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Landfill leachate treatment by an anaerobic process enhanced with recyclable uniform beads (RUB) of seaweed species of *Gracilaria*

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ABSTRACT

Landfill leachate containing heavy metals was subjected to anaerobic treatment by using an upflow anaerobic sludge blanket (UASB) reactor combined with recyclable uniform beads (RUB) of seaweed species of *Gracilaria*. During the treatment of leachate without the RUB, the organic loading rate (OLR) was increased in stages from 0.125 to 0.833 kg chemical oxygen demand (COD) $m^{-3}d^{-1}$ and further increased to 2.50 kg COD $m^{-3}d^{-1}$ by reducing the hydraulic retention time from 4 to 1 d. Results showed that the COD removal efficiency declined from 65.70% to 9.33% when the OLR was increased from 0.125 to 2.50 kg COD $m^{-3}d^{-1}$. The removal of cadmium (Cd), nickel (Ni), and iron (Fe) was almost constant, regardless of the OLR (around Cd [36%], Ni [32%], and Fe [29%]). However, when the leachate was treated with UASB + RUB, a complete removal (100%) of Cd, Ni, and Fe was witnessed. Fourier-transform infrared spectrometer spectra of RUB pre and post leachate treatment indicated clearly that RUB was the major component that worked to remove the heavy metals. The functional groups that were responsible for the removal of heavy metals were hydroxide (O–H), amine (N–H), carboxylic (C=O), amide (N=O), sulfinyl (S=O), and sulfides (C=S).

Keywords: Recyclable uniform beads (RUB); Seaweed extract; Upflow anaerobic sludge blanket (UASB); Landfill leachate; Heavy metals; *Gracilaria* sp.

1. Introduction

Landfilling is the primary method of waste disposal in developed and developing countries [1]. Although this method has numerous benefits, one of its disadvantages is leachate production, which must be properly managed. Without proper treatment, landfill leachate will greatly increase water pollution as it can penetrate through soil and subsoil. Leachate contains water, organic, and inorganic chemical substances as well as recalcitrant chemicals, for instance, excessive phosphate, nitrates, and metal salts, including heavy metals [2]. Heavy metals such as cadmium

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(Cd), chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), zinc (Zn), and iron (Fe) are commonly found in leachate [3]. The main sources contributing to the presence of heavy metals are typical home appliances, such as fluorescent tubes, garden pesticides, batteries, waste oil, paint, and electronic waste [4]. Heavy metals may be potentially phytotoxic and pose a threat to animals and human health via food chain.

Heavy metals and other toxic compounds are removed during the final treatment process in the landfill. Current leachate treatment options include recycling, on-site treatment, biological treatment, chemical oxidation, and discharge to a municipal water treatment facility, or a combination of the above. However, biological treatment is still widely used in landfill leachate treatment. Biological treatment is divided into aerobic and anaerobic systems. Under aerobic conditions, microorganism biodegrades organic compounds to CO_2, whereas biodegrades to biogas (a mixture of CO_2 and CH_4) and biomass in anaerobic conditions [5]. Due to its limitations such as sludge bulking in the conventional aerobic system which apparently disturbs the treatment of leachate, the anaerobic treatment is now becoming a possible substitute due to its general advantages over aerobic systems. One of the problems during treatment by using anaerobic process is the inhibition of the biological treatment of toxic compounds found in leachate such as heavy metals. It is well documented that heavy metals are toxic to anaerobic microorganisms and may affect the process performance of landfill leachate treatment using this type of reactors. Accordingly, this study investigates the landfill leachate treatment by combining the anaerobic reactor (upflow anaerobic sludge blanket [UASB]) with naturally found seaweed for process optimization. None of the prior art explored the performance of landfill leachate treatment using the combination of seaweed and the anaerobic reactor. Moreover, there are very limited studies which explored the removal of heavy metals from landfill leachate using anaerobic reactors.

Seaweeds, widely known as macroalgae, are predecessors of all terrestrial plants. Macroalgae can be divided into three major categories: brown, green, and red macroalgae. Seaweeds lack structures such as roots and leaves, which are typical of terrestrial plants. Thus, seaweed does not utilize nutrients in the same way as terrestrial plants do. Seaweeds have a rootlike structure called holdfast, which serves to attach the seaweed to substrates [6]. The rest of the seaweed structure is called thallus. The thallus contains pigments for photosynthesis. Because seaweeds lack roots for nutrient uptake, the thallus or thalli are used to obtain nutrients from the surrounding waters by diffusion and active transport [7]. This particular feature of seaweed allows it to absorb nutrients in the form of salts and metals, which are available in seawater. Abdel-Raouf et al. [8] reported that seaweed, in its natural and extracted form, is able to absorb heavy metals from synthetic wastewater and metallurgy wastewater. Naturally found seaweed provides an attractive and cost-effective solution for heavy metals removal from landfill leachate. The red seaweed (*Gracilaria* sp.) mainly constitutes polysaccharide agarose and carrageenan; thus, it has a high potential for heavy metals accumulation. Metal ion uptake by biomass occurs through interaction with the cell walls. This is due to the presence of various functional groups, such as carboxyl, amino, sulfate, and hydroxyl groups, which act as binding agents, and involve ionic interactions and complex formations between metal cations and ligands on the surface of the seaweeds.

