ABSTRACT

Contamination of soil by organic chemicals, (mostly hydrocarbons) is prevalent in oil producing and industrialized countries of the world. This may pose a great threat to the environment and human being at large. Different treatment methods have been employed to remediate contaminated soil. Bioremediation, a strategy that uses biological means to degrade, stabilize, and/or remove soil contaminants can be an alternative green technology for remediation of hydrocarbon-contaminated soil. This investigation consists of studies with the primary aim of enhancing biodegradation of used lubricating oil in soil with the supplementation of organic wastes. Three organic wastes (brewery spent grain (BSG), banana skin (BS) and spent mushroom compost (SMC)) and two economically viable plants (*Jatropha curcas and Hibiscus cannabinus*) were utilized to enhance biodegradation of used lubricating oil in soil contaminated with different concentrations of used lubricating oil.

Bioremediation of soil contaminated with 5%, 10% and 15% (w/w) used lubricating oil amended with 5% and 10% BSG, BS and SMC were studied for the period of 84 days under laboratory condition. The highest percentage of oil biodegradation (91.8%) was recorded in soil contaminated with 5% used lubricating oil amended with 10% BSG. The least percentage of oil biodegradation (29%) was recorded in soil contaminated with 15% used lubricating oil amended with 5% SMC. Maximum oil biodegradation was recorded in soil contaminated with 5% oil, while medium degradations were recorded in 10% oil contaminated soil. However, partial oil degradation was recorded in soil contaminated with 15% oil concentration throughout the study period.

Organic wastes supplementations of used lubricating oil contaminated soil were studied under natural condition for a period of 12 months. Biodegradation of used lubricating oil was higher in all the different concentration of oil pollution (5%, 10% and 15%) compared to the study conducted under laboratory condition at the end of the study period. Complete biodegradation of C_7 to C_{14} and substantial degradation of C_{15} to C_{28} hydrocarbon fractions were recorded in organic wastes amended soil. BSG amended soil recorded the highest percentage of total petroleum hydrocarbon (TPH) and hydrocarbon fractions biodegradation throughout the twelve months period of study. Thus suggesting the high potential possessed by BSG in enhancing biodegradation of hydrocarbons by indigenous microorganisms.

Phytoremediation of soil contaminated with 2.5% and 1% (w/w) used lubricating oil using *J. curcas* and organic wastes amendments was undertaken for a period of 180 days under laboratory and natural condition. 56.5% and 67.3% loss of used lubricating oil was recorded in *Jatropha* remediated soil without organic wastes amendment for 2.5% and 1% contamination, respectively. However, addition of organic waste (BSG) to *Jatropha* remediation rapidly increased the removal of used lubricating oil to 89.6% and 96.6%, in 2.5% and 1% oil contamination, respectively under laboratory condition. Percentage of oil loss in *Jatropha* remediation studied under natural condition was lower than those studied under laboratory condition at the end of 180 days. *Jatropha* root did not accumulate hydrocarbons from the soil, thus suggesting that the mechanism of the oil degradation was via rhizodegradation.

Phytoremediation with *H. cannabinus* was conducted for 90 days under natural condition. At the end of 90 days, the highest percentage oil loss of 86.4% and 91.8% were recorded in

soil contaminated with 2.5% and 1% oil and amended with BSG, respectively. However, only 52.5% and 58.9% oil loss were recorded in unamended soil contaminated with 2.5% and 1% oil, respectively. *Hibiscus* did not accumulate hydrocarbon from soil, but recorded appreciable bioaccumulation of Fe and Zn in the root and stem.

Potential of hydrocarbon utilizing bacteria and yeast isolated from oil contaminated soil to degrade used lubricating oil was studied in broth culture for 28 days at 30 ± 2^0C. Four isolates (*Pseudomonas aeruginosa, Micrococcus luteus, Trichosporon mucoides* and *Candida tropicalis*) were used for the study. The highest percentage (40.6%) of TPH and hydrocarbon fractions degradation was recorded by *C. tropicalis* followed by *T. mucoides* throughout the study period, compared to those recorded by *P. aeruginosa* and *M. luteus.* Thus, pointing out the potential of the yeast species in biodegradation of used lubricating oil.

The results of these studies demonstrated the potential of BSG and the two plants (*J. curcas and H. cannabinus*) as a good substrate for enhanced remediation of hydrocarbon contaminated soil.

ABSTRAK

Pencemaran tanah dari bahan kimia organik (terutamanya sebatian hidrokarbon) adalah lazim dan biasa berlaku di negara perindustrian dan pengeluar minyak dunia. Ini mendatangkan ancaman kepada alam sekitar dan manusia. Pelbagai kaedah rawatan digunakan untuk tujuan proses pemulihan dan pembaikan tanah yang tercemar.Pemulihan secara biologi atau *Bioremediation* merupakan satu strategi secara biologi untuk mensusut, menstabil atau/dan menyingkir bahan pencemar dari tanah.Ia boleh menjadi alternatif teknologi hijau untuk proses pemulihan dan pembaikan tanah yang tercemar oleh hidrokarbon. Siasatan ini melibatkan kajian dengan tujuan utama untuk menambahbaik proses biodegradasi minyak pelincir terpakai dari tanah dengan bantuan sisa organik. Tiga jenis sisa organik berbeza iaitu bijirin dari proses pembuatan bir (brewery spent grain), kulit pisang (banana skin) dan kompos cendawan terpakai (spent mushroom compost) dan dua jenis tumbuhan iaiu *Jatropha curcas* dan *Hibiscus cannabinus* digunakan untuk menambahbaik proses "biodegradation" dalam tanah yang telah tercemar dengan minyak pelincir terpakai pada kepekatan yang berbeza.

