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Research Article

Partially Acetylated Sugarcane Bagasse for Wicking Oil from Contaminated Wetlands

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Sugarcane bagasse was partially acetylated to enhance its oil-wicking ability in saturated environments while holding moisture for hydrocarbon biodegradation. The water sorption capacity of raw bagasse was reduced fourfold after treatment, which indicated considerably increased hydrophobicity but not a limited capability to hold moisture for hydrocarbon biodegradation. Characterization results by Fourier transform infrared (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD), and surface area analyzer suggested that treated bagasse exhibited enhanced hydrophobicity and surface area. Oil wicking test results indicate that treated bagasse is more effective in wicking oil from highly saturated environments than raw bagasse and suggest that application of this material in remediation of oil spills in highly saturated wetlands is promising.

Keywords: Crude oil, Oil wicking, Porous materials, Soil contamination, Sugarcane bagasse

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1 Introduction

Sugarcane bagasse is an agricultural byproduct from the cane sugar refining process. Huge amounts (millions of metric tons per year) of this waste are produced worldwide. However, only a fraction of this material is actually reused as fuels in sugar factories and as raw material for pulp and paper products [1]. One of the useful features of this organic material is that it can absorb by capillary forces an amount of oil and/or water greater than its own weight. In addition, this natural material can be completely degraded in nature by biological, physical, chemical, and photochemical processes [2]. In the past two decades, the reuse of agricultural byproducts as oil sorbents has received growing attention due to their low cost and biodegradability [3]. Most agricultural byproducts are derived from plants such as bagasse, coir, kenaf, rice straw, sisal, and sawdust, all of which have been investigated for oil spill cleanup applications [4]. The main drawbacks of these plant-derived sorbents are a relatively low oil sorption capacity, low hydrophobicity, and poor buoyancy compared to synthetic sorbents such as polypropylene [5, 6].

Once plant-derived sorbents are applied to saturated environments, preferential water sorption is favored over the sorption of oil because these sorbents are generally hydrophilic in

nature. An earlier microcosm study with ammoniated bagasse revealed that the effectiveness of the sorbent was severely limited when the water level covered the sorbent layer, regardless of sediment particle sizes and oil contamination levels [7]. Lee et al. [8] reported that the oil sorption capacity of kenaf and cotton decreased significantly when they were presoaked with water. The major constituents of sugarcane bagasse are 40% cellulose, 30% hemicelluloses, and 20% lignin [9]. The former two components are recognized as hydrophilic and the latter is hydrophobic. Cellulose has many hydroxyl groups, which are responsible for hydrophilic functionality in the forms of various alcohols, carboxyl acids, and phenols.

Hydrophobicity (or oleophilicity) is one of the major determinants of sorbent properties influencing the effectiveness of oil sorption in the presence of water. The effectiveness of the sorbent in saturated environments would be enhanced if the density of the hydroxyl functional group decreased. This functional group is the most reactive and abundant site in cell wall polymers of lignocellulosic material such as sugarcane bagasse [10]. The acetylation reaction is one of the most common techniques used for the hydrophobic treatment of lignocellulosic material (e.g., wood) by a substitution reaction of a hydroxyl group (hydrophilic) into an acetyl group (hydrophobic). This reaction is usually carried out by heating lignocellulosic material in the presence of acetic anhydride with or without a catalyst [11]. Various catalysts have been used for enhancing the efficiency of acetylation reactions. Pyridine and 4-dimethylaminopyridine (DMAP) have been commonly applied for acetylation for many years [12, 13]. However, they are too toxic and/or expensive for commercial use. Sun et al. [14] recently reported that acetylation of sugarcane bagasse

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with *N*-bromosuccinimide (NBS) as catalyst in a solvent-free system was a convenient and effective method. In addition, they claimed that modified bagasse applied in an oil-water system presented an enhanced oil sorption capacity exceeding that of commercial synthetic fibers.

The use of plant-derived sorbents combined with biodegradation offers a simple and inexpensive remediation method for oil spills in wetlands. The addition of sorbents on the contaminated surface can be done without significant ecosystem damage. Once the oil is wicked out of the anaerobic subsurface by sorbents, hydrocarbons degrade rapidly under aerobic conditions by indigenous oil-degrading microorganisms. However, the effectiveness of sorbents in wicking oil is often limited by the hydrophilic nature of sorbent materials. If hydrophobic properties of these materials can be enhanced, these sorbents theoretically would be made much more effective for treating oil-contaminated wetlands and marshes. The purpose of this study is to modify sugarcane bagasse by partial acetylation to improve its oil-wicking ability in saturated environments while holding a minimum moisture for hydrocarbon biodegradation. The changes before and after treatment were investigated using various characterization techniques. Several oil-wicking experiments were performed in microcosms using different water levels to assess the effectiveness of treated bagasse in remediation of oil-contaminated wetlands.

