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Utilization of the White-rot Fungus, *Trametes menziesii* for Landfill Leachate Treatment

(Penggunaan Kulat Busuk Putih, *Trametes menziesii* untuk Pengolahan Bahan Larut Lesap Tanah Isian)

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ABSTRACT

The study monitored the characteristics of the leachate collected from ten different landfills and presented the experimental work for the treatment of leachate by immobilized Trametes menziesii. *Variation in biological oxygen demand (BOD), chemical oxygen demand (COD) and ammoniacal nitrogen (NH_3-N) showed that the age of the leachate has a significant effect on its characteristics and composition. The BOD_5/COD ratio tends to decrease as the age of leachate increases, varying from 0.71 for a relatively 'fresh' leachate to 0.62 for an older (more stabilized) one. Variations in the characteristics of the leachate suggested that these leachates are difficult to treat. The principal pollutants in the leachate samples were organic and ammonia loads. Treatment of leachate using immobilized* Trametes menziesii *achieved 89.14 and 2.11% removals for leachate BOD_5 and COD, respectively. These findings suggested that using immobilized* Trametes menziesii *can remove promising percentage of BOD and COD leachate.*

Keywords: BOD; COD; leachate; white-rot fungi

ABSTRAK

Pencirian bahan larut lesap daripada 10 tanah isian berbeza dan hasil eksperimen dalam pengolahan bahan larut lesap oleh kultur pegun Trametes menziesii *telah dikaji. BOD_5, COD dan NH_3-N yang bervariasi menunjukkan bahawa usia bahan larut lesap memberikan kesan yang signifikan ke atas ciri dan kandungan bahan larut lesap. Nisbah BOD_5/COD menunjukkan pengurangan apabila usia bahan larut lesap meningkat, ia 0.71 bagi bahan larut lesap yang 'muda' dan 0.62 bagi bahan larut lesap yang lebih berusia. Ciri-ciri bahan larut lesap yang bervariasi menyebabkan ia sukar untuk diolah. Bahan cemar yang utama dalam bahan larut lesap adalah bahan organik dan ammonia. Pengolahan bahan larut lesap oleh kultur pegun* Trametes menziesii *mencatat 89.14 dan 2.11% pembuangan BOD_5 dan COD bahan larut lesap. Penemuan ini mencadangkan bahawa penggunaan kultur pegun* Trametes menziesii *berupaya untuk menyingkirkan bahan larut lesap BOD_5 dan COD dalam peratusan yang menggalakkan.*

Kata kunci: Bahan larut lesap; BOD; COD; kulat busuk putih

INTRODUCTION

Increasing of solid wastes has become a major problem for the environment. Jemec et al. (2012) reported that according to EUROSTAT statistics from the year 2007, approximately 106 million tons (which yielding 522 kg/capita) of municipal waste was land-filled in the whole European. Land-filling is still a most common disposal alternative of municipal solidwaste in most countries like China (Ding et al. 2001) and Kuwait (Al-Muzaini 2006). Meanwhile in Malaysia, landfilling and disposing of wastes in non-sanitary landfill had been and is expected to remain as the most common method for the disposal of municipal solid wastes. According to Kamaruddin et al. (2014), among 261 landfills in Malaysia, more than 80% of them are open dump. A major problem arising from landfills is the discharge of leachate. Due to the high amounts of precipitation, large quantities of leachate (liquid discharge from solid waste) from landfills in tropical climates are to be expected. According to Edi Munawar and Fellner (2013), an annual leachate generation rate of more than 1000 litres per m^2 was frequently observed in tropical countries.

