African Journal of Biotechnology Vol. 10(63), pp. 13841-13851, 17 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1720
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Isolation and characterization of Jatropha oil-degradation by *Enterococcus faecalis* and *Burkholderia cenocepacia* W-1 under anaerobic condition

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Accepted 19 September, 2011

Ground contamination by oily substances led to several environmental issues. This work focused on the isolation and characterization of micro-organisms which possess the capability of degrading Jatropha oil, a feed stock of biodiesel production, under anaerobic condition. The active isolated strains were tested for morphological, physiological and genotypic characteristics. Based on 16S rRNA sequence analysis, the *Enterococcus faecalis* and *Burkholderia cenocepacia* W-1 were selected due to their ability to produce large clear zones. Further studies were carried out for oil degradation using minimal M9 salt media supplemented with 1% Jatropha oil as the sole carbon source. Lipolytic activity assay showed that the highest activity was obtained at 532 U/L. In addition, GC/MS analysis revealed that different compounds from the head space and supernatant included several hydrocarbons. However, free fatty acids formed during hydrolytic activity resulted in the decrease of pH, and also retarded bacterial growth probably due to the toxicity of the fatty acids on the cells. The consequence of this study is the benefit of reduced environmental problems from Jatropha oil contamination as well as obtained useful gaseous biofuels.

Key words: Jatropha oil, anaerobic biodegradation, phylogenetic tree, lipolytic activity, hydrocarbons.

INTRODUCTION

Jatropha is a multipurpose small tree, belonging to the Euphorbiaceae family, which has its native distributional region in Central America. Nowadays, it is commonly found and utilized throughout most of the tropical and subtropical regions of the world (de Oliveira et al., 2009). Several properties of the crop, especially its high oil content, led to its use as feed stock for biodiesel production in order to substitute the depleting fossil fuels. The chemical composition of Jatropha oil strongly depends on interactions with both environment and genetics. Typically, the oil contains more than 75% unsaturated fatty acid. The fatty acid composition is dominated by oleic (C18:1) and linoleic acid (C18:2), respectively. However, the maturity stage of the fruits at the moment of collection is reported to influence the fatty acid composition of the oil (Archten et al., 2008). During biodiesel production process, ground and sewage contamination by the oil leads to several environmental issues especially blockage of the drainage system because of the difficulty to digest lipid substances. So far, many investigations have been realized on microbial degradation of lipids under anaerobic conditions (Ahring, 2003). In an anaerobic environment, lipids are first hydrolyzed by extracellular lipase to produce glycerol and long-chain fatty acids which are further hydrolyzed through the β-oxidation pathway. Many works of microbial degradation of lipid substances were dedicated to environment protection (Dudd et al., 1998; Hatamoto et al., 2007; Li et al., 2005; Matsumiya et al., 2007; Sousa et al., 2007; Sugimori et al., 2002). In addition, lipids are also attractive substrates for biogas production due to

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their high theoretical methane yield in comparison with other organic substrates. Recently, experimental works have focused on enhancement of biogas production with the present of lipid substances (Azbar et al., 2009; Cirne et al., 2007; Grossi et al., 2001; Hansen et al., 1999; Li et al., 2005).

Recently, bacteria that are able to degrade oily substances under anaerobic condition are isolated and identified. The use of rRNA sequences for classification and identification of microorganisms is now a regular technique which provides a powerful tool to determine the phylogenetic and evolutionary relationships of micro-organisms. This research work aimed to screen potential micro-organisms having the capability of utilizing lipid from Jatropha under anaerobic condition. Lipolytic activities were also investigated. Finally, attempts were made on investigations of hydrocarbon formation during anaerobic biodegradation of lipid from both gaseous headspace and supernatant.

MATERIALS AND METHODS

Sample and chemicals

All chemicals were of analytical grade. Soil-contaminated samples were aseptically collected from Jatropha oil press factories in Nakhon Ratchasima and in Chiang Mai, Thailand. All samples were kept in anaerobic jars and brought to our laboratory for further analysis. The composition of mineral salt medium was as follows (g/L): Na_2HPO_4 6.3, KH_2PO_4 3.0, NaCl 0.5 and $(NH_4)_2SO_4$ 1.0 in 950 mL of distilled water (pH 7.0), sterilized at 121°C for 15 min. Subsequently, the following solutions were added into the medium: 1 M $MgCl_2$ 2 mL, 1 M $CaCl_2$ 10 µL, 0.01 M $FeSO_4$ 1 mL, Vitamin solution 1 mL, and Micronutrient 1 mL which is composed of Thiamine 50 mg, D-biotin 10 mg, Choline chloride 10 mg, Folic acid 10 mg, Niacinamide 10 mg, D-panthothenic acid 10 mg, Pyridoxal 10 mg and Riboflavin 1 mg, respectively. The medium was supplemented with 1% (w/v) Jatropha oil as the sole carbon source.

Screening of microorganisms and cultivation procedures

Bacterial colonies developed on the agar plates were individually picked and re-streaked on fresh agar plates by dilution-streaking technique in order to obtain single colony, or pure culture. The purity of the cultures grown on Jatropha oil was finally checked by phase-contrast and scanning electron microscopy. For all samples, 5 g of soil-contaminated sample were transferred into 50 ml of sterile LB medium prior to place in an anaerobic chamber at 30°C for 5 days. Single colony streak of each pure isolate was obtained by repeated streaking on minimal M9 salt agar medium (Atlas, 1981) supplemented with 1% (w/v) Jatropha oil, and anaerobically incubated by using an anaerobic chamber filled with gas packs (Anaerocult®, Merck, Germany). Different bacteria were isolated from the agar plates, and those with a clearing zone were recorded and preserved. The obtained cultures were incubated under anaerobic conditions for 2 months at 30°C in minimal salts medium.