This study aims to optimize the anaerobic treatment by using a UASB reactor and utilizing seaweed extract as an adsorbent of heavy metals for leachate treatment. The research-specific objectives for accomplishing the aims are as follows: to evaluate the efficiency of UASB reactor in the landfill leachate treatment at various organic loading rates (OLRs) and to assess the performance of the UASB reactor with the addition of seaweed extract (recyclable uniform bead [RUB]). Most of the previous studies on the treatment of landfill leachate using anaerobic reactor concentrated on the removal of chemical oxygen demand (COD), ammoniacal nitrogen, and color, but neglected the heavy metal degradation in the process. To date, there is no reported study on the use of UASB combining with seaweed extract for the treatment of landfill leachate containing heavy metals.

2. Materials and methods

2.1. UASB reactor

The UASB (Fig. 1) used in this experimental study was 18 cm in internal diameter and 110 cm in height, with an active volume of 20 L. The reactor had a 3-phase separator baffle (pore diameter of 2 mm) placed 2 cm below the effluent ports to prevent floating granules from being washed out with the effluent. Sampling ports were placed at 8-cm intervals (lowest being 21 cm from the base) that allowed biological solid and liquid samples to be withdrawn from the sludge bed. The influent wastewater entered through a 2.7-cm down comer tube at the head plate that extended to within 105 cm of the reactor base and allowed the feed to flow upward through the sludge bed. A temperature controller and heater were installed to maintain the reactor temperature at 37°C.

2.2. Landfill leachate

The landfill leachate was obtained from an ageing leachate treatment pond at Jinjang transfer station, in Selayang, Selangor, Malaysia, and had the following characteristics: pH = 8.0, COD = 2,500 mg L^{-1}, As = 9.40 mg L^{-1}, Fe = 12.8 mg L^{-1}, Ni = 0.50 mg L^{-1}, and Cd = 0.43 mg L^{-1}. The leachate used in this study was collected at once and stored, then was used throughout the study. Thus, the characteristics were constant.

2.3. UASB operations

The reactor was seeded with anaerobically digested sewage sludge (Bunus Sewage Treatment Plant, Kuala Lumpur). 7.5 L of sieved sludge (by using a 2-mm mesh) was filled into the UASB, and the remaining volume being filled with tap water. This amount of sludge substantially contributed to the solid requirement in the reactor system after settling. After seeding, the head plates were attached, and the headspace above each compartment was flushed with nitrogen gas to displace residual air in the system before introducing the feed. The reactor was allowed to stabilize at 37°C for 7 d without further modification. The start-up of the reactor was carried out by using dilute leachate with a very low COD concentration. Once the reactor attained a steady state