Landfill leachate is formed by water that passed through the waste layers and thus contains various types of pollutants. The subsequent movement of the landfill leachates into the surrounding soil, ground water or surface water could lead to severe pollution. Landfill leachate is a complex mixture of inorganic and organic substances. Kjeldsen et al. (2002) summarized the most common constituents of leachates based on several biological and chemical analyses performed on landfill leachates that come from different industrial origins. These include dissolved organic matter, inorganic macro components (e.g., Ca^{2+}, Mg^{2+}, K^+ and Fe^{2+}), heavy metals and xenobiotic organic compounds originating from household or industrial chemicals e.g. aromatic hydrocarbons, phenols, chlorinated aliphatics and pesticides (Baun et al. 2004). However, in general, leachate may contain high concentrations of dissolved organic matter and inorganic macro components which may vary due to varieties of influencing factors. Jemec et al. (2012) stated that the components of leachate depend on the stabilization stage of the landfill and seasonal variation. They reported that based on physicochemical characterization, the properties of leachates collected at different periods of the year vary considerably. In addition, Kim et al. (2003) stated that the characteristics of leachate depends on factors such as hydrogeology, waste composition, amount of rainfall, the landfill method and the age of the landfill (e.g. active or closed landfill).

Leachate composition is an indication of the type of waste disposed and the processes which occur within the landfill (Slack et al. 2005). Al-Muzaini (2006) stated the complex chemical and biological reactions that take place in a landfill site make it difficult to predict the quality of leachate at any given landfill site. However, Marttinen et al. (2002) stated that the characteristic of the leachate is one of the factors used to determine the applicability of a treatment in most cases. Therefore, the characteristic of the leachate is a critical factor in establishing a corresponding effective treatment process. Hence, to treat leachate, it is very important to determine the characteristic of that leachate.

Several options have been put into practise for leachate treatment, presenting varying degree of efficiency (Kamaruddin et al. 2014). Biological processes based upon suspended-growth biomass, were proved to be effective for the removal of organic carbon and nutrients content (Zoubolis et al. 2001). White-rot fungi are of current interest to be used for the bioremediation of a broad spectrum of persistent xenobiotics. White-rot basidiomycetous fungi have been implicated in the transformation of a large amount of organopollutants structurally related to lignin for example *Pycnoporus sanguineus*, *Coriolus pubescens* and *Trametes* sp. in degradation of lignosulphonates (Eugenio et al. 2008) and *Phanerochaete chrysosporium*, *Pleurotus* sp. and *Trametes versicolor* in mineralizing polycyclic aromatic hydrocarbons (PAH) (Pointing 2001). Besides that, Polak and Jarosz-Wilkołazka (2010) also stated that the use of fungal cultures to transform various chemical compounds had been reported in several studies. White rot fungi were able to produce extracellular lignin peroxidase (LiP) and manganese-dependent peroxidase that is essential for lignin degradation (Leonowicz et al. 1999). These enzymes are able to oxidize a variety of high-priority aromatic pollutants such as polycyclic aromatic hydrocarbons, chloromatics and polyaromatic dyes (Kotterman et al. 1996). Saetang and Babel (2009) have applied immobilized *T. versicolor* to treat landfill leachate in column experiments since the immobilization is expected to improve the mass transfer of oxygen and nutrients for fungal mycelia by providing a large attaching surface area. Besides that, Saetang and Babel (2010) stated that the advantages of using immobilized microorganism for pollutant degradation is due to their economically cheaper, easier to handle and the immobilized fungus is reusable for several batches.

In this paper we focused on investigating the level of selected parameters in the raw leachate collected from various types of landfills and compare the changes of leachate composition from both old (closed) landfills and landfills still in use (active landfills). With the information of leachate component obtained, biological method by immobilized fungi was applied for leachate treatment in order to meet the discharge standards.

Materials and Methods

LANDFILL DESCRIPTION

Leachates samples were collected from ten different landfills listed in Table 1. The landfills received wastes within the municipality area. Three of the landfills (Nos. 1-3) were engineered/sanitary (i.e. with liners and leachate collection systems), while seven others (Nos. 4-10) were dumpsites/non-sanitary (i.e. without liners and leachate collection system) landfills. Among three sanitary landfills, two were active (Nos. 1-2) and another one was a closed landfill (Nos. 3). Among the seven non-sanitary landfills, landfills Nos. 4-5 were active landfills and landfills Nos. 6-10 were closed landfills.

SAMPLING PROCEDURE

Leachate samples were collected from existing ponds. Leachate collection ponds servicing the whole landfill were sampled at all the sanitary landfills (Nos. 1-3). At the non-sanitary landfills no such collection ponds were installed hence, samples were collected from leachate flowing out below the Landfills Nos. 4-10. At all landfills, the sampling bottles were lowered into the pond in order to collect leachate. All samples were collected in plastic bottles. The samples were packed in cool boxes (8-15°C) and were transported to the laboratory for analysis.

