# Biodegradation of *Vernonia galamensis* Seed Oil by *Acinetobacter* and *Pseudomonas* sp.

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**The biodegradability of *Vernonia galamensis* seed oil (VO) has been demonstrated with two environmental bacterial strains, *Acinetobacter Iwoffi* (HU 3955), and *Pseudomonas* sp. (HU 4020). A time-dependent increase in the degradative activities of both bacteria species was apparent. There was *ca.* 60% decrease in the amount of VO over an eight-day incubation period. Additionally, lipolytic activity was evident from the amount of free fatty acid (FFA) that was generated. The percent FFA of the residual oil were 82% for the *Pseudomonas* strain, and 62% for the *Acinetobacter* strain. The weight per epoxy value of the VO in the fermentation medium remained relatively constant over the incubation period, suggesting the lack of preference for either the epoxidized or nonepoxidized acids present in VO.**

KEY WORDS: *Acinetobacter* sp., biodegradation, *Pseudomonas* sp., seed oil, *Vernonia galamensis*.

*Vernonia galamensis* is a herbaceous plant native to the semi-arid regions of Africa. Several recent publications have highlighted the economic potential of its naturally epoxidized triglyceride seed oil (1–9). Commercial cultivation is now underway in Costa Rica and Zimbabwe, in addition to screening experiments in Arizona, Ethiopia and Kenya. The seed contains about 40% epoxidized triglyceride oil, of which vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenoic) acid constitutes 72–80%. Thus, the uniqueness of the oil is derived from its high epoxy triglyceride content, which potentially lends it to a variety of industrial applications, ranging from coatings to chemical feedstock (3,7).

While most of the studies have been directed toward industrial utilization of the unique chemical properties of *Vernonia galamensis* oil (VO), there have been no reported studies on its biodegradation. Consequently, the present communication is designed to examine the ability of two environmental bacterial strains to degrade VO under aerobic conditions.

## EXPERIMENTAL PROCEDURES

*Bacteria strains.* The bacterial strains were *Acinetobacter Iwoffi*, HU 3955; and *Pseudomonas* sp., HU 4020. They were originally isolated from soil and shown to express an array of lipolytic activities in a semi-quantitative enzyme assay (10).

*Medium and culture conditions.* The medium was essentially a solution of minimal salt that was supplemented with 10% VO as the only carbon source. The solution contained the following ingredients per liter; $K_3PO_4$, 7 g; $K_2HPO_4$, 2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 1 mL of 1% solution; and $FeSO_4 \cdot 7H_2O$, 0.5 mL of 0.1% solution. The pH was adjusted to 6.8, and the oil (10% vol/vol) was asceptically added to the medium immediately after autoclaving at 121°C for 15 min. Two sets of control experiments were used, one containing only the medium and the oil, and another containing only the medium and the bacterial strain. All chemicals except the oil were obtained from Sigma Chemical Company, St. Louis, MO. The crude vernonia oil (acid value = 3.99, weight per epoxy = 434) was obtained from mechanical pressing of enzyme-deactivated seed. Enzyme deactivation of seed was performed by a procedure similar to that previously reported (2). Overnight cultures of the bacteria growing on Tripticase Soy Agar (TSA) were harvested and washed twice in phosphate-buffered saline. The cells were resuspended in the same solution and adjusted to an optical density of 0.5 at 600 nm with a Bausch and Lomb Spectronic 20. From this standardized culture, a 0.1-mL sample was used to inoculate 100 mL of the medium in a 250-mL Erlenmeyer flask, and the cultures were incubated at 25°C in a gyratory shaker at 100 rpm. Each day, an experimental culture and two controls were processed and analyzed as outlined below.

*Determination of total bacterial load.* The bacterial cells were separated from the fermentation mixture by centrifuging the samples at 10,000 × *g* for 10 min at 4°C. The resulting pellets were used for the determination of the total bacterial load by the standard plate count method. This was accomplished by diluting the pellets in phosphate-buffered saline and plating in duplicate onto TSA. The number of colony-forming units per milliliter (cfu/mL) of the sample was determined with a Darkfield Quebec Colony Counter (Cambridge Instrument, Inc., Buffalo, NY), after incubation at 30°C for 2 days.

*Extraction of residual oil.* The fermentation mixture, obtained after the removal of the bacterial cells (see above), was cooled and acidified with 0.5 mL of concentrated hydrochloric acid and then extracted twice with 200-mL portions of dichloromethane. The combined organic fraction was dried with anhydrous sodium sulfate (62.5 g/100 mL) and stripped under vacuum to afford golden-colored oil.