2 Materials and Methods

2.1 Materials

Sugarcane bagasse was obtained from the Louisiana State University Agricultural Center. Prior to use, it was oven-dried overnight at 105 °C to remove moisture and microorganisms. Acetic anhydride (95% purity) and *N*-bromosuccinimide (95% purity) were purchased from Acros Chemicals. Previously weathered Bonny Light crude oil was obtained from the U.S. EPA and used as the oil contaminant. Fine-grain sand was obtained by sieving bulk sand (40 × 100 mesh U.S. standard) into specific size ranges (60 × 80 mesh U.S. standard). It was washed with 1 N nitric acid to remove impurities and oven-dried at 105 °C. Distilled water was added to various levels in the microcosms. To ensure that no biodegradation occurs during the experiment, sodium azide was added to the water at a concentration of 5 mg L⁻¹.

2.2 Preparation of the Hydrophobic Sorbent

Raw bagasse was treated with acetic anhydride in the absence or presence of the catalyst, *N*-bromosuccinimide. The amounts of substrate and reactant were combined in a ratio of 1:20 (g dried sugarcane bagasse/mL acetic anhydride), and the amount of the catalyst was 1% (% liquid, g mL⁻¹) as suggested by Sun et al. [14]. Reaction temperature and reaction time varied from 110 to 130 °C and 2 to 3 h, respectively. The mixture of raw bagasse, acetic anhydride, and the catalyst was placed in a round bottom flask. The oil bath was placed on top of the heating equipment to maintain a constant temperature. After

the target temperature was reached, the flask was placed in the oil bath and the reflux condenser was fitted. The reaction was performed under automatic temperature control by using a digital hot plate (IKA works) equipped with temperature sensor (±1 °C). After the reaction time was reached, the excess amount of the hot reagent was decanted off. The byproduct and the unreacted agent were washed with ethanol and acetone. Filtering was followed to separate the dissolved catalyst from the treated bagasse, which was dried in the oven before use.

2.3 Characterizations of the Sorbents

Under each reaction condition, the weight percent gain after the reaction was used as a measure of the extent of substitution because this reaction is a single site substitution without polymerization [9]. The weight percent gain was calculated using the following formula:

$$\text{Weight Percent Gain (\%)} = \frac{(\text{Weight after reaction} - \text{Weight before reaction})}{\text{Weight before reaction}} \times 100$$

Fourier transform infrared spectroscopy (FT-IR) (Spectrum one, Perkin Elmer) analysis of raw bagasse and treated bagasse was followed by the KBr pellet method. The water sorption capacity, an important characteristic in saturated environments, of each sorbent was measured in triplicate with time. Each sorbent material was applied to the water surface in a 100-mL beaker and allowed to absorb water for specific soaking times (10, 20, 40, and 80 min). After the specified soaking time, the wet sorbent was drained on the filter paper for 10 min under vacuum filtration. The water sorption capacity of the sorbent was calculated as follows:

$$\text{Water Sorption Capacity (g g}^{-1}\text{)} = \frac{\text{amount of wet sorbent (g)} - \text{amount of dry sorbent (g)}}{\text{amount of dry sorbent (g)}}$$

A porosimetry analyzer (Tristar 3000, Micrometrics) was used to measure the surface area by nitrogen adsorption and desorption. The morphological change before and after the reaction was investigated with an environmental scanning electron microscope (Philips, XL 30-ESEM FEG). Specimens were prepared by attaching individual fibers with carbon tape and platinum-coating the fibers. Patterns of crystal structure were obtained by an X-ray diffractometer (Philips, X'Pert XRD) using Cu K α radiation ($\lambda = 0.154$ nm).

2.4 Oil-Wicking Tests in Abiotic Microcosms

Cylindrical glassware, 10 cm in height and 10 cm in diameter, was used to enclose the microcosms. Each microcosm consisted of three layers: a clean sand layer, an oil-contaminated

sand layer, and an overlying distilled water and/or sorbent layer (from the bottom to the top). Microcosm conditions included three water table levels (on top of the clean sand layer, the oiled sand layer, and the overlying sorbent layer) and two levels of sorbent type (treated or untreated). First, the glass microcosms were filled with water, and sand was gently poured into the water until it was saturated. A stainless-steel screen (100×100 mesh) was placed on the top of the clean sand layer as a separator. Atop this layer, an oil-sand mixture (50% of oil saturation) was compacted to a depth of 1 cm. The sorbent was placed on top of the oil-contaminated layer up to 2 cm in height. Water was subsequently added to the microcosms to accomplish the desired coverage. Parafilm and aluminum foil were used to seal the top of the microcosms. The prepared microcosms were kept in the dark, and oil wicking was allowed to take place for three months. At time zero, three representative samples were collected from the clean sand, sorbent, and oil-sand mixture. They were used to calculate the initial amount of oil in each layer of the microcosms. The final sampling event took place after three months. Samples were extracted with dichloromethane using a Soxhlet extraction apparatus and separatory funnels. The extraction efficiency was checked by spiking of surrogate compounds after extraction. Samples with surrogate recovery of 70–130% were taken.

