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Degradation of Residual *Jatropha* Oil by a Promising Lipase-Producing Bacterial Consortium

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The potential of using lipase-producing bacteria in degrading the residual oil from *Jatropha curcas* L. biodiesel wastewater was assessed. Nine bacterial isolates obtained from mangrove areas in the Philippines and seven isolates from biodiesel wastewater were qualitatively and quantitatively screened for lipase activities. The three most promising bacteria (*Arthrobacter* sp. BOcMJL-12, *Bacillus* cereus BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2), with lipase activities of 19.33, 35.67 and 19.50 U/mL, respectively, were selected and evaluated, singly or in combination, for residual *Jatropha* oil degradation efficiency. The highest oil degradation efficiency of 94.84% was attained using the three isolates in a consortium. The conditions for oil degradation (medium composition, initial medium pH, initial substrate concentration and culture incubation time) by the three bacteria in combination were also partially optimized using the Random Balance Designs. The oil degradation assay, under partially optimized conditions (Medium #5, pH 7 and 1% residual *Jatropha* oil substrate), revealed that the mixed culture containing the three most promising bacteria could degrade up to 96.99% of residual *Jatropha* oil after 7-8 d of incubation with continuous agitation. This bacterial consortium may be used as a promising strategy for the treatment of lipid-rich wastes.

Key Words: bacterial consortium, *Jatropha*, lipase, partial optimization, residual oil

INTRODUCTION

The ever increasing price of petroleum and continuing global environmental concerns have been the main reasons for exploring the use of cleaner burning fuels. In the Philippines, the use of biofuels is mandated by the government under Republic Act 9367 (Biofuels Act of 2006). This Republic Act requires all liquid fuel for vehicles to have local biofuel components to ensure the availability of alternative and renewable, clean energy without much detriment to the environment (http://www.senate.gov.ph/republic_acts/ra%209367.pdf). By using biofuels such as bioethanol and biodiesel, greenhouse gas emissions can be minimized (Von Wedel 1999). However, high demands for petroleum alternatives will entail the generation of tremendous amounts of biodiesel wastewater, which may then result to difficulties in wastewater disposal (Suehara et al. 2005, Matsumiya et al. 2007). Such environmental concerns can possibly be addressed by tapping microbial resources in order to degrade wastes that are rich in lipids. The application of lipase-producing microorganisms to treat lipid-rich wastewater may provide an interesting strategy to eliminate expensive and harsh wastewater pre-treatments. This paper reports the use of bacterial consortia in the degradation of residual *Jatropha* oil. The phenotypic and genotypic characterization and identification of the three most promising bacterial isolates in the selected consortium have been described separately (Creencia et al. 2013).

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MATERIALS AND METHODS

Test organisms. A total of 16 bacteria were screened for lipase activities both by qualitative (Tween 80 hydrolysis and Rhodamine B plate assay) and quantitative (titrimetric) methods. Nine of these bacteria (BOrMAW-1, BOcMFW-2, BOrMEW-4, BOrMFS-5, BBoB1L2-6, BOcMJL-9, BOcMGL-12, BOcMJL-12 and BOcMJL-14) were isolated from Bohol and from Occidental and Oriental Mindoro, Philippines mangrove sites. *Pseudomonas aeruginosa* BIOTECH 1824 (ATCC 9027^T), a known lipase-producer, was used as positive control (Kouker and Jaeger 1987). Seven bacteria (BDF-1 to BDF-7) were isolated from *Jatropha curcas* L. biodiesel wastewater using the following procedure : About 2% (v/v) of the wastewater was inoculated into a 600 mL Erlenmeyer flask containing an enrichment medium which was composed (on a per liter basis) of 10 g of $(NH_4)_2SO_4$, 5 g of $MgSO_4 \cdot 7H_2O$ and 1 g of KH_2PO_4 (Matsumiya 2007). The set-ups were incubated with shaking (150 rpm) at room temperature for up to 3 days or until turbidity was observed. A loopful from the broth culture was streaked onto a Tryptone Glucose Yeast Extract Agar (TGYA) plate for isolation and purity check.