ANALYSIS OF LEACHATE

The leachate was characterized based on several pollution parameters as required by Environmental Quality Act (EQA) (2009) i.e. biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN), total suspended solid (TSS), ammoniacal-nitrogen (NH_3-N), pH and also heavy metals such as: magnesium, lead, copper, iron, zinc and cadmium. The techniques used for sampling and analyses were in accordance with the Standard Method for the Examination of Water and Wastewater (APHA 1998). The Hach DR 2800 spectrophotometer was used for the determination of chemical concentration. The experiment was replicated three times to obtain an average. All the experiments were undertaken at 20±2°C.

FUNGI AND SUB-CULTURE OF FUNGI

Trametes menziesii was obtained from the Mycology Laboratory, Institute of Biological Sciences, University

TABLE 1. General characteristics of the Municipal solid wastes (MSW) landfills included in the study

Landfill No.	Location	Type of landfill (closed/active)	Landfill area (acre)	Period of operation	Type of waste
1	*Jeram, Kuala Selangor	Sanitary (active)	160	2007-	Domestic waste (95%), Others (5%)
2	*Tanjung 12, Sepang	Sanitary (active)	160	2010-	Domestic waste (96%), Industrial (3%), Others (1%)
3	*Air Hitam, Puchong	Sanitary (closed)	100	1995-2006	Domestic waste
4	Sg Besar, Sabak Bernam	Non-sanitary (active)	10	NA	Domestic waste
5	Bukit Beruntung, Hulu selangor	Non-sanitary (active)	NA	NA	Domestic waste
6	Teluk Kapas, Rantau Panjang,	Non-sanitary (closed)	32.4	2000-2003	Domestic waste
7	Teluk Gong, Pandamaran	Non-sanitary (closed)	19.42	1986-2000	Domestic waste
8	Kundang, Selayang	Non-sanitary (closed)	NA	NA	Domestic waste
9	Batu 20, Rawang	Non-sanitary (closed)	NA	NA	Domestic waste
10	*Kubang Badak, Kuala Selangor	Non-sanitary (closed)	30	2006-2007	Domestic waste

Sanitary: Engineered landfill with liners and leachate collection system; Non-sanitary: Uncontrolled landfills without liners and leachate collection system; NA: Not available. (Source: Department of Local Government 2003; *Sanitary landfills 2009)

of Malaya, Malaysia. The fungi were maintained on malt extract (MEA) (Oxoid) agar slants and the inoculum was prepared by sub-culturing onto MEA grown for 7 days at 28±2ºC. Sub-culture was done once a week to obtain active fungi.

MYCELIAL SUSPENSION AND IMMOBILIZATION OF FUNGI ON ECOMAT

Mycelial suspension Four plugs (6- mm^2 diameter) of a 7-day old fungal colony growing in MEA media in Petri plates were transferred into 250 mL Erlenmeyer culture flasks containing 100 mL of Glucose-yeast-malt-peptone (GYMP) growth medium under sterile conditions. The GYMP growth medium contained the following: $MgSO_4.7H_2O$ (1.00 g/L); KH_2PO_4 (1.00 g/L); K_2HPO_4 (1.00 g/L); NH_4Cl (1.00 g/L); Glucose (15.00 g/L); Peptone (8.00 g/L); Yeast Extract (8.00 g/L) and Malt Extract (8.00 g/L). Inoculated flasks were then agitated on an orbital shaker for 48 h at 28±2ºC at 150 rpm.

Ecomat sterilization 50 pieces of Ecomat were put into 500 mL beaker. The beaker was covered with aluminium foil and then sterilized in autoclave for 1 h prior to use.

Fungi immobilization on Ecomat Four pieces of sterilized Ecomat and 5 mL of mycelia suspension were added to 250 mL Erlenmeyer culture flasks containing 50 mL of GYMP growth medium. The flasks were agitated at 100 rpm on an orbital shaker. The Ecomat covered with fungal mycelium within 4 days were used for the study.

