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Kapok (*Ceiba pentandra* (L.) Gaertn.) Fibers Packed in Nylon Nets as Sorbent for Diesel Oil Spill and its *ex-situ* Bioremediation

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ABSTRACT

The effects of oil spill on the ecosystem and human lives are unprecedented. Early response and containment of the oil spill is the best approach in reducing the environmental impacts. This study assessed the diesel oil absorption capacity of Kapok fiber packed in Nylon net then tested the ability of a consortium of bacterial species reported to have oil degrading properties. To evaluate the conditions for the application of ex situ bioremediation, the hydrocarbon conversion was determined qualitatively by monitoring some possible degradation products with hexadecane as reference.

Kapok (Ceiba pentandra (L.) Gaertn.) fibers packed in Nylon net were found effective in adsorbing diesel oil with a sorption capacity of 15.5 g g^{-1} fibers. A consortium of Bacillus megaterium, Corynebacterium flavescens, Micrococcus luteus and Pseudomonas putida with nutrient amendment (0.15 g N and 0.03 g P $gram^{-1}$ oil) was used to determine preliminary oil biodegration. Microbial population was sustained for six weeks and all species were found to contribute in the degradation process. Biosurfactant production was also observed in the seawater media. Gas chromatographic analysis showed some degradation products of the adsorbed diesel oil after one week of treatment. The use of Kapok sorbents for Tier 1 and 2 oil spill clean-up and its bioremediation done ex situ to degrade the diesel oil hydrocarbons can be an option.

Key words: *oil spill, Kapok sorbents, diesel hydrocarbon degradation, bioremediation*

INTRODUCTION

One of the most prevalent organic pollutants in the marine ecosystem is crude oil that enters the waters regularly in substantial amount. Petroleum can be released naturally through seeps or springs where liquid and gaseous hydrocarbons accumulated underground leak out or accidentally from the various stages in oil production and its distribution. Seeping oil does not seem to have that much effect on the environment because they are highly dispersed. However, oil spills from off-shore oil rigs and tankers have great impacts and causes long-term and possible irreversible damage to the environment and the livelihoods of people.

The response strategy implemented by the Philippine Coast Guard is based on Tier classes. Tier 1 is when the spill is less than 1,000 L and the spiller is expected to undertake the clean-up using their own resources. When the spill is between 1,000 and 10,000 L, Tier 2 applies and the Coast Guard District Command will act. Once spill escalates to Tier 3, exceeding 10,000 L, the National Oil Spill Contingency Plan (NOSCP) will be activated. If the oil already reached a coastline, clean-up will be performed by personnel under the jurisdiction of the Coast Guard with the assistance from the Department of Environment and Natural Resources (DENR).

The environmental impacts of an oil spill and the damages it can create are unprecedented as well as the human health risks it poses. The apparent effect of oil spills is habitat destruction that may lead to loss of biodiversity. Oil slicks may cover the bodies of sea birds, sea turtles and marine mammals and possibly suffocate fishes since oxygen dissolution is restricted. Ingestion or absorption may cause organ dysfunction due to accumulation in tissues. Worst, toxicity of the oil components could directly kill organisms. Oil spill incidents in the country can be searched in the website of the Philippine Coast Guard (http://www.coastguard.gov.ph). Major oil spill includes the rupture of the National Power Corporation power barge at Barangay Semirara, Antique on December 18, 2005 causing a massive oil spill of approximately 220,000 L of bunker fuel and the M/T Solar 1 incident in Guimaras last August 11, 2006 with 2M L of bunker fuel. But there are a lot of small to medium oil spills occurring all over the country.

Early response and containment may reduce the impacts of an oil spill. In 2007, the National Response Team (NRT) of the United States Environmental Protection Agency (USEPA) reported that "the first line of defense in cleaning up oil spills on surface waters consists of mechanical countermeasures such as booms and skimmers". Sorbents are classified as absorbent booms compared to the conventional curtain booms for containment that are non-absorbent. The National Oil and Hazardous Substances Pollution

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Contingency Plan (NCP) defines sorbents as essentially inert and insoluble materials used to remove oil and hazardous materials from water through the mechanisms of absorption or adsorption or both. There are three categories of sorbents provided by the NCP based on material: (1) Organic products such as cellulose fibers, straws, cobs and feathers, (2) Mineral compounds like volcanic ash or perlite, vermiculite and zeolite, and (3) Synthetic products including polypropylene, polyethylene, polyurethane and polyester. Sorbents may also differ in form, as roll, film, sheet, pad, blanket, web (Type I adsorbent), as unconsolidated particulate material (Type II adsorbent) and as pillows where the adsorbent material is contained by an outer fabric or netting (Type III).

The different forms of sorbents have been reviewed by *Birkedal, Stromquist & Huebner* (*2010*) and stated that Type I adsorbents and Type III adsorbents if applied properly would pose little or no impacts to superficial archeological remains as well as on buried resources. In addition, "the prompt use would be beneficial to keep the spill substances from penetrating the soil where they could contaminate archeological remains". Type II adsorbents, on the other hand, might pose more threat due to difficulty in the retrieval process. On the other hand, the advantage of organic materials over synthetic is an ecologically-sound method for disposal after use. Also, these materials would be relatively inexpensive and readily available. Utilization of such materials can as well create a market for unvalued resources.

Kapok (*Ceiba pentandra* (L.) Gaertn.) belongs to the family Malvaceae (reclassified from Bombacaceae) and commonly grows in Southeast Asia, Sri Lanka, some parts of East Asia, and Africa. In the Philippines, *Kapok* (or white silk cotton as its English name) is also known as "Buboi" and the fibers are usually used as stuffing for bedding and upholstery since it is fluffy, lightweight, non-allergenic, non-toxic, resistant to rot, and odorless. The chemical composition of *Kapok* according to *Kobayashi et. al.* (*1977*) as cited by *Lim and Huang* (*2007*) is 64 % cellulose, 13 % lignin and 23 % pentosan. Being an all natural material, it has an advantage of biodegradability. It also has a waxy cutin on the fiber surface making them water repellent. Further characterization of *Lim and Huang* (*2007*) revealed that water cannot penetrate *Kapok* fibers easily and this is very important if it will be used as oil sorbent for oil spill clean-up.

The alteration of organic molecules by microorganisms is biodegradation. But this process can be accelerated through bioremediation, which is the manipulation of the conditions in the contaminated environment (*Hoff 1993*). There are two major techniques for bioremediation, one is the addition of a known oil-degrading biota or bioaugmentation and the other is biostimulation or the addition of limiting nutrients for the indigenous microbial populations (*Tyagi, da Fonseca & de Carvalho 2010*). However, the potential impact of bioaugmentation and biostimulation in the marine ecosystem may result to uncontrollable growth of non-target organisms. Bioremediation (*ex situ*) is a very promising technology for treatment of hazardous substances such as petroleum.