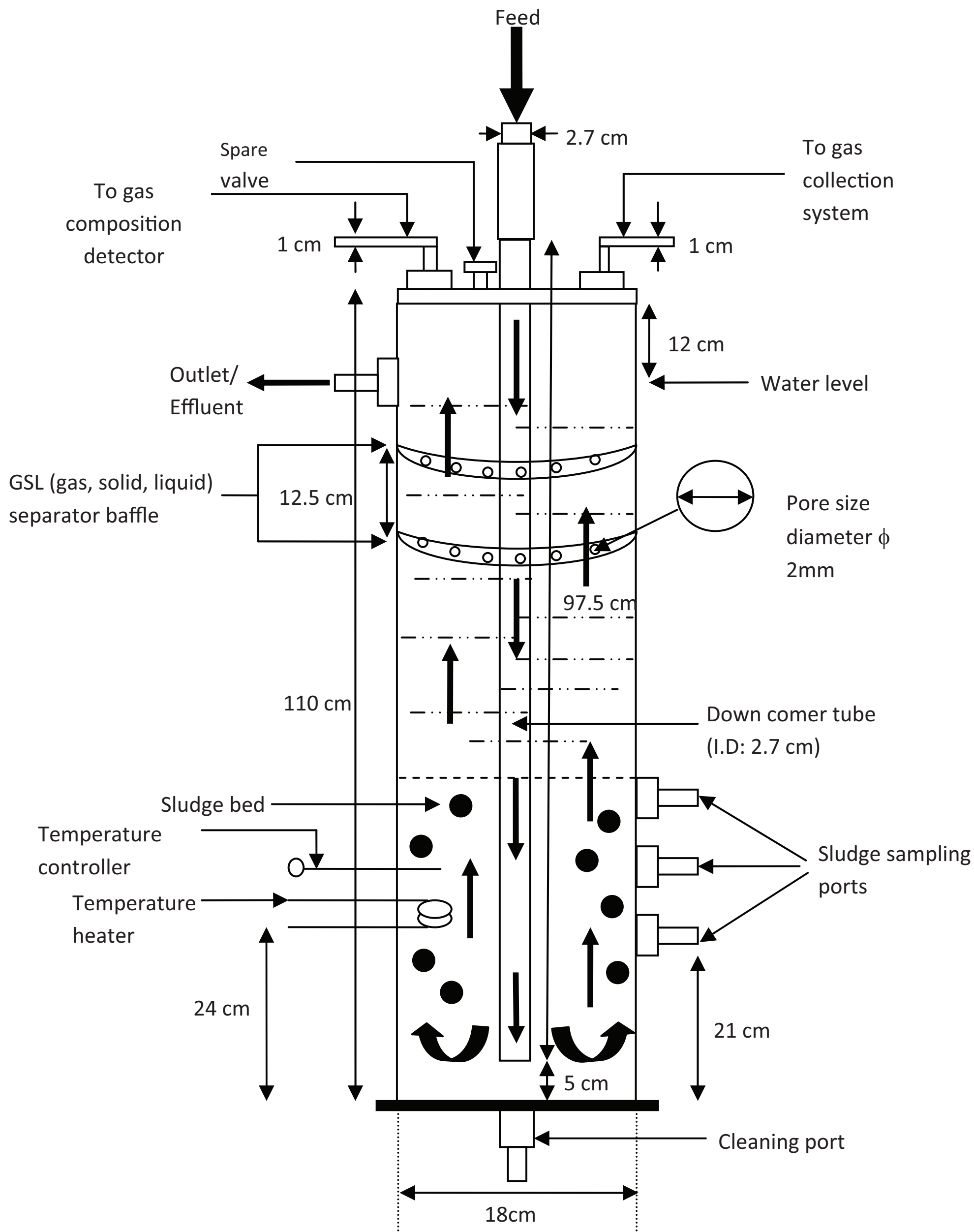

Fig. 1. Schematic diagram of the UASB and feed flow.

condition (>80% COD removal), the feed (leachate) concentration was gradually increased by reducing the amount of water added. The OLR was increased stepwise from 0.125 to 0.833 kg COD m^{-3} d^{-1} and increased further to 2.5 kg COD m^{-3} d^{-1} by reducing the hydraulic retention time (HRT) from 4 to 1 d. Finally, the OLR was again reduced to 0.375 kg COD m^{-3} d^{-1} (HRT 4 d) to determine the ability of the reactor to recover the treatment efficiency (Table 1). The OLR was returned to low level to observe the reactor capability to return to stable condition. The optimum macronutrient to COD ratio was maintained at COD:N:P = 250:7:1 by adding N100 (Bio-Systems Corporation Asia Pacific Sdn Bhd) macronutrient supplement. The choice of this nutrient was based on inadequate nutrients in the landfill leachate. There were no excessive nutrients added to the feed as N100 was first

Table 1
Summary of reactor operating conditions during the investigation of OLR on leachate treatment process

Feed COD (mg L^{-1})	OLR (kg COD m^{-3} d^{-1})	HRT (d)	Duration (d)
500	0.125	4.0	1–20
1,500	0.375	4.0	20–40
2,500	0.625	4.0	40–60
2,500	0.833	3.0	60–75
2,500	1.25	2.0	75–85
2,500	2.50	1.0	85–90
1,500	0.375	4.0	90–105

diluted 10 times of its original concentration. In addition, the reactor was operated by using these nutrients, previously used for the treatment of palm oil mill effluent and showed stable reactor operations. Average values of the measured parameters quoted for each OLR were based on three data points taken when the reactor achieved a steady state.

2.4. Sampling and analysis

Sample analysis, such as COD, pH, and volatile fatty acid (VFA), was conducted according to standard methods [9]. The total biogas volume was determined by using an optical gas-bubble counter. The biogas composition was determined by using a portable gas analyzer (GA2000, Geotechnical Instruments). Heavy metal analysis of the leachate was conducted by using an atomic absorption spectrometry (AA-7700, Shimadzu Corp.).

2.5. Recyclable uniform bead

The dried local seaweed species of *Gracilaria* sp. was made into gels of different concentrations and tested for several properties, such as gel strength, gelling and melting temperature, and alginate yield before proceeding to bead development. The following sections describe the gel properties analysis.

2.5.1. Agar yield

Agar yield was examined according to the method described by the Food and Agriculture Organization (FAO) [10]. A fresh sample of seaweeds was weighed (wet weight), and 100 g of the fresh sample was dried in various conditions, such as air drying, solar drying, and oven drying at 60°C and 100°C temperatures. Then, the dry weight of each sample was determined. A 10 g sample from each drying condition was obtained and boiled in 500 mL water for 1 h. The extract produced from the boiling was then filtered through a glass microfiber filter and allowed to cool. The filtrate was frozen and oven dried for 24 h at 60°C. The final weight of the dried sample was measured to determine the agar yield under various drying conditions.

2.5.2. Gel strength

Gel strength was measured via a penetration test according to Marinho-Soriano and Bourret [11] and Kumar and Fotedar [12]. The gel strength was determined by a plunger with 1-cm^2 surface area and by penetrating the gel at a speed of 1 mm s^{-1} to a depth of 5 mm.

2.5.3. Gelling temperature and melting temperature

These experiments were performed according to methods described by Kumar and Fotedar [12] and Freile-Pelegrin and Murano [13]. A spherical glass bead was placed onto the gel, which was about to be cooled during the cooling phase of the agar yield study. Then, the gel was allowed to cool down together with the glass bead in it and the gel matrix, together with the glass bead entrapped in it, was reheated. Thus, the melting point of the gel is the temperature at which the glass bead entrapped in the gel matrix completely sinks into the gel and reaches the bottom of the container.

2.5.4. Alginate yield

Alginates are polysaccharides found in seaweeds that have carboxyl group in their molecules for metal chelation and absorption of chemical molecules. This study is based on the methods illustrated by the FAO [10]. A sample of 10 g of fresh seaweeds was soaked in a sodium bicarbonate solution. The alginic acid salts will precipitate in the alkaline sodium bicarbonate solution. This alkaline solution was then filtered by using a microfiber glass filter paper. Then the filter paper was washed with hydrochloric acid. The precipitated insoluble alginic acid was collected on the filter paper. By calculating the difference between the initial weight of the filter paper and the final weight of the filter paper, the alginate yield can be determined.

