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# Degradation of Cashew Nut Shell Liquid by *Pseudomonas* sp Isolated from Soil

[1]T. Rajeswari, [1]B. Padmapriya, [1]K. Teesha and [2]P. Kavitha Kumari

[1]Department of Microbiology, Karpagam University, Coimbatore-21, Tamilnadu, India
[2]C.E.P.C. Laboratory and Technical division, Kollam - 691 001, Kerala, India

**Abstract:** Cashew nut shell liquid (CNSL) is a naturally occurring phenolic compound and it is toxic to human skin on contact. To suppress the toxic effect, it is essential to decarboxylate and refine it by using biological method. In this study, *Pseudomonas* sp, *Arthrobacter* and one reference strain *Pseudomonas aeruginosa* (MTCC 424) were used. The growth and degradation efficiency on CNSL was determined by taking optical density and using Gas Chromatography analysis respectively. Among the three bacteria, *Pseudomonas* sp. was found to be produce highest degradation rate.

**Key words:** Cashew nut shell liquid · *Pseudomonas* species · *Arthrobacter* species · *Pseudomonas aeruginosa* · Biodegradation

## INTRODUCTION

Cashew (*Anacardium occidentale*) belongs to the family Anacardiaceae, which includes many economically important tropical and subtropical trees and shrubs. *Anacardium occidentale* (cashew) containing the principal component phenols, Anacardic acid, cardol, 2-methyl cardol and cardanol is a unique source of materials useful both for industrial technology, in semi-synthesis and for biological/pharmaceutical applications. The mesocarp of cashew nut consists of honey comb network of cells containing a viscous liquid called cashew nut shell liquid (CNSL). It is one of the sources of naturally occurring phenols. It obtained from the shell of a cashew nut. About 30-35% CNSL is present in the shell, which amounts to approximately 6%of the nut. Paramashivappa *et al*. [1] reported that commercially available cashew (*Anacardium occidentale*) nut shell liquid (CNSL) mainly contains the phenolic constituent's anacardic acid, cardol and cardanol. There are various processes for the extraction of CNSL from the raw nuts. Hot oil bath, expeller and kiln solvent extraction processes. The most common method is the "Hot oil" process, which was first introduced in India by M/s Peirce hesile and company limited in 1975.

According to Igbinosa *et al*. [2] environmental conditions may significantly affect the rate of biodegradation reported for a compound in the environment. Phenol was oxidized under anaerobic conditions. In most cases, organic compounds are more readily degraded in the presence of oxygen (aerobic conditions) than when oxygen is not present (anaerobic or anoxic conditions) [3]. Zhang *et al*. [4] observed the biodegradation of crude oil by *Pseudomonas aeruginosa* in the rhamnolipids which produced 15.4 gram per litre rhamnolipid cultured in a basal mineral medium using glycerol as a sole carbon source. They concluded that either rhamnolipid or glycerol could facilitate the biodegradation of crude oil by *Pseudomonas aeruginosa*.

Annadurai *et al*. [5] reported that biodegradation of phenol using *Pseudomonas pictorum* (23328) a potential biodegrading of phenol was investigated under different operating conditions. Tyman. *et al*. [6] described that Cashew nut shell liquid (CNSL) is a natural product that consists of a mixture of phenolic compounds was separated in to cardanol, cardol and 2-methyl cardol using column chromatography. The separation was aimed at recovering cardanol that can be used in the synthesis of cation exchange resin. Bak and Widdel [7] studied that only few pure cultures of denitrifying bacteria that is able to use phenol as a sole source of carbon and energy. Rebecca *et al*. [8] studied biodegradation of hydrocarbon partitioned by an *Arthrobacter* species in to an organic solvent. The organism mineralized naphthalene and n-hexadecane dissolved in 2, 2, 4, 4, 6, 8, 8-heptamethyl nonane. General uses for Gas chromatography that are perform dynamic separation and identification of volatile organic compounds and several inorganic permanent

**Corresponding Author:** T. Rajeswari, Department of Microbiology, Karpagam University, Coimbatore-21, Tamilnadu, India. E-mail: rajeswarithamarai@rediffmail.com.

gases. It also performs quantitative and qualitative determination of compounds in mixtures. This study, therefore, aimed to suppress the toxic effect of CNSL by degrading it using biological method. Three different strains were checked for the degradation rate of CNSL using spectrophotometric and Gas chromatographic analysis.

## MATERIALS AND METHODS

Cashew nut shell liquid (CNSL) was used for the biodegradation study. The CNSL containing soil was collected from near the cashew nut factory at Kollam, Kerala, India. The soil was collected in a sterile polythene bag by using sterile spatula.