EXPERIMENTAL DESIGN OF LEACHATE TREATMENT BY IMMOBILIZED *TRAMETES MENZIESII*

Leachate characterization before treatment Leachate sample used in this study was collected from the pond of untreated leachate at the sanitary landfill. The leachate was filtered to remove suspended solids before measurement and was analyzed for pH, COD, BOD_5 and NH3-N.

Leachate treatment by immobilized T. menziesii The experiment was carried-out in 250 mL Erlenmeyer culture flasks; 125 mL of leachate was treated with immobilized *T. menziesii* on Ecomat. The flasks were then agitated on an orbital shaker for 28 days at 28±2ºC at 150 rpm. At weekly time intervals (day 7, 14, 21 and 28), the value of BOD_5, COD, pH and ammoniacal nitrogen were analyzed. All processes were done under sterile conditions at ambient temperature.

Determination of leachate characteristics after treatment Removal of BOD, COD and NH_3-N were investigated after fungal treatment and the results were compared with the initial value.

RESULTS AND DISCUSSION

Characteristics of the leachate content is one of the most important criteria to be determined before establishing the most suitable method for treating and disposing of any given pollutant. These analytical methods are required to assess the polluting strength of the waste. According to the EQA Standard 1974 (2009) and Waites et al. (2001), the tests usually include the determination of BOD, COD, TSS and TS. However, other tests may also be performed in order to determine the levels of specific components such as nitrogen, phosphorus and heavy metals.

Landfills can be either non-sanitary or sanitary landfill. This preliminary study was conducted in order to get the base data of leachate characteristics collected from different landfills that either closed or active and either non-sanitary or sanitary landfill. Table 1 shows that the landfills were in various range of operation duration time. It is between 3 and 27 years. The sources of solid waste in Malaysia usually generated by residential, commercial, institutional, construction, municipal services, treatment plant site, industrial and agriculture. Unfortunately, most of the wastes are dumped at non-sanitary landfill with improper record on the total amount and type of waste due to the uncontrolled manner of waste disposal during the early 1980's except for sanitary landfills.

The characterization of the landfill leachates in terms of general chemical parameters showed that there are huge differences between the landfills (Table 2). For instance, the total nitrogen concentrations were varied from 6.0 to 1700 mg/L, the total suspended solids were varied from 10 to 3000 mg/L and correspondingly the observed concentrations of ammoniacal nitrogen were varied from 0.94 mg/L up to the extremely high concentration of 3200 mg/L in the leachate from Landfill No. 3. The concentration of total organic carbon was 12.0–45070 mg/L and not detected in the leachate from Landfill No. 9, while the pH-values were varied from 6.29 to 8.39. The value of BOD_5 was lower for all the leachate collected from non-sanitary landfills than in leachate collected from sanitary landfills. The values of BOD_5 for leachate from closed non-sanitary landfills were 171±18.36 mg/L (Landfill No. 6), 369±32.97 mg/L (Landfill No. 7), 81±6.24 mg/L (Landfill No. 8) and from closed sanitary landfill is 2497±221.31 mg/L (Landfill No. 3). Leachate from active non-sanitary Landfill No. 4 has BOD_5 value of 1160 ± 98.49 mg/L, while leachate from active sanitary Landfill No. 1 and Landfill No. 2 have BOD_5 value of 11360±703.42 mg/L and 1971±16.46 mg/L, respectively. Similar patterns were shown for the COD value for leachate from non-sanitary and sanitary landfill.

The results suggested that leachates from the non-sanitary landfills (Nos. 4–10) generally had lower values than sanitary for all tested parameters, except for the pH-values. This may be due to the method of leachate collection and other different factors such as amount and composition of waste. However, these results are within the ranges generally observed in landfills (Kjeldsen et al. 2002). In addition, Chu et al. (1994) stated that chemical properties of leachate samples from different landfills vary widely. They were affected by the amount of waste disposal on landfill, composition and moisture content of the refuse; hydrogeology and climate of the site; age and height of the landfill and season of the year. On top of that, it is also found that the leachate produced from landfills is a high strength of organic wastewater which, when discharged directly to a municipal wastewater treatment plant, may cause corrosion of the pump station, difficulty in maintaining constant effluent chlorine residual, and sludge bulking and settling problems (Deng 2007). Biological activity within landfill influence chemical concentration levels of the landfill. Particularly, at the onset of biodegradation processes the high organic and moisture contents resulted in an extremely strong leachate,