Pemulihan biologi atau *bioremediation* tanah yang telah tercemar oleh minyak pelincir terpakai pada 5%, 10% dan 15% (berat/berat) ditambah dengan 5% dan 10% bijirin dari proses pembuatan bir (brewery spent grain), kulit pisang (banana skin) dan kompos cendawan terpakai (spent mushroom compost). Kajian dilakukan selama 84 hari dalam keadaan makmal. Peratus paling tinggi susutan biologi minyak sebanyak 91.8% telah dicatatkan dalam tanah yang tercemar dengan 5% minyak pelincir terpakai dengan 10% tambahan bijirin dari proses pembuatan bir (brewery spent grain). Manakala peratus paling rendah susutan biologi minyak sebanyak 29 % telah dicatatkan dalam tanah yang tercemar

dengan 5% minyak pelincir terpakai dengan 10% tambahan kompos cendawan terpakai (spent mushroom compost). Proses “biodegradation” minyak maksimum dicatatkan dalam tanah yang tercemar dengan 5% minyak, sementara “biodegradation” sederhana dicatatkan pada 10% tanah yang dicemari minyak. Walaubagaimanapun. hanya sebahagian biodegradation dicatatkan dalam tanah yang tercemar dengan 15% kepekatan minyak sepanjang tempoh kajian.

Tambahan sisa organik kepada tanah yang tercemar dengan minyak pelincir terpakai dikaji dibawah keadaan semulajadi untuk tempoh selama 12 bulan. Biodegradasi minyak pelincir terpakai adalah tinggi untuk semua kepekatan pencemar minyak yang berbeza (5%, 10% dan 15%) berbanding bawah keadaan makmal pada penghujung tempoh kajian. Biodegradasi adalah lengkap untuk C_7 kepada C_{14} dan susutan berikut C_{15} kepada C_{28} pecahan hidrokarbon dicatat dalam tanah yang ditambah sisa organik. Tambahan bijirin dari proses pembuatan bir (brewery spent grain) dalam tanah mencatatkan peratusan paling tinggi dari segi Jumlah Hidrokarbon Petroleum, “Total Petroleum Hydrocarbon” (TPH) dan “biodegradation” bagi pecahan hidrokarbon sepanjang tempoh kajian selama 12 bulan. Bijirin dari proses pembuatan bir (brewery spent grain) dicadangkan mempunyai potensi cerah dalam menambahbaik proses biodegradasi hidrokarbon melalui mikoorganisma asli.

Phytoremediation kepada tanah yang tercemar dengan 2.5 % dan 1 % (berat/berat) minyak pelincir terpakai menggunakan *J. curcas* dan tambahan sisa organik dilakukan untuk tempoh 180 hari dalam keadaan makmal dan semulajadi. Sebanyak 56.5 % dan 67.3 % minyak pelincir terpakai hilang dicatat untuk tanah ditambah *Jatropha* tanpa tambahan sisa organik untuk pencemar 2.5 % dan 1%. Walaubagaimanapun, tambahan sisa organik bijirin dari proses pembuatan bir (brewery spent grain) kepada pemulihan *Jatropha* dalam

penyingkiran minyak pelincir terpakai meningkat sebanyak 89.6% dan 96.6% secara mendadak pada 2.5% dan 1% pencemar minyak bawah keadaan makmal. Peratusan minyak yang hilang dalam pemulihan *Jatropha* dikaji di bawah keadaan semulajadi adalah rendah dibandingkan dengan keadaan makmal pada penghujung hari ke-180. Akar *Jatroph*a tidak menyerap dan mengumpul hidrokarbon dari tanah, maka dicadangkan mekanisma susutan minyak adalah melalui "rhizodegradation".

Eksperimen *Phytoremediation* menggunakan *H. cannabinus* dijalankan selama 90 hari dibawah keadaan semulajadi. Pada penghujung hari ke-90, peratusan paling tertinggi kehilangan minyak dicatatkan adalah 86.4% dan 91.8% dalam tanah tercemar dengan 2.5% dan 1% minyak dengan tambahan bijirin dari proses pembuatan bir (brewery spent grain). Hanya 52.5% dan 58.9% minyak hilang dicatatkan dalam tanah tercemar tanpa tambahan dengan 2.5% dan 1%. Seperti *Jatropha*, *Hibiscus* tidak mengumpul hidrokarbon dari tanah tetapi rekod menunjukkan pengumpulan secara biologi atau *bioaccumulation* Fe dan Zn berlaku pada akar dan batang.

Bakteria dan Yis yang berpotensi tinggi menggunakan hidrokarbon telah diasingkan dari tanah yang tercemar dengan minyak untuk menyingkirkan minyak pelincir terpakai dikaji melalui "broth culture" selama 28 hari pada 30 ± 2^0 C. 4 isolasi (*Pseudomonas aeruginosa, Micrococcus luteus, Trichosporon mucoides* and *Candida tropicalis*) digunakan untuk kajian ini. Peratusan TPH paling tinggi 40.6 % dan penyingkiran pecahan hidrokarbon direkod oleh *C. tropicalis* diikuti *T. mucoides* sepanjang tempoh kajian, berbanding yang direkod oleh *P. aeruginosa* dan *M. luteus.* Secara tidak langung menunjukkan potensi spesis yis dalam *biodegradation* minyak pelincir terpakai. Keputusan melalui kajian ini menunjukkan potensi bijirin dari proses pembuatan bir (brewery spent grain) dan dua jenis

tumbuhan (*J.curcas* dan *H. cannabinus*) sebagai substrat yang baik untuk menambahbaik pemulihan pada tanah yang tercemar dengan hidrokarbon.

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