Potential of *Chromolaena odorata* and Indigenous Bacterial Consortium for Oily Wastewater Treatment

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Abstract: This study was carried out to investigate the possibility of using *Chromolaena odorata* as absorbent and to ascertain as to whether indigenous bacteria has the potential to degrade hydrocarbon from oily wastewater and thereby reducing its toxicity and other negative impacts to the environment particularly on aquatic life. The potential of *Chromolaena odorata* and indigenous bacteria to remove oil from wastewater was evaluated by measuring total suspended solid (TSS) concentration. The result shows that, about 55% of oil substances were absorbed or removed after 4 hours of *Chromolaena odorata* application with the optimum dosage of 4mg/L. Growth percentage of bacterial consortium and single pure strain on medium containing oily wastewater was studied. It was found that bacterial consortium, the mixture of *Bacillus cereus* (KA) and *Acinetobacter* (TB) shows higher growth among other bacterial consortium and single pure cultures and this consortium was used for further analysis. In addition, the results have shown that, bacterial consortium (KA+TB) exhibited high efficiency to remove TSS at the optimum dosage of 1 mg/L. Absorption and biodegradation rate was increased after being treated with *Chromolaena odorata* and bacterial consortium mixture. The mixture of *Chromolaena odorata* powder and bacterial consortium in 4:1 ratio was obtained to significantly removed TSS by 75% in 24 hours.

Keywords: Bacterial consortium, *Chromolaena odorata, Bacillus cereus* (KA), *Acinetobacter* (TB), TSS, oily wastewater.

INTRODUCTION

Some of the automotive workshops do not have access to an integrated wastewater treatment plant (WWTP) to treat oily wastewater which was generated from automotive workshop activities. Currently, this oily wastewater was discharged directly into stream and eventually to river systems. The organic and in-organic chemicals in oily wastewater that was exceeded the maximum permissible limit set under Interim National Water Quality Standard for Malaysia (INWQS) and Standard B, Environmental Quality (Sewage) Regulations, 2009 was believed to pose negative impacts to the environment especially aquatic life. There are also long-term effects on ecosystems related to the release of toxic components over a prolonged period as the oil breaks up and the concentration of toxicants in organisms towards the top of the food chain increases [1]. Oily wastewater from these automotive workshops must be treated before being released into the environment in order to avoid pollution. In addition, oily waste containing petroleum compound are considered to be recalcitrant to microbial degradation and persist in ecosystems because of their hydrophobic nature and low volatility and thus they pose a significant threat to the environment [2]. Biodegradation of oily waste is involved the mechanism for removing the toxicity and non- volatile component of oil from environment. Meaning that, oily waste degrader microorganisms would be able to breakdown oily waste compound into harmless compound such as carbon dioxide. Microorganisms have enzyme systems to degrade and utilize oily waste as a source of carbon and energy [3].

Several works on the biodegradation of hydrocarbon have been reported including report on the isolation of hydrocarbon degrading microorganisms. In addition, petroleum hydrocarbon can be degraded by various microorganisms such as fungi, yeast and bacteria [4]. In order to enhance the hydrocarbon biodegradation rate, the hydrocarbon degrading mixed culture could be prepared by combining number of microorganisms to establish a defined mixed culture [5].

Bacterial consortium or mixed cultures is defined as a two culture or more bacteria in which each organism's benefits from the other. Mixed culture was prepared by combining a number of microorganisms in order to enhanced biodegrading capabilities. Even then, the selection of strain forming a defined mixed cultured should be based on the efficiency of microorganisms to metabolize petroleum compound [5]. This study also has been carried out to assess the interaction between microorganisms able to degrade different types of hydrocarbon.

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Absorption is a method to remove organic and inorganic pollutant in wastewater using purification and separation techniques. In this method, the solid waste product was used as absorbent for removing the pollutant material from the wastewater [6]. The example of sorbent based on an agricultural by product had been used for wastewater treatment which including orange peel [7], banana pith [8], sawdust [9], powdered waste sludge [10], wheat shells [11], wheat straw [12] and wheat bran [13]. The application of organic material such as volcanic rock, compost, straw, bagasse and etc can help to accelerate degradation of toxic compound [14]. However, the application of *Chromolaena odorata* as adsorbent and removal of pollutant material from oily wastewater has not been reported.

Chromolaena odorata is one of the world's worst tropical weeds. It is a member of the tribe Eupatorieae in the sunflower family Asteraceae. The weed goes by many common names including Siam weed, devil weed, French weed, communist weed, hagonoy, co hoy etc. *Chromolaena* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, anti-microbial, cytoprotective and many other medicinally significant properties [15].

