

Biodegradation of palmarosa oil (green oil) by *Serratia marcescens*

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ABSTRACT: Biodegradation of oil depends on the nature of the oil, the type of microbial community and a variety of environmental factors. Green oils are being used as consumer goods and as raw materials in industries such as food processing, pharmaceutical and cosmetic. Microbial contaminations of green oils have been the cause of degradation problems. *Serratia Marcescens* produced cytochrome oxidase, catalase, Dextrose, Lactose, Manose and sorbitol enzymes were the main reason for the degradation of palmarosa oil. Changes of colour and turbidity was also the evidence for green oil degradation by bacteria. More oxygen included protons ($O-CH_2$) group was produced in the presence of bacterial species and the addition of oxygen took place during bacterial degradation of palmarosa oil. The biodegradation of palmarosa oil by *Serratia marcescens* have been carried out using High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic spectroscopy (NMR) analysis. Carboxyl group present in the palmorasa oil is utilised as a sole carbon sources for the *Serratia marcescens*.

Key words: Biodegradation, green oil, palmarosa oil, *serratia* sp.

INTRODUCTION

Shogren (2003; 2004) Isinguzo (2005) reported that the biodegradation behaviour of polymerized green oils which were used in agriculture. The suitability of the respirometric BOD oxitop method for determining the biodegradability of oils in ground water using forestry hydraulic oils as model compounds (Kuokkanen, 2004). Liu-LJ (2002) studied the selective enzymatic degradation of poly and poly blend films. The proteins can degrade amorphous but cannot degrade crystalline materials. In contrast, *Pseudomonas* lipase can degrade both amorphous and crystalline. More literature have been discussing about the microbial degradation of hydrocarbon (Atlas, 1981; Kinney, 1968; Widdel, 1980). palmarosa oil is a pale yellow or pale olive liquid with a sweet, floral rosy odour and various back notes or top notes according to the quality and age of the oil. World production of green oil raised from 18×10^6 tones in 1960 (Karleskid, 1992) to 84×10^6 tonnes in 1999 (Dabat and Lencon, 1999). This upward trend is mostly linked with the growing demand of importing countries like China and India with increasing population and standard of living. Microbial contamination of green oil has been the cause of degradation problems throughout the world for many years and more recently

the frequency and severity of the cases appears to have been increasing dramatically (Gwenaella Bucas, 2002, Sevim, 1995, Zhengkai, 2004, Philp mercaria, 2004). In the present study Palmarosa green oil is selected which is much useful to us as cosmetic and the degradation study has been carried out.

MATERIALS AND METHODS

Palmorasa oil is collected from locally available market. Bushnell – Hass broth (Hi-Media), Nutrient agar and *Serratia* bacterial strain were used for the green oil degradation. The experimental system consists of 200 mL of BH broth, 5 mL of *Palmorasa* oil and 3 mL of bacterial strain. A control was identically maintained but without the inoculation of bacterial strain. To confirm the green oil degrading effect by the *serratia* sp, after 60 days incubation periods, one loopfull of culture from respective system were taken and strucked on nutrient agar plate and were incubated for 24 h at 37 °C temperature and the bacterial counts were observed. After 25 days immersion, the residual green oil of the entire flask was extracted with an equal volume of dichloromethane. Evaporation of solvent was carried out in a hot water bath at 40 °C. The 1μL of the resultant solution was analyzed. The number of compounds in palmorasa oil and the degradation has

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been identified by using HPLC model (Shimadzu Japan model running 100% methanol HPLC grade) and 254 nm wavelength at a flow rate of 1ml per minute. Elution, collection fractions, analysis and regarding of data are performed in an automatic analyser.

FTIR Por Elmer, UK make, Paragon 500 model was used to detect the functional group of the *Palmarosa* oil before and after degradation. The spectrum was taken in the mid IR region of 400-4000/cm with 16 scan speeds. The samples were fixed with KBr spectroscopic pure chloroform in the ratio of 1:100 and pellets were fixed in the sample holder and the analysis were carried out. NMR analysis was carried out using Perlin Elmer spectrometer to detect the protons of the nuclei in the *Palmarosa* oil was dissolved in deuterated chloroform solvent and tetra methyl silane was used as a reference standard various physical and biochemical tests were carried out from *serratia* bacterial sp.