Quantitative growth experiments

The time course and degradation balance of Jatropha oil degradation were measured in 50 mL bottles containing 30 mL of minimal M9 salt media. Cultures were shaken by hand for few seconds per day and incubated without agitation. After 10 days of incubation, visible changes in the medium appeared, and an appreciable increase of the bacterial density in medium was observed. In order to measure cultures growth, 2 mL aliquots were periodically taken from the aqueous phase followed by centrifugation, and re-suspension in an equal volume of phosphate buffer. The optical density (OD) was measured at 660 nm. The metabolites (produced by the degradation in the medium) content was measured immediately after sampling.

PCR amplification of 16S rRNA genes

DNA templates for PCR amplification were prepared by the method previously described (Marmur, 1961). The reaction mixture (100 µL total volume) for the PCR was composed as follows: 15 ng of template DNA, 2.0 µM of two primers, 2.5 units of Taq polymerase, 2.0 mM $MgCl_2$, 0.2 mM dNTP and 10 µL of 10×Tag buffer, pH 8.8, containing $(NH_4)_2SO_4$, which was composed of 750 mM Tris-HCl, 200 mM $(NH_4)_2SO_4$ and 0.1% Tween 20. The partial 16S rRNA nucleotide sequence was determined. The 16S rRNA gene was amplified by PCR using primers as described by Brosius et al. (1981). A PCR product for sequencing 16S rRNA regions was prepared by using the following two primers: forward primer 20F (5'-GAG TTT GAT CCT GGC TCA G-3'), positions 9-27 on 16S rRNA by the *E. coli* numbering systems, and reverse primer 1500R (5'-GTT ACC TTG TTA CGA CTT-3'), positions 1509-1492 on 16S rRNA by the *E. coli* numbering system (Brosius et al., 1981). The PCR reaction consisted of an initial denaturation step at 94°C for 3 min, followed by 25 cycles with denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min and primer extension at 72°C for 2 min, with a final extension at 72°C for 3 min. The PCR products were analyzed by 0.8% (w/v) agarose gel electrophoresis.

Purification and 16S rRNA sequencing analysis

Purification of 16S rRNA gene PCR products was carried out with the QIAquick® PCR purification kit (Qiagen, Gernamy). For sequencing, the purified PCR products were carried out with an ABI PRISM® BigDye™ Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Direct sequencing of both single-banded and purified PCR product was carried out. The following primers were used: forward primer 520F (5′-CAG CAG CCG CGG TAA TAC-3′) with position 519-536, reverse primer 520R (5′-GTA TTA CCG CGG CTG CTG-3′) with position 539-519, forward primer 920F (5′-AAA CTC AAA TGA ATT GAC GG -3′) with position 907-926, and reverse primer 920R (5′-CCG TCA ATT CAT TTG AGT TT -3′) with position 926-907 (Brosius et al., 1981). The reaction mixture (10 µL total volume) was composed as follows: 5 ng of template DNAs, 2.0 µL of BigDye™ terminator ready reaction mixture, 5 ng of DNA template, 1.6 pM of sequencing primer, and 1.5 µl of 5× BigDye ™ sequencing buffer, filled with deionized water. The PCR reactions were carried out as follows: an initial denaturation step at 96°C for 30 s by 25 cycles of denaturation at 96°C for 10 sec, primer annealing at 50°C for 5 sec and primer extension at 60°C for 4 min. The sequences were then compared to those in GenBank (National Center for Biotechnology Information; www.ncbi.nig.gov).

Residual oil content, lipolytic activity and protein concentration assay

Jatropha oil content was determined using a modified partition-gravimetric method. The aqueous phase was extracted with chloroform and methanol (2:1, v/v). Organic solvents were

subsequently evaporated by nitrogen to obtain the total oil extract. Free fatty acids were removed to yield the neutral oil by titration with NaOH followed by gentle washing. The remaining oil residue was then completely dried by incubation at 37°C for 24 h and the weight of extracted oil was measured. The assay of lipolytic activity was carried out using p-nitrophenyl laurate (Sigma, USA) as substrate at pH 8.0, 65°C with the reaction time of 20 min (Fuciños et al., 2005). One unit of lipolytic activity is equivalent to the amount of enzyme that produced 1 µM of p-nitrophenol per min. In addition, the concentration of protein was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as standard.

GC/MS/MS analysis of metabolites

The headspace-gas samples were withdrawn using a 1 mL syringe prior to analysis. For liquid samples, bacterial cells were removed by centrifugation at 6,000 g for 5 min. The supernatants were subsequently extracted with the same volume of diethyl ether. The extracts were further analyzed by using a gas chromatography coupled with a flame ionization detector (model CP-3800; Varian, USA). A capillary column (FactorFourTM; Varian, USA) with 30 m length, 0.25 microns film thickness, and a diameter of 0.25 m was used to analyze the samples. A quadruple mass spectrometer detector (model 1200 L; Varian, USA) was also employed to further identify individual hydrocarbon metabolite. The oven temperature profile was set as follows: initial temperature was held at 30°C for 7 min before it was ramped to 250°C at the rate of 15°C/min. The temperature was then held for 12 min. Injector temperature was set at 300°C. Various hydrocarbon metabolites were identified by interpretation of mass spectra compared with standard C8 to C20 aliphatic alkanes.

RESULTS AND DISCUSSION

Isolation of strains and phenotypic characteristics

In the minimal M9 salt agar containing Jatropha oil as the sole carbon source, few bacterial colonies are able to grow and result in the formation of clear zones, whereas many colonies had no clear zone. In this study, we focused on two bacterial strains namely W-1 and W-2 which produced the largest clear zones on the agar plates. By repeated picking up and dilution of the colony of two strains, pure cultures of these strains were successfully obtained. More biochemical and physiological tests were carried out to identify the strains precisely, and their specific names were tentatively given only at this point. Microscopic examination of the two strains (W-1: gram-negative, and W-2: gram-positive) revealed the presence of short cocci cells. Cells of both strains were non-motile and cocci shaped approximately 1 to 3 mm cell diameter. Microscopic examination did not reveal any evidence of spores or of swimming motility.