TABLE 2. Characteristics of landfill leachates with respect to general chemical parameters

Parameter	*Standard	Landfill No.				
		1	2	3	4	5
BOD_5 (mg/L)	20	11360 ± 703.42	1971 ± 16.46	2497 ± 221.31	1160 ± 98.49	1120 ± 73.43
COD (mg/L)	400	16000 ± 1130.62	6050 ± 44.44	4000 ± 312.77	2982 ± 308.64	2371 ± 222.71
TSS (mg/L)	50	130 ± 13.45	570 ± 27.40	800 ± 14.53	3000 ± 146.27	320 ± 22.91
NH_3-N (mg/L)	5	21.3 ± 3.18	17.2 ± 1.73	3200 ± 185.00	29.0 ± 3.61	2.2 ± 4.00
TOC (mg/L)	NA	4700 ± 145.26	11100 ± 1056.42	45070 ± 1044.46	281 ± 16.70	247 ± 13.00
TN (mg/L)	NA	98 ± 13.45	50 ± 6.08	1700 ± 149.66	119 ± 6.24	54 ± 5.57
pH	6.0 – 9.0	8.05 ± 0.05	8.07± 0.03	8.19 ± 0.17	8.39 ± 0.11	7.27 ± 0.07
Parameter	*Standard	Landfill No.				
		6	7	8	9	10
BOD_5 (mg/L)	20	171 ± 18.36	369 ± 32.97	81 ± 6.24	56 ± 8.18	925 ± 6.00
COD (mg/L)	400	455 ± 33.15	385 ± 12.29	165 ± 16.09	285 ± 37.24	2880 ± 128.55
TSS (mg/L)	50	50 ± 15.72	50 ± 6.08	10 ± 4.36	130 ± 13.86	200 ± 17.58
NH_3-N (mg/L)	5	2.6 ± 0.82	10.0 ± 2.65	0.94 ± 0.19	5.0 ± 2.00	650 ± 8.89
TOC (mg/L)	NA	12 ± 2.00	27 ± 4.00	15 ± 2.65	ND	23000 ± 836.48
TN (mg/L)	NA	6 ± 2.00	102 ± 21.63	8 ± 2.65	32 ± 9.16	600 ± 43.30
pH	6.0 – 9.0	6.76 ± 0.05	7.54 ± 0.06	7.02 ± 0.17	6.29 ± 0.25	8.30 ± 0.11

ND: Not detected
NA: Not available
* Source: The EQA (2009)

which can affect the leachate treatment facility (Bilgili et al. 2007).

Besides comparing the characteristics of leachate from different types of landfills, the characteristics of leachate from active and closed landfills were also compared. The comparison was significant to identify the status of the pollutant resulting from the landfills even though it has been closed for several years. This is due to Kjeldsen et al. (2002) who stated that even after a landfill stops accepting waste and a final cover is placed over the landfill, the waste will continue to decompose. As an example, Chu et al. (1994) reported that ammoniacal nitrogen remained the mean values between 500 and 1500 mg/L for at least 50 years.

From the results shown in Table 3, it shows that the characteristics of leachate from two active landfills i.e. sanitary (No. 1) and non-sanitary (No. 4) while, two were closed landfills i.e. sanitary (No. 3) and Landfill No. 10 (non-sanitary). The composition of leachate shows that almost all the studied parameters of the leachate from closed landfills were lower than the leachate from active landfills except pH and ammoniacal nitrogen. These may be due to biological and chemical composition of the landfill. Measurement of BOD and COD shows the organic acid content in leachate. The value of BOD_5 for the leachate of closed landfills (No. 3: 2497±221.31 and No. 10: 925±6.00 mg/L) were lower than leachate of active landfills (No. 1: 11360±703.42 and No. 4: 1160±98.49 mg/L). Similar findings were obtained for the COD value where the values for leachate from closed landfills i.e. No. 3: 4000±312.77 and No. 10: 2880±128.55 mg/L were lower than COD value for leachate from active landfills i.e. No. 1: 16000±1130.63 and No. 4: 2982±308.64 mg/L. This study showed that the leachate from closed landfills of both sanitary and non-sanitary has lower biological oxygen demand (BOD_5) and chemical oxygen demand (COD) content compared with leachate from active landfills. This finding is parallel with the report by Kang et al. (2002) which stated that, organic concentration (measured as BOD and COD) decreased as the landfilling age increased. In addition, Miller and Clesceri (2003) stated that for waste sites (landfills) of longer standing (inactive or closed), COD and BOD levels in leachate may be less than the value for young leachate (which collected from active landfill). However, they generally exceed levels found in wastewaters, thus cannot be discharged to the surrounding environment.