Actively-growing cells of the bacteria were maintained by re-streaking onto fresh Marine Agar (MA) for the mangrove isolates, and on Tryptone Glucose Yeast Extract (TGYA) agar for the indigenous wastewater bacteria and the positive control. All bacterial isolates were preserved by liquid-drying (L-drying) and by glycerol-broth stocks at -80˚C (Malik 1991). Likewise, the bacteria were accessioned at the Philippine National Collection of Microorganisms (PNCM) under BIOTECH accession numbers 10477 (*Bacillus cereus* BDF-2), 10478 (*Pseudoaltermonas* sp. BOcMFW-2), 10479 (*Arthrobacter* sp. BOcMJL-12), U-1212 (BDF-1), U-1213 (BDF-3), U-1214 (BDF-4), U-1215 (BDF-5), U-1216 (BDF-6), U-1217 (BDF-7), U-1218 (BOrMAW-1), U-1219 (BOrMEW-4), U-1220 (BOrMFS-5), U-1221 (BBoB1L2-6), U-1222 (BOcMJL-9), U-1223 (BOcMGL-12) and U-1224 (BOcMJL-14).

Tween 80 hydrolysis. A loopful of the isolate was streaked onto Sierra's agar plate medium with 1.5% NaCl and incubated at 30°C for up to 7 d. The appearance of opaque zones around the bacterial colony was indicative of hydrolysis of Tween 80 (polyethylene sorbitan monooleate) substrate (Kouker and Jaeger 1987, Jaeger et al. 1994, Hasan et al. 2000).

Rhodamine B agar assay for lipase activity. Sterile filter paper disks (8 mm in diameter) were laid on agar plate medium containing 0.8% of Nutrient Broth, 0.4% NaCl, 1% agar, 2.5% olive oil and 10 mL of Rhodamine B (1 mg/mL), at appropriate distances. Ten microliters of the supernatant from the cultivation medium was dispensed in each paper disk. Lipase activity was detected by the appearance of an orange fluorescent halo under UV light at 350 nm after 24 to 26 h of incubation at 37°C (Haba et al. 2000).

Titrimetric lipase assay. Quantification of the lipase enzyme produced by the bacterial isolates selected from the qualitative screening was determined based on the Khyami-Horani Method. A loopful of each test culture was inoculated into the Khyami-Horani broth and incubated with shaking (150 rpm) at room temperature (30°C) for up to 72 h. Cells were pelleted by centrifugation at 10,000 rpm for 5 m. The reaction cocktail contained 5 mL olive oil emulsion (25 mL olive oil and 75 mL 2% polyvinyl alcohol), 4 mL phosphate buffer (50 mM, pH 7), 110 mM $CaCl_2$ and 1 mL sample (cell-free supernant from the Khyami-Horani broth). The assay was done every 24 h for 3 d. Lipase activity was determined based on the amount of alkali that was used to neutralize the free fatty acids released with time (Khyami-Horani 1996).

Residual Jatropha biodiesel wastewater oil degradation assay. Ten milliliters of bacterial culture suspension (McFarland standard #4 = 1.2 x 10^9 CFU/mL) was inoculated into 100 mL of basal mineral medium consisting of 7.0 g $NaNO_3$ (NA), 2.0 g K_2HPO_4, 1.0 g KH_2PO_4, 0.1 g KCl (KC), 0.5 g $MgSO_4 \cdot 7H_2O$ (MG), 0.01g $CaCl_2$ (CA), 0.012 g $FeSO_4 \cdot 7H_2O$ (FE), 1.0 g yeast extract (YE), and 0.05 mL trace elements (TE) solution (Haba et al. 2000). The medium was supplemented with 2.0% (w/v) residual oil (JO) from *Jatropha* biodiesel wastewater. The set-ups were incubated with shaking (150 rpm) at room temperature (30°C) for 7 d. One milliliter triplicate samples were obtained and assayed every 24 h. The amount of residual oil was estimated by the n-Hexane Extraction Method and was determined as the difference between the weight of the flask containing the oil and the weight of the flask alone (US EPA 1999). Oil degradation efficiencies of both pure cultures and mixed cultures were evaluated. The three most promising bacterial isolates were selected and used for further residual *Jatropha* oil degradation studies under partially optimized conditions.

