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BIOREMEDIATION OF POLY AROMATIC HYDROCARBONS BY SELCECTED STAINS OF BACTERIAS

R. Padmini[1] and Dr. J. Thirumagal*[2]

[1]M.Phil. Research Scholar, K. M. G. College of Arts and Science, Gudiyattam.
[2]Research Supervisor in Biochemistry, K. M. G. College of Arts and Science, Gudiyattam.

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***Corresponding Author**
Dr. J. Thirumagal
Research Supervisor in Biochemistry, K. M. G. College of Arts and Science, Gudiyattam.

ABSTRACT

Bioremediation is the Biochemical dissolution of materials by bacteria or other biological means. Although often conflated, biodegradable is distinct in meaning from compostable. The ultimate task of the work is to develop a methodology to reduce nitrogen discharge from tannery effluent by microbial mediated nitrification and denitrification. To attain the task microorganisms were isolated from the deliming water and air. The efficiency was assured by estimating the level of nitrogen content. It was found that all organisms were capable of performing the denitrifying reaction. The obtained isolates were inoculated in synthetic wastewater and their growth characteristics were studied by varying the pH between 4 to 10. The growth of organisms was maximum in between pH 7 to 9. The isolates were inoculated in deliming water (real life wastewater). Two efficient isolates were selected DN3 and DN5. The level of nitrogen was estimated in the deliming water by kjeldahl method and noted as 280mg/l. After inoculating the two isolates and 4 days incubation again nitrogen content was checked. The level of nitrogen was reduced for DN3 by 55mg/l and for DN5 by 98mg/l. The isolate DN5 was screened for the utilization of PAHs.

KEYWORDS: Bioremediation, PAHs, Pseudomonas Bacterial Strains, Denitrification.

INTRODUCTION

Bioremediation is the Biochemical dissolution of materials by bacteria or other biological means. Although often conflated, biodegradable is distinct in meaning from compostable. While biodegradable simply means to be consumed by microorganisms and return to compounds found in nature, "compostable" makes the specific demand that the object break down in a compost pile. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process.

Isolation of Bacteria

Bacterial species for the present work were collected from air and deliming water. five isolates were obtained, shown in fig 1.1(a, b, c, d, e).

Morphology

Isolate -1 (DN1): Colonies were white and circular in shape.

Fig. 1.1 (a).

Isolate- 2 (DN2): Colonies were yellow and irregular in shape.

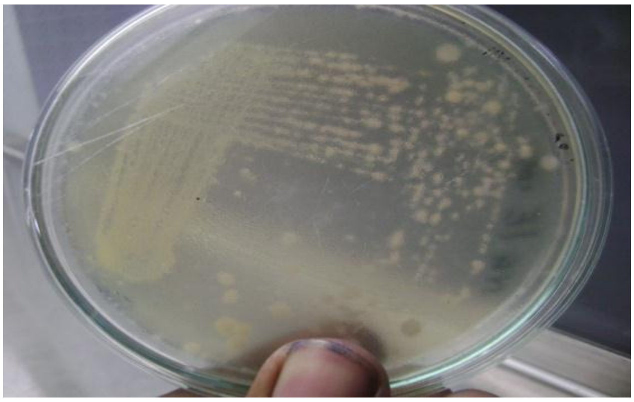


Fig. 1.1 (b).

Isolate -3 (DN3)

Colonies were white and irregular in shape.

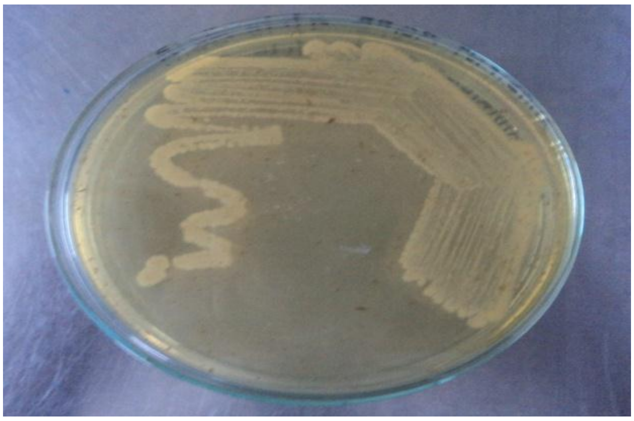


Fig. 1.1 (c).