The concentration and mass of target compounds in each sample were quantified using GC-MS (HP-5890 series II with mass-selective detector). The oven temperature was programmed to increase from 45 °C to 200 °C at 4 °C min⁻¹ and then to increase from 200 °C to 310 °C (held for 10 min) at 10 °C min⁻¹. The temperatures of inlet and detector were 290 °C and 320 °C, respectively. Chromatographic separation was achieved with an SPB-5 capillary column (Supelco). Ultra-high-purity helium gas (99.999% pure) was used as a carrier gas at a flow rate of 1 mL min⁻¹, and the mass-selective detector was operated in selective ion monitoring (SIM) mode with chemical ionization. GC-MS results were quantified with calibration curves maintained within 75–125% of check standards. Mass closure in each microcosm was determined as the sum of alkanes (C₁₀–C₃₅), pristane, phytane, hopane, 2-, 3-, and 4-ring PAHs, and pyrogenic PAHs (5- and 6-rings). The effectiveness of the sorbent was determined by the changes in each layer between time 0 and three months. The formulas for the mass closure and effectiveness of the sorbent are provided below:

Mass closure =

$$\frac{(\text{total mass of target compounds in all layer})_{t=3 \text{ months}}}{(\text{total mass of target compounds in all layer})_{t=0}} \times 100$$

Effectiveness =

$$\frac{(\text{total mass of target compounds in the sorbent layer})_{t=3 \text{ months}}}{(\text{total mass of target compounds in all layer})_{t=0}} \times 100$$

3 Results and Discussion

3.1 Effect of Reaction Conditions on Weight Percent Gains

As a result of the acetylation reaction, bagasse gained weight through the substitution of higher-molecular-weight functional groups (acetyl group) for low-molecular-weight functional groups (hydroxyl group). A separate test with raw bagasse confirmed that the weight loss during the purification step was almost negligible (<0.5%). The weight percent gains (WPGs) under various reaction conditions are summarized in Tab. 1. No significant differences ($p > 0.05$) in WPGs were observed between the instances where no catalyst was used (7.8–9.2%) and when the raw catalyst (NBS) was involved (8.2–9.6%). To improve the effectiveness of the catalyst, NBS powder was recrystallized by heating a mixture of 10 g of raw NBS and 100 mL of distilled water to 100 °C followed by slow cooling. Clear crystals of NBS are obtained from orange-color powder after recrystallization. When the recrystallized catalyst was used, WPGs are enhanced significantly to 14.5–20.5% (sample 7–10). These increases in WPGs indicate that the impurity (e.g., moisture) of the catalyst played a significant role in limiting the effectiveness of the catalyst.

Table 1. Weight percent gains of treated bagasse under various reaction conditions.

Sample No.	Reaction temperature [°C]	Reaction time [h]	Catalyst	Weight percent gain [%]
1	120	2	Without catalyst	7.8
2	130	2	Without catalyst	8.5
3	130	3	Without catalyst	9.2
4	120	2	Raw NBS	8.2
5	130	2	Raw NBS	8.8
6	130	3	Raw NBS	9.6
7	110	2	Recrystallized NBS	14.5
8	120	2	Recrystallized NBS	18.2
9	130	2	Recrystallized NBS	20.0
10	130	3	Recrystallized NBS	20.5

Samples 7 and 8 were considered as the candidates of a desirable sample since either low-acetylated or high-acetylated bagasse would not be suitable for our purpose (oil wicking combined with hydrocarbon biodegradation). Therefore, the water sorption capacities of raw bagasse and treated bagasse (samples 7 and 8) were measured. Fig. 1 presents the amount of water sorbed over time by untreated bagasse and by treated bagasse with time.

Raw sugarcane bagasse can hold significant amounts of water due to hydrogen bonding between hydroxyl groups and water molecules. Water sorption by raw bagasse increases with time and reaches a saturation point at 4 g of water/g of dry sor-