Partial optimization of conditions for oil degradation. The treatment (a pure or mixed bacterial culture) exhibiting the highest oil degradation efficiency relative to the control set-up was used to optimize medium composition, initial medium pH, initial substrate concentration and culture incubation time for oil degradation. Flasks containing 100 mL basal mineral medium were inoculated with 10 mL bacterial culture suspension equivalent to McFarland standard #4 and were incubated with shaking (150 rpm) at 30°C. Oil degradation efficiency was assayed as described previously.

Effect of medium composition

Improvement of medium composition was done by Random Balance Designs (Recknagel et al. 1988). An 8-component medium based on basal mineral salts medium was partially improved by using a 6-point experimental design. This resulted to the development of 6 different medium formulations,

wherein the original basal medium was considered as the basic point or reference medium (zero level). Variation intervals were first determined for each medium ingredient. The lower and upper levels of the components were derived from the reference medium by either addition or subtraction of the amount of variation for each component.

Effect of initial medium pH

The selected medium composition was adjusted to an initial pH of 6, 7 or 8 to determine the optimum pH condition for oil degradation. The initial medium pH in which the oil degradation efficiency was the highest was noted.

Effect of substrate concentration

The effect of initial substrate concentration on oil degradation efficiency was determined using different concentrations of residual *Jatropha* oil (1%, 2%, 3%, 4%, 5%, 10%, 15% and 20% w/v).

Effect of culture incubation time

The set-ups were incubated with shaking (150 rpm) at room temperature (30°C). Oil degradation efficiencies were monitored overtime. Oil degradation assay by hexane extraction was done every 24 h for up to 8 d.

Experimental design and statistical analysis

All quantitative data in this study were statistically analyzed using Analysis of Variance (ANOVA) under a completely randomized design (CRD). Means were compared using the Least Significant Difference Test.

RESULTS AND DISCUSSION

Lipase-producing bacterial isolates

All 16 isolates produced a zone of precipitate on Sierra's medium indicative of Tween 80 hydrolysis. However, a positive reaction in this medium does not confirm the presence of true lipases or glycerol ester hydrolases since other lipolytic enzymes such as esterases can also hydrolyze the Tween 80 substrate resulting to the same opacity response (Kouker and Jaeger 1987). The use of the fluorescent dye Rhodamine B in a plate assay which utilizes emulsified olive oil can distinguish true lipases from esterases (Jaeger et al. 1994). The former have a unique feature of acting at the interface between the aqueous and the non-aqueous phase. Specifically, a lipase reaction occurs in an emulsified solution containing water and water-insoluble substrates, as that in the case of the Rhodamine B Plate Assay. Formation of an orange fluorescent halo around the inoculation site upon UV irradiation, indicative of substrate hydrolysis, constitutes a positive reaction (Kouker and Jaeger 1987). All sixteen isolates and the positive control exhibited orange fluorescence on the Rhodamine B plate, indicating that these isolates produced true lipases (Figure 1).

