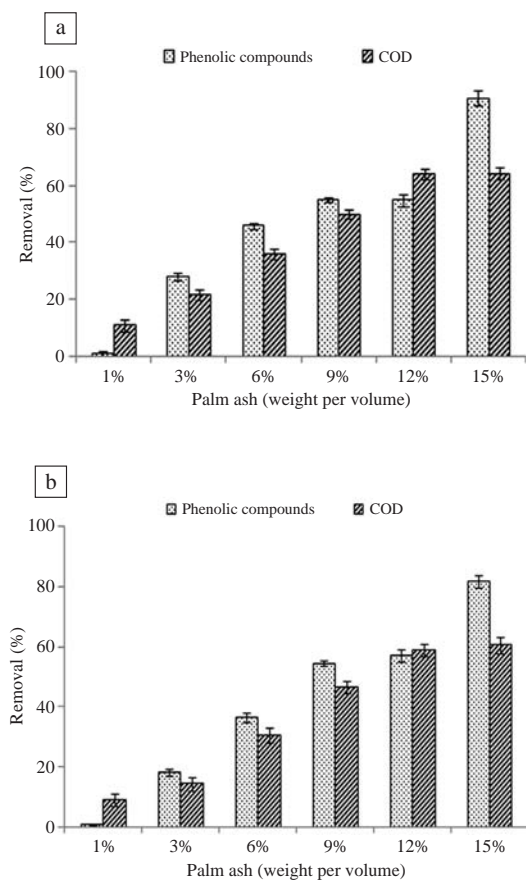


Figure 6 Profile of biological treatment method with co-palm ash absorption after 1 hr reaction time: (a) Phenol removal with initial pH adjustment (pH = 4) and (b) Organic removal (chemical oxygen demand; COD) with no initial pH adjustment (pH = 9.15). (Error bars show \pm SD).

The results above indicated that the addition of 9–15% palm ash with the initial pH adjusted to 4.0 gave a high percentage of decolorization and colorless effluent. In fact, the colorless effluent could also be achieved without adjusting the initial pH providing 12–15% palm ash was added.

The characteristics of BEPOM before and after treatment using the biological method with Super LDD.1 for 2 d in combination with palm ash (9–12% w/v) for 1 h are given in Table 3. After treatment, the color, phenol and COD were removed 81.6–84.7%, 75.0–90.8% and 86.1–88.9%, respectively, and the pH increased to 8.2–8.8. Above all, the final effluent was colorless (Figure 7). The final values of phenol ($1\text{--}3\text{ mg.L}^{-1}$) and COD ($120\text{--}160\text{ mg.L}^{-1}$) met the Discharged Effluent Standard (Ministry of Industry of Thailand, 1996).

CONCLUSION

Super LDD.1 and Super LDD.2 were more efficient in the decolorization of biogas effluent than Super EM under microaerobic conditions. The optimum inoculum size of both Super LDD.1 and Super LDD.2 was 20% (v/v), and Super LDD.1 was found to be more efficient in decolorization and phenol removal than Super LDD.2 under aerobic conditions without nutrient supplementation. Treatment by a biological

Table 3 Removal of color, phenol and chemical oxygen demand (COD) of the biogas effluent from palm oil mill treated by mixed cultures (Super LDD.1) and 9–15% (w/v) palm ash after 1 hr reaction.

Parameter	Biogas effluent from palm oil mill	After treatment	Standard of discharged effluent
pH	7.5-7.9	8.2 \pm 0.5	5-9
Color (PtCo)	1,630-1950	135 \pm 56	-
COD (mg.L ⁻¹)	1,800-2,500	126.8 \pm 30	<120
Phenol (mg.L ⁻¹)	21.2-32.60	1.8 \pm 1	<1.0
Apparent color	Dark brown	Colorless	-

PtCo = Platinum-cobalt unit of measurement from the Standard Methods for the Examination of Water and Wastewater (American Public Health Association (1998)).

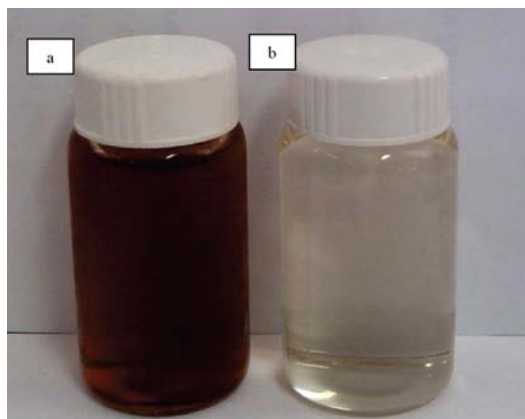


Figure 7 Color comparison of biogas effluent from palm oil mill: (a) biogas effluent and (b) effluent after treatment with Super LDD.1 and palm ash.

method (20% Super LDD.1 for 2 d) and a physical method (9–12%, w/v palm ash for 1 hr) gave 84.7% decolorization, 90.8% phenol removal and 88.9% COD removal.

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