There are several pathways for hydrocarbon biodegradation but the mineralization of most compounds found in crude oil occurs aerobically. *Fritsche and Hofrichter* (*2005*) provided some essential characteristics of aerobic microorganisms for them to utilize organic substances. One is the capability to produce biosurfactants or oil emulsifiers to optimize contact between the microbial cells and the hydrocarbon molecule which are generally water-insoluble i.e. this is in addition to other mechanisms of microorganisms to adhere, like the synthesis of alginate and other exopolymers that together with the type IV pili forms a biofilm on oil droplets (*de Lorenzo 2006*). Another is that they possess the enzymes, oxygenases and peroxidases that catalyze the initial intracellular attack which is an oxidative process; the activation and incorporation of oxygen is very important. Their peripheral degradation pathways convert the organic compounds into intermediates of the central intermediary metabolism which in this case is the tricarboxylic acid cycle. The metabolites produced (acetyl-CoA, succinate, pyruvate) serve as precursor for biosynthesis and growth of cell biomass; required sugars are synthesized via glucogenesis.

The use of bioremediation for oil spill clean-up in the Philippines has very limited studies that in fact there is still no published research yet. Though, *Barraquio* in *1976* has already discovered microorganisms that can degrade crude oil. He has successfully isolated *Corynebacterium* sp., *Brevibacterium* sp. and *Pseudomonas* sp. from Manila Bay and tested their oil degradation potential by obtaining the percent oil conversion based on *Reisfeld's* (*1972*) method. After more than two decades, two separate studies were conducted to isolate hydrocarbon-degrading bacteria and verify their bioremediation potential. *Dela Cruz* (*1997*) tested water and sediment samples from Manila Bay and identified *Vibrio* sp., *Alcaligenes* sp., *Acinetobacter* sp. and Flavobacterium indologenes while samples from Pasig River gave him *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Bacillus* sp. and *Micrococcus* sp. Microorganisms isolated by *Talorete* (*1997*) from the Manila Bay North Harbor were *Flavobacterium* sp., *Acinetobacter* sp., *Vibrio* sp. and Coryneform.

This study investigated the potential of *Kapok* fibers packed in Nylon net as oil sorbent for spill clean-up of the marine environment. Hydrocarbon degrading bacteria, previously identified from various sites in the Philippines was used for the treatment of adsorbed oil. Oil biodegradation was done with seeding and nutrient amendments. Degradation products were analyzed using gas chromatography.

The study was delimited only to laboratory work for the applicability of *Kapok* sorbents for marine oil spills. Actual field testing was not done since simulation of an oil spill may create an environmental problem.

The study offers an alternative way of cleaning up small to medium size marine oil spills. The use of Kapok fibers packed in Nylon net as oil sorbents was found to be more cost-effective compared to the synthetic material being utilized today since the sorption capacity is comparable but the cost is lower and being a natural material is an advantage. The employment of *ex situ* bioremediation technique as treatment for disposal of used sorbents would be safer to avoid additional harm and damage to the ecosystem and community hence, its acceptability would not be an issue.

MATERIALS AND METHOD

This study was performed at the National Institute of Molecular Biology and Biotechnology (BIOTECH) of the University of the Philippines Los Baños. The laboratory scale experiment was performed to provide the details on the degradation of the diesel hydrocarbons and the fate of the microorganisms for possible bioremediation. A field scale was also set-up in parallel to see if the end results would be the same.

Diesel Oil Sorption

Kapok fibers (**Figure 1a**) were bought as pillows from Ibaan, Batangas public market. *Kapok* seeds were removed from the fibers prior to packing in the prepared Nylon nets (**Figure 1b**). For the laboratory scale experiments, eighteen (18) 6.5 x 12 x 1 cm nylon nets were used and filled with about 7 g fibers or approximately 0.09 g cm^{-3} packing density (*Lim and Huang 2007*). For the field scale, three (3) 10 x 100 x 1 cm nets were filled with about 90 g *Kapok* fibers.

Diesel oil was bought from the Caltex Station located along the National Road of Barangay Batong Malake, Los Baños, Laguna. This was pre-weathered according to *Duke et. al.* (*2000*) wherein roughly 10 L of oil was placed in a pool of seawater that was approximately 0.1 m deep and was exposed for 24 hr. Seawater was obtained from Batangas Bay at 13°45'00.91"N and 121°02'23.38"E which was around 150 m away from the Batangas International Port.

The prepared *Kapok* sorbents in the net were placed in a wire basket and soaked for one hour in a pool of filtered seawater with the weathered diesel oil. After the allotted time for adsorption, the wire basket was lifted out and the packed sorbents were allowed to drain for one minute as suggested by *Lim and Huang* (*2007*). The weights of the drained **Kapok** sorbents were recorded to obtain the estimated amount of adsorbed oil. The density of diesel at room temperature was measured using a 25 mL pycnometer

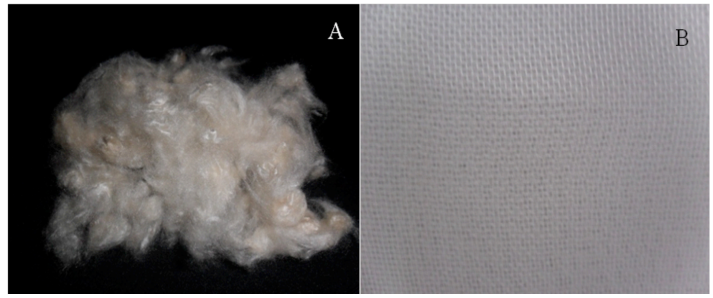

Figure 1. *Kapok* fiber (a) and Nylon Net (b) used for packing the sorbent.

to calculate for the approximate volume of adsorbed oil. Three (3) *Kapok* sorbents were analyzed for the initial hydrocarbon composition of the diesel oil prior to treatment.

Biodegradation of Adsorbed Diesel oil

Cultures of *Bacillus megaterium* (BIOTECH 1046), *Corynebacterium flavescens* (BIOTECH 1413), *Micrococcus luteus* (BIOTECH 1829), and *Pseudomonas putida* (BIOTECH 1504) were bought from the Philippine National Collection of Microorganisms (PNCM) of the National Institute of Molecular Biology and Biotechnology (BIOTECH). Microbial consortium of these four bacteria was used to assess its efficiency to degrade the diesel oil hydrocarbons.

Equal volumes of a 24-h culture of each bacterium were mixed to produce the inoculums. Pure cultures were plated to observe the cultural characteristics, the optical density and the viable plate count after serial dilutions for each species were also obtained to estimate the initial cell count prior to mixing. About 10 mL of the prepared inoculum was sufficient to promote biodegradation (*Anel 1998*) at a ratio of 10 mL inoculum: 7 g kapok fibers.

The experiment was done continuously by destructive batch sampling. A total of 15 set-ups were prepared for the laboratory scale. Treated samples were in triplicate while controls (no treatment except for aeration) were in duplicate. The *Kapok* sorbents with diesel for the laboratory scale were placed in 1 L beakers containing 200 mL seawater. Five beakers for each sampling period were set and sampling was done after the first week, third week, and sixth week. For the field scale, only two samples were treated with one control. The field scale *Kapok* sorbents with diesel were placed in three aquariums with 5000 mL seawater. Samples were incubated at room temperature.