The aims of this paper are to assess the potential of *Chromolaena odorata* to absorb hydrocarbon in oily wastewater and the ability of indigenous bacteria to degrade hydrocarbon.

MATERIAL AND METHODS

Oily Wastewater Sampling

Oily wastewater samples were collected from several automotive workshops in Kota Bharu, Kelantan, Malaysia. Oily wastewater samples were collected in sterile sampling bottle which was cleaned by using sulfuric acid (H_2SO_4). Sample was kept in refrigerator at 4ºC before analysis being conducted.

Preparation of *Chromolaena odorata* as the Absorbent

The stem and leaf of *Chromolaena odorata* was washed in order to remove dust and soil. The stem and leaf of *Chromolaena odorata* was oven dried for two days at 50ºC. Dried stem and leaf of *Chromolaena odorata* was then grinded and sieve through 0.4mm size sieve before it could be use for experiment.

Absorption Test

Absorption tests were performed in batches by using six-place conventional jar- test which consisted of 6 beakers (50 mL). Each beaker was added with 50 mL of oily wastewater and added with difference concentration of *Chromolaena odorata*: 1mg/L, 2 mg/L, 3 mg/L, 4 mg/L and 5mg/L. Beaker with 50 mL of oily wastewater without *Chromolaena odorata* was used as control. The experimental process involved 3 minutes of rapid mixing at 200rpm followed by slow mixing for 30 minutes at 50 rpm and finally the mixture was left for 20 minutes to settle down before TSS was measured.

Optimum Growth of Bacteria Test

The potential of indigenous bacteria for hydrocarbon utilization were isolated by spread plate technique using 1 mL aliquots of appropriate dilution unto nutrient agar plate. Isolated pre cultured were tested for their ability to grow on mineral salt medium (MSM) with 1 v/v of oily wastewater as sole of carbon sources. All isolated bacteria was cultivated in 25mL universal bottle containing 10 ml liquid MSM supplement with 1% v/v oily wastewater as a sole carbon sources. The cultures were incubated shaking (150 rpm) for 24 hours at 30ºC. The growth of bacteria was monitored by measuring the optical density at 600nm (OD 600 nm) in order to determine the potential of bacteria with high utilization of hydrocarbon. Single bacterial species as well as consortium were used to determine optimum growth of bacteria.

Determination of Optimum Ratio of Bacterial Consortium and *Chromolaena odorata*

In this study, *Bacillus cereus* and *Acinetobacter* sp. were mixed together as bacterial consortium (1:1) to determine ability to degrade hydrocarbon in mineral salt medium (MSM). The experiment was carried out in triplicate for 24 hours. Bacterial consortium was incubating at 30^{o}C with 150 rpm agitated. Each bottle was added with 10mL of MSM containing 4mg/L of *Chromolaena odorata* and 1% v/v of oily wastewater as carbon sources. In order to determine the optimum ratio of *Chromolaena odorata* and bacteria, different concentration of bacteria consortium (0, 0.5, 1.0, 1.5 mg/L) was added to the prepared sample. TSS was measured to determine the optimum ratio of bacterial consortium and *Chromolaena odorata.*

Degradation Test

The mixture of *Chromolaena odorata* and bacterial consortium with ratio 4:1 was used to determine the biodegradation rate of hydrocarbon from oily wastewater. Two beakers were prepared to conduct the experiment; one beaker contained 100 mL of oily wastewater, while the other one contained 100 mL of oily wastewater and mixture of *Chromolaena odorata* and bacterial consortium. Both prepared sample were incubated at 30ºC temperature for 24 hours in incubator shaker at 150 rpm. TSS was measured in order to determine the effectiveness of *Chromolaena odorata* powder and bacterial consortium mixture in treating oily wastewater.

RESULTS AND DISCUSSION

Chromolaena odorata was selected as absorbent material in this study because it has several advantages for example, easy to found and collected, its stem has a sponge with high absorbent properties and easy to growth.

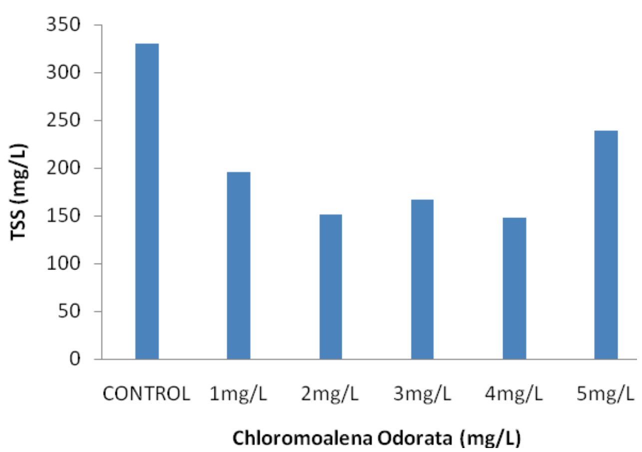

Figure 1: Total Suspended Solid (TSS) concentrations for blank and samples after being treated with different concentrations of *Chromolaena odorata*.