The changes in leachate biodegradability are mainly reflected by the BOD_5/COD ratio, as BOD_5 is a direct measurement of the treatability of wastewater by the application of biological processes. Ratios of leachate BOD_5/COD can be used to predict the effectiveness of various biological and physical-chemical processes for leachate treatment (Samudro & Mangkoedihardjo 2010). Table 3 shows the BOD_5 to COD ratio was reduced from 0.71 of young-landfill (Landfill No. 1) to 0.62 of old-landfill (Landfill No. 3). This represents the decrease in biodegradability of the leachates with respect to their age. However this small amount of differences may be due to the factor that the closed landfill was only stopped receiving the waste just three years before the sample collection. These observations are coherent with findings by El-Fadel et al. (2002) that stated the BOD/COD ratio can be considered as a measure of the biodegradability of the organic matter, which typically decrease with time. Therefore, according to Tatsi and Zouboulis (2002) the observed decrease in BOD_5/COD ratio represents a more complete oxidation of organic carbon; hence, it becomes less readily available as an energy source for microbial growth.

Meanwhile, the Table also show that the content of ammonium-nitrogen for leachate collected from closed landfills (No.10: 650 ± 8.89 mg/L) was higher compared with the amount of leachate from active landfills (No.1: 21.3 ± 3.17 mg/L). The ammonia concentration in aged leachate showed higher concentration than in young leachate that may be due to biological activities such as deamination of amino acids during destruction of original organic compounds (Faeiza et al. 2004). Tatsi and Zouboulis (2002) stated the great majority of total Kjeldahl nitrogen (TKN) content was found in ammoniacal form. Therefore, ammonia is the principal pollutant of ‘old’ leachate samples. Table 3 shows the ranged of pH was from 8.05 to 8.30. The pH for the leachate from active sanitary landfill (No. 1) was 8.05±0.05 while the pH value for the leachate from closed sanitary landfill (No. 3) was 8.19±0.17. The results showed that the pH of leachate increased in the closed landfills (=older age landfill). These results are consistent with several other previous reports on the same subject. Faeiza et al. (2004) reported a study in Hong Kong detected that pH for 3.5 years of closed landfills ranged from 7.2 to 8.0 and pH for a 1.5 year closed landfill was only at 5.8. The finding was supported by Chu et al. (1994) who stated that pH of leachate increased with time due to the decrease of the concentration of partially ionized free volatile fatty acid over the age.

However, the result for other parameters such as total suspended solids and total nitrogen were varied in leachate. Similar results were found by Tatsi and Zouboulis (2002) where the concentration of the total dissolved solids (TDS) fluctuates widely.

The results showed that the concentrations of all tested heavy metals in the leachate landfill were below the standards levels, except for Pb (0.5±0.17 mg/L) and Fe (10.41±0.74 mg/L) for Landfill No. 10. The comparison of heavy metals concentrations in leachate from active landfill (Landfill No. 1) and closed landfill (Landfill No. 3) shows that the concentration of heavy metals was higher in leachate from closed landfill than in leachate from active landfill except for Fe. The concentrations of Pb were 0.11±0.01 mg/L for Landfill No. 3 and 0.06±0.02 mg/L for Landfill No. 1; the concentrations for Zn were 0.19±0.03 mg/L for Landfill No. 3 and 0.18±0.03 mg/L for Landfill No. 1 and the concentrations for Mg were 180.79±5.31 mg/L for Landfill No. 3 and 23.01±0.90 mg/L for Landfill No. 1. Meanwhile, the concentrations of Fe were 2.62±0.11 mg/L for Landfill No. 3 and 4.44±0.11