Nutrients, N and P, were amended by spraying evenly a prepared fertilizer solution containing 710 mg $(NH_4)_2SO_4$ and 137 mg Na_2HPO_4 per gram oil (C:N:P ratio, *Philp et. al. 2005*) for six weeks. Incorporation of oxygen for the field scale was accomplished by aeration using an air pump with an output of 2500 cc min^{-1} for 10 min every day while an orbital shaker at 100-150 rpm for 30 min was used for the laboratory scale. Seawater in replacement of the fertilizer solution was added to the control.

Analysis of Degradation Products

Degradation products of the microbially-treated diesel oil were determined by gas chromatography after six weeks (*Maki et. al. 2003*). Four samples were taken both for hydrocarbon analysis and microbial count: initial, after one week, three and six weeks. The weights of the Kapok sorbent as well as the viable plate counts were obtained for each sampling time for the laboratory scale, while only the final viable plate count was taken from the field trial. However, microbial growth was checked after the third week of the experiment. Dominant colonies and morphology of the bacteria that remained and survived the experiment were determined.

Treated kapok fibers were also taken for ultrastructure analysis by Transmission Electron Microscopy using a HITACHI TEM-H300. A digital light microscope (Olympus MIC-D) was also used to describe the internal structure of the Kapok fiber before, during and after contact with diesel oil. A Hitachi Scanning Electron Microscope (SEM-S510) was used to characterize the surface of the fiber after treatment.

The *Kapok* sorbent was manually squeezed to recover the adsorbed oil which was centrifuged at 3000 rpm for 10 min to separate the oil from the water and other impurities. The liquid where the *Kapok* sorbents were soaked was extracted five times with 10 mL n-heptane. Both the oil and the n-heptane extracts were analyzed for the hydrocarbon content by gas chromatography. All samples were purified by solid-phase extraction (SPE) as recommended by *Wang* (*2000*) using the Supelco LC18 column topped with 0.5 cm of anhydrous Na_2SO_4 prior to gas chromatography. The SPE column was preconditioned with methanol prior to sample clean-up. Sample was dissolved in absolute ethanol (20 mg mL^{-1}) and an aliquot was quantitatively transferred to the SPE column. The sample was eluted using n-Heptane (3 mL) and 1:1 toluene: n-heptane (4 mL) to extract the saturated and aromatic hydrocarbons, respectively. The combined solution was concentrated in a stream of nitrogen and reconstituted with 0.5 mL n-heptane prior to gas chromatography (Shimadzu GC-14B) using a 30 m x 0.22 mm ID (0.25µm film) BPX5 capillary column (SGE Analytical Science, Australia), with the following conditions: carrier gas: high purity nitrogen; injector and detector temperatures: 330 °C and 380 °C, respectively; oven temperature program: 2 minutes hold at 50 °C; ramp to 350 °C at 6 °C min^{-1} and hold for 20 minutes. A C_{16} alkane as reference (Hexadecane, 10 µg mL^{-1}) was used to monitor the extent of degradation of the oil components.

RESULTS AND DISCUSSION

Fate of Oil in the Marine Environment

Diesel, specifically petrodiesel was used in this experiment since most boats and ferries use it as fuel. The chances of its spillage in the sea are high. Petrodiesel is produced from the fractional distillation of crude oil between 200 °C to 350 °C with a typical composition of C_8 to C_{21} which can be paraffins, naphthenes, and aromatics. In the classification provided by *Speight* (1991) based on the American Petroleum Industry (API) gravity, diesel is considered as "heavy" crude oils, which have API between 10 to <20°. The pre-weathering was done to simulate the condition of oil when sorbents would be applied during spill incidents, oil had spread over the water surface and exposed to the sun.

When petroleum is spilled into the sea, physical changes occur even before biodegradation begins. The oil spreads over the surface of the water and is subjected to many modifications and the composition of the petroleum changes. Evaporation of the low molecular weight fractions, dissolution of the water soluble components, mixing of oil droplets with seawater and photochemical oxidation contribute to natural weathering. n-Alkanes shorter than C_{14} and aromatic fractions boiling below 250 °C are removed by evaporation with some portions of the aromatics being dissolved exerting great toxicity on aquatic organisms. Exposure to sunlight can affect petroleum in two ways, light-induced polymerization and/or photodegradation according to *Harayama et. al.* (*1999*). Another observation is an increase in the polar fraction and a decrease in the aromatic fraction. The aliphatic components, on the other hand, are believed to be photochemically inert. Though in the presence of the aromatic or polar components of petroleum and anthraquinone in seawater, photosensitized oxidation may occur and convert the n-alkanes to terminal n-alkenes and low molecular weight carbonyl compounds (*Ehrhardt & Weber 1991* cited in the same paper). (*Harayama, Kishira, Kasai & Shutsubo 1999*).

The seawater used was obtained from the Batangas Bay where the Batangas International Port (locally known as the Batangas Pier) and the Shell Tabangao Refinery are located. The area was assumed to be constantly exposed to oil contamination. It was also assumed that hydrocarbon degrading bacteria are present and will assist in bioremediation. However, no microbial test was performed to identify the specific microorganisms that exist. Some physico-chemical parameters were measured: Temperature is 28.2 °C ± 0.0, pH is 8.24 ± 0.02, Salinity is 3.24 % ± 0.00, Conductivity is 49.5 mS cm^{-1} ± 0.0, Turbidity is 2 NTU ± 0 and Dissolved Oxygen is 6.04 mg L^{-1} ± 0.10. The Port Area is relatively clean and well-maintained; very minimal oil slick was visible, no solid waste were disposed on the water and sea breeze was still refreshing and without stench.

Evaluation of Kapok as Oil Sorbent

Oil sorbents work via two different mechanisms, absorption wherein the liquid is distributed throughout the molecular structure causing the material to swell, and adsorption wherein the liquid adheres on the surface of the material through pores and capillaries (*USNRP 2007*). *Kapok* is considered as an adsorbent through capillary action since it has a hollow tubular structure (**Figure 2**). The lumen of the fiber is approximately 10 µm while the external diameter is about 14 µm. A digital light microscope image also showed the absorbed oil droplets inside the lumen of the *Kapok* fiber (**Figure 3**) which provide an evidence for its adsorptive capacity.

The *Kapok* fibers packed in a Nylon net, which according to the USNRP classification will fall under Type III adsorbents, provide an advantage of easy handling and recovery. The fiber can be therefore be utilized as sorbent for oil spills, being inexpensive and readily available, since *Kapok* trees naturally grow in the country but bear fruits only once a year. Hence, the fibers can be collected during the summer season and stored in nylon nets in preparation for an oil spill disaster. Another factor is the low interest of people in planting *Kapok* since its sole known purpose is for stuffing pillows and stuffed toys which is now being replaced with synthetic materials such as foam and fiberfills.