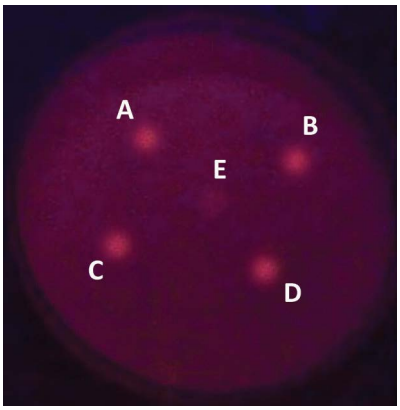

Figure 1. Appearance of an orange fluorescent halo on Rhodamine B plate under UV light at 350 nm after 24 to 26 h of incubation at 37°C. (A: positive control, B: *Bacillus cereus* BDF-2, C: *Arthrobacter* sp. BOcMJL-12, D: *Pseudoalteromonas* sp. BOcMFW-2 and E: uninoculated)

Majority of the isolates exhibited their peak lipase activities on the 48th h of incubation (Table 1). Isolates BDF-2, BOcMFW-2 and BOcMJL-12 produced the highest lipase activities (35.67 U/mL, 19.50 and 19.33, respectively) at this period compared to the positive control (*Pseudomonas aeruginosa* BIOTECH 1824) which produced only 4.5 U/mL after 72 h. These activities are considerably higher than those obtained from a study by Khyami-Horani (1996) wherein only about 2.00 U/mL lipase activity was obtained after 10 h incubation in the same medium and assayed using the same method. The highest lipase activity producer (identified to be *Bacillus cereus* BDF-2 in Creencia et al. 2013), Occidental Mindoro mangrove sites. These three promising isolates were, therefore, selected for the oil degradation assay.

Evaluation of oil degradation efficiencies

The set-up containing the consortium of three bacteria (*Arthrobacter* sp. BOcMJL-12, *Bacillus cereus* BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2) yielded the highest oil degradation rate (94.84%) compared to the positive control (69.03%) and any of those single bacterial culture or combinations of two cultures (Table 2). It is important to note though that isolate *Pseudoalteromonas* sp. BOcMFW-2 singly produced a high oil degradation activity of 91.91% which was not significantly different from that produced by the consortium. The single culture of *Bacillus cereus* BDF-2 and that of *Arthrobacter* sp. BOcMJL-12 resulted to relatively low degradation rates (i.e., 73.13% and 63.01%, respectively). Isolate *Bacillus cereus* BDF-2 when combined

Table 1. Lipase activities (U/mL) of the 16 lipase-producing bacterial isolates recovered from *Jatropha curcas* L. biodiesel wastewater and mangrove sites in Bohol and Mindoro, Philippines as determined using the titrimetric method.

ISOLATE	LIPASE ACTIVITY* (U/mL)**		
	24 h	48 h	72 h
Biodiesel Wastewater Isolates			
BDF-1	0.000^{b}	00.00^{e}	1.170^{d}
BDF-2 ***	0.000^{b}	**35.67^{a}**	00.00^{e}
BDF-3	0.000^{b}	3.660^{d}	00.00^{e}
BDF-4	0.000^{b}	2.000^{de}	00.00^{e}
BDF-5	0.000^{b}	3.170^{d}	00.00^{e}
BDF-6	0.000^{b}	4.670^{d}	00.00^{e}
BDF-7	0.000^{b}	4.330^{d}	00.00^{e}
Mangrove Isolates			
BOrMAW-1	0.000^{b}	8.830^{c}	00.00^{e}
BOcMFW-2***	0.000^{b}	**19.50^{b}**	00.00^{e}
BOrMEW-4	0.000^{b}	00.00^{e}	10.50^{a}
BOrMFS-5	0.000^{b}	4.750^{d}	00.00^{e}
BBoB1L2-6	0.000^{b}	17.00^{b}	00.00^{e}
BOcMJL-9	0.000^{b}	16.67^{b}	1.670^{d}
BOcMGL-12	0.000^{b}	2.330^{de}	9.000^{b}
BOcMJL-12***	0.670^{a}	**19.33^{b}**	1.500^{d}
BOcMJL-14	0.000^{b}	00.00^{e}	9.830^{ab}
***Pseudomonas aeruginosa* BIOTECH 1824 (positive control)**	0.000^{b}	00.00^{e}	4.500^{c}
***Uninoculated* (negative control)**	0.000^{b}	00.00^{e}	00.00^{e}