**Isolation of Bacteria:** 1g of CNSL contaminated soil sample was weighed aseptically and added to the 99ml of Butter field's phosphate buffer [4] and mixed the sample. It was designated as$10^{-2}$ dilution. From this $10^{-2}$ dilution serially diluted tills $10^{-4}$ dilutions. From this each dilution 1ml each added in to petriplates in duplicates. Mineral medium [5]. 1% CNSL was poured in to Petri plates and mixed the medium and allowed to solidify. After the pour plate technique the plates were incubated at 30°C for 48hrs.After incubation period the isolated colonies were streaked in to the nutrient agar plates for purification and identification. The isolated colonies were transformed to nutrient agar slants and stored for further studies.

**Identification of Bacteria:** Identification of the bacteria was done based on the bacterial identification chart [9].

Culture was stabbed onto Hugh and Leifson's medium and incubated one set aerobically and another set anaerobically. After incubation, observed for the acid production (yellow color) that indicates metabolism of glucose.

The isolated cultures were streaked onto the Nutrient agar containing 5% glycerol and incubated at 37°C for 48 hrs. After incubation the colonies were observed in dark room under luminescence. Additional test for *Pseudomonas* sp. was further identified as per IS standard.

**Biodegradation Study:** Biodegradation is the process in which the organic substances are broken down by living organisms. The CNSL degradation was determined by using spectrophotometrically with Shimadzu UV-1601 visible spectrophotometer. Bacterial growth was measured at 600nm with visible spectrophotometer.

**Selection of Bacteria for Biodegradation Study:** From the CNSL contaminated soil, bacterial strains were isolated using Mineral medium with CNSL. *Pseudomonas* sp and *Arthrobacter* sp., were isolated and those strains were used for degradation study. The pure culture *Pseudomonas aeruginosa* MTCC-424 was used as reference strain for comparing the degradability efficiency.

**Experimental Set up [10]:** 2% filter sterilized CNSL was added to 100ml mineral media and 1ml each of the overnight cultures were inoculated and incubated at 30°C for 17 days. Degradation was maintained together with control and for each organism, the duplicate also maintained.

**Growth Estimation:** Bacterial growth was measured spectrophotometrically at 600nm with Shimadzu model UV-1601 visible spectrophotometer from $6^{th}$ to $17^{th}$ day.

**Determination of CNSL Degradation:** CNSL degradation was performed by Gas chromatographic (GC) analysis. GC analysis was performed with the extracted sample. The $6^{th}$ day and $17^{th}$ day samples were taken for GC. The samples were extracted by using organic solvent. Degradation of different compounds of CNSL was determined by the reduction in peak areas of the compounds at particular retention time.

**Sample Extraction:** 5ml of sample was taken in a test tube and 2ml of hexane was added. A separate layer was formed for the sample and hexane. The extracted layer (hexane layer) was collected in to screw cap tube and covered with Aluminium foil. This extraction was used for further studies.

**Gas Chromatographic Analysis of CNSL [11]:** Degradation of CNSL was determined by Gas chromatographic analysis. GC in FID (Gas chromatographic- Flame Ionized Detector) system was used for the analysis. Helium gas was used, as a mobile phase and column gas was used stationary phase. In addition hydrogen gas and air was also used.

## RESULTS AND DISCUSSION

Cashew nut shell liquid (CNSL) was a byproduct obtained during the processing of cashew nuts, was used in the manufacture of industrially important products. There are many bacteria using CNSL as a sole source of carbon and energy.

Table 1: Colonies Formed on Mineral Medium with Cnsl

| S.No | Type of colony | Number of colony | Organism |
|---|---|---|---|
| 1 | Small white colored regular colony | 15 | *Pseudomonas* species |
| 2 | Small transparent irregular colony | 09 | *Arthrobacter* species |

Table 2: CNSL Degradation by Gas Chromatography

| | | Peak areas | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Pseudomonas aeruginosa* - MTCC 424 (Control) | | *Pseudomonas* sp. | | *Arthrobacter* sp. | |
| S.No. | Retention time | 6th | 17th | 6th | 17th | 6th | 17th |
| 1 | 1.74 | 105 x $10^4$ | 79 x $10^4$ | 240 x $10^4$ | 67 x $10^4$ | 41 x $10^4$ | 85 x $10^4$ |
| 2 | 1.82 | 61 x $10^4$ | 26 x $10^4$ | 80 x $10^4$ | 50 x $10^4$ | 30 x $10^4$ | 29 x $10^4$ |
| 3 | 1.97 | 8 x $10^4$ | 0.8 x $10^4$ | 18 x $10^4$ | 9 x $10^4$ | 2 x $10^4$ | 1 x $10^4$ |
| 4 | 2.08 | 9 x $10^4$ | 7 x $10^4$ | 24 x $10^4$ | 9 x $10^4$ | 4 x $10^4$ | 7 x $10^4$ |
| 5 | 2.16 | 4 x $10^4$ | 2 x $10^4$ | 11 x $10^4$ | 3 x $10^4$ | 1 x $10^4$ | 3 x $10^4$ |
| 6 | 2.32 | 4 x $10^4$ | 3 x $10^4$ | 10 x $10^4$ | 3 x $10^4$ | 8 x $10^4$ | 4 x $10^4$ |
| 7 | 2.63 | 0.8 x $10^4$ | 0.4 x $10^4$ | 1 x $10^4$ | 0.3 x $10^4$ | 1 x $10^4$ | 0.4 x $10^4$ |