*Determination of free fatty acids.* The acid value and percent free fatty acids (FFA) were determined by titrating the residual oil against standardized alcoholic potassium hydroxide solution to a phenolphthalein end point.

*Determination of weight per epoxy.* The weight per epoxy (WPE) of the residual oil was determined titrimetrically. A 250-mL Erlenmeyer flask, equipped with a magnetic stirrer bar, was charged with 25 mL of dichloromethane and 15 mL of tetramethylammonium bromide (TEAB) in dichloromethane and glacial acetic acid (100 g TEAB, 400 mL each of dichloromethane and glacial acetic acid). With continuous stirring, this solution was titrated against 0.1N perchloric acid to the first blue color that persisted for 30 s. About 0.600 g sample of the residual oil was then added to the blank solution and subsequently titrated against 0.1N perchloric acid to the first blue-green color that persisted for 30 s. The WPE was calculated based on the volume of perchloric acid used. (WPE = wt of sample × 1,000/vol of perchloric acid × 0.1).

*Gas chromatographic/mass spectrometric (GC/MS) analysis.* GC/MS analysis of the original VO and the residual oil was carried out as previously published (11,12).

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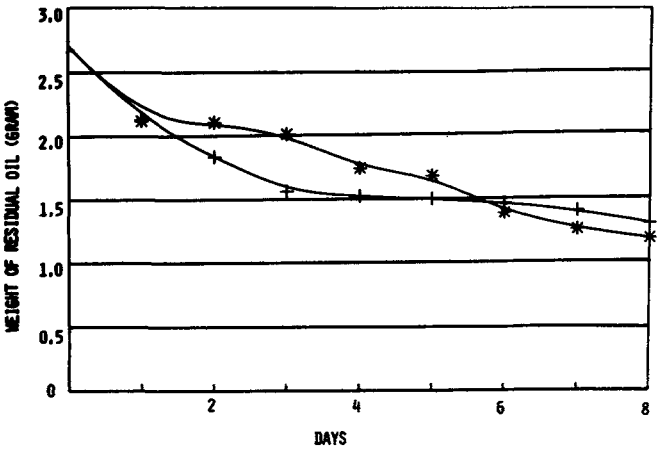


FIG. 1. Biodegradation of *Vernonia galamensis* seed oil. The degradation by two bacteria sp., *Acinetobacter* (+) and *Pseudomonas* (*), is represented by the amount of residual oil over an 8-d incubation period. Initial amount of oil was 2.7 g. Experiments were performed in duplicate (standard deviation = ± 0.013).

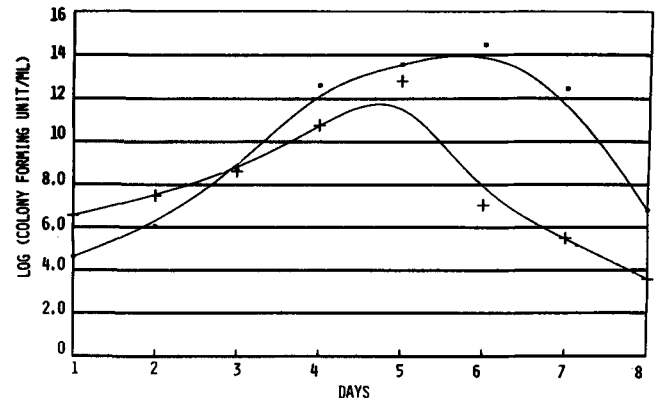


FIG. 2. Rate of bacterial growth in the Vernonia oil-supplemented fermentation medium. *Acinetobacter* sp. (+), *Pseudomonas* sp. (*). Experiments were performed in duplicate (standard deviation = ± 0.011).

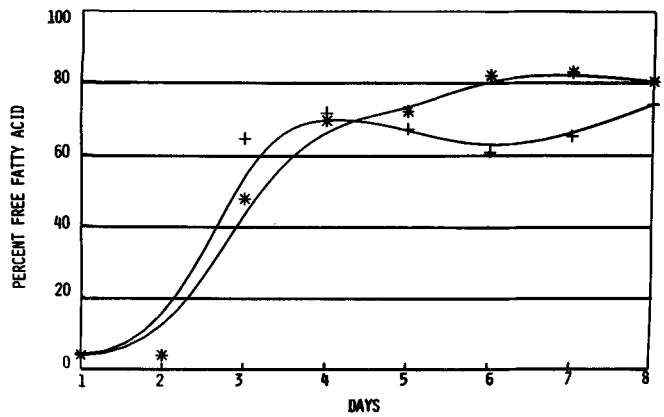


FIG. 3. Percent free fatty acid of residual oil in the Vernonia oil-supplemented fermentation medium. *Acinetobacter* sp. (+), *Pseudomonas* sp. (*). Experiments were performed in duplicate (standard deviation = ± 0.014).