TABLE 3. Comparison of leachate characteristics from active and closed landfills based on selected pollution parameters

		Sanitary		Open dump	
		(Active)	(Closed)	(Active)	(Closed)
Parameter /Landfill No.	Standard	1	3	4	10
Biochemicals oxygen demand (BOD) @ 20ºC, 5 Days (mg/L)	20	11360 ± 703.42	2497 ± 221.31	1160 ± 98.49	925 ± 6.00
Chemical oxygen demand (COD) (mg/L)	400	16000 ± 1130.62	4000 ± 312.77	2982 ± 308.64	2880 ± 128.55
BOD_5/COD	NA	0.71 ± 0.08	0.62 ± 0.09	0.39 ± 0.05	0.32 ± 0.07
Total suspended solids (TSS) (mg/L)	50	130 ± 13.45	800 ± 14.55	3000 ± 146.27	200 ± 17.58
Ammoniacal nitrogen (NH_3N) (mg/L)	5	21.3 ± 3.17	3200 ± 185.00	29.0 ± 3.60	650 ± 8.89
Total carbon (TOC) (mg/L)	NA	4700 ± 145.26	45070 ± 1044.46	281 ± 16.70	23000 ± 836.48
Total nitrogen (TKN) (mg/L)	NA	98 ± 13.45	1700 ± 149.66	119 ± 6.24	600 ± 43.30
pH @ 25°C	6.0 – 9.0	8.05 ± 0.05	8.19 ± 0.17	8.39 ± 0.11	8.30 ± 0.11
HEAVY METAL					
Leads as Pb (mg/L)	0.1	0.06 ± 0.02	0.11 ± 0.01	NA	0.5 ± 0.17
Cadmium as Cd (mg/L)	0.01	ND < 0.002	ND < 0.002	NA	ND < 0.002
Copper as Cu (mg/L)	0.2	0.02 ± 0.01	ND < 0.01	NA	0.12 ± 0.02
Iron as Fe (mg/L)	5	4.44 ± 0.11	2.62 ± 0.11	NA	10.41± 0.74
Zinc as Zn (mg/L)	2	0.18 ± 0.03	0.19 ± 0.03	NA	0.80 ± 0.05
Magnesium as Mg (mg/L)	NA	23.01 ± 0.90	180.79 ± 5.31	NA	186.62 ± 10.76

ND: Not detected; NA: Not available

mg/L for Landfill No. 1. These observations were in contrast with the finding by Tatsi and Zouboulis (2002) which stated inorganic contaminants also follow the trend of decreasing concentrations with increasing leachate age and stability. Cotman and Gotvajn (2010) reported the concentration of lead decreased with the increasing age of landfill. Besides that, Tatsi and Zouboulis (2002) stated that the concentration of metals in leachate samples were affected by the initial amounts that existed in domestic solid wastes, but they can also be leached by the degradation processes within the landfill. 'Fresh' leachate samples showed a higher degree of metal solubilization due to lower pH values caused by the biological production of organic fatty acids. The difference in the findings between this study and the study of Tatsi and Zouboulis (2002) may be due to the difference in climate and source of waste disposal. However, as the landfill age increases, the pH values increases too. This may be caused by a decrease in the concentration of free fatty acids which, due to anaerobic consumption.

This study noted that the principal pollutants in the leachate samples were organic loads; therefore, the best treatment method to treat this leachate is biological treatment. The biological treatment of concentrated leachate was carried-out in flask containing 100% leachate by immobilized *T. menziesii* on Ecomat for 28 days. The result of weekly analysis showed that the value of BOD_5 was reduced as the incubation time increased. It shows that the removal of BOD_5 increased until Day 28 with the highest percentage of 93.48%. It was observed that significant removal of BOD_5 occurred at Day 7, 82.00% and gradually increased at Day 14 with BOD_5 of 87.78% and 89.14%, respectively. Similarly, the removal of COD occurred at Day 7 at 24.66% and at Day 21 with COD removal of 2.11%. Table 4 also showed that the value of NH_3-N increased through-out the experiment until at Day 28 and indicating that no removal of NH_3-N occurred. The value of pH shows that the longer the incubation time, the leachate growth medium will became more alkaline.