It is interesting that these two strains are facultative anaerobes, which are characterized by their ability to exist under both aerobic and anaerobic conditions, and allow to achieve more stable population numbers and metabolic (in this case, catabolic) activity within a wide oxidation–reduction potential range. Strain isolation involves the processes of enrichment and spreading of the bacteria on mineral salt agar containing Jatropha oil. Strains that grow on the agar plates are able to degrade the substrate, and produce clear zones on the plates. Bacterial populations could develop over time to metabolize these compounds. From these finding, the two strains, W-1 and W-2, which had the ability of degrading substrate were selected for further investigations in order to study these two bacteria physiology and metabolism characteristics.

Genotypic characterization of the isolated strains

A preliminary estimation of their degradation activity and taxonomic distribution were used as criteria in the selection of code strains W-1 and W-2 for further investigations. The taxonomic position of bacteria has, however, not been known with certainty. We circumvented this problem by using selective PCR amplification, and subsequent sequencing of the 16S rRNA genes directly from the isolated bacteria. Genomic DNA of two isolates were extracted and used as DNA template for PCR amplification. The single amplified DNA fragments of approximately ±1,500 bp size were generated as shown in Figure 1. The two strains were identified on the basis of their 16S rRNA homologies with entries in the GenBank-EMBL databases. Experimental results showed that 2 isolated microorganisms were able to grow on Jatropha oil as the sole carbon source under anaerobic condition. The 2 isolated strains were named W-1 and W-2 prior to further analysis for genotypic characteristics using 16S rRNA technique. When examined by BLAST similarity analysis, 16S rRNA sequence from the isolate (W-1 and W-2) was produced and closely related to *Burkholderia cenocepacia* and *Enterococcus faecalis* with a homology of 98 (GenBank database: accession number JF701797) and 100% (GenBank database: accession number JF701796), respectively. However, the first isolate strain belonged to the group of *Burkholderia cepacia* complex which is composed of at least nine different closely related species. This high degree of homology can be classified into putative *Burkholderia cenocepacia* W-1, but this species requires sequencing of housekeeping genes in order to confirm its identification.

Jatropha oil degradation profiles

The time profiles of oil degradation under anaerobic condition by both bacterial strains were investigated. Figure 2 displays the Jatropha oil degradation rate, pH, biomass concentration and lipolytic activity, respectively. In this work, supernatant of the medium culture was subjected to lipase activity assay. *E. faecalis* revealed its higher ability in degradation of Jatropha oil compared to *B. cenocapacia* W-1 throughout the experiment. After

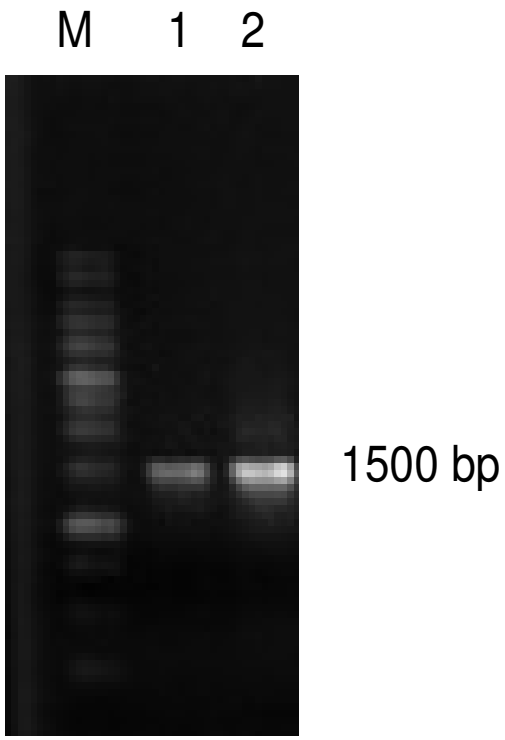

Figure 1. Agarose gel electrophoresis of 16S rRNA-PCR fragment obtained from the amplification of genomic DNA of the two isolates: Lane M (Kb DNA ladder); Lane 1 (isolate W-1); Lane 2 (isolate W-2).

incubation, *E. faecalis* started to grow on Jatropha oil with a lag phase of approximately 10 days. Subsequently, cell growth entered the exponential phase where cell concentration of *E. faecalis* rapidly increased from 0.42 to 3.35 g/L for 36 days followed by a slow growth rate before reaching the plateau of 3.92 g/L after 52 days. *B. cenocapacia* W-1 possessed a longer lag phase of approximately 15 days, and cell concentration increased until the final concentration of 3.58 g/L was obtained. Time course of lipolytic activity and changes in the composition of residual oil were also investigated (Figure 2C and D). It is a fact that the activity of lipase results in liberation of free fatty acids from triglycerides which affect the growth of the bacteria, but the study of this inhibition is beyond the scope of this work.

Lipolytic activity of both strains increased rapidly during the exponential growth phase to reach the maximum value of 420 U/L for *E. faecalis* and 400 U/L for *B. cenocepacia* W-1, respectively. However, lipolytic activity for the latter case decreased sharply after reaching the highest point to the final value of approximately 250 U/L. The drop in the lipolytic activity is probably due to the depletion of the Jatropha oil substrate. In addition, Jatropha oil was degraded efficiently and followed the same trend as a growth characteristic. Slow oil degradation rate was observed at approximately 15%, respectively. Oil degradation rate value rapidly increased from 10 to 80% and then increased more slowly to 96% at the end of the experiment. It is a fact that two strains can grow on medium containing Jatropha oil as a sole carbon source. From the time profile, pH of strain *B. cenocepacia* and *E. faecalis* were continually decreased from the initial value of 7.0 to the final value of 6.0 and 5.5 (after 60 days), respectively. The exponential growth phase was relatively short, as seen in the linear plot of the cell concentrations versus time. Therefore, only an approximate determination of the doubling time in exponential phase was possible in the quantitative growth experiments, yielding a value of approximately 12 days.