*one unit of enzyme activity corresponds to the liberation of 1 µmol of fatty acid from the oil substrate per minute
** means with same letter designations are not significantly different at α=0.050 by the Least Significant Difference Test (LSDT)
*** identified to be *Bacillus cereus* BDF-2, *Pseudoalteromonas* sp. BOcMFW-2 and *Arthrobacter* sp. BOcMJL-12 (Creencia et al. 2013)

Table 2. Oil-degradation efficiencies of the single- and mixed bacterial culture-treatments of the most promising lipase-producing isolates cultivated with continuous agitation on basal mineral medium at 30°C for 7 d.

CULTURE SET-UP	OIL DEGRADATION EFFICIENCY (%)*
Arthrobacter sp. **BocMJL-12 (A)**	63.01^{cd}
Bacillus cereus **BDF-2 (B)**	73.13^{bc}
Pseudoalteromonas sp. **BocMFW-2 (C)**	**91.91^{a}**
A+C	79.38^{b}
B+C	55.66^{d}
A+B	68.70^{bc}
A+B+C	**94.84^{a}**
Pseudomonas aeruginosa BIOTECH 1824	69.03^{bc}

* means with same letter designations are not significantly different at α= 0.050 by the Least Significant Difference Test

with *Pseudoalteromonas* sp. BOcMFW-2 resulted to a very low degradation rate of 55.66%, but with the addition of *Arthrobacter* sp. BOcMJL-12, resulted to a very significant increase in degradation ability. A microbial consortium consisting of *Aerobacter aerogenes, Bacillus subtilis, Cellulomonas biazotea, Nitrosomonas* sp., *Nitrobacter winogradskyi, Pseudomonas denitrificans, P. stutzeri* and *Rhodopseudomonas palustris* had been used as bioadditive to treat wastewater with high lipid content under an oxygen concentration of 4-8 mg/L for 12 to 72 h and using a bacterial cell density of 10^7 to 10^9 cells/mL (Gardon and Lebesque 1991 as cited by Cammarota and Freire 2006). Tano-Debrah et al. (1999) isolated 15 bacterial species from wastewater samples from grease-traps of restaurants in Japan. An inoculum composed of these isolates was developed to degrade eight types of fats and oils from animal and plant origin. The mixed culture was able to degrade up to 73% olive oil after 7 days of incubation at 30°C.

The specific roles of each organism in synergism may not be defined but it is possible that one species may have the ability to remove toxic substances that may repress the enzyme activity of the other. It is likewise possible that the 2nd species may be able to degrade the compound that the first could only degrade partially (Mukred et al. 2008). Cooperation between the three bacteria may have occurred resulting to a significantly higher lipase activity. Inefficiency of one bacterium to exhibit an enzymatic activity can be compensated by the capability of the other. This will possibly ensure the stability of the lipase-producing bacteria to carry out degradation of the substrate when inoculated into the actual wastewater sample wherein conditions are unfavorable. For these reasons, the mixed culture set-up was selected in further tests.

The advantages of using mixed cultures as opposed to pure cultures in biotreatment have been widely demonstrated. The specific roles of each organism in synergism may not be defined but it is possible that one species may have the ability to remove toxic substances that may repress the enzyme activity of the other. It is likewise possible that the 2nd species may be able to degrade the compound that the first could only degrade partially. Cooperation between the three bacteria may have occurred resulting to a significantly high lipase activity. Inefficiency of one bacterium to exhibit an enzymatic activity can be compensated by the capability of the other. This will possibly ensure the stability of the lipase-producing bacteria to carry out degradation of the substrate when inoculated into the actual wastewater sample wherein conditions are unfavorable. For these reasons, the mixed culture setup was selected in further tests.