RESULTS

Based on the colony morphology, physical and biochemical test, *Serratia* sp is a gram negative, rod shaped bacteria and grows under room temperature. The biochemical tests show positive to the citrate utilisation, voges prokauer, gelatine hydrolysis, cytochrome oxidase, catalase test and oxidation and fermentation test presented in the Table 1. Based on the biochemical tests, *serratia* sp produced enzymes like catalase, oxidase, tetrarase, lactose, mannose and sorbital. These enzymes are mainly involved in the degradation of green oil. The initial bacterial load was 4.8×10^5 CFU/mL, while degradation the count

increased to 2.0×10^6 CFU/mL. It indicates that *Serratia* sp has taken energy from the green oil. Fig. 3 and Table 2 indicate the log value of the bacterial load.

The HPLC of palmarosa oil shows three peaks. It indicates that oil contains three numbers of compounds. In the *Serratia* bacterial strain inoculated system with palmarosa oil also shows three peaks but the peak height and percentage of area were changed. It indicates the palmarosa oil degraded by *Serratia* sp in Figs. 4 and 5.

Fig. 6 indicates that the IR spectrum of pure palmarosa oil in BH medium and Fig. 7 indicates the IR spectrum of degraded Palmarosa oil by bacteria. The pure palmarosa oil in BH medium shows the characteristic bands at 3354/cm (OH group), 2967/cm and 2916/cm (C-H aliphatic stretch), 1740/cm and 1722/cm (C=O stretch for acid group), 1668/cm (C=C conjugated diene) 1442/cm and 1378/cm (C-H def in methyl group), 1233/cm, 1181/cm and 1094/cm (C=O stretch in C-O-C group), 996/cm, 830/cm, 777/cm and 743/cm sbstituted benzene. But presence of new peaks 3261/cm OH group. 1635/cm (C=C conjugated diene), 652/cm (Chloride position of the peaks and intensity of the peaks were varied. This is because of the bacterial degradation.

The NMR spectrum of pure palmarosa oil is shown in Fig. 8. Some major peaks at 0.3 chemical shifts indicate the presence of aliphatic protons. The other peaks at 4-6 chemical shifts indicate the presence of oxygen-included protons (O-CH₂) it may be due to the oil containing flavonoids group. But in the case of palmarosa oil with *Serratia* bacterial inoculated system the new peak was absent at 4-6 chemical shifts (Fig. 9).