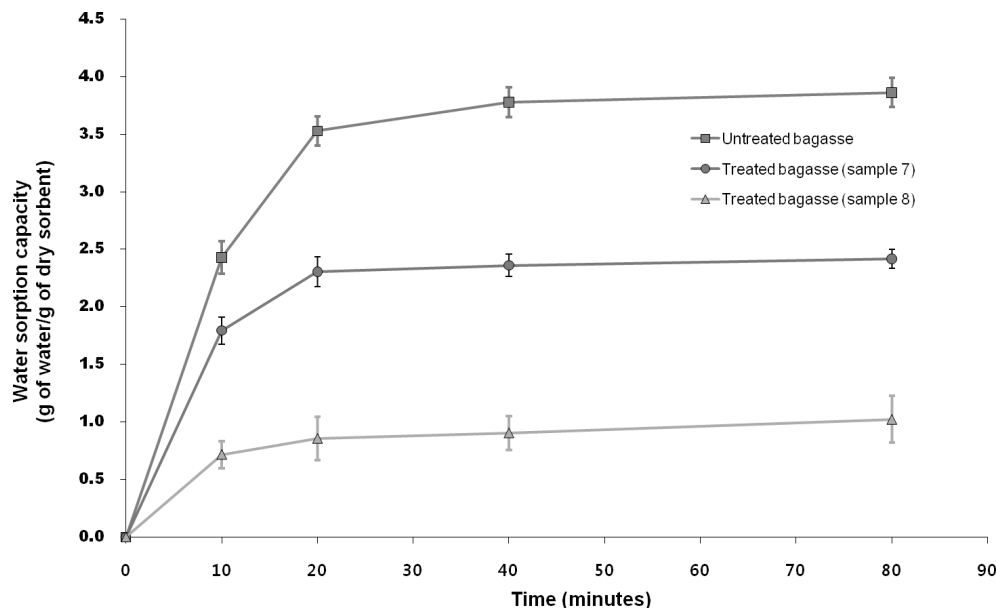


Figure 1. Water sorption capacity of untreated and treated bagasse (samples 7 and 8) with time.

bagasse. On the other hand, samples 7 and 8 reach the saturation point at 2.3 and 1 g of water/g of dry sorbent. Treated bagasse sorbs less water than untreated bagasse due to an enhanced hydrophobicity. Reduced water sorption capacity and enhanced surface area (discussed in Sect. 3.4) in treated bagasse would result in more accessible surface area to oil and microorganisms. Brietenbeck and Grace [15] noted that optimal microbial activity under aerobic condition generally occurs at 50–80% of water holding capacity (% moisture in wet sorbent). The water-holding capacity of sample 8 corresponds to about 50%, while the capacities of the others correspond to 70–80%. Sample 8 has the highest hydrophobicity in terms of WPGs while it holds the minimum moisture required for

hydrocarbon biodegradation. Thus, the treatment condition was chosen at a temperature of 120 °C and at a reaction time of 2 h with recrystallized catalyst.

3.2 FT-IR Analysis

Fig. 2 illustrates the FT-IR spectra of untreated bagasse (spectrum 1) and treated bagasse (spectrum 2). Major changes before and after treatment are a reduced peak at 3450 cm^{-1} (O–H, hydroxyl group) and increased peaks at 1750 cm^{-1} (C=O in acetyl group), 1240 cm^{-1} (–C–O– in acetyl group), and 1380 cm^{-1} (–C–CH₃ in acetyl group). These changes in the

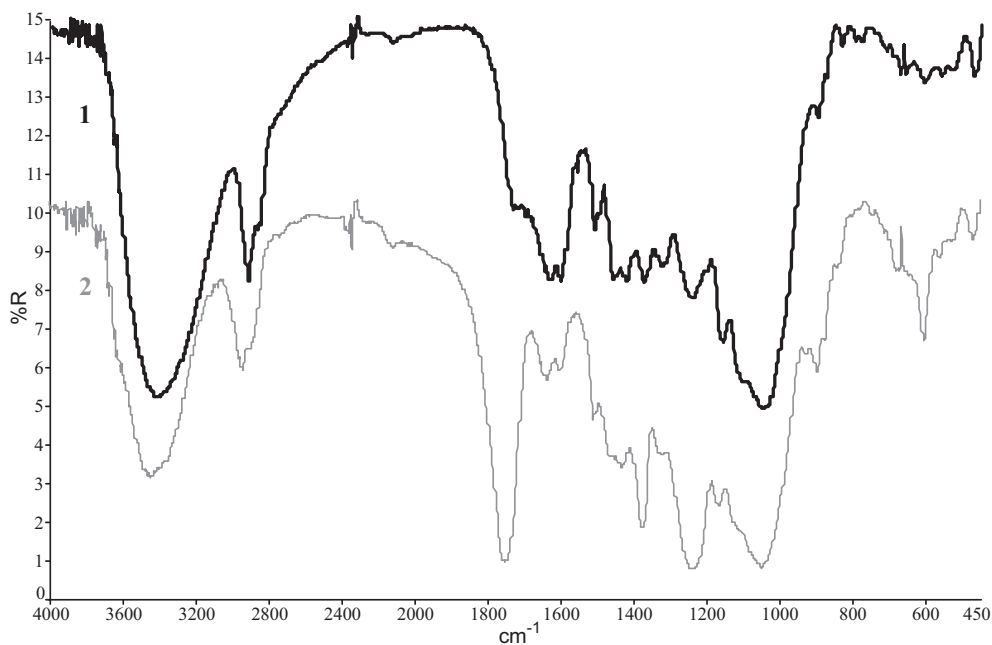


Figure 2. FT-IR spectra of (1) untreated bagasse and (2) treated bagasse at 120 °C for 2 h in the presence of acetic anhydride and recrystallized NBS catalyst.

FT-IR spectra are consistent with those of acetylated cellulosic materials reported by other researchers [16–18]. The results indicate that the acetyl functional group has been successfully attached to bagasse at the expense of the hydroxyl group. The absence of peaks at 1700 cm^{-1} and $1840\text{--}1760\text{ cm}^{-1}$ in spectrum 2 indicate that the byproduct (acetic acid) and unreacted reactant (acetic anhydride) are successfully removed during the purification step [17].