Characterization of lipolytic activity of culture supernatant

Since *E. faecalis* showed a high oil degradation performance, additional works were carried out in order to characterize the lipolytic activity of the enzyme under different operating conditions. Influence of temperature on lipolytic activity is shown in Figure 3(A). The culture supernatant exhibited lipolytic activity for all over the temperature which ranged from 25 to 60°C of 5°C interval. From the initial value of 277 U/L at 25°C, lipolytic

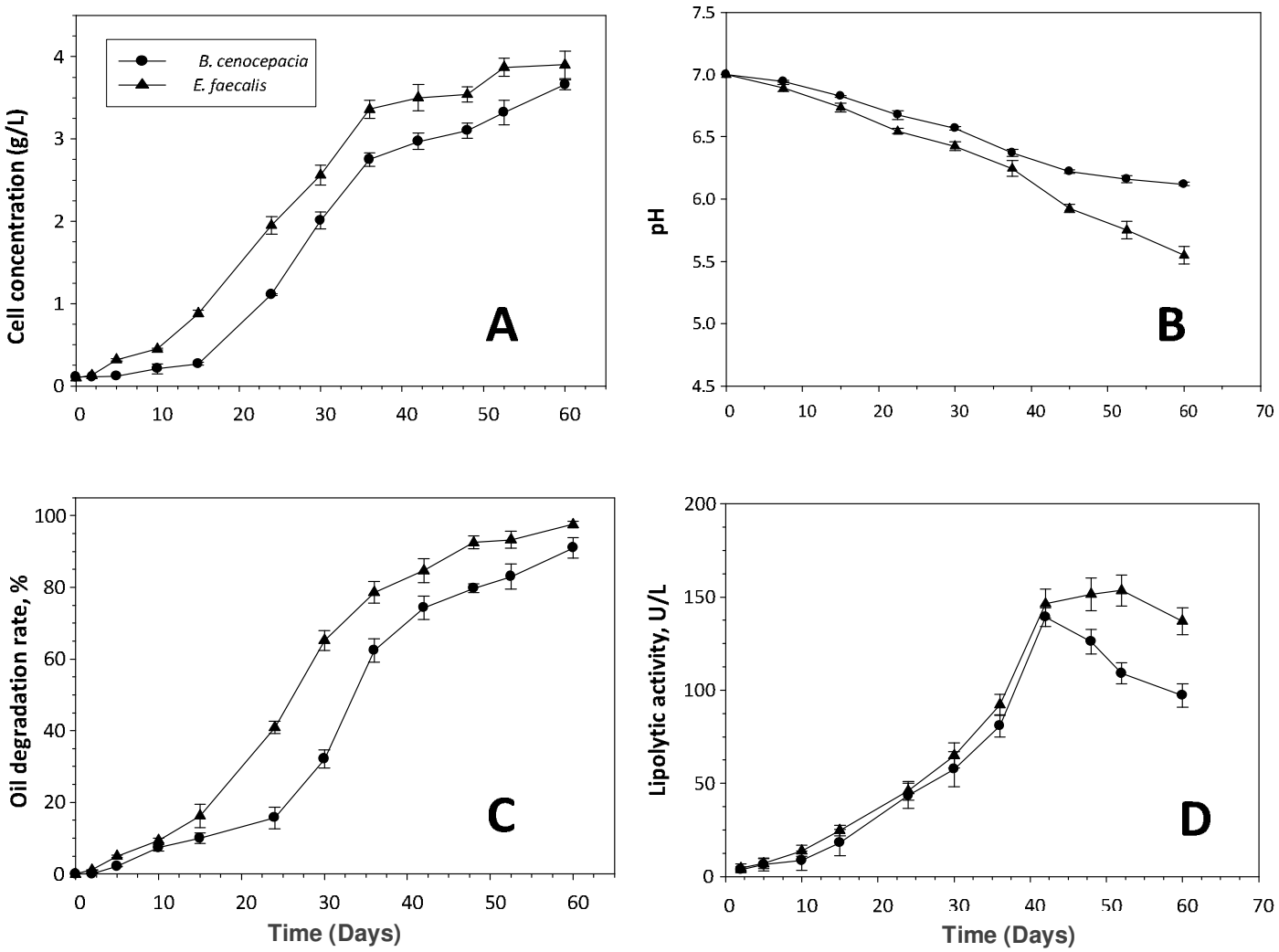

Figure 2. Time profiles of growth, oil degradation rate, pH, and lipolytic activity of bacterial strain *B. cenocepacia* W-1 (circle) and *E. faecalis* (triangle) on mineral M9 salt medium containing 1% Jatropha oil. The data are means (symbols) ± SD (error bars) obtained from three independent measurements of the same samples from representative cultures.

activity rapidly increased to its highest point of over 475 U/L at 50°C before it gradually decreased to approximately 90% at 60°C. The maximum value was observed at 50°C. Figure 3(B) shows the relationship between lipolytic activity and pH, keeping the temperature constant at 50°C. The graph clearly shows that lipolytic activity strongly depends on the pH. At pH 4.0, enzyme activity was lower than 20 U/L which accounted for only about 5% of the maximum value of 425 U/L at pH 8.0. With the increase of the pH, enzyme activities were measured as approximately 30, 65 and 80% at pH 5, 6 and 7, respectively. After reaching its highest value, enzyme activity decreased by approximately 90 and 80% of the highest value at pH 9 and 10, respectively. From both graphs, experimental results clearly illustrated that pH possessed a more profound effect on lipolytic activity than temperature. In addition, pH of the culture constantly decreased during incubation due to liberation of free fatty acids. As a result, the low pH greatly influenced the Jatropha oil degradation efficiency. However, free fatty acids are the main substrate for formation of hydrocarbon products. It is suggested that pH should be controlled, especially for a larger scale experiment, by using an alkali solution in order to keep a high lipolytic activity.