The ability of *Chromolaena odorata* in absorbing oil from oily wastewater is shown in Figure **1**. The result shows that, TSS concentration decreased after *Chromolaena odorata* was added to the oily wastewater. *Chromolaena odorata* showed the highest absorption of oil (55.1%) at the dosage of 4mg/L and the absorption process reached its equilibrium in 4 hour of contact. This observation implies that the sponge in *Chromolaena odorata* stem played an importance role in absorption of oil from oily wastewater and *Chromolaena odorata* stem has a good potential as absorbent due to its capability to absorb the organic and inorganic pollutant in oily wastewater.

Several works on the application of agricultural waste or plant materials as low cost absorbent have been reported including saw-dust [16], apple residues [17], leaves [18], ash [19], etc. The effectiveness of oil palm fibre as low cost absorbent for removal of nickel (Ni) from wastewater was studied [20]. The result of his study shows that, the treated fibre of oil palm was an effective absorbent for the removal of Ni from wastewater and the Ni adsorption pattern followed the Freundlich isotherm. His study also revealed that the optimum agitation time was 90 minutes at pH 7.

Banana peel also shows high potential as a promising adsorbent for phenolic compound [21]. The result of this study similarly revealed that banana peel exhibited a high adsorption capacity of phenolic compound which was able to reduce about 60% to 80% of it in olive mill water.

The physical and chemical interactions between cell wall ligands and adsorbents by ion exchange, complexation, coordination, chelation, physical adsorption and micro-precipitation can be explained by the binding mechanisms of heavy metals through biosorption [22].

In order to evaluate the growth potential of the single pure strain and consortium culture to grow on mineral salt medium supplemented with oily wastewater as carbon sources, turbidity was chosen as the criteria upon which performance was assessed.

The result in Figure **2** showed that the mixture of KA and TB culture was able to grow well in the presence of hydrocarbon substrates and exhibited greatest growth compared to other cultures. In summary, this result has shown that a microbial consortium of KA and TB was found to utilize hydrocarbon better than single pure strain. The result from this study was consistent with what was reported by [23, 24] where they found that a microbial consortium isolated from samples improved the performance for degradation of phenol when compared to single pure strain.

The biodegradation efficiency of mixed culture has been reported by several authors [25]; [26]; [5]; [24]; all of whom has suggested that the mixed cultures were more efficient that the pure cultures at reducing the total amount of diesel fuel. Furthermore, as the two strain defined culture was able to degrade 90% of the aliphatic fraction they concluded that the combination of several microorganisms with different biodegrading capabilities was more efficient compared with the action of single strain in oil degradation [26].