The results obtained showed the ability of white-rot fungi *T. menziesii* to remove BOD_5 and COD but not NH_3-N. This finding is coherent with Kim et al. (2003) who reported the use of white-rot fungus *P. chrysosporium* for the biological removal of organics measured as COD. White-rot fungi have been shown to degrade a wide variety of environmental pollutants, including PCP (Walter et al. 2005). Coulibaly et al. (2003) noted that white-rot fungi have been attracting a growing interest for the biotreatment (removal or destruction) of waste water ingredients such as metals, inorganic nutrients and organic compound. This may be due to their capacities to adapt to severe environmental constraints. Basidiomyceteous white-rot fungi are capable of degrading a variety of environmentally pollutants including leachate. This specialty arises

TABLE 4. Percentage removal of 100% leachate BOD_5, COD, NH_3-N and pH changes by *T. menziesii* immobilized on Ecomat at weekly intervals for 28 days incubated at room temperature, shaking at 150 rpm

Incubation time	Parameters	Levels in untreated leachate	Levels in treated leachate	Percentage removed (-), increased (+)
Day 7	BOD_5 (mg/L)	11265.00	2028.00 ± 97.04	-82.00
	COD (mg/L)	14600.00	11000.00 ± 991.36	-24.66
	NH_3-N (mg/L)	20.4	23.47 ± 0.80	+15.05
	pH	7.93	8.82 ± 0.37	+
Day 14	BOD_5 (mg/L)	11371.00	1390.00 ± 41.04	-87.78
	COD (mg/L)	14260.00	14390.00 ± 62.45	+0.91
	NH_3-N (mg/L)	15.45	17.00 ± 1.51	+10.03
	pH	7.90	9.16 ± 0.14	+
Day 21	BOD_5 (mg/L)	11530.00	1251.67 ± 54.34	-89.14
	COD (mg/L)	13240.00	12960.00 ± 474.45	-2.11
	NH_3-N (mg/L)	26.60	28.33 ± 1.59	+6.50
	pH	7.86	9.23 ± 0.18	+
Day 28	BOD_5 (mg/L)	11290.00	736.67 ± 12.47	-93.48
	COD (mg/L)	14740.00	15790.00 ± 246.82	+7.12
	NH_3-N (mg/L)	18.40	39.90 ± 2.46	+116.85
	pH	7.99	9.38 ± 0.26	+

– indicates reduced (removed); + indicates increased values expressed are means ± s.d. of triplicate measurements

from the production of powerful oxidative and non-specific extracellular enzymes known as peroxidases. The peroxidases families are best recognized by lignin peroxidases (LiP), manganese peroxidases (MnP) and aryl alcohol oxidase (AAO) (Pozdnyakova et al. 2011). Previous result (Noorlidah et al. 2013) shows that white-rot fungus *Ganoderma australe* was able to produce ligninolytic enzymes such as LiP, MnP and Laccase. These enzymes were known for their ability to degrade a variety of environmental pollutants that include leachate. The ability of these enzymes to degrade a variety of industrial pollutants, environmental pollutants and leachate was also supported by Turkdogan-Aydinol et al. (2011).

CONCLUSION

Our study indicated that large variations exist in the characteristics of leachates from different types of landfills (in this case closed and active landfills) in terms of their physicochemical parameters. All the parameters studied were lower in leachate from closed landfills than leachate from active landfills except pH and ammoniacal nitrogen. Consequently, these results showed the difficulty in leachate treatment. With reference to the discharge limit of 400 mg/L for COD and 5 mg/L for NH_3, it was suggested that these landfills leachate need further treatment before they can be discharged to the nearby environment. This study showed that the principal pollutants in the leachate samples were organic loads; therefore, biological treatment is the best treatment method. The application of biological treatment using immobilized *T. menziesii* showed a promising potential to be used in leachate treatment since significant removal of BOD_5 (89.14%) and COD (2.11%) were observed after 28 days.

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