2.5.5. Gel characterization by Fourier-transform infrared spectrometer (FTIR)

A concentrated solution of the gel was prepared in a suitable solvent (CH_2Cl_2). A small amount (2–5 mg) of the gel was directly placed on the plates and one drop of solvent was added. The potassium bromide plates were thoroughly cleaned after this procedure to prevent contamination of future samples. The windows were wiped with a tissue and then washed several times with a solvent, then ethanol. The polishing kit in the lab was used to polish the window surface. The cleaned surface was clear and free from scratches. The infrared spectra of the gel were determined by using an FTIR (Agilent Cary 600 Series FTIR). The gel was ground with spectroscopic (IR) grade potassium bromide (KBr) powder and then pressed into 1-mm pellets for FTIR measurement in the wavenumber range of 600 and 4,000 cm^{-1} via 16 scans.

2.6. Development of RUB

After a methodical study of the properties of seaweeds, agar development of spherical beads can begin. The spherical shape provides a high surface-area-to-volume ratio, which increases the efficiency of treating pollutants from leachate. Cold used oil spherification method was used to develop the seaweed beads. Based on the results of the gel test and jar test, the best seaweed species and optimum gel concentration were used to make beads. An optimum amount of dried seaweed was introduced into a 1-L beaker, and 0.4 mL of water was added. The mixture was left to boil for 1 h. After 1 h, the hot extract was filtered and left to cool for 15 min. The cooled extract solution was immediately introduced into the cold used oil (refrigerated for 24 h prior to the experiment) by using a syringe to produce spherical beads. Due to the low temperature and insolubility in oil, the boiled extract will gelify in spherical shapes at the bottom of the container.

2.7. Spherical bead filter

From the results of gel strength, gelling temperature, and melting temperature, a spherical bead filter was designed.

Based on the working temperature range, a suitable material to store the filter medium was plexiglass. This is due to the heat insulating properties of plexiglass. Another advantage of plexiglass is that it is transparent; thus, the operation of the filter medium could be observed by the eye. The filter was cylindrical in shape, with a base of 5-cm diameter and 30-cm height. This filter design was optimal to support stacks of spherical beads on top of each other without crushing the beads. The bead had a diameter of 0.5 cm, and it was loosely packed to allow feed flow without resistance, and at the same time, the loosely packed beads would also ensure that there was no clogging in the filter medium.

3. Results and discussion

3.1. Treatment of leachate by UASB

The average pH variations in the UASB when the OLR was gradually increased from 0.125 to 2.50 kg COD m^{-3} d^{-1} (Fig. 2(a)). The pH levels were stable (pH 8.37–7.53) in the UASB until the reactor OLR exceeded 0.625 kg COD m^{-3} d^{-1}. During this period, the VFA concentration was observed to be low (25–250 mg L^{-1} HOAc). At a reactor OLR of 0.833 kg COD m^{-3} d^{-1}, the pH in the reactor dropped to 6.77 due to the rapid production of VFA resulting from the increased acidogenic activity. A further increase in the OLR to 1.25 and 2.50 kg COD m^{-3} d^{-1} diminished the pH of the reactor to 6.10 and 5.48, respectively. However, when the reactor OLR was reduced back to 0.375 kg COD m^{-3} d^{-1}, the pH in the reactor recovered to 8.37, indicating that the acidogenesis and methanogenesis had recovered to previous levels under low OLR conditions. From the pH data, it can be assumed that the metabolic processes differed between each OLR of the UASB system, causing each OLR to favor a unique population of microorganisms. As displayed in Fig. 2(a), the VFA concentration in the reactor was lower than 300 mg L^{-1} HOAc, considered acceptable for a UASB reactor system [14]. The VFA concentration was observed to be low when operated at an OLR in the range of 0.125–1.25 kg COD m^{-3} d^{-1}. Increasing the OLR beyond 1.25 kg COD m^{-3} d^{-1} resulted in higher VFA concentrations in the effluent. A drastic increase in VFA concentration was observed (255.7 mg L^{-1} HOAc) at OLR of 2.5 kg COD m^{-3} d^{-1}. When the OLR was reduced to 0.375 kg COD m^{-3} d^{-1}, the VFA concentration began to decline and stabilized to 157.3 mg L^{-1} HOAc. Reduced contact time between the substrate and biomass in UASB favored the activity of acidogens, leading to decreased methanogen activity in the reactor [15]. Some of the variations in the VFA profiles may be influenced by the presence of inhibitory substances, such as heavy metals [16]. At an OLR of 0.125 kg COD m^{-3} d^{-1} (HRT 4 d), the average COD removal efficiency was 65.70% as per Fig. 2(b). The increase of the OLR from 0.375 to 1.250 kg COD m^{-3} d^{-1} resulted in a decreasing COD removal efficiency until 9.33% was observed at an OLR of 2.50 kg COD m^{-3} d^{-1}. It is unlikely that this was caused by limitations in the UASB reactor as this reactor was shown to achieve over 90% COD removal at high OLR (e.g., more than 20 kg COD m^{-3} d^{-1}) [17]. However, matured landfill leachate containing a high proportion of recalcitrant and complex organic carbon content may limit the UASB performance at high OLR. Moreover, heavy metals concentrations in the feed (leachate) may also contribute to the poor performance of the reactor system [18]. Organic matters washed out from the reactor in the form COD may have contributed to the overall low removal of COD.