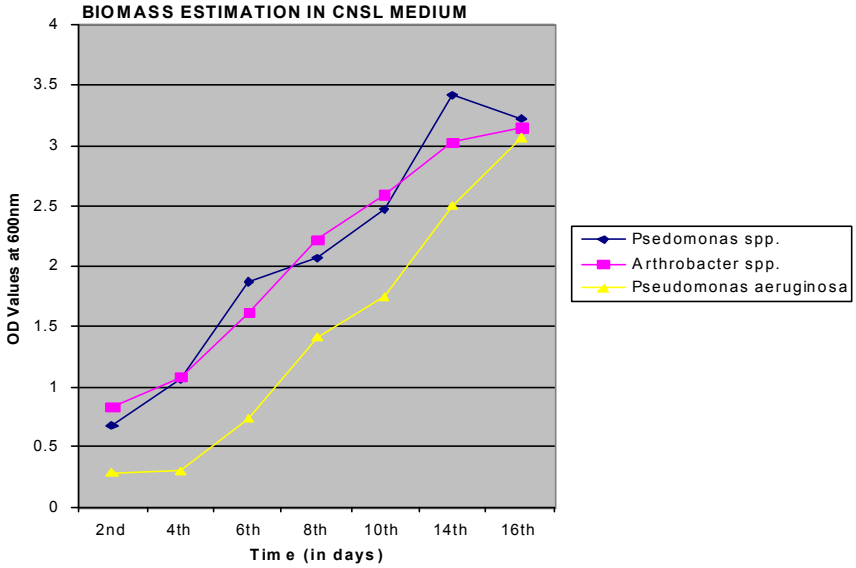


Fig. 1: Biomass estimation in CNSL medium

**Isolation and Identification of Bacteria:** To isolate and enumerate the microorganism from CNSL contaminated soil, Mineral medium with CNSL was used. Bacterial colonies were identified by the bacterial identification chart [9] and additional tests. Two types of colonies were isolated (Table 1) and were identified as *Pseudomonas* species and *Arthrobacter* species by performing Gram staining and further biochemical tests.

**Biodegradation Study:** For further studies, *Pseudomonas* and *Arthrobacter* species were used for the biodegradation study. The bacterial growth was determined by spectrophotometric assay (Figure 1). The CNSL degradation was determined by Gas chromatographic methods and the physical appearance of the media. On the initial day of the incubation period the CNSL was floated above the media and after the middle of the incubation period, the CNSL was appeared like small bubbles. From the OD values shown in Figure 1 indicates that *Pseudomonas* species utilized more CNSL than the *Arthrobacter* species and *pseudomonas aeruginosa*. The degradation of CNSL was determined by Gas chromatographic profile the peaks obtained at particular retention time indicated a particular compound (Table 2).

Kar *et al*. [12] demonstrated that *Arthrobacter* species can degrade phenol much faster than other microbes are reported degrade toxic compounds. According to Kotresha and Vidhyasagar. [13] *Pseudomonas aeruginosa* strain (MTCC 4996) isolated from a pulp industrial effluent-contaminated site was capable of degrading phenol up to a concentration of 1,300 mg $L^{-1}$ within 156 h. Complete degradation was observed at pH values ranging from 6.0 to 10.0 and temperatures from 15 to 45°C, with an optimum pH of 7.0 and optimum temperature of 37°C.

In conclusion, Cashew nut shell liquid (CNSL) is a naturally occurring phenolic compound. It is the byproduct of the cashew nut processing industry. It is generally believed that, it is toxic to human skin on contact, but many bacteria can use CNSL as sole source of carbon. In the present study, the bacterial growth in CNSL medium and degradation of CNSL were determined by spectrophotometrically. Based on the results, it was concluded that, *Pseudomonas* species degraded the CNSL more efficiently than *Arthrobacter* species and *Pseudomonas aeruginosa* MTCC-424.

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