Partial optimization of conditions for oil degradation

The medium combination that yielded the highest oil degradation efficiency after 7 d (96.03%) was Medium #5 (Table 3). It is to be noted, however, that its efficiency was very close to that

Table 3. Summary effect of the six different medium formulations evaluated on residual *Jatropha* oil degradation efficiency by the mixed culture of isolates *Arthrobacter* sp. BOcMJL-12, *Bacillus cereus* BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2 cultivated with continuous agitation (150 rpm) in basal mineral medium (pH 7) at 30°C for 7 d.

HABA MEDIUM COMBINATION	OIL DEGRADATION EFFICIENCY (%)*
1	47.49[d]
2	78.97[ab]
3	65.29[bcd]
4	77.62[abc]
5	**96.03**[a]
6	48.13[cd]

* means with same letter designations are not significantly different at α= 0.050 by the Least Significant Difference Test
Medium 1: 3% JO, 10.5 g NA, 0.5 g YE, 0.2 g KC and 0.02 g CA
Medium 2: 1% JO, 3.5 g NA, 1.5 g YE, 0.2 g KC, 0.024 g FE and 0.1 mL TE
Medium 3: 3% JO, 3.5 g NA, 1.5 g YE, 0.2 g KC, and 1.0 g MG
Medium 4: 1% JO, 10.5 g NA, 0.5 g YE, 1.0 g MG, 0.024 g FE and 0.1 mL TE
Medium 5: 1% JO, 10.5 g NA, 1.5 g YE, 0.02 g CA, 0.024 g FE and 0.1 mL TE
Medium 6: 3% JO, 3.5 g NA, 0.5 g YE, 1.0 g MG and 0.02 g CA

for the basal medium (which was automatically 100%, being the reference point). In this medium, the concentration of the substrate and C-source, KCl and $MgSO_4 \cdot 7H_2O$ were decreased, while that of $NaNO_3$, yeast extract, $CaCl_2$, $FeSO_4 \cdot 7H_2O$ and trace element solution were increased, relative to the original basal mineral medium composition. Specifically, Medium #5 contained (in g/L): 1 g oil substrate (JO), 10.5 g $NaNO_3$ (NA) 1.5 g yeast extract (YE), 0.02 g $CaCl_2$ (CA), 0.024 g $FeSO_4 \cdot 7H_2O$ (FE) and 0.1 mL trace elements (TE) solution. Two components, KCl (KC) and $MgSO_4 \cdot 7H_2O$ (MG), were omitted.

Results indicated that oil degradation by the bacterial consortium after 7 d was highest (90.29%) when the initial pH of the medium was maintained at pH 7 (Table 4).This may indicate that the lipases produced by the three bacteria were most active at neutral pH. At pH 8, oil degradation efficiency was 82.67% while that at pH 6 was 75.83%. It was previously reported that a more stable emulsion formation can occur at alkaline pH than at acidic pH (Casa 2001). Studies by Chen *et al.* (1998) and Zhen-Qian and Chun-Yun (2009) also revealed that lipase can be produced optimally at 30°C and at pH 7 for certain strains of *Acinetobacter* sp. and *Enterobacter* sp.

There was generally an inversely proportional relationship between the substrate concentrations used and oil degradation efficiency. With increasing substrate concentration, the oil degradation efficiency decreased. Among those tested, 1% oil substrate was the substrate concentration that was degraded most efficiently by the bacterial consortium (Table 5). This is relatively close to the concentration of oil and grease present in the *Jatropha* biodiesel wastewater which is 5,604 ppm or

Table 4. Effect of three initial medium pH levels on residual *Jatropha* biodiesel wastewater oil degradation efficiencies by the mixed culture-treatment of isolates *Arthrobacter* sp. BOcMJL-12, *Bacillus cereus* BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2 cultivated with continuous agitation (150 rpm) in basal mineral medium #5 at 30°C for 7 d.