3.3 SEM Analysis

The SEM image of a cross section for treated bagasse is displayed in Fig. 3a and for untreated bagasse in Fig. 3b. Raw bagasse fiber has bundles of lumens which can transport and hold oil (and/or water) in capillaries. The lumen sizes varied between 10 and $100\text{ }\mu\text{m}$, which can be classified as macropore (IUPAC). Most of the lumens in treated bagasse keep their shape except the ones disrupted on cutting, since bagasse fiber becomes brittle after the treatment. The shape of the lumens,

depicted by two perpendicular arrows in Figs. 3a and b, in treated bagasse is well-developed in both directions, while that in untreated bagasse is not. This difference indicates that lumens undergo swelling during treatment. The surface of treated bagasse has many perforations (indicated by arrows in Fig. 3c) with a size of a couple hundred nanometers. However, these holes are rarely observed in the surface of raw bagasse, except occasional damages (indicated by arrows in Fig. 3d) during handling and storage. The size of perforation is much smaller than those attributable to physical damages, even though they are irregular in shape and size. The activity of chemicals (acetic anhydride or acetic acid) is possibly responsible for the perforations. Perforations on the surface and/or swelling of lumens would increase the surface area of treated bagasse, which is discussed in Sect. 3.4. In addition, Figs. 3c and d demonstrate that untreated bagasse has rougher surfaces than treated bagasse. This difference is likely due to the presence of crystallites in untreated bagasse, as discussed in Sect. 3.5.

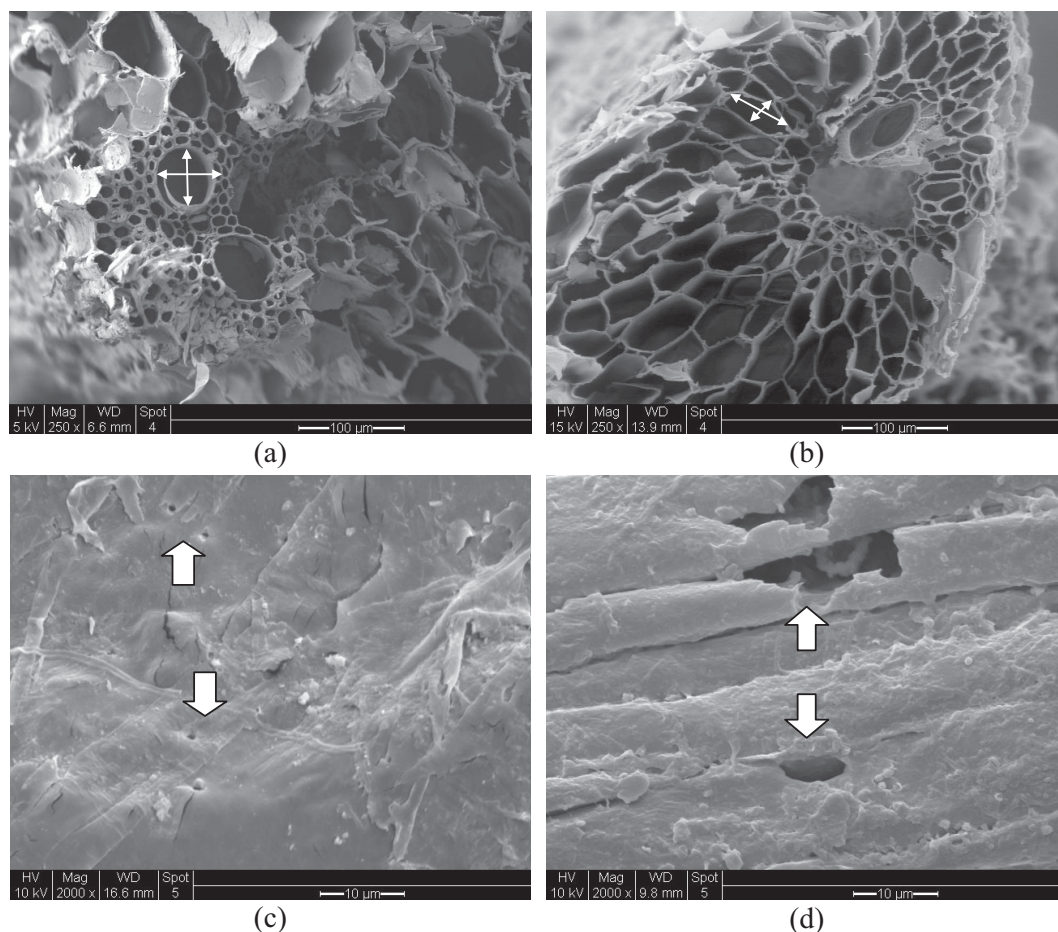


Figure 3. SEM images of cross section ($250\times$) and surface ($2000\times$) in untreated and treated bagasse: (a) cross section in untreated bagasse, (b) cross section in treated bagasse (lumen shape described by two perpendicular arrows), (c) surface in untreated bagasse (perforations indicated by white arrows), (d) surface in treated bagasse. Physical damages are indicated by white arrows.