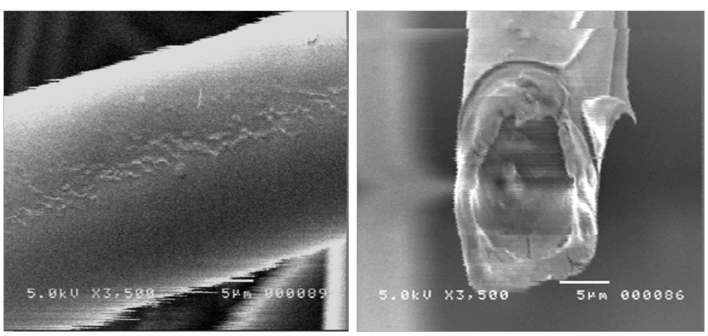

Figure *2.* SEM micrograph showing the ultrastructure of the *Kapok* fiber.

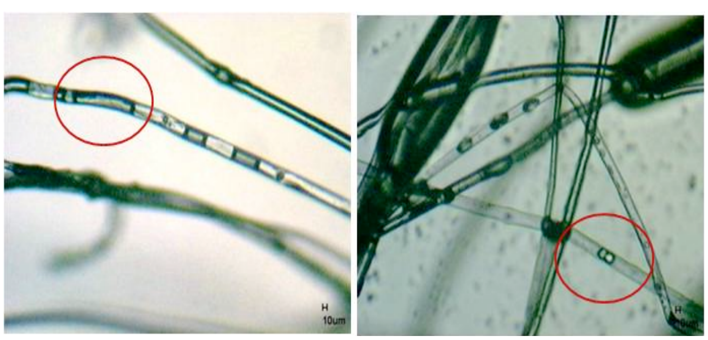

Figure 3. *Kapok* fiber showing the adsorbed oil droplets inside the hollow structure.

Several factors would affect the capability of the packed *Kapok* as oil sorbent applied to marine oil spills. The ideal characteristics of good oil sorbent given by *Lim and Huang* (*2007*) were used to evaluate the suitability of the packed *Kapok* as oil sorbent. Fast oil sorption rate, high buoyancy and low water pickup were demonstrated by the *Kapok* fiber during the experiment. One hour was allotted for adsorption. Though, *Lim and Huang* (*2007*) stated that only a minimum of 15 minutes was needed for oil sorption to reach equilibrium for less dense oil like diesel, longer time was selected to test the buoyancy and water pick up of the *Kapok* sorbent. The sorbent did float for some time even if it was already saturated with oil. This further proved that the *Kapok* fiber did not absorb much water due to its hydrophobic property. When the sorbent was allowed to drip, it was observed that very minimal liquid to almost none drained out. The selectivity of *Kapok* for oil over water was also confirmed from the appearance of recovered oil from the sorbent labeled "initial" which was almost pure. This sorbent was allowed to adsorb the weathered oil but did not undergo biodegradation. The selectivity of the fiber for oil and being water repellant can be attributed to its cellulosic nature and the presence of waxy cutin (*Lim and Huang 2007*).

The oil sorption capacity using the 0.09 g cm^{-3} packing density was 15.5 g g^{-1}, which was higher than the results (7.9 g g^{-1}) reported by *Lim and Huang* (*2007*). This may be due to the difference in total exposed surface area since they used cylindrical test cells. The sorption capacity reported by *Lim and Huang* (*2007*) was the basis for the volume of the pre-weathered oil but the expected amount of oil to be adsorbed was doubled for allowance. Most of the diesel oil spilled over water was adsorbed or absorbed and that the *Kapok* sorbent for the field scale adsorbed more than the calculated oil sorption capacity (22.9 g g^{-1}). **Figure 4** depicts how effective is Kapok for clean up, about 400 g of fibers adsorbed the 10 L diesel oil which proves its usefulness for oil spill disasters. In addition, *Kapok* definitely has a high oil retention capacity because even after six weeks of soaking and shaking, most of the oil still remains adsorbed (**Table 1)**.

Kapok has a good reusability since the adsorbed oil can easily be removed by just squeezing and highest recovery

Figure 4. *Kapok* sorbent in seawater showing with diesel oil before (A) and after (B) adsorption.

Table 1. Percent recovery of adsorbed diesel oil.

Sample	Mean Wt. of Oil (g)	*Mean Vol. of Oil (mL)	Approx. Vol. of Squeezed Oil (mL)	Recovery (%)
Initial	120.96	145	120	83
First week	92.81	111	80	72
Control	94.77	113	90	75
Third week	112.85	135	90	67
Control	108.04	129	100	78
Sixth week	110.24	132	85	64
Control	119.80	143	110	77

*The density of oil used was 0.8368 g mL^{-1} measured by a pycnometer.

obtained was 83 % (**Table 1**). However, the sorption capacity for every use will eventually be reduced since some of the oil remained trapped in the lumen. In addition, after oil was squeezed out, the Kapok fibers lumped together (**Figure 5**). *Lim and Huang* (*2007*) reported that four cycles of sorption/desorption of diesel oil was obtained by the *Kapok* sorbent; beyond this, the capacity remains constant. However, for a more consistent desorption method, they use centrifugation instead of squeezing to recover the diesel oil. Squeezing is preferred in this experiment for practical application; its reusability would be less than four then.

Assessment of the Biodegradation Process

The experiment on bioremediation was not done *in situ* to avoid probable damage to the environment. Also, it is thought off that the best response for spilled oil is an early containment and to be treated *ex situ*. Water or any liquid media e.g. nutrient broth, is necessary for the microorganisms to access the oil through emulsification. The use of nutrient broth as well as artificial seawater would not be feasible if the procedure would be applied in large scale. The only pretreatment done on seawater before use was filtration to remove any suspended organic matter.

The growth of microorganisms can only happen if they have the necessary precursors or the essential nutrients for metabolism and other cell activities. The elements carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P), sulfur (S), potassium (K) and sodium (Na) are among

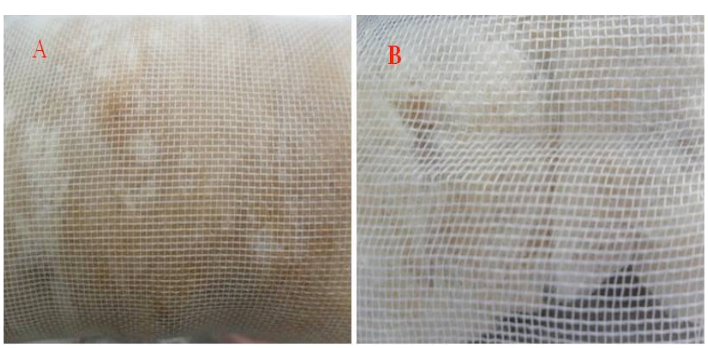

Figure 5. *Kapok* sorbent with adsorbed diesel oil (A) and after the oil was squeezed out (B).

the macronutrients needed by bacteria. Carbon is the most abundant component of cells so it is required in large amounts. In this experiment, the diesel oil serves as the main source of C for the microorganisms. Molecular oxygen (O_2) serves as a reactant in the biochemical processes and its incorporation is catalyzed by the enzymes called oxygenases (*Fritsche and Hofrichter 2005*). Hence, the experimental procedure includes everyday aeration via shaking or pumping.