The average methane composition fluctuated from 38.50% to 9.53% as per Fig. 2(c), likely due to the changes in the OLR, because the methanogenic bacteria are sensitive to the changes in feed OLR. As an indirect measure of biomass fluctuations in the reactor, the suspended solids (data not provided) in the reactor correlated well with the methane generation. The methane composition profile followed the COD removal efficiency, where the concentration was reduced concomitantly with COD removal. A similar trend was also observed for the VFA profile. Abbassi-Guendouz et al. [19] demonstrated that VFA decreased when methane composition decreased in an anaerobic treatment process. The methane profile has a close relationship with pH where a decrease in the pH affects the methane generation [20]. Overall, the methane percentage was low first of all due to the fact that matured leachate contains a less organic fraction. Besides that, the high pH value could also be the reason for lack of methanogenic activity.

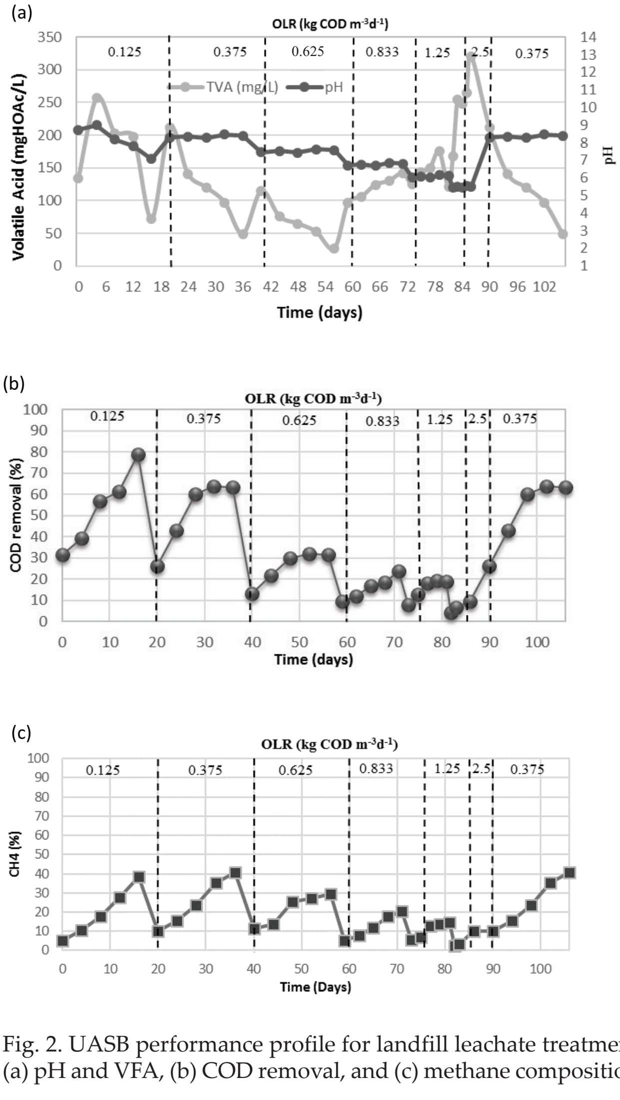

Fig. 2. UASB performance profile for landfill leachate treatment: (a) pH and VFA, (b) COD removal, and (c) methane composition.

3.1.1. Heavy metal removal

The effect of OLR on the heavy metals removal is shown in Table 2. It can be seen that the removal of Cd, Ni, and Fe was almost constant, regardless of the OLR (around Cd [36%], Ni [32%], and Fe [29%]). The stable population of bacteria appears to tolerate the introduction of these metals into the reactor system when the OLR gradually increased from 0.125 to 2.5 kg COD m^{-3} d^{-1} and decreased back to 0.375 kg COD m^{-3} d^{-1}. In contrast, the degree of removal at low OLR's (e.g., 40% at 0.125 and 0.833 kg COD m^{-3} d^{-1}, respectively), but at high OLR (e.g., 2.5 kg COD m^{-3} d^{-1}), was dramatically decreased (3.83% and 7.81%, respectively). When the OLR was decreased back to 0.375 kg COD m^{-3} d^{-1}, some removal was still evident (around 26%), signifying that the anaerobic microorganisms in the reactor were capable of recuperating from the shock load. The removal of heavy metals could have occurred by either bioaccumulation or accumulation in sludge [21]. Bioaccumulation was less likely to be possible, thus, the pollutants might have accumulated in the sludge. One important observation in this study was the consistency in heavy metal removal (%) at all the OLR's investigated. Even though the heavy metal removal efficiency is constant, the concentration of feed and effluent varies at each OLR. It can be seen that the heavy metal concentration of feed and effluent increased when the OLR was gradually increased from 0.125 to 2.500 kg COD m^{-3} d^{-1}. The constant removal (%) of heavy metals probably to the nature and the capability of the reactor performance which limits the removal to a certain degree, for example, 40% removal at OLR 0.125 to 8.833 kg COD m^{-3} d^{-1}.