3.4 Surface Area Measurement

Nitrogen adsorption/desorption isotherms of untreated and treated bagasse are presented in Figs. 4a and b. Both of the isotherm curves correspond to the type II model (IUPAC) which is macroporous. More hysteresis is observed in the isotherm of raw bagasse than treated bagasse, because the pore shape of naturally occurring material typically deviates from ideal assumption. The decreased hysteresis in the isotherm of treat-

ed bagasse indicates an improvement of accessible area after treatment. The surface area of untreated and treated bagasse was calculated based on the BET model theory with a good correlation coefficient ($R^2 = 0.99$) and intercept constant as shown in Figs. 4a and b.

Generally, the surface area of natural cellulosic fiber such as dried flax and hemp is lower than $1 \text{ m}^2 \text{ g}^{-1}$ [19]. The measured surface area is about $0.9 \text{ m}^2 \text{ g}^{-1}$ for untreated bagasse and $1.4 \text{ m}^2 \text{ g}^{-1}$ for treated bagasse. It is noticeable that the surface area of treated bagasse is about 47 times higher than that of fine-grain sand (60×100 mesh U.S. standard, $0.03 \text{ m}^2 \text{ g}^{-1}$) reported in the literature [20]. Treated bagasse has a 1.56 times higher surface area than untreated bagasse. One plausible explanation is that, as observed in SEM analysis, the surface area increased (i) by the swelling of lumens and/or (ii) by the perforations on the surface.

3.5 XRD Analysis

XRD patterns of untreated bagasse and treated bagasse are presented in Fig. 5. Two broad peaks appear at 16° and at 23° in the crystalline patterns of untreated bagasse, which are the typical XRD patterns of α -cellulose [21]. Significantly reduced intensity peaks are observed in the crystalline patterns of treated bagasse, which indicate that the structure of raw bagasse was transformed to an amorphous structure after the acetylation reaction. Acetylation of cellulose materials often causes decrease in crystallinity [22–24]. The major part of cellulose is in crystalline form (about two-thirds) due to intra- and intermolecular hydrogen bonding of hydroxyl groups [25]. These crystallites mainly have a hydrogen bonding with the hydroxyl group and are attacked by acetic anhydride to form acetylated cellulose in the amorphous structure. The substitution of an acetyl group for a hydroxyl group reduces the density of hydrogen bonding because an acetyl group offers a more bulky branch (a lower ability to form hydrogen bonding) than a hydroxyl group [26]. To summarize, the characterization results imply that the treated sorbent has desirable properties for wicking oil and for promoting hydrocarbon biodegradation.