The amounts of N and P must be in proportion with the available concentration of the hydrocarbon. Ammonium sulfate was used to provide nitrogen in the form of ammonium (NH_4^+) and the weights used were computed to give 0.15 g N per gram of oil. Sulfate (SO_4^{2-}), however, can supply the needed S. The other salt, disodium hydrogen phosphate, was the source of phosphorus in the form of phosphate (PO_4^{3-}) and 0.03 g P per gram of oil was the ratio followed. High concentration of both N and P can limit growth according to *Dela Cruz* (*1997*), therefore the C:N:P ratio given by *Philp et. al.* (*2005*), which was the one used in the experiment, seemed to be adequate. The inorganic forms of the elements are the most common in nature and they are readily used by the cells to synthesize amino acids, nucleic acids, fatty acids, organic acids and sugars for cell growth and replication.

The microorganisms used were *Bacillus megaterium*, *Corynebacterium flavescens*, *Micrococcus luteus*, and *Pseudomonas putida*. These genera of bacteria were chosen since they were reported by *Barraquio* (*1976*), *Dela Cruz* (*1997*), and *Barredo* (*1998*) to degrade hydrocarbons. Summary of the characteristics observed from the Transmission Electron Microscope is given (**Table 2**). These will be used for the identification of the bacteria that dominated and survived after the experiment both in the laboratory scale and the field scale. The inoculums used were a mixture of a 24 h culture of the four species of bacteria. To have an estimate of the initial cell count of each, the optical density was measured at 600 nm and the viable plate counts were obtained (**Table 3**). The Kapok sorbent for the laboratory scale experiment which was placed in 200 mL seawater was inoculated with 10 mL of this resulting bacterial consortium and the cell count was computed at 8.4 x 10^7 CFU mL^{-1}. The viable plate count was obtained every sampling period: after one week, after three weeks and after six weeks. The trend of the microbial population is shown below (**Figure 6**).

Microbial Growth and Activity

The population of the microorganisms slightly increased in the first week. All the inoculated bacteria seemed to have survived the first week of treatment based from the observed cultural characteristics. Most of the plates for the control contained a spreader and growth is very minimal. Three weeks later, the population somewhat declined. Colonies that dominated are still the same. Microscopy was performed to verify the organisms and the representative colonies were prepared for gram staining. Based on the results, all four species are still present. The last and final sampling was done after six weeks and the population seemed to plateau. The observed colonies are still the same. Transmission electron microscope was used to see the actual bacterial

Table 2. Cell morphology observed in the TEM of the microorganisms used.

Species	Cell size and shape
Bacillus megaterium	Rods with flagella, 1.2-1.5µm x 2-5µm
Corynebacterium flavescens	Cuboidal, 1.0-1.3µm size
Micrococcus luteus	Sphere, 0.5-1.0µm dia
Pseudomonas putida	Rods with flagella, 0.5-1.0µm x 1.5-3.0µm

Table 3. Initial plate cell count of each bacterium.

Species	Optical density λ=600nm	Viable plate count, CFU mL^{-1}
Bacillus megaterium	1.345	8.50 x 10^8
Corynebacterium flavescens	1.619	6.05 x 10^8
Micrococcus luteus	2.233	2.25 x 10^9
Pseudomonas putida	1.808	3.36 x 10^9

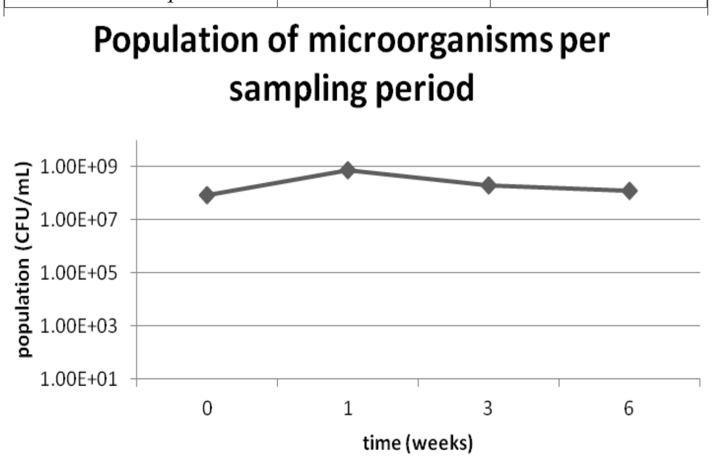

Figure 6. The population of the microbial consortium obtained during the three sampling periods.

composition that participated in hydrocarbon degradation. Based on the morphology and size, the larger bodies can be *C. flavescens* while the smaller one with a more perfect circular shape is *M. luteus*. Rods are shown with lots of tiny spheres that can possibly be spores (presumably *B. megaterium*) and some salt deposits. The smaller rods with flagellum may be *P. putida* and some cells in clusters. Thus, it can be deduced that all inoculated bacteria can tolerate diesel oil and are involved in hydrocarbon degradation, though the rods are outnumbered by the cocci.

The experimental set-up for the simulated field scale was not sampled as frequent as that for the laboratory scale. It was just checked for growth after three weeks and viable plate count was only carried out when the treatments were finished. However, most of the plates have TNTC or the colonies are too numerous to count. In doing viable plate count, only from 25 to 250 colonies are said to be a valid count. Population is too high and dilutions prior to plating were not enough. It can also be observed that the plate for the control has microbial growth; these can be from the seawater or from the environment. TEM was also done to determine which organism persisted. Almost the same type of bacterium participated in the field scale as that in the laboratory scale based on morphology. Large and medium spherical bodies were observed as well as a rod shape cell and a lot of tiny spheres were also seen. The consortium of *B. megaterium*, *C. flavescens*, *M. luteus*, and *P. putida* metabolized the diesel oil with addition of nutrients for their growth.

The liquid medium where the treated *Kapok* sorbent was soaked started to become turbid after two days of inoculation and becomes more unclear after a week. Turbidity is considered an indicator of population growth. In addition, the inoculated is more turbid than the control. Another observation is the increased production of froth both in the laboratory scale and field scale experiments. In microbial cultures, froth can suggest gas production during metabolism. In this case, it is also proposed that this foam is due to the emulsification via formation of biosurfactants. Two species used in this experiment, *B. megaterium* and *P. putida*, are well studied for biosurfactant production.

Biosurfactants emulsify the hydrocarbons, therefore increasing the solubility and availability of the oil to microbial degradation. According to *Cheng, Jian and Wang* (*2008*), emulsification properties can be measured in terms of emulsification activity (EA) and emulsification stability (ES). EA is directly related to oil droplet size, the smaller the size the greater the activity. ES, on the other hand, is a measure of time when the emulsion separates back into oil and water phases since it is thermodynamically unstable. In this study, the amount of surfactant increases with time (**Figure 7**). The corresponding microscopic images of the emulsions (**Figure 8**) showed that oil globules decreased in size.