Formation of metabolites during anaerobic degradation

As more bacteria capable of Jatropha degradation are isolated and studied (Matsuoka et al., 2009), the potential for anaerobic biodegradation will be easier to assess. However, a better understanding of the details of anaerobic metabolism (for example, formation of by-products and substrate interactions) are required to optimize anaerobic biodegradation, and to reliably predict

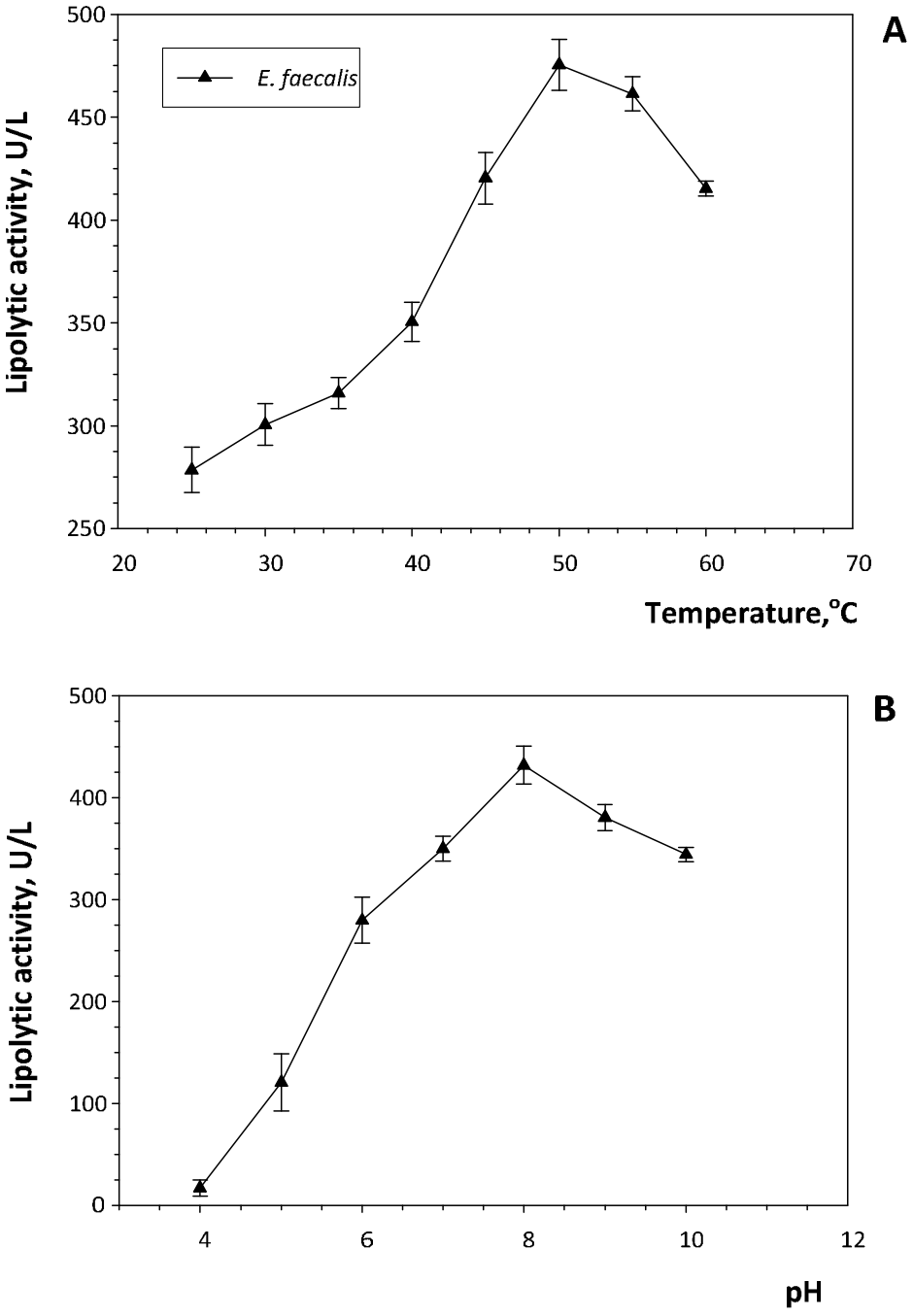

Figure 3. Influences of temperature, and pH on the lipolytic activity secreted by *E. faecalis*. Experiments on the effect of temperature were carried out at constant pH of 7.0 whilst temperature was set at 50°C during the effect of pH experiments.

its consequences. In control experiments without cells, Jatropha oil almost remained in the medium after incubation. Additional studies were carried out for hydrocarbon formation in both headspace and supernatant during 60 days of the incubation time. Gaseous samples revealed that several compounds were detected including methane, ethane, propane and carbon dioxide, respectively. These compounds are the major constituents in biogas production system. However, gas production rate was extremely slow compared to conventional biogas production system. This result was probably due to limitation of other nutritious compounds. Figure 4 reveals profiles of metabolites formed from the 2 strains compared with standard aliphatic alkanes with a