Isolate -4 (DN4)

Colonies were orangish and circular in shape.

Fig. 1.1 (d).

Isolate -5 (DN5)

Colonies were white and round in shape.

Fig. 1.1 (e).

Microscopical Observations

Gram staining

- DN1 Gram negative rod
- DN2 Pleomorphic cocci
- DN3 Gram positive rod
- DN4 Pleomorphic rod
- DN5 Gram positive cocci

Biochemical Characterization of Bacteria

Table 5.1.

Isolates	DN1	DN2	DN3	DN4	DN5
Catalase test	+	+	-	+	-
Oxidase test	+	+	-	+	-

Nitrification Test: The nitrification test has been performed. Organisms (DN3, DN5) were incubated in ammonium sulfate broth and nitrite broth.

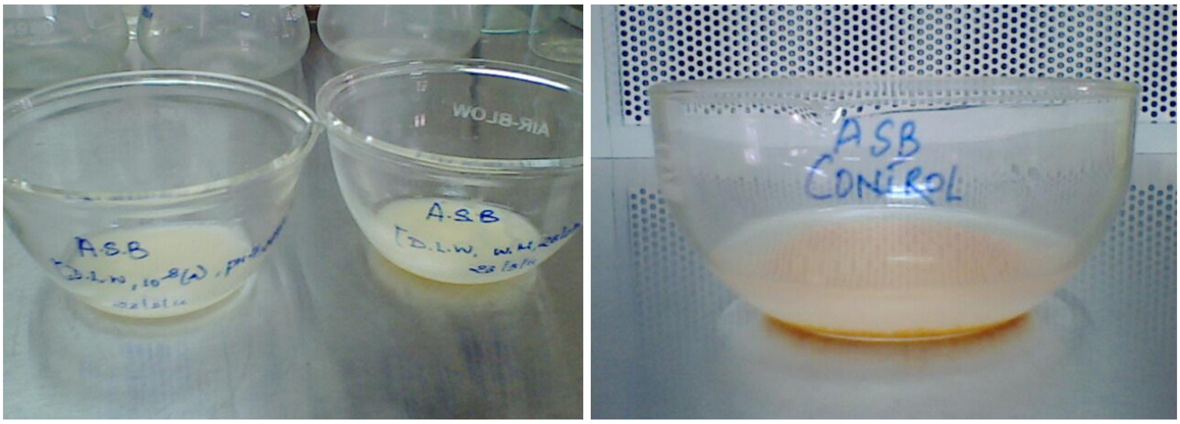
Conversion of ammonia to nitrite

- Medium-Ammonium sulfate broth

1. Reagent-Nessler reagent

Result

Absence of color after adding Nessler reagent, indicates the absence of ammonia.



(a) DN3 & DN5 **(b) CONTROL**

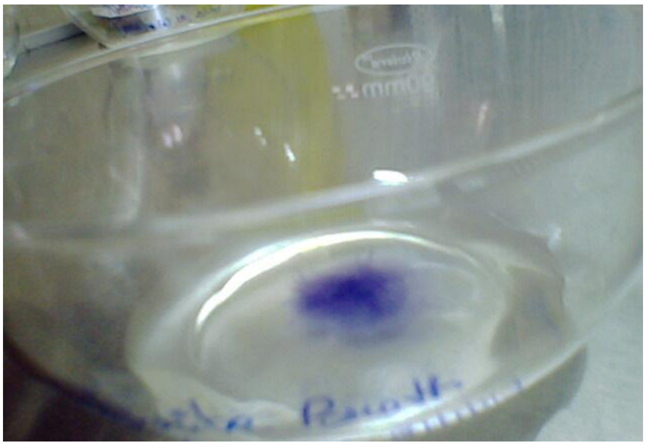
Fig. 1.2 Nessler reagent test.

2. Reagent - Trommsdorf reagent

Result

Formation of blue color, indicates the presence of nitrite and absence of ammonia.