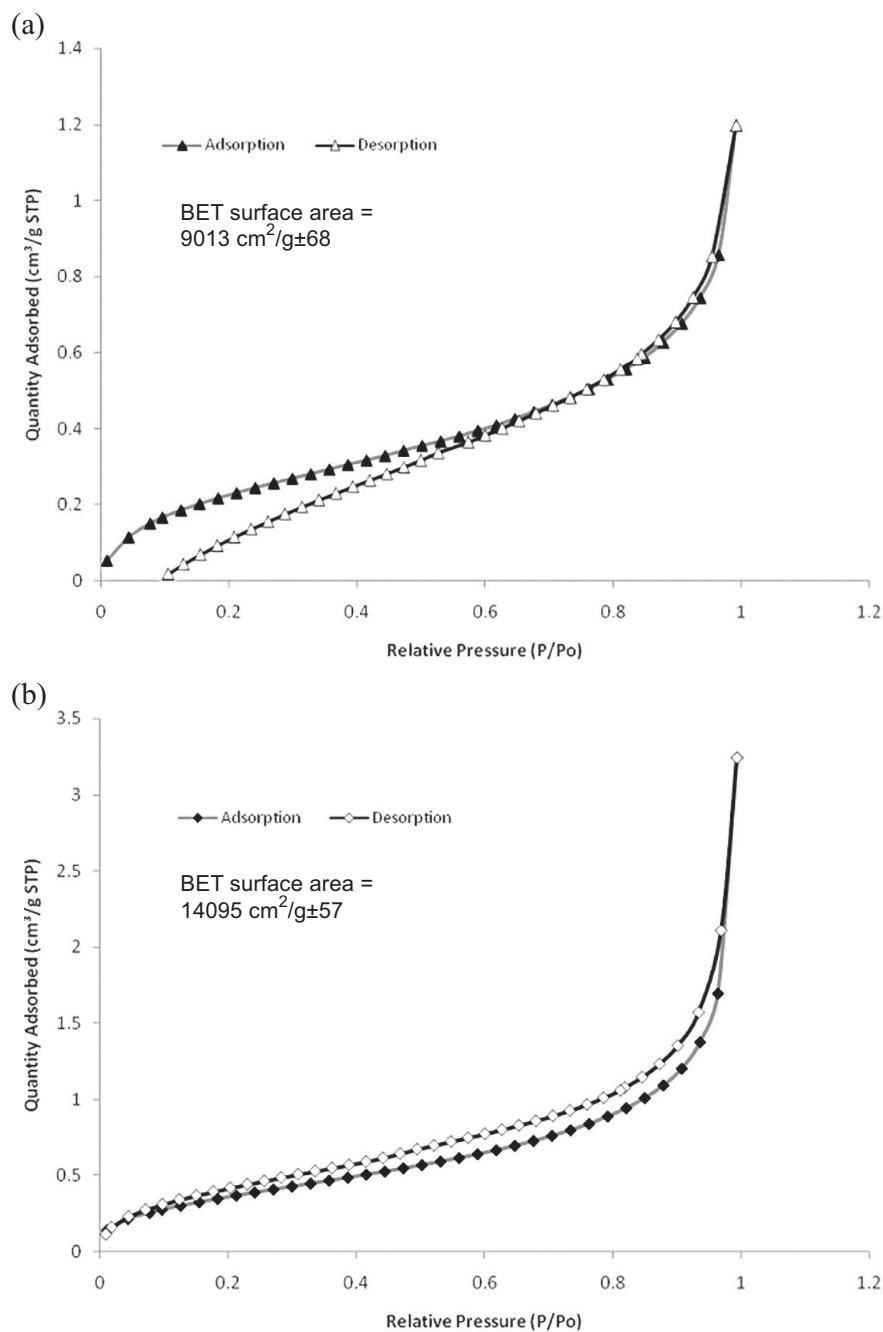


Figure 4. Nitrogen adsorption/desorption isotherm of (a) untreated bagasse and (b) treated bagasse.

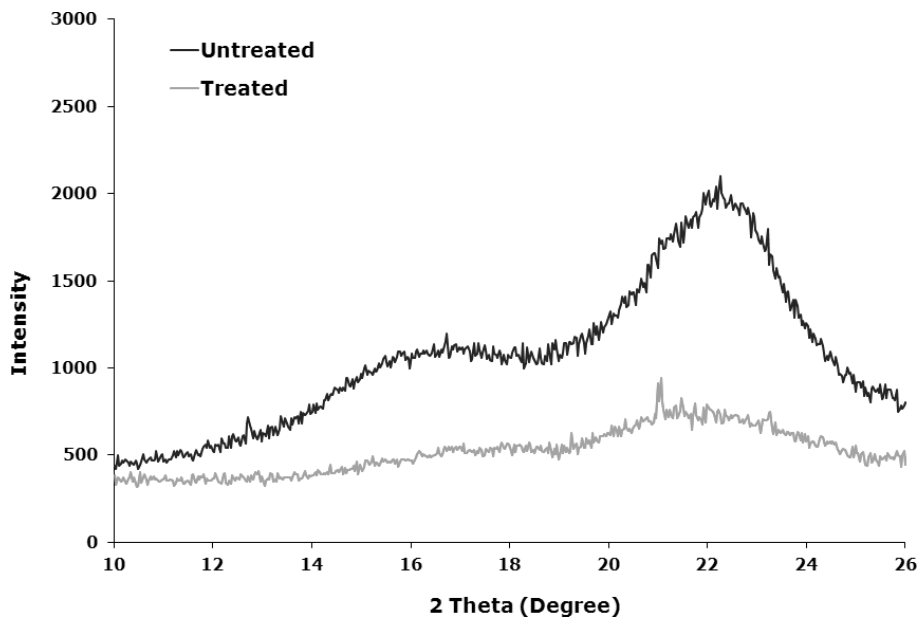


Figure 5. XRD patterns of untreated (top) and treated bagasse (bottom).

3.6 Oil-Wicking Tests in Abiotic Microcosms

Fig. 6 illustrates the oil-wicking effectiveness of untreated bagasse and treated bagasse. When the water level is on top of the clean sand layer, the effectiveness of untreated bagasse and treated bagasse are both about 85%. When the water level is on top of the oiled sand layer, the effectiveness of untreated bagasse is 72% and that of treated bagasse 80%. When water covers the sorbent layer, the effectiveness of untreated bagasse is 10% while that of treated bagasse is up to 30%. The difference in effectiveness between treated and untreated bagasse becomes more significant as the water coverage increases.

After the 3-month wicking period, 91–95% of total target petroleum hydrocarbons were recovered in terms of mass