The amount of recovered diesel oil can be one of the bases that it is being utilized by the microorganisms, or on the contrary, the oil just remains in the seawater medium. How do the microorganisms access the oil? Can they really penetrate the *Kapok* fiber or just in the periphery and use the oil in the emulsion? Does the oil come out of the *Kapok* during shaking?

The SEM micrographs of the treated *Kapok* showed that the microorganisms can grow on the walls of the kapok fiber (**Figure 9**). Analysis of the hydrocarbon composition of the adsorbed diesel would further prove where and how degradation occurred.

Analysis of Degradation Products

Diesel oil or any petroleum products is composed of various hydrocarbons. In this study, hydrocarbon degradation was analyzed using gas chromatography

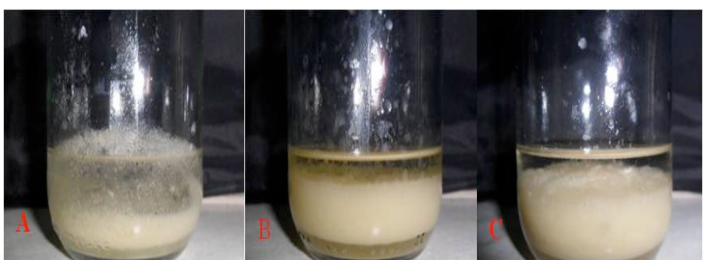

Figure 7. Emulsion formed when the treated seawater was extracted with n-heptane and increasing the amount after one week (A), three weeks (B) and six weeks (C).

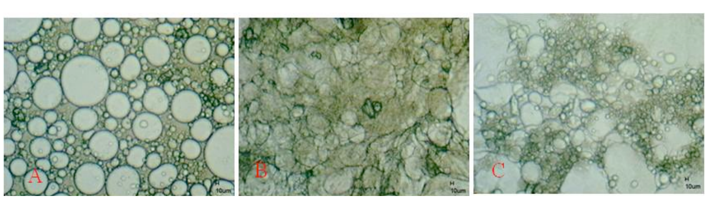

Figure 8. Oil emulsion using Mic-D. The size of the globules decreases as the biosurfactant concentration increases with time.

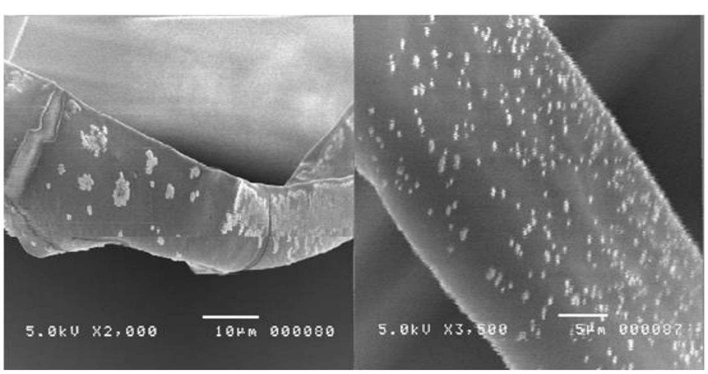

Figure 9. SEM micrographs showing clusters of bacteria on the surface of the treated *Kapok* fiber.

(**Figure 10**). Hexadecane was used as a reference for the degradation as well as peaks that would indicate lower molecular weight hydrocarbons as degradation products (**Figure 11**). A total run time of 75 minutes per sample was carried out. The retention times of the solvent vary from one sample to another in a wide range, about two minutes. This can be attributed to the timing of injection since it was done manually and to the condition of the column. The hexadecane standard has a retention time of 26.608 minutes while when spiked in the sample, it was resolved at 25.94 minutes and relatively the fifth major peak.

In gas chromatography, internal standards are necessary in order to quantized the analyte and evaluate a trend. Also, to fully identify the components of a sample, it is best to use Mass Spectrophotometer as detector. But since the experiment is just exploratory and preliminary in nature, the analysis would be just qualitative based on theory that hydrocarbons with lower molecular weight compared to hexadecane ($C_{16}H_{34}$) would elute before it and these are possible degradation products. Analysis showed that the components of the adsorbed oil are comparable to the unweathered diesel oil with no additional major peaks suggesting that no significant reaction occurred between the oil and the fibers. Chromatograms of the treated oil illustrate that 13 major components are still present; however the trend in their quantity cannot be obtained. There are four additional major peaks common to all samples with lower retention time therefore lower molecular weight compared to hexadecane (**Table 4**).

Most of the diesel oil remained inside the fiber

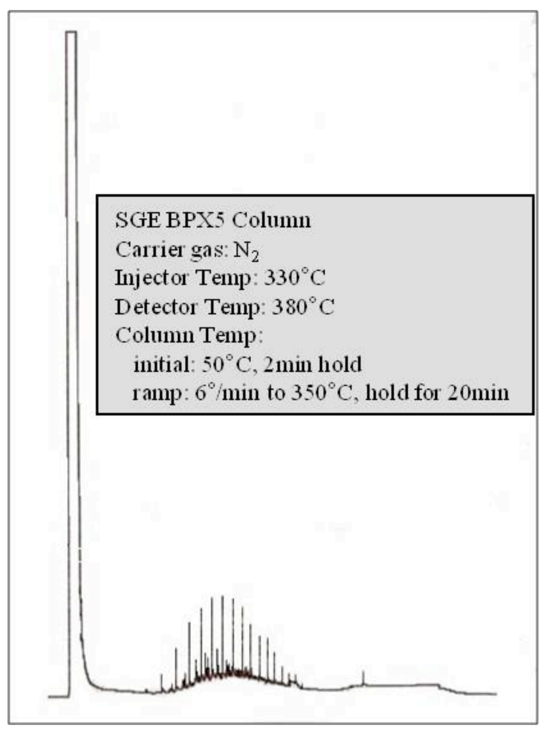

Figure 10. Chromatogram obtained from the untreated diesel oil sample.

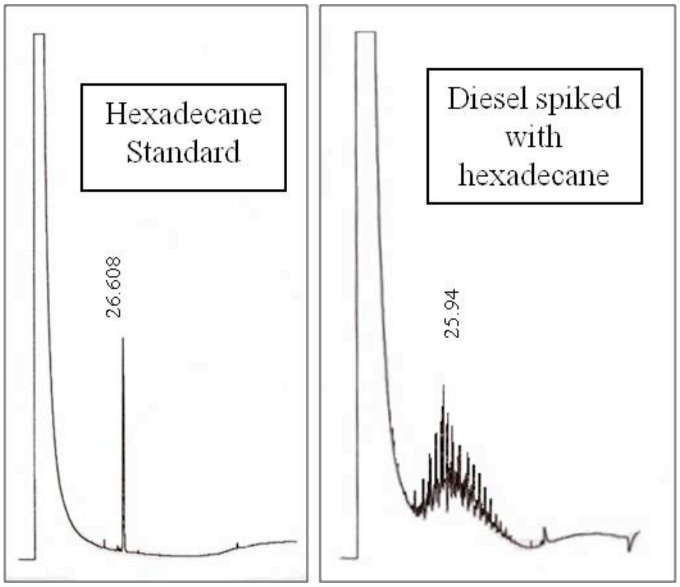

Figure 11. Chromatograms of hexadecane and spiked diesel oil.

throughout the duration of the experiment; hence, the degradation that occurred in the oil suggests that the microorganisms feed on it inside the fiber. The analysis of the n-heptane extracts would provide evidence if some of the oil leaks out during shaking or by merely microbial activity (**Table 5**). Hexadecane was not detected in the samples collected after one week signifying very minimal amount is in the seawater medium. However, it was present in the seawater medium after three weeks and still exists after the sixth week in the treated samples but not in the control. No possible degradation products were detected after one week but degradation have occurred after three weeks. The same results were obtained for the field scale after six weeks though all components are in greater amounts.