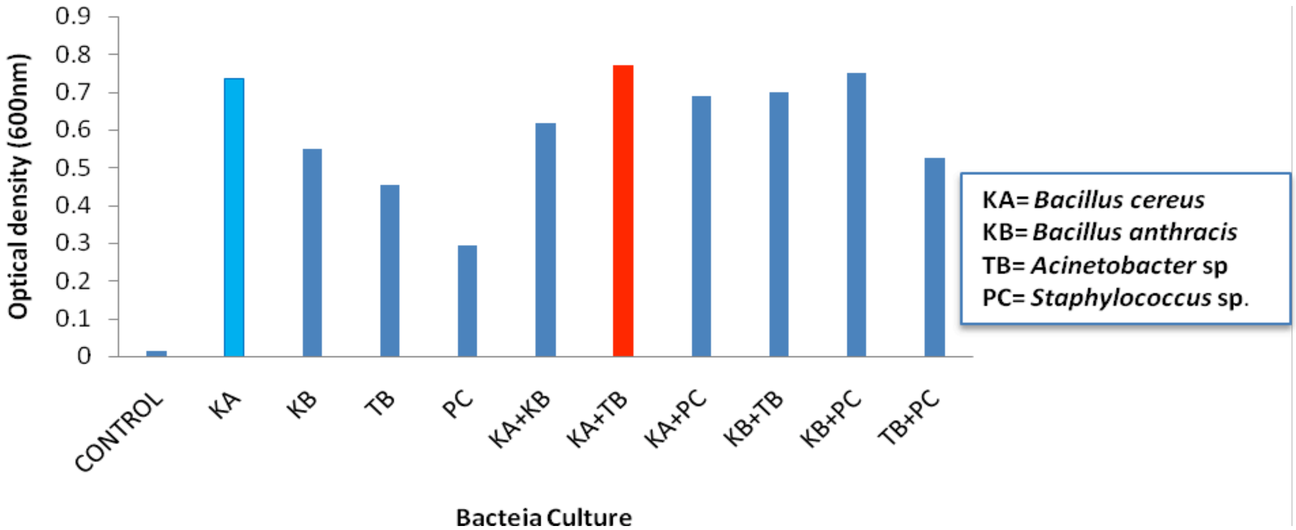

Figure 2: Growth of bacteria consortium and single pure strain in MSM medium containing 1% v/v oily wastewater incubated at 30°C with shaking at 150rpm.

The presence of bacillus strain in mixed culture can contribute to the higher hydrocarbon consumption compared to other mixed cultures without bacillus strain [27]. The ability of *Bacillus* spp. to be more tolerant to high level of hydrocarbon in soil is due to their resistant endospores [28]. In this study, the presence of bacillus sp. in mixed cultures also showed better results compared to other mixed cultures which has no bacillus presence.

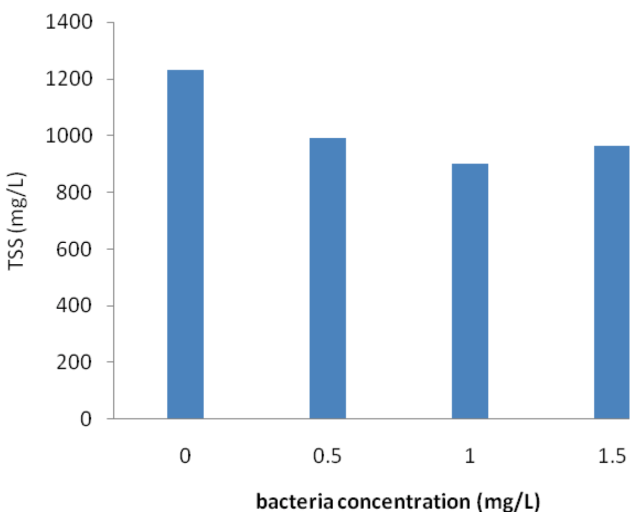

Figure 3: Total suspended solid (TSS) concentrations for samples after being treated by different concentrations of mixed culture.

The results in Figure **3** show that the optimum concentration of bacterial consortium (KA + TB) is 1 mg/L in 4 mg/L of *Chromolaena odorata* or in other words the optimum ratio of *C. odorata* and indigenous bacterial consortium is 4:1 which is four parts of *C. odorata* and one part of indigenous bacterial consortium.

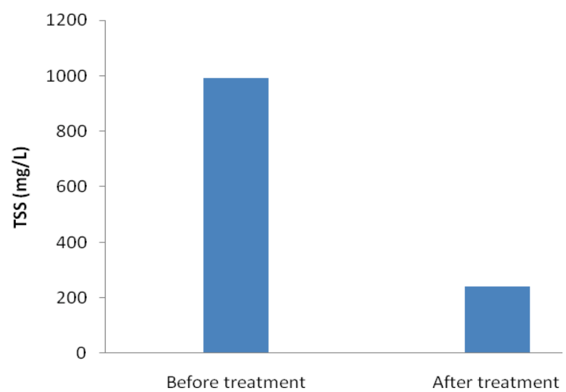

Figure 4: Total Suspended Solid (TSS) concentrations for 100 mL sample before and after being treated by *Chromolaena odorata* containing bacteria mixture.

The concentration of total suspended solids was measured to determine the effectiveness of *C. odorata* and indigenous bacterial consortium mixture to treat oily wastewater. The result shown in Figure **4** illustrated the TSS concentration of oily wastewater after being treated with *C. odorata* and indigenous bacterial consortium mixture compared to that of oily wastewater before treatment. Based on this result, the concentration of TSS was reduced by about 75% after being treated with *C. odorata* and indigenous bacterial consortium mixture. This combination of the *C. odorata* and indigenous bacterial consortium mixture was successful in removing the organic and inorganic pollutants from oily wastewater.

CONCLUSION

The preliminary findings from this study demonstrate that *Chromolaena odorata* exhibited convincingly the ability to absorb pollutants from oily wastewater and further revealed that degradation of oil from oily wastewater increased with the combination of effective bacterial consortium and *Chromolaena odorata*.

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