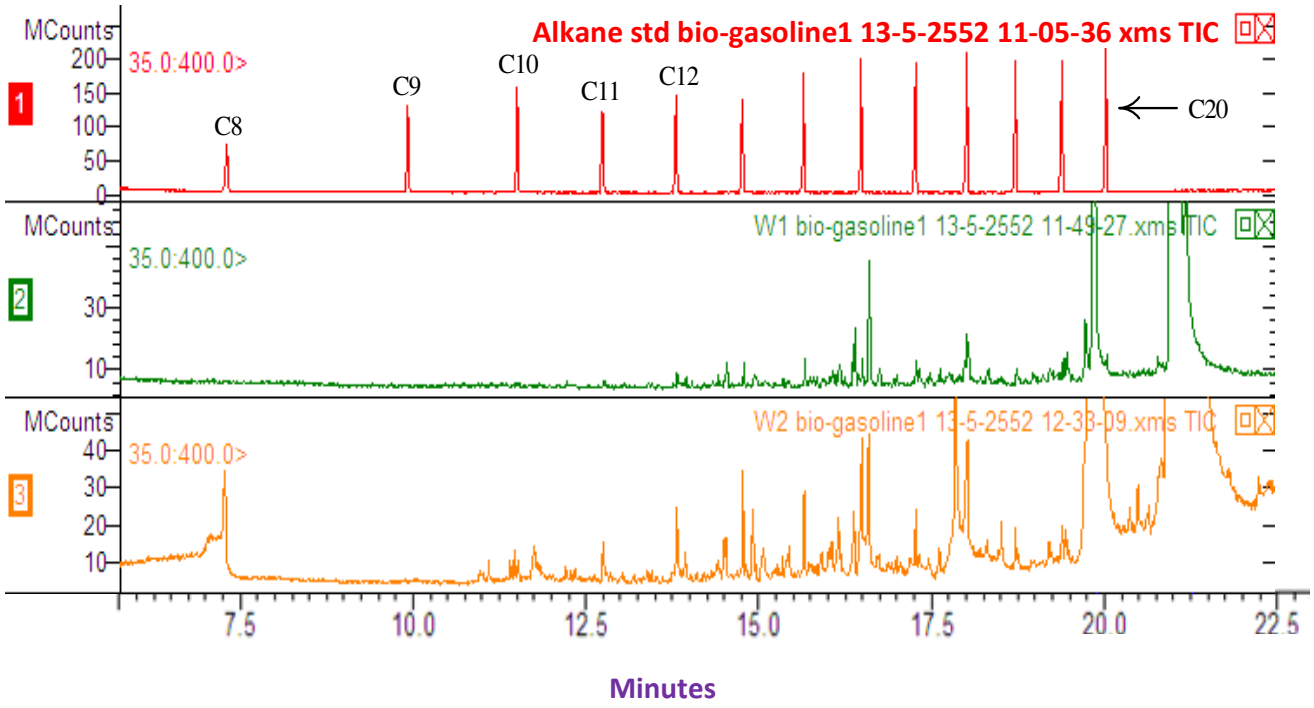

Figure 4. Chromatograms of extract samples derived from *B. cenocepacia* W-1 (2) and *E. faecalis* (3) after 60 days of incubation compared with standard aliphatic Alkanes (1) of n-octane (C8) to n-Eicosane (C20).

range of number of carbon between 8 to 20 atoms. Standard aliphatic alkanes are shown in Figures 3-A, whilst extract samples of *B. cenocepacia* W-1 and *E. faecalis* (W-2) are shown in Figures 4-B and 4-C, respectively. The chromatograms clearly showed that many hydrocarbon metabolites were formed during incubation. More than 40 metabolites were detected in the case of *B. cenocepacia* W-1, whilst *E. faecalis* produced more than 60 hydrocarbon metabolites. The experimental results also revealed, in addition to the obtained hydrocarbon metabolites, long-chain, branched-chain and some other aromatic hydrocarbons with the number of carbons in the molecule in a range of 8 to 18 atoms. Some representative metabolites detected by CG/MS are shown in Figure 5. Formation of aromatic hydrocarbons is possibly due to degradation of some other lipid components: such as, sterol and oil soluble pigments. Aliphatic alkanes products might be degraded from long chain fatty acid. In addition, other organic compounds were identified especially different fatty acids which are the product of enzymatic hydrolysis process of triglyceride by lipase. 1-methyl-naphthalene, penta-decane and undecane were indicated as typical meta-bolites (Figure 5). From the analysis of chromatograms and mass spectrum of each compound at the end of the incubation, it was shown that *B. cenocepacia* W-1 degraded Jatropha oil to yield hydrocarbon metabolites (C12 to C20) including 64.29% of alkane hydrocarbons, 7.1% of aromatic hydrocarbons and 28.61% of fatty acids, respectively. On the other hand, *E. faecalis* produced hydrocarbon metabolites including 68.15% of alkane hydrocarbons, 18.52% of aromatic hydrocarbons, and 13.33% of fatty acids, respectively. Although, these hydrocarbon metabolites were detected by GC-MS analysis, their concentrations were very low and were not accumulated in the medium probably due to serial degradation by enzymatic systems. The profile of hydrocarbon metabolites formed during incubation by *E. faecalis* was significantly different from the one of *B. cenocepacia* W-1, as the metabolite products contained smaller number of carbon atoms. Table 1 and 2 summarizes the hydrocarbon metabolites detected from the supernatant of the two bacteria. These hydrocarbon products implied that it could be useful for biological fuel production in the future. The experimental results also suggested that it may be possible to use anaerobic rather than aerobic condition as a treatment strategy. These organisms can oxidize organic substrates releasing large quantities of energy, comparable to the oxidative system in anaerobes. Anaerobic condition could be much less expensive than the more commonly considered aerobic approach, which is costly and energy intensive due to the need for vigorous agitation and aeration in order to introduce sufficient quantities of O_2. However, investi-gation of metabolic pathways of these metabolites is extremely complicated and beyond the scope of this work.