(c) DN3 **(d) CONTROL**



(e) DN5

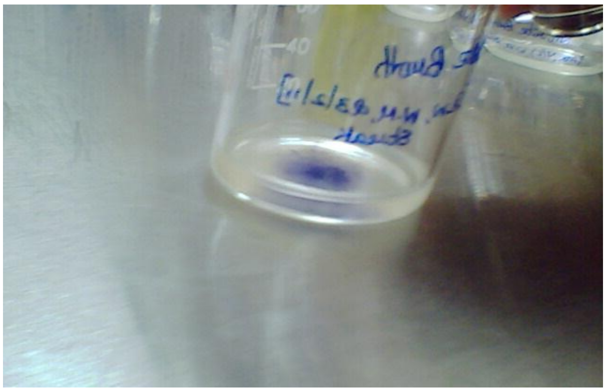
Fig. 1.3 Trommsdorf reagent test.

Conversion of nitrite to nitrate

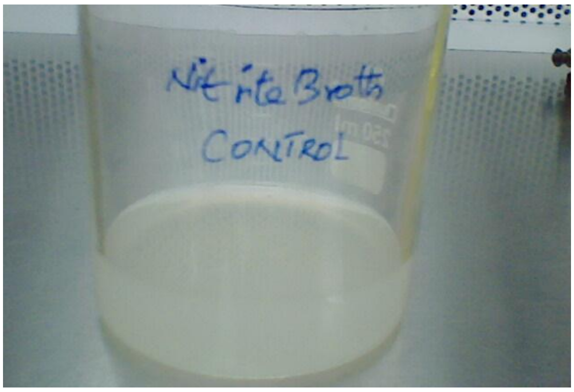
- Medium - Nitrite broth
- Reagent - Diphenylamine reagent

Result

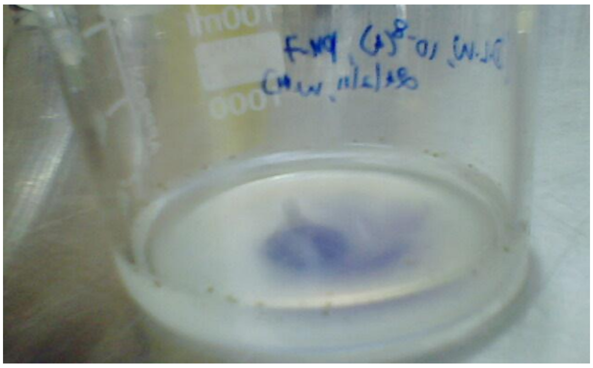
Formation of deep blue ring after adding diphenylamine reagent , shows the presence of nitrate.



(f) DN3



(g) Control



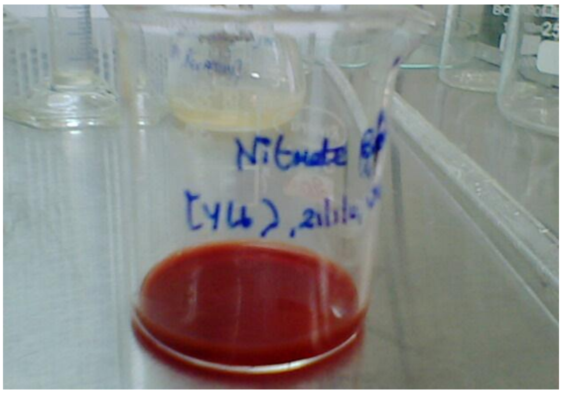
(h) DN5

Fig. 1.4 Diphenylamine test.

Denitrification Test: The denitrification test for 6 isolates have been performed. Organisms were grown in nitrate broth.

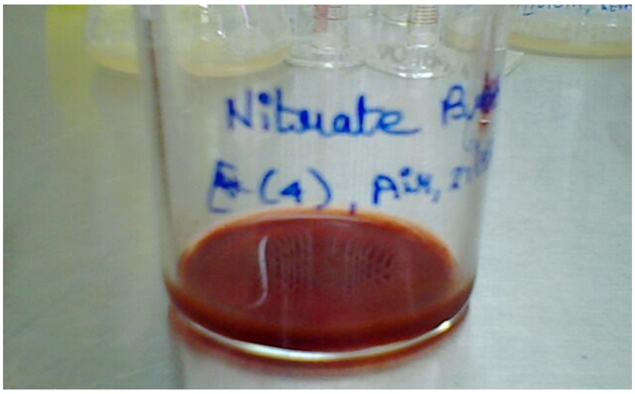
- Medium - Nitrate broth
- Reagent - Sulphanilic acid and alpha naphthylamine

Result: Formation of cherry red color, indicates the presence of nitrite i.e. nitrate is reduced to nitrite.