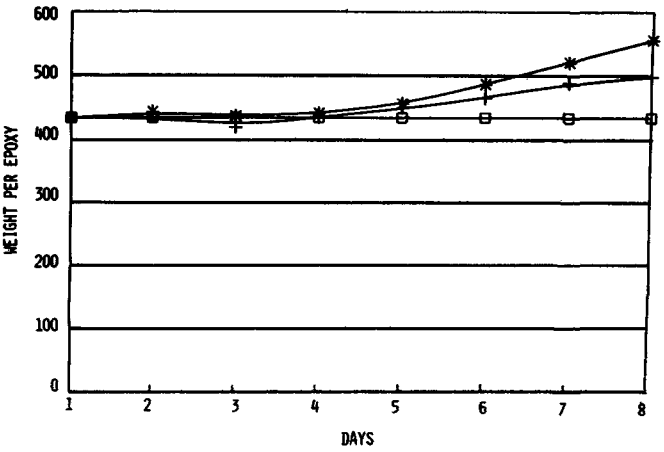


FIG. 4. Weight per epoxy of the residual oil in the Vernonia oil-supplemented fermentation medium, *Acinentobacter* sp. (+), *Pseudomonas* sp. (*), Control (□). Experiments were performed in duplicate (standard deviation = ± 0.001).

## RESULTS AND DISCUSSION

Initially, several bacterial strains were screened for activities relative to FFA and residual oil content at different pH values (5.5, 6.8, 8.0), and a pH of 6.8 was found to be the optimum. The bacterial strains were able to grow in a minimal medium that was supplemented with *Vernonia galamensis* oil as the only source of carbon. However, only two strains that demonstrated maximum activities were selected for further quantitative studies. The two strains belong to the bacterial genera *Acinetobacter* and *Pseudomonas*, several members of which are well-known for their ability to biodegrade various organic molecules (13). Figure 1 illustrates the extent to which the oil was degraded as represented by the rate of decrease in oil content with incubation time. That the bacteria strains were utilizing VO as a carbon source was evident from the fact that the amount of oil in the control experiment (containing only medium and VO) remained relatively constant over the 8-d incubation period. This conclusion was further corroborated by a lack of bacterial growth in the control culture that contained only the medium and the bacteria. Although the amounts of recovered residual oil from the two sets of bacteria cultures were comparable, there was slight variation in the rates of bacterial growth (Fig. 2). Maximum growth rates were achieved after four days for the *Acinetobacter* sp., and after six days for the *Pseudomonas* sp. However, the degradative activities continued after the maximum bacterial growth rate as evident in the continued decrease in the residual oil (Fig. 1). At this point, it is unclear whether the bacteria degraded the triglyceride oil prior to or after lipolysis. However, subsequent analysis of the FFA content of the residual oil indicated a time-dependent increase in the percent FFA (Fig. 3). The *Pseudomonas* sp. showed the maximum FFA content (*ca.* 82%) within seven days of incubation, while the *Acinetobacter* sp. showed 62% FFA in six days.

With regard to the bacteria having any potential preference between vernolic acid moieties and nonepoxidized acid moieties, the weight per epoxy of the residual oil (Fig. 4) clearly demonstrates the nondiscriminating nature of

the bacteria for the component acids in VO. Thus, the WPE values remain relatively constant over the incubation period. This conclusion was further supported by the GC/MS analysis of the methylated and transesterified residual oil, which showed a typical VO fatty esters profile (11). The GC/MS analysis of the residual oils also reveal the presence of a minor component, the structure of which is yet undetermined, with a GC retention time indicative of a degradative product that could possibly result from any of the C-18 fatty acids present in VO.

Finally, the results of this study have shown for the first time that *Vernonia galamensis* seed oil is biodegradable. Although no attempt was made at this time to characterize other possible products resulting from the bacterial degradation, it is conceivable that microbial activities on the oil could lead to the bioconversion of VO to other useful industrial products. Efforts are underway to explore this latter potential.

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