In conclusion, anaerobic digestion of vegetable oils not

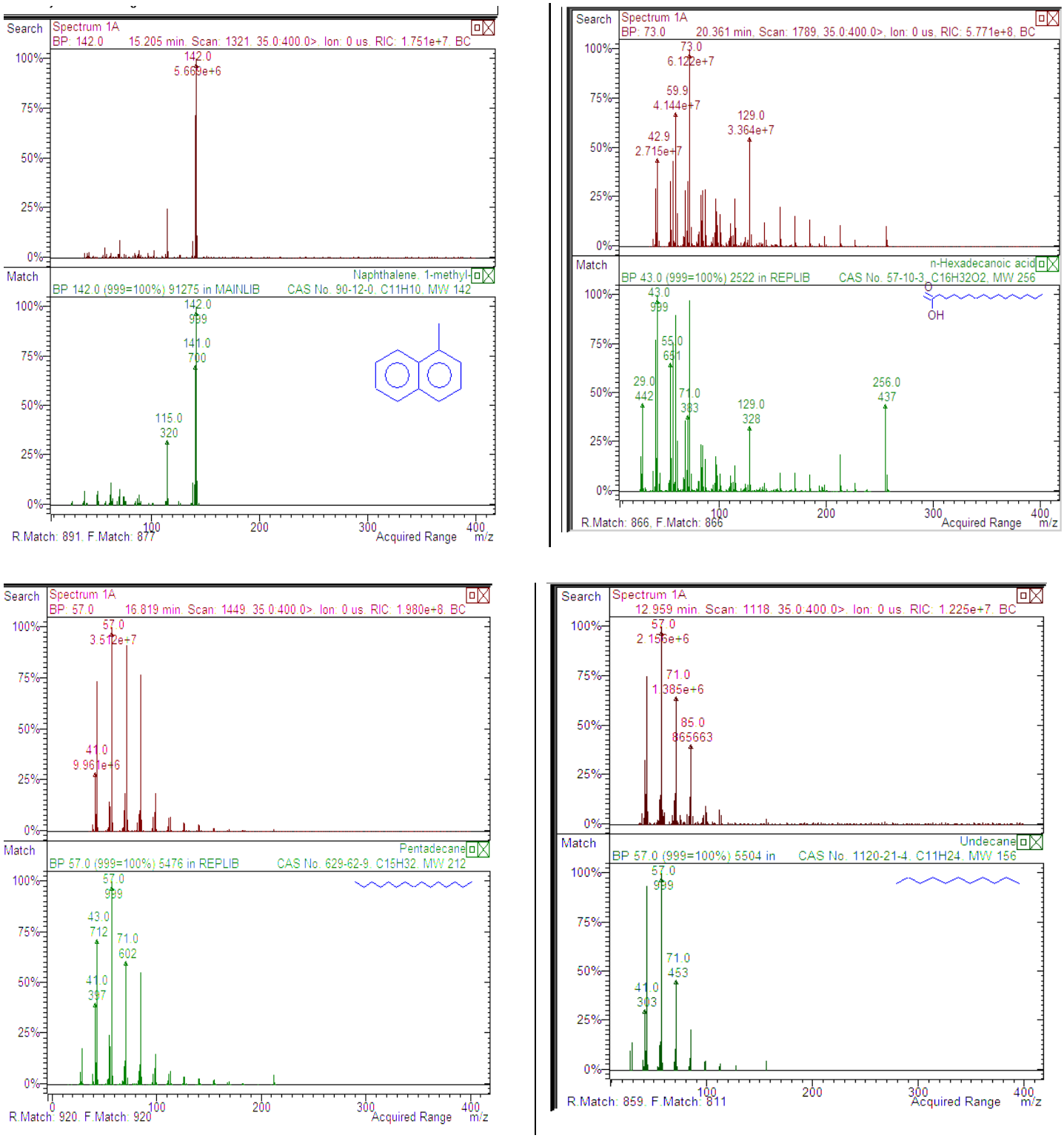

Figure 5. Mass spectral fragmentation pattern of typical compounds detected during the analysis of the extract. 1-methyl naphthalene (top left), Palmitic acid (top right), pentadecane (bottom left), and undecane (bottom right), respectively.

only reduces environmental problems to inland water, but also produces different kinds of hydrocarbon that could be used as biofuels. However, the high rates of oil degradation and products formation are the key parameters for a success operation. Although, both strains exhibited high lipolytic activities, the oil degradation rates

Table 1. Hydrocarbon metabolites derived from Jatrophal oil biodegradation by *B. cenocepacia* W-1. and E. faecalis

Microorganism	Product metabolites	Molecular weight	Formula
B. cenocepacia	Borane, diethyl(decyloxy)	226	$C_{14}H_{31}BO$
	Butylated hydroxytoluene	220	$C_{15}H_{24}O$
	Cyclohexane,1-(1,5-dimethylhexyl)-4-(4-methylpentyl)	280	$C_{20}H_40$
	Heptadecane	240	$C_{17}H_{36}$
	Hexadecane	226	$C_{16}H_{34}$
	2-methyl undecane	170	$C_{12}H_{26}$
	1-(2-hydroxypropyl)-Naphthalene	186	$C_{13}H_{14}O$
	Octadecane	254	$C_{18}H_{38}$
	Oxalic acid, 6-ethyloct-3-yl hexyl ester	314	$C_{18}H_{34}O_4$
	Pentadecane	212	$C_{15}H_{32}$
	Sulfurous acid, dodecyl hexyl ester	334	$C_{18}H_{38}O$
	Tetradecane	198	$C_{14}H_30$
	2,6,10,14-tetramethyl-pentadecane-6-ol	284	$C_{19}H_{40}O$
	Tridecane	184	$C_{13}H_{28}$
E. faecalis	Benzene, 1,3,5-trimethyl	120	C_9H_{12}
	Benzene,1-methyl-3-propyl-	134	$C_{10}H_{14}$
	Benzeneethanol,.alpha.,.alpha., .beta.-trimethyl-	164	$C_{11}H_{16}O$
	Borane, diethyl(decyloxy)-	226	$C_{14}H_{31}BO$
	Butylated hydroxytoluene	220	$C_{15}H_{24}O$
	Cyclohexane, pentyl	154	$C_{11}H_{22}$
	Cyclopentanecarboxylic acid,4-methylene-2-phenyl, methyl ester, trane-	216	$C_{14}H_{16}O_2$
	Decane	140	$C_{10}H_{22}$
	1,2-Diheptylcyclopropene	236	$C_{17}H_{32}$
	2,3-Dihydroxypropylelaidate	356	$C_{21}H_{40}O_4$
	2,6-Dimethyldecane	170	$C_{12}H_{26}$
	Dodecane,2,6,11-trimethyl-	212	$C_{15}H_{32}$
	Dodecane, 3-methyl	184	$C_{13}H_{28}$
	Eiconane	282	$C_{20}H_{42}$
	Furane, 2-pentyl	138	$C_9H_{14}O$
	Hexadecane	226	$C_{16}H_{34}$
	Hexyl octyl ether	214	$C_{14}H_{30}O$
	Nanodecane	268	$C_{19}H_{40}$
	Nephthalene,1-(2-hydroxypropyl)	186	$C_{13}H_{14}O$
	Nephthalene, 2,3-dimethyl	156	$C_{12}H_{12}$
	Octadecane	254	$C_{18}H_{38}$
	Oxalic acid, 6-ethyloct-3-yl, isobutylester	286	$C_{16}H_{30}O_4$
	Tetradecane	198	$C_{14}H_{30}$
	Tetradecane,2,6,10-trimethyl-	240	$C_{17}H_{36}$
	2,6,10,14-Tetramethyl pentadecane-6-ol	284	$C_{19}H_{40}O$
	Tridecane	184	$C_{13}H_{28}$
	Undecane	156	$C_{11}H_{24}$