3.2. Leachate treatment by UASB and RUB

After successfully creating RUB and placing it into the adsorbent holder, a pre-treatment step to enhance the treatment of leachate and then conducting UASB treatment was performed. In this phase, the reactor was operated at a fixed HRT (4 d) and OLR (0.375 kg COD m^{-3} d^{-1}), and performance was evaluated and compared before and after the combination of RUB with the UASB. The pH variations when the leachate was treated by UASB and UASB + RUB are illustrated in Fig. 3(a). The pH levels were generally stable (7.98–7.01) in the treatment of leachate by UASB and in the treatment of leachate by UASB + RUB (7.51–6.64). pH is an important parameter in anaerobic treatment performance, and many studies have shown that the optimum pH for the anaerobic digestion is in the alkaline region [22]. The anaerobic process could be advantageous to methanogenic bacteria when the pH ranges from 6.5 to 7.5 [23]. From the pH profile, it can be assumed that the UASB + RUB treatment of leachate takes place at a more suitable pH for methanogenic bacteria activity as compared with UASB treatment of leachate.

VFA concentrations were better in UASB + RUB than in UASB treatment (Fig. 3(b)). This was evident because the VFA values registered were lower after UASB + RUB treatment of leachate than UASB treatment. The VFA concentration is an indicator of feed utilization by anaerobic microorganisms. When there is a buildup of VFA in the anaerobic system, it is probably an indication of the anaerobic microorganism's failure to utilize the VFA as feed. Conversely, the results of UASB + RUB and UASB treatment leachate revealed that there was no obstruction to VFA utilization by anaerobic microorganisms as all the VFA registered readings within the range that indicated a healthy anaerobic system [24]. However, the addition of RUB enhanced the leachate treatment by almost 50% as compared with the average value of VFA. Results of COD removal by UASB + RUB and UASB treatment of leachate are illustrated in Fig. 3(c). Both treatment systems attained a constant COD removal after Day 20, however, the values attained had set them apart. The UASB + RUB treatment of leachate has an edge over UASB treatment of leachate as it could achieve removal up to 93%. The remainder of untreated COD in the effluent probably belongs to inorganic constituents or originated from bacterial waste generated during the UASB + RUB treatment process itself. The addition of RUB to the UASB system improved the COD removal by 15%. The average methane composition at steady state was 71.3% and 64.17% (Fig. 3(d)) for UASB + RUB and UASB treatment of leachate, respectively. The methane composition profile followed the COD removal efficiency pattern,

Table 2
Effect of OLR on heavy metal removal

Heavy metal concentration ($mg\ L^{-1}$) and removal (%)		OLR (kg COD m^{-3} d^{-1})						
		0.125	0.375	0.625	0.833	1.250	2.500	0.375
As	Feed	0.47	1.92	2.35	3.13	4.70	9.40	1.92
	Effluent	0.28	1.15	1.41	1.88	3.82	9.04	1.42
	Removal	40.00	40.00	40.00	40.00	18.72	3.83	26.04
Cd	Feed	0.02	0.07	0.11	0.14	0.22	0.43	0.07
	Effluent	0.01	0.04	0.07	0.09	0.14	0.27	0.04
	Removal	36.36	36.92	37.04	36.36	36.28	36.28	36.92
Ni	Feed	0.025	0.075	0.125	0.167	0.25	0.50	0.075
	Effluent	0.017	0.05	0.084	0.112	0.168	0.336	0.05
	Removal	32.00	33.33	32.80	32.93	32.80	32.80	33.33
Fe	Feed	0.64	1.92	3.20	4.26	6.40	12.80	1.92
	Effluent	0.45	1.35	2.25	3.00	4.50	9.00	1.35
	Removal	29.69	29.69	29.69	29.69	29.70	29.70	29.69