Fig. 1: Photomicrograph of before degradation of palmarosa oil

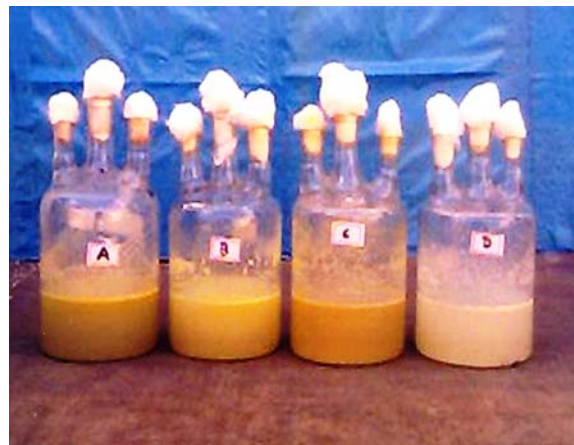


Fig. 2: Photomicrograph of after degradation of palmarosa oil

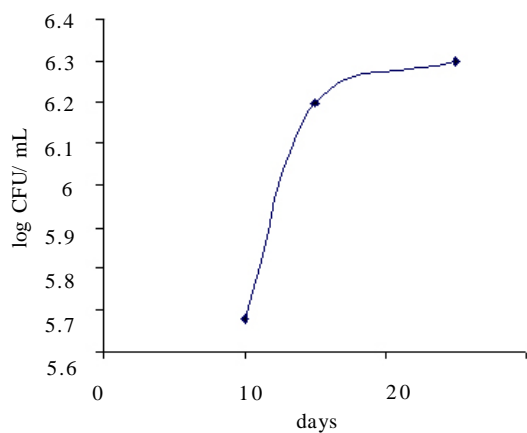


Fig. 3: Growth curve for the palmarosa oil in *S. marcescens* bacterial culture

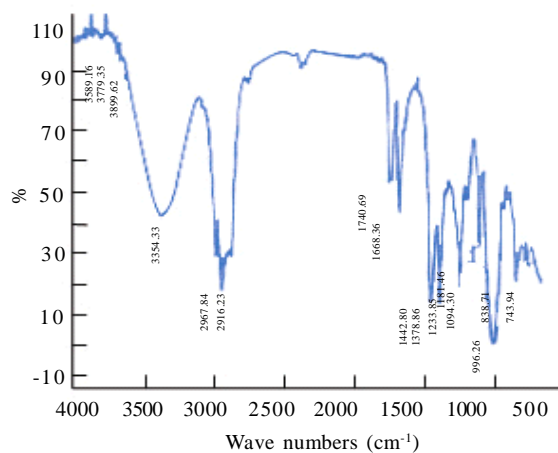


Fig. 6: FTIR spectrum of pure palmarosa oil

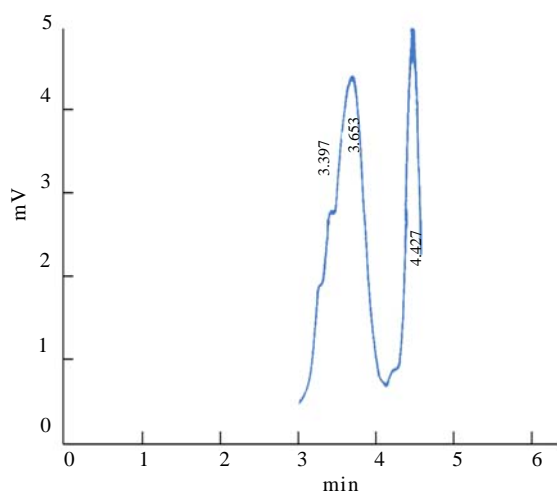


Fig. 4: HPLC spectrum of pure palmarosa oil

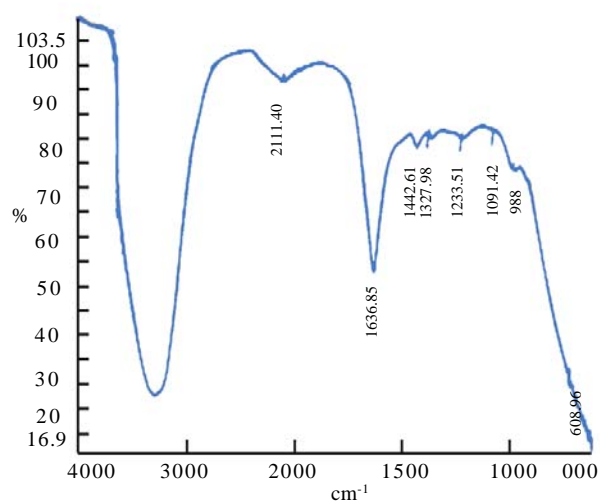


Fig. 7: FTIR spectrum of after 60 days inoculation with *S. marcescens* with palmarosa oil

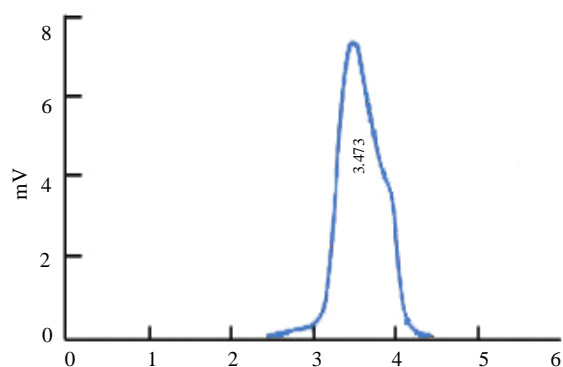


Fig. 5: HPLC spectrum of after 60 days inoculation with *S. marcescens* with palmarosa oil