were slow and took nearly 2 months to complete the operation. Therefore, acceleration of the digestion process is highly encouraged. One possibility is the degradation of Jatropha oil supplemented with some other wastes which are rich in nitrogenous compounds; for example, food waste, and animal manure. Co-

Table 1. Hydrocarbon metabolites derived from Jatrophal oil biodegradation by *Burkholderia cenocepacia* W-1.

Microorganisms	Product metabolites	Molecular weight	Formula
B. cenocepacia	2-methyl undecane	170	$C_{12}H_{26}$
	Tridecane	184	$C_{13}H_{28}$
	1-(2-hydroxypropyl)-Naphthalene	186	$C_{13}H_{14}O$
	Tetradecane	198	$C_{14}H_30$
	Borane, diethyl(decyloxy)	226	$C_{14}H_{31}BO$
	Pentadecane	212	$C_{15}H_{32}$
	Butylated hydroxytoluene	220	$C_{15}H_{24}O$
	Hexadecane	226	$C_{16}H_{34}$
	Heptadecane	240	$C_{17}H_{36}$
	Octadecane	254	$C_{18}H_{38}$
	Sulfurous acid, dodecyl hexyl ester	334	$C_{18}H_{38}O$
	Oxalic acid, 6-ethyloct-3-yl hexyl ester	314	$C_{18}H_{34}O_4$
	2,6,10,14-tetramethyl-pentadecane-6-ol	284	$C_{19}H_{40}O$
	Cyclohexane,1-(1,5-dimethylhexyl)-4-(4-methylpentyl)	280	$C_{20}H_40$

Table 2. Product metabolites from anaerobic Jatrophal oil degradation by *Enterococcus faecalis*.

E. faecalis	Benzene, 1,3,5-trimethyl	120	C_9H_{12}
	Furane, 2-pentyl	138	$C_9H_{14}O$
	Decane	140	$C_{10}H_{22}$
	Benzene,1-methyl-3-propyl-	134	$C_{10}H_{14}$
	Undecane	156	$C_{11}H_{24}$
	Cyclohexane, pentyl	154	$C_{11}H_{22}$
	Benzeneethanol,.alpha.,.alpha., .beta.-trimethyl-	164	$C_{11}H_{16}O$
	2,6-Dimethyldecane	170	$C_{12}H_{26}$
	Tridecane	184	$C_{13}H_{28}$
	Nephthalene,1-(2-hydroxypropyl)	186	$C_{13}H_{14}O$
	Dodecane, 3-methyl	184	$C_{13}H_{28}$
	Tetradecane	198	$C_{14}H_{30}$
	Cyclopentanecarboxylic acid,4-methylene-2-phenyl, methyl ester, trane-	216	$C_{14}H_{16}O_2$
	Nephthalene, 2,3-dimethyl	156	$C_{12}H_{12}$
	Borane, diethyl(decyloxy)-	226	$C_{14}H_{31}BO$
	Hexyl octyl ether	214	$C_{14}H_{30}O$
	Dodecane,2,6,11-trimethyl-	212	$C_{15}H_{32}$
	Butylated hydroxytoluene	220	$C_{15}H_{24}O$
	Hexadecane	226	$C_{16}H_{34}$
	Oxalic acid, 6-ethyloct-3-yl isobutylester	286	$C_{16}H_{30}O_4$
	Tetradecane,2,6,10-trimethyl-	240	$C_{17}H_{36}$
	1,2-Diheptylcyclopropene	236	$C_{17}H_{32}$
	Octadecane	254	$C_{18}H_{38}$
	Nanodecane	268	$C_{19}H_{40}$
	2,6,10,14-Tetramethyl pentadecane-6-ol	284	$C_{19}H_{40}O$
	Eiconane	282	$C_{20}H_{42}$
	2,3-Dihydroxypropylelaidate	356	$C_{21}H_{40}O_4$

digestions of oily substances with these supplements have been addressed by several workers (El-Mashad and Zhang, 2010; Nayono et al., 2009; Luostarinen et al., 2009; Nielsen and Ahring, 2006). Finally, direct contact with *B. cenocepacia* should be avoided since the strain has been reported as a potential pathogen (Ortega et al.,

2007).

Conclusion

B. cenocepacia W-1 and *E. faecalis* are the two bacteria with the highest capability for anaerobic Jatropha oil degradation. Free fatty acids were liberated from triglyceride prior to further utilization as sources of energy and growth. Hydrocarbon formations in the gaseous headspace and medium were the result of enzymatic reactions. However, their concentrations were much diluted, and it took more than 2 months to complete the oil degradation process. Therefore, a bioprocess development especially nutritional modification is necessary in order to improve the yield and volumetric productivity.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the help of Mrs. Hua Yanling, the center for scientific and technological equipment, Suranaree University of Technology, for GC/MS analysis of samples.

This research work was financially supported by the National Science and Technology Development Agency (NSTDA) under contract number F-31-312-19-01.

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