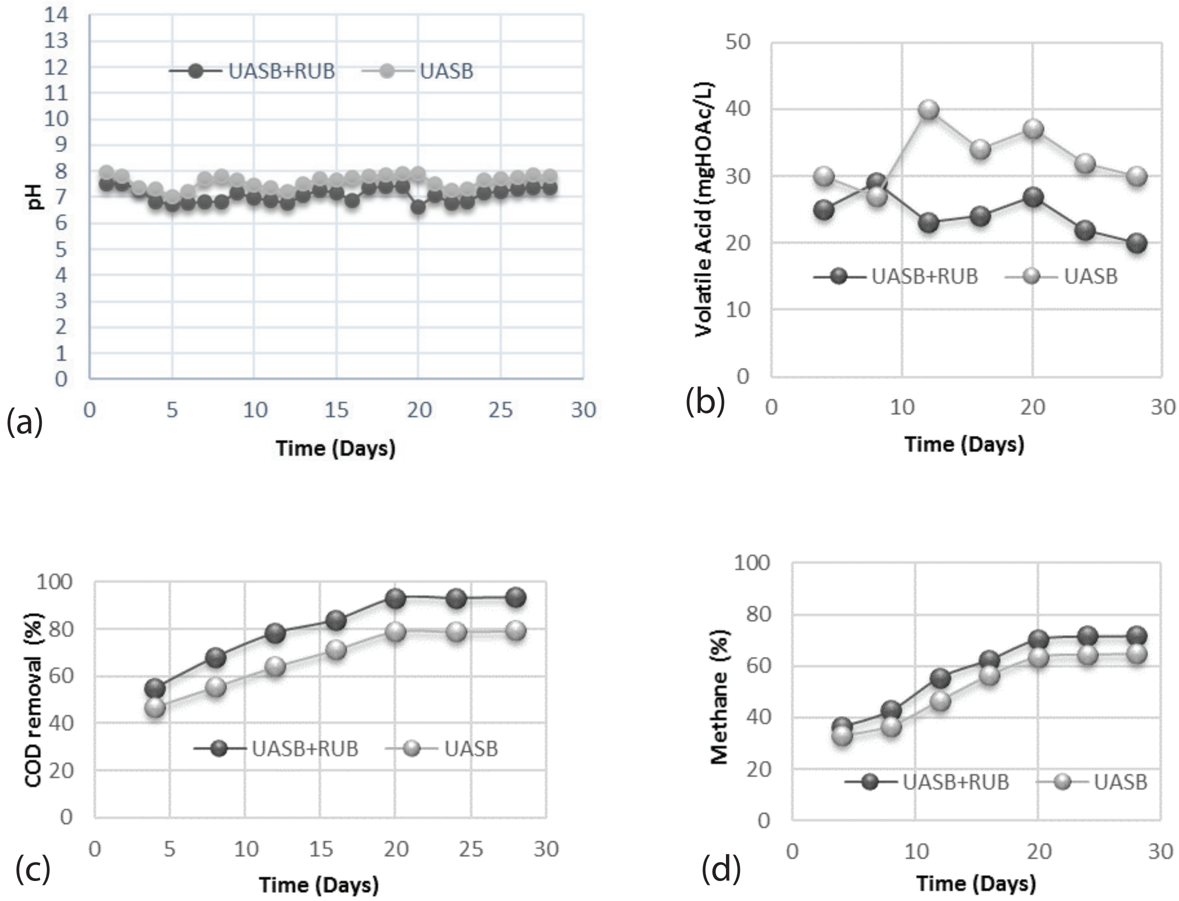

Fig. 3. UASB performance profile before and after the treatment with RUB: (a) pH, (b) volatile acid, (c) COD removal, and (d) methane composition.

whereby, it steadily increased up to 20 d and then stabilized for both treatment systems. The combination of RUB with UASB increased the methane composition by 7.13% on average at steady state.

3.2.1. *Heavy metal removal*

Table 3 shows the heavy metals removal in leachate by UASB + RUB and UASB. This indicated that the anaerobic microorganisms utilized the pollutants or it was absorbed by the RUB. This explains the trend observed in heavy metals speciation in this study (data not provided), whereby UASB + RUB always displayed a higher efficiency in heavy metals removal from leachate as compared with UASB. UASB treatment of leachate registered poor treatment effectiveness for heavy metals, as the removal for As, Cd, Ni, and Fe was 40.00%, 36.36%, 32.00%, and 29.69%, respectively (Table 3). However, when the leachate was treated with UASB + RUB, the removal efficiency of As, Cd, Ni, and Fe drastically increased to 95.4%, 100%, 100%, and 100%, respectively. *Gracilaria* sp. based adsorbents are capable of adsorbing 0.15 to 0.76 mmol g^{-1} of Cd and 0.2 mmol g^{-1} of Ni, which is equivalent to 0.058 to 0.2939 mg L^{-1} of Cd and 0.0773 mg L^{-1} of Ni [7].

Table 3
Heavy metal removal profile by UASB + RUB and UASB

Treatment type	Heavy metal removal (%)			
	As	Cd	Ni	Fe
UASB	40.00	36.36	32.00	29.69
UASB + RUB	95.4	100	100	100

3.2.2. *FTIR spectra of RUB pre and post leachate treatment*

This section discusses the results obtained from FTIR spectroscopy analysis of RUB before and after they were used in leachate treatment. The spectral overlay of RUB prior to the leachate treatment at the beginning RUB extracted from the adsorbent holder after the leachate treatment at the end of the experiment was illustrated in Fig. 4. The increase of absorbance in 3,500–3,000 cm^{-1}, 1,700–1,500 cm^{-1}, 1,200–1,000 cm^{-1}, and 661 cm^{-1} indicates clearly that RUB is the major component that has worked to remove the heavy metals.

From Table 4 it is evident that functional groups or compounds that are present in the RUB are the major adsorbing agents. A study by Yanagisawa et al. [25] also confirms the presence of all the aforementioned functional groups and compounds that are present in the *Gracilaria* sp. sample. RUB made of *Gracilaria* sp. proves to be a good adsorbent for the