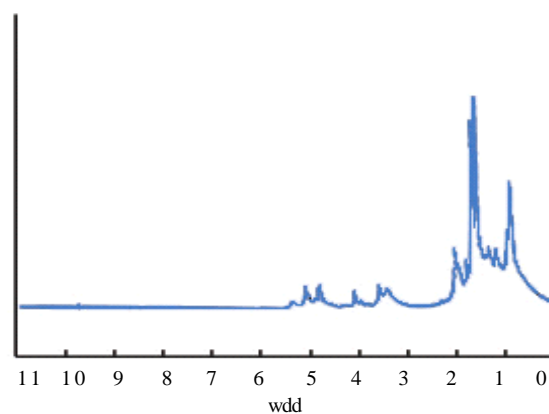


Fig. 8: NMR spectrum of pure palmarosa oil

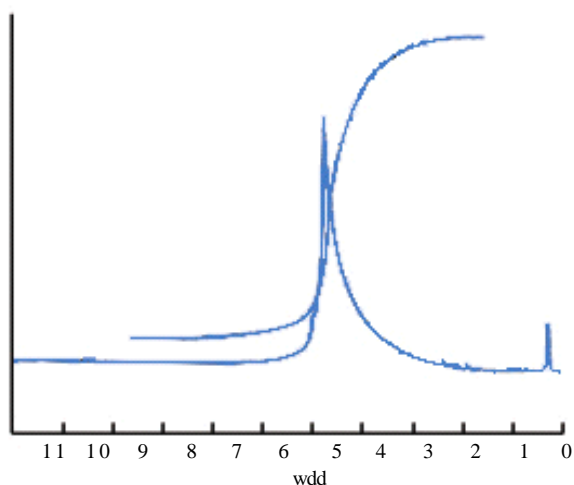


Fig. 9: NMR spectrum of after 60 days inoculation with *S. marcescens* with palmarosa oil

Table 1: Morphological, physical and biochemical tests for the *Serratia marcescens*

Species No.	Test	Results
Colony morphology	Surface	Smooth
	Pigments	-
	Gram's reaction	-
	Cell Shape	Rods
Spore Production	Arrangement	Single
	Endospores	-
	Motility	+
	Temperature	+
Physiological Tests	25°C	+
	37°C	+
	pH	+
	5.7	-
Biochemical Tests	6.8	-
	Growth on Mac Conkey Agar	+
	Indole test	-
	Methyl red test	-
	Voges Proskauer test	+
	Citrate utilization	+
	Case in hydrolysis	-
	Starch hydrolysis	-
	Gelatin hydrolysis	+
	H ₂ S production	-
	Cytochrome oxidase	+
	Catalase test	+
Acid production from carbohydrates	Oxidation / Fermentation	+
	Dextrase	+
	Lactose	+
	Mannose	+
	Sorbitol	+

It may be due to the bacterial degradation in palmarosa oil. The more oxygen-included protons (O-CH₂) group was produced in the presence of bacterial species. The addition of oxygen was took place during

bacterial degradation of palmarosa oil. The effect of ferric hydroxide on the anaerobic biodegradation of green oil in fresh water sediments was studied (Lietal, 2004). Cavagnaro, *et al.* (1988) have reviewed the biodegradation of food oils and grasses. Several articles speculate that soy oil should biodegrade more readily than mineral oil (Ticer, 1988).

Table 2: Enumeration of bacterial colonies (*Serratia marcescens*) during green oil degradation

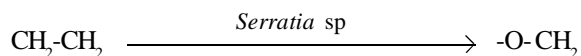
Sample	Biodegradation periods (days)	Bacterial counts (CFU/mL)	Log value (CFU/mL)
200 mL BH Broth + 5 mL Palmarosa oil	10	4.8×10^5	5.68
+ 3 mL <i>Serratia marcescens</i> bacterial culture	30	1.6×10^6	6.20
	60	2.0×10^6	6.30

Elimination or petroleum-based resin and reduced pigment requirements due to the light vehicle colour, should provide a competitively priced alternative to petroleum-based inks. These ink vehicles along with the commercial ones were subjected to biodegradation. The vegetable-oil vehicles degrade and more completely than commercial soy or mineral-oil based vehicles. Both mono and mixed cultures of microorganisms, which are commonly found in soil, were used. Greater increase in biodegradation with time was observed in pure vegetable oil.

DISCUSSION AND CONCLUSION

Arthrobacter is capable of completely transforming added. Tyrosol to the corresponding 4-hydroxy phenylacetic acid and after 139 h of fermentation no traces of tyrosol can be identified G.Knuppa *et al.*, (1996). In contrast, *Bacillus ponilus* was unable to completely biodegrade tyrosol over 139 h.

Biodegradation of green oil in fresh water sediments exhibits self-inhibitory characteristics when it occurs under methanogenic conditions but not under iron reducing conditions. The basis of the protective effect of iron was investigated by comparing its effect on oil biodegradation rate the toxicity of oil amended sediments to those of clay and calcium, which reduce the toxicity of oil-derived long chain fatty acids by adsorption and precipitation respectively. This present work reveals that carboxyl group present in green oil is utilized as a sole carbon source for the *Serratia* species and the degradation took place as follows;



The following observations have been made from the work reported in the present study

1. Based on the biochemical tests, *Serratia* sp. produced enzymes like catalase, oxidase, Dextrase, Lactose, mannose and Sorbital. These enzymes are mainly involved in the palmarosa oil degradation process.
2. From the HPLC, FTIR and NMR results indicate the palmarosa oil degraded by *Serratia* sp.
3. The present work reveals that carboxyl group present in the green oil is utilized as a carbon source for the *Serratia* sp. and the degradation took place.

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