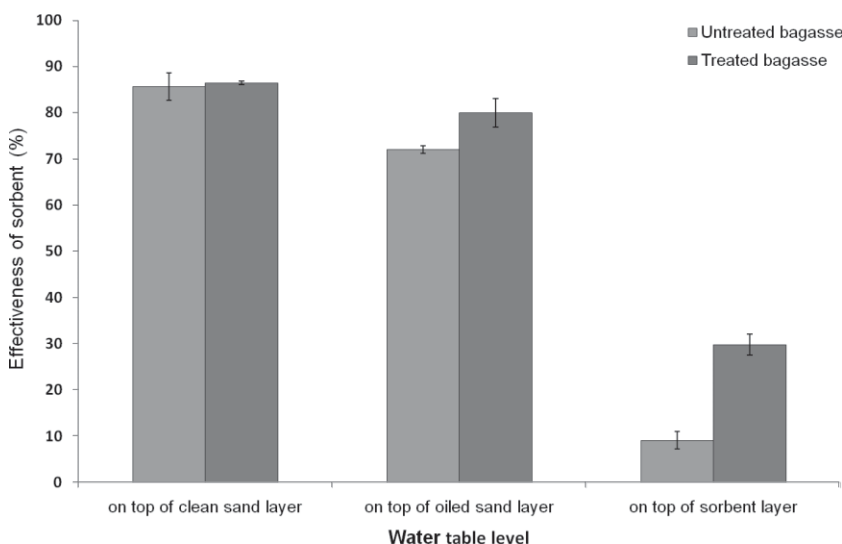


Figure 6. Oil-wicking effectiveness of sorbent in different water table levels.

closure. Considering the effectiveness between the selected sorbents, the difference in mass closure may cause a biased comparison. Therefore, statistical analyses by a two-sample T-test are performed on the pairs of mass closure and on the pairs of effectiveness. A hypothesis ($\mu_{\text{untreated}} = \mu_{\text{treated}}$) is tested for the pairs of mass closure and two hypotheses ($\mu_{\text{untreated}} = \mu_{\text{treated}}$ and $\mu_{\text{untreated}} > \mu_{\text{treated}}$) are tested for the pairs of effectiveness. The effectiveness of untreated bagasse and treated bagasse at a specific water table can be compared without a significant bias, since mass closures of the compared microcosms are not significantly different ($P > 0.05$). When the water level is on top of the clean sand layer, the effectiveness of untreated bagasse and that of treated bagasse are not significantly different ($P > 0.05$).

The effectiveness of treated bagasse is significantly higher ($\mu_{\text{untreated}} \geq \mu_{\text{untreated}}$, $P < 0.05$) than that of untreated bagasse when the water covered either the oiled-sand layer or the sorbent layer. Therefore, it is concluded that the acetylated bagasse is more effective in wicking oil from more saturated environments than untreated bagasse.

4 Conclusions

Sugarcane bagasse was treated by acetylation reaction to improve its abilities for wicking oil and for promoting biodegradation in saturated environments. The changes before and after treatment were investigated by several characterization techniques. The substitution of an acetyl group for a hydroxyl group during treatment was evidenced by FT-IR analysis and weight percent gain measurement. Nitrogen adsorption test results indicated that the BET surface area increased by 1.56 times after treatment.

The possible reasons for this increase are swollen lumens and/or the perforated surface of sugarcane bagasse during treatment as observed by SEM imaging. X-ray diffraction patterns demonstrated that this treatment was accompanied by structural changes (crystalline \rightarrow amorphous) due to a reduced density of hydrogen bonding with the hydroxyl group. The water sorption capacity of sugarcane bagasse was reduced from 80% to 50% after treatment, which indicated a considerable hydrophobicity but not a limited capability to hold moisture required for hydrocarbon biode-

gradation. The changes before and after treatment observed by characterization techniques agreed with the changes reported in the literature (referred to in Sect. 3) regarding acetylation of vegetable fibers (including sugarcane bagasse). Oil-wicking test results proved that treated bagasse had a higher potential of wicking oil from saturated environments than diduntreated bagasse. To summarize, experimental results implied that treated bagasse had desirable properties for wicking oil and for promoting hydrocarbon biodegradation in saturated environments.

The benefit of treated bagasse obtained in this study would be better understood if compared with works by other researchers. Said et al. [27] evaluated the oil sorption capacity of raw bagasse and that of hydrophobic bagasse (grafted by fatty acid) in the absence of water. They claimed that raw bagasse is more cost-effective for removing oil in the absence of water. In the present study, the oil-wicking test result in the lowest water level agrees with their claim. Sun et al. [9] reported that a highly acetylated bagasse (up to 24.7% of WPGs) was useful to remove oil spill in the presence of water due to high selectivity of oil to water. They assumed that a highly hydrophobic bagasse did not capture water and, therefore, suggested its application for capturing oil from open water. Under our treatment condition, a partially acetylated (18.2% of WPGs) bagasse with 50% of water-holding capacity was obtained. Either raw bagasse or highly hydrophobic bagasse would not be suitable for the application in bioremediation of oil-contaminated wetlands. Raw bagasse holds a significant amount of moisture that may limit the oil-wicking ability and oxygen diffusion required for hydrocarbon biodegradation. On the other hand, highly acetylated bagasse repels water, so that it cannot hold the minimum amount of moisture required for the hydrocarbon biodegradation. Therefore, the use of partially acetylated bagasse for wicking oil and for promoting hydrocarbon biodegradation in saturated environments due to an improved hydrophobicity and oil-wicking ability is suggested. In particular, the application of this material would be useful for remediation of oil-contaminated environments with a high degree of water saturation, with deficiency of oxygen for hydrocarbon biodegradation, and with a strong binding between oil and sediment.

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The authors have declared no conflict of interest.

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