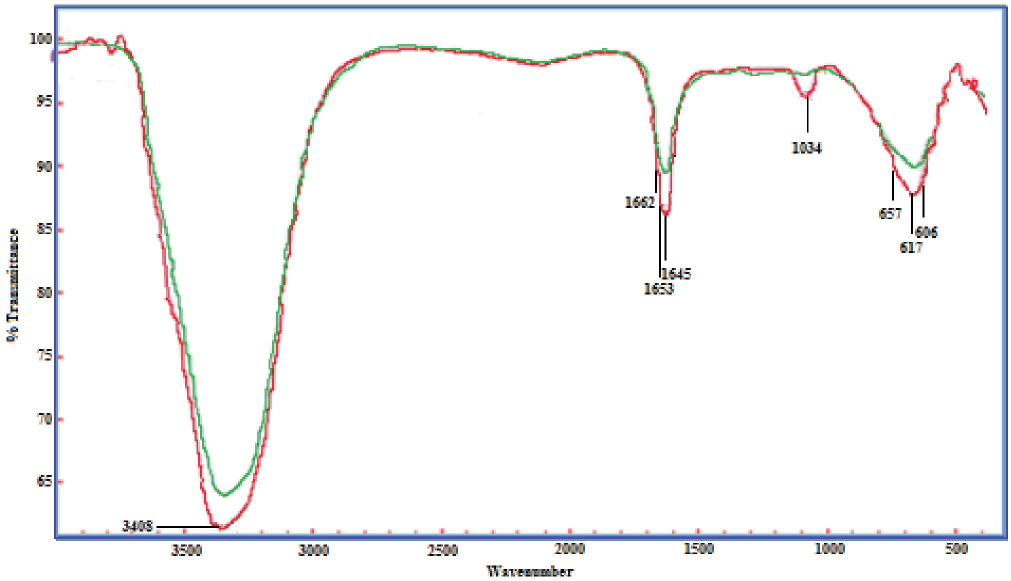

Fig. 4. Overlay of FTIR spectroscopy of RUB pre and post leachate treatment (red: posttreatment, green: pretreatment).

Table 4
Characterization of RUB using FTIR

Gracilaria sp. Absorption frequency (cm^{-1})	Functional groups	Compound
3,408	N–H stretching and O–H stretching	Amino acids and polysaccharides
1,662, 1,653, and 1,645	C=O stretching, N=O asymmetric, and stretching (nitrate)	Ester and pectin
1,560	C=C stretching	Lignin
1,457 and 1,430	C–O stretching and O–H bending	Cutin
1,246	C–C–O and S=O stretching	Lignin
1,193	C–O stretching (phenols) and C–F stretching	Cellulose
1,102	C–F stretching and Si–O	Cellulose and carbohydrates
1,034	S=O stretching (sulfonides)	Starch and polysaccharides
657, 617, and 606	C–S stretching and C=S stretching (sulfides)	Sulfates

pollutants present in matured leachate sample. This is evident from the results (Fig. 4 and Table 4) of FTIR study of the bead before and after adsorption study. The results revealed that there were four major chemical components responsible for pollutants removal from the matured leachate. The first region was found to be 3,408 cm^{-1}, where it indicated the N–H or amine, O–H or hydroxide bond which can be found in polysaccharides or water molecules. This region was found to possess prominent absorbance as compared with other regions in the FTIR result, which shows that it is the most abundant functional group present in the RUB. Consequently, the order of functional group abundance was followed by C=O or carboxylic, N=O or amide, S=O or sulfinyl, and C=S or sulfides. The comparison was made respective to the absorbance area acquired by each functional group. The results revealed that pollutants (heavy metals) could be adsorbed by more than one functional group.

Despite the multifaceted adsorbent nature of the functional groups in the RUB, the common factor which acts as the driving force of the adsorption process is the lone pair of electrons present in the functional group. Lone-pair electrons signify the pair of valence electrons which are not shared with an adjacent atom. According to Lewis structure of functional group and quantity of lone pair of electrons [26], functional group with the lone-pair electrons is sulfinyl (S=O) which has four lone pair of electrons, followed by hydroxides (O–H), and amides (N=O) which has three lone pair of electrons, carboxylic (C=O) functional group has two lone pair of electrons and amine (N–H) and sulfide (CS) have one lone pair of electrons each. Heavy absorbance at the O–H and N–H regions of the FTIR spectra indicates that the dominance of this functional is in abundance. Apart from the sulfinyl presence, sulfide is also heavy in terms of absorbance in FTIR spectra. That is evident when there is a sharp increase in absorbance of RUB after treatment spectra (Fig. 4). In Fig. 4, it was also clear that the C=O and N=O also have a sharp increase in absorbance in RUB after treatment spectra. From this result, we could only conclude that O–H, N–H, C=O, N=O, S=O, and CS are responsible for the removal of pollutants in matured leachate. Further study is required to determine which functional group has the most significant role in the removal of these pollutants from the matured leachate.

4. Conclusions

This research was initiated with the main aim of optimizing the anaerobic treatment by using a UASB reactor for leachate treatment by utilizing seaweed extract as an adsorbent of heavy metals. From the experimental results, there appears to be considerable potential for the UASB combined with the RUB system to be implemented on site for treatment of matured landfill leachate. However, a techno-economic feasibility study of the treatment system should be conducted in future to calculate the actual cost of the process at the landfill site. Moreover, an experiment should also be conducted to recover the heavy metals that were adsorbed by the seaweed. The treatment efficiency of the reactor was affected at an OLR of 2.50 kg COD m^{-3} d^{-1}, probably due to the recalcitrant nature of the wastewater containing high levels of heavy metals at elevated OLR. At high OLR, the concentration of heavy metals may have increased many folds, which may have inhibited the methanogens. The UASB + RUB treatment of leachate has the edge over UASB treatment of leachate as it could achieve removal up to 93%. UASB + RUB always has a higher efficiency in heavy metals removal from leachate as compared with UASB. The FTIR study of RUB after treatment revealed that there was an increase of absorbance and clearly indicated that RUB was the major component that has worked to remove the heavy metals.

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