Practical Applications

The sorbent material and the casing used in this experiment are not new to the oil spill supplies industry. However, there are still no marketed oil sorbent packaging as the one assembled in this research. Among the few that are posted at the worldwide web and resembles the netted *Kapok* (**Table 6**). It is assumed that there is no local production of oil sorbents and that oil spill equipments used by the Philippine Coast Guard are imported. The *Kapok* sorbent used in the experiment (10 x 100 x 1 cm packed with 90 g fiber) roughly cost PhP 70.00 (see **Table 7** for the costing) and the adsorbed oil per sorbent is approximately 2.4 L but possibly higher. This is very promising and comparable to the marketed oil sorbents. The prices provided do not include shipping costs. Since oil

Table 5. Monitoring of Hexadecane in the n-Heptane extract.

Sampling time	**RT Range, min**	
	Sample	Control
Laboratory scale		
First week	ND	ND
Third week	25.737-25.948	ND
Sixth week	25.753-26.023	ND
Field scale		
Sixth week	28.465-29.152	ND

ND = none detected

Table 4. Major peaks resolved from the treated Diesel oil in both the laboratory scale and field scale experiments.

Peak ID	**Oil from initial samples RT range, min**	**Oil from treated samples RT range, min**
P1	19.788-19.957	19.498-19.967
P2	20.990-21.575	20.125-20.437
d1	ND	21.983-22.253
d2	ND	23.060-23.347
P3	23.745-23.942	23.390-23.667
P4	24.710-24.873	23.890-24.230
d3	ND	24.875-25.133
d4	ND	25.158-25.417
P5	25.677-26.157	25.760-26.017
P6	26.748-26.967	26.733-26.992
P7	27.632-28.468	27.572-27.823
P8	28.558-29.307	29.353-29.602
P9	29.425-30.250	30.287-30.538
P10	31.072-31.965	31.090-31.342
P11	32.682-33.685	32.755-33.017
P12	34.217-35.308	34.258-34.507
P13	35.795-36.852	35.752-36.008

P5 is peak for hexadecane; d1, d2, d3 and d4 are peaks of possible degradation products. ND = none detected spill response is based on Tier classes, the local governments as well as communities must be aware and capable of responding. If this *Kapok* fibers-packed in Nylon nets oil sorbent can be endorsed to these institutions, they can readily clean bodies of water that are contaminated with oil.

In applying the *Kapok* sorbent on the field, the amount needed is very important. In an incidence in Culasi, Panay Island last July 31, 2011, it was reported that 5,000 L of diesel fuel can be possibly spilled. In order to contain this using the *Kapok* sorbent, roughly 2 kg of fibers would be required and if the same dimensions of the Nylon net would be used, this is equivalent to 23 packs. This will cost around PhP 1,610.00. However, if the marine oil spill would be as large as that of in Guimaras Island last August 11, 2006 wherein 2 M liters of bunker fuel were spilled, enormous amount of *Kapok* would be needed. But using siphons would be more effective at this size of spill, though the sorption capacity of *Kapok* for more viscous oil like engine oil is higher compared to diesel fuel, 8.6 g g^{-1} versus 7.9 g g^{-1} at the same packing density (*Lim and Huang 2007*).

Oil recovery by squeezing the *Kapok* is definitely the more desirable characteristic over the existing oil sorbents. Especially for oil companies, financial losses can be reduced. If it will be used in large scale oil clean-up, the size of the assembly can be increased similar to a boom but the packing density must be retained. Also, it can be in other form such as mats or pads, however handling and transfers may be a problem. Another advantage of *Kapok* is that no further treatment is necessary as long as the fruits are mature prior to use and fibers are dry. Packing the sorbents that are loose or particulate in structure is necessary for manageability. Nylon nets (1 mm x 1 mm mesh size) are readily available in fishing supply stores. Due to its durability, it can be use several times as long as it is not physically damaged. It can be refilled with new *Kapok* fibers hence the disposal of the used *Kapok* fibers would be the concern. But since it is a natural product, its biodegradability can be harnessed.

The degradation of the adsorbed oil involved a microbial consortium. If this technique will also be adapted, proper training for the handling of microorganisms is necessary. Though the bacteria used are not pathogenic and biohazards, mishandling can still pose risks both to humans and the environment. If the community will be allowed to apply the *Kapok* sorbent on oil spill, the disposal or treatment must still be supervised by the Philippine Coast Guard.

CONCLUSION

Many oil spill incidents occur in the country but most of them are undocumented and possibly no intervention was employed because of their extent of contamination. However, oil spill contamination in the marine environment, regardless of its volume, would still have its impacts. The *Kapok* sorbent used in this research would definitely be of help in performing small to medium size (less than 10, 000 L)

Table 6. Comparison of marketed oil sorbents with *Kapok* sorbent.

Manufacturer	Material	Dimension/*Price	Capacity
SpillTech oil boom (Item no. WB520SN)	polypropylene	12.70 x 609.60 cm US$25,19 per boom	56.8 L per boom
Dawg, Inc. sorbent sock (Item no. DAWG 300)	polypropylene	7.62 x 121.92 cm US$8.15 per sock	3.8 L per sock
Annapolis Valley Peat Moss Company (Cansorb)	Canadian Sphagnum Peat Moss	Not available	eight times its weight depending on the hydrocarbon
PCI Products Company	Corn cob filler	7.62 x 121.92 cm US$1.65 per sock	1.9 L per sock
Kapok sorbent	*Kapok*	10 x 100 cm US$1.63 per sock	2.4 L

Table 7. Variable costs of producing a unit of *Kapok* sorbent (10 x 100 x 1 cm packed with 90 g fiber).

Materials	Unit Cost	Quantity	Cost per Sorbent
Kapok fibers	PhP 0.17/g	90 g	P15.30
Nylon net	PhP 40.00/yd	0.4 yd	P16.00
Thread	PhP 8.00/100 m	25m	P2.00
Electricity	PhP 5.00/KWh	1 KWh	P5.00
***Labor**	Unit cost	Quantity	Cost per sorbent
Cutting and sewing	PhP 31.65/hr	0.5/hr	PhP 15.80
Filling	PhP 31.65/hr	0.5/hr	PhP 15.80
Total	PhP 69.90		

oil spill clean-up due to its low-cost and availability. In addition, its application is very simple. It can be used for containment and collection of the spilled oil. Furthermore, for the disposal of the used *Kapok* fibers, biodegradation of the adsorbed diesel oil can be done, since partial degradation of the hydrocarbons was observed for six weeks. However, a more extensive study for degradation is necessary. Aside from bioremediation, other methods for disposal of the *Kapok* sorbent may be developed.