INITIAL MEDIUM pH	OIL DEGRADATION EFFICIENCY (%)*
6	75.83[c]
7	**90.29**[a]
8	82.67[b]

*means with same letter designations are not significantly different at α= 0.050 by the Least Significant Difference Test

0.5604% which, in turn, indicates that the mixed culture-treatment may be applied to biodiesel wastewater. This also confirms the results in the medium improvement conducted wherein Medium #5 containing 1% oil was the best medium for oil degradation. On the other hand, the medium which had the greatest amount of substrate (20%) yielded the lowest oil degradation efficiency (71.42%). Increased substrate concentration may have repressed lipolysis by reducing the availability of oxygen as well as the surface area-to-volume ratio of the oil-water interface.

Generally, oil degradation efficiency gradually increased with increasing culture incubation time (Table 6). At day 1, the ability to degrade the *Jatropha* oil was at its lowest efficiency (66.52%). Extending the incubation time up to the 8th day resulted to the highest degradation rate of 96.99% which was, however, not significantly different from the 96.54% efficiency obtained at day 7. With the partial optimization conducted, the oil degradation efficiency of the mixed bacterial culture increased only by

Table 5. Effect of initial substrate concentration on the residual *Jatropha* oil biodiesel wastewater degradation efficiency of the mixed culture-treatment of isolates *Arthrobacter* sp. BOcMJL-12, *Bacillus cereus* BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2 cultivated withcontinuous agitation (150 rpm) in basal mineral medium #5 (pH 7) at 30°C for 7 d.

CONCENTRATION OF RESIDUAL *Jatropha* OIL (% w/v)	OIL DEGRADATION EFFICIENCY (%)*
1	97.86[a]
2	90.52c
3	92.51[b]
4	92.52[b]
5	81.46[d]
10	89.37[c]
20	71.42[e]

*means with same letter designations are not significantly different at α= 0.050 by the Least Significant Difference Test

Table 6. Effect of culture incubation time on the residual *Jatropha* biodiesel wastewater oil degradation efficiency of the mixed culture-treatment of isolates *Arthrobacter* sp. BOcMJL-12, *Bacillus cereus* BDF-2 and *Pseudoalteromonas* sp. BOcMFW-2 cultivated with continuous agitation (150 rpm) in basal mineral medium #5 containing 1% w/v *Jatropha* oil (pH 7) at 30°C for 8 d.

INCUBATION TIME (DAYS)	OIL DEGRADATION EFFICIENCY (%)*
1	66.52^{f}
2	84.14^{e}
3	84.57^{de}
4	87.16^{cd}
5	90.04^{bc}
6	90.38^{b}
7	96.54^{a}
8	96.99^{a}

*means with same letter designations are not significantly different at α= 0.050 by the Least Significant Difference Test

about 2% (from 94.84% under unoptimized conditions). It is most likely that the unoptimized medium and incubation conditions were actually already near optimum. However, an increase in oil degradation efficiency after 8 d could also be possible. Based on a study by Mongkolthanaruk and Dharmsthiti (2002), a mixed culture composed of *P. aeruginosa* LP_{602}, *Acinetobacter calcoaceticus* LP_{009} and *Bacillus* sp. B_{304} had been effective in lowering the Biological Oxygen Demand (BOD) and lipid content of a wastewater sample within 12 d of treatment under aerobic conditions. A culture incubation time longer than 8 d is, therefore, recommended to be tested to confirm the relationship between oil degradation efficiency and culture incubation time.

CONCLUSION

The data obtained from this study indicate that with provision of optimum conditions (i.e., Medium #5, pH 7, 1% residual *Jatropha* oil substrate, and 7 to 8 d culture incubation), the use of a high lipase-producing bacterial consortium composed of isolates BOcMJL-12, BDF-2 and BOcMFW-2, which were previously identified as *Arthrobacter* sp., *Bacillus cereus* and *Pseudoalteromonas* sp., respectively, has a very good potential in degrading residual *Jatropha* oil in biodiesel wastewater. Because of their significantly high oil degradation efficiencies, they can be considered a promising strategy for the biotreatment of lipid-rich wastes.

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