RECOMMENDATIONS

The endorsement of this oil sorbent by the Philippine Coast Guard to local communities and LGUs would really help in the promotion though the bioremediation part still needs further study and definitely financial support for further experiment. It is recommended to do a more detailed study on the degradation of diesel but it is suggested that the absorbed oil be removed first before biodegradation. However since the *Kapok* fiber is inert towards diesel oil and it has a very high percent recovery, the collected oil can then be reused. The degradation procedure can be improved by employing microorganisms capable of utilizing the fiber itself. This is important for the proper disposal of the used fibers. Since *Kapok* is 35 % cellulose, 22 % xylan and 21.5 % lignin (*Hori 2000*), organisms such as *Sporocytophaga* and *Cytopahaga*, *clostridia* and *actinomycetes* are the most common, can be used to digest cellulose while *Amphibacillus* attacks the xylan. However, if biodegradation would be employed, proper training for handling the microorganisms is necessary for biosafety purposes. But biodegradation is not the only way for disposal, the new and emerging technology of briquettes is another possible solution. The used *Kapok* fiber with the adsorbed oil can be processed to biomass briquettes and be a substitute for fossil fuels. Further research on this matter is thus recommended.

Application in large scale or increasing the size of the assembly or changing the form e.g. mats should be studied further with a special consideration on how it will be retrieved and squeezed since the *Kapok* becomes heavy upon saturation with oil. Machines or any contraption would really be needed to pull the assembly out of the water.

REFERENCES

Añel, R. M. D. 1998. Determination of the crude oil degradation capacity by a selected microbial consortium. BS Thesis. University of the Philippines Diliman.

Barraquio, W. L. 1976. Crude oil degradation by microorganisms. MS Thesis. University of the Philippines Los Baños.

Barredo, C. G. 1998. Bioremediation application of selected microbial strains in simulated oil spills in the Pasig River. BS Thesis. University of the Philippines Diliman.

Birkedal, T., Stromquist, L & Huebner, B. 2010. Review of NRT-RRT Factsheet - “Guidance on the Application of Oil Sorbents and Solidifiers” from the Perspective of Archeology and other Cultural Resource Types. Retrieved December 5, 2011 from http://dnr.alaska.gov/parks/oha/oilspill.htm (Alaska Department of Natural Resources, Division of Parks and Recreation, Office of History and Archaeology).

Cheng, K. B., Jiian, Z. & Wang, Z. 2008. Emulsification properties of bacterial biosurfactants native to the Yellow River Delta on Hexadecane and Diesel oil. Research Paper. Clarkson University. Retrieved from http://web2.clarkson.edu.

De Lorenzo, V. 2006. “Blueprint of an oil-eating bacterium.” Nature Biotechnology 24(8), 952 – 953. doi:10.1038/nbt0806-952

Dela C, J. (1997). Isolation, identification and bioremediation potential of oil-degrading bacteria from Manila Bay and Pasig River. BS Thesis. University of the Philippines Diliman.

Duke, N. C., Burns, K. A., Swanell, R. P. J., Dalhaus, O. & Rupp, R. J. 2000. “Dispersant use and a bioremediation strategy as alternative means of reducing impacts of large oil spills on mangroves: The Gladstone Field Trials.” Marine Pollution Bulletin, 41(7-12), 403-412. doi:10.1016/S0025-326X(00)00133-8

Fritsche, W. & Hofrichter M. 2005. “Aerobic degradation of recalcitrant organic compounds by microorganisms.” In Jördening, H. & Winter, J. Environmental Biotechnology: Concepts and Applications. Germany: Wiley-VCH Verlag GmbH & Co. KGaA. pp 203-216.

Harayama, S., Kishira, H., Kasai, Y. & Shutsubo, K. 1999. “Petroleum degradation in marine environment.” Journal of Molecular Microbiology and Biotechnology, 1(1), 63-70. www.horizonpress.com/jmmb/v1/v1n1/10.pdf

Hoff, R. 1993. “Bioremediation: an overview of its development and use for oil spill cleanup” Marine Pollution Bulletin, 26(9), 476-481. doi:10.1016/0025-326x(93)90463-T

Hori, K., Flavier, M.E., Kuga, S., Lam, T.B.T. & Iiyama, K. 2000. “Excellent oil absorbent kapok [Ceiba pentandra (L.) Gaertn.] fiber: fiber structure, chemical characteristics, and application.” Journal of Wood Science, 46, 401-404.

Lim, T. T. & Huang, X. 2007. “Evaluation of Kapok (Ceiba pentandra (L.) Gaertn.) as a natural hollow hydrophobic-oleophilic fibrous sorbent for oil spill cleanup.” Chemosphere, 66(5), 955-963. doi:10.1016/j.chemosphere.2006.05.062

Olavario, A. C. Response to HNS spills: PCG’s emerging new challenge. Retrieved August 20, 2011 from http://www.coastguard.gov.ph (Maritime Environmental Protection).

Philp, J. C., Whiteley, A. S., Ciric, L. & Bailey, M. J. 2005. “Monitoring bioremediation. In R. M. Atlas & J. C. Philp

(Eds.), Bioremediation: Applied Microbial Solutions for Real-World Environmental Cleanup. Washington DC: ASM Press. pp 237-268.

Philippine Coast Guard Law of 2009. Retrieved September 6, 2011 from http://www.lawphil.net/statutes/repacts/ra2010/ra_9993_2010.html

Pritchard, P. H. 1991. "Bioremediation as a technology: Experiences with the Exxon Valdez oil spill." Journal of Hazardous Materials, 28, 115-130. doi:10.1016/0304-3894(91)87011-P

Rojo, F. 2009. "Degradation of alkanes by bacteria." Environmental Microbiology, 11(10), 2477-2490. doi: 10.1111/j.1462-2920.2009.01948.x

Talorete, T. P. N. 1997. Marine Hydrocarbon-degrading bacteria from the Manila Bay North Harbor and their bioremediation potential. MS Thesis. University of the Philippines Diliman.

Tyagi, M., Da Fonseca, M. M. R., & De Carvalho, C. C. C. R. 2010. "Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes." Springer Science+Business Media, 22(2), 231-241. doi:10.1007/s10532-010-9394-4

United States National Response Team (USNRP), Science and Technology Committee. (2007). NRT-RRT Factsheet Application of Sorbents and Solidifiers for oil Spills. Retrieved December 5, 2011 from http://www.nrt.org.

Wang, Z., Fingas, M. & Sigquin, L. 2000. "Characterization and source identification of an unknown spilled oil using fingerprinting techniques by GC-MS and GC-FID." LCGC, 18 (10), 1058-1067. http://www.chromatographyonline.com

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