(i) DN3

(j) CONTROL



(k) DN5

Fig. 1.5 Nitrate reduction test.

Synthetic Wastewater

Synthetic wastewater was prepared and DN3 and DN5 were inoculated. The growth was noted by varying the pH (4,5,6,7,8,9,10) after 3 days, shown in table 2.

Table 5.2 Growth of DN3 isolate

S/no	pH	OD(600 nm)
1	4	0.3734
2	5	0.4324
3	6	0.4802
4	7	0.5341
5	8	0.7541
6	9	0.8221
7	10	0.5217

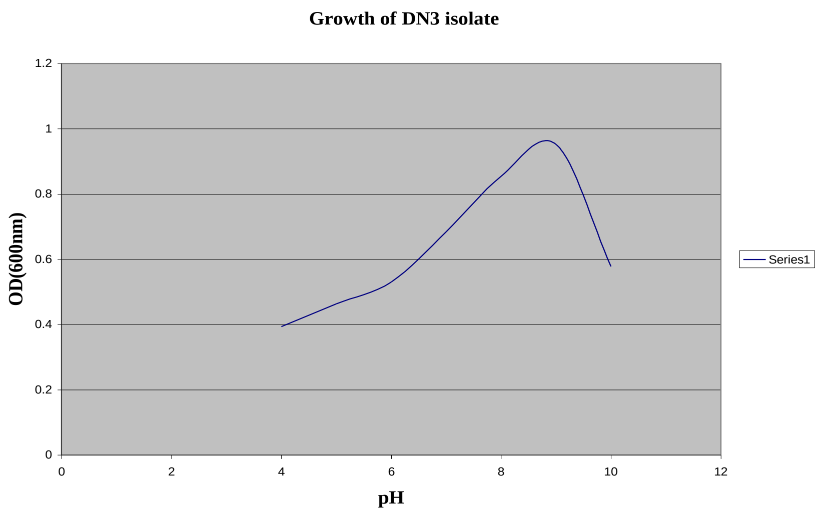


Fig. 1.6.

Table. 1.3 Growth of DN5 isolate.

S. No	Ph	OD(600nm)
1	4	0.2231
2	5	0.2645
3	6	0.3456
4	7	0.5623
5	8	0.6513
6	9	0.8445
7	10	0.5043

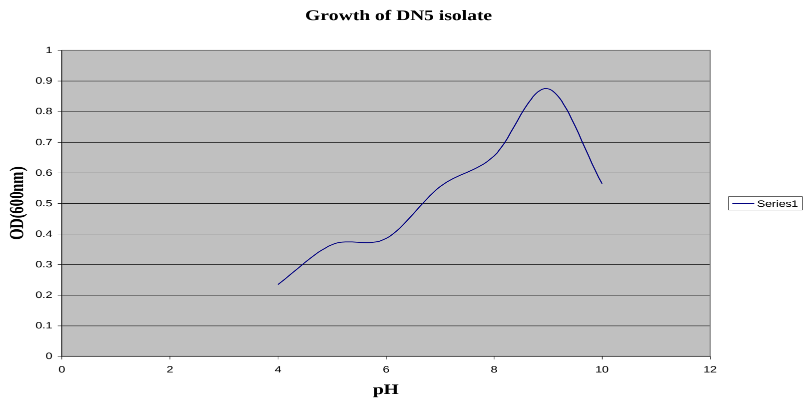


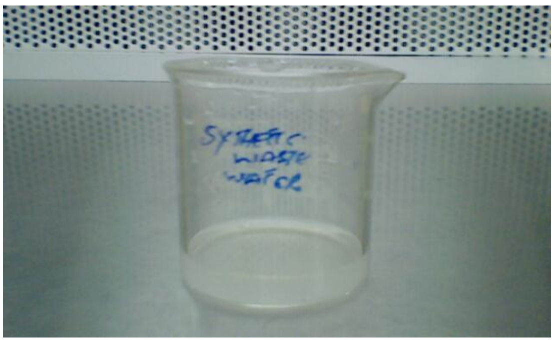
Fig. 1.7.

The graph indicates that the growth of the organisms were maxium in the pH range of 7 to 9 for DN3 and 8 t0 9 for DN5. Obtained isolates DN3 & DN5 were inoculated in synthetic wastewater for 4 days. The following tests were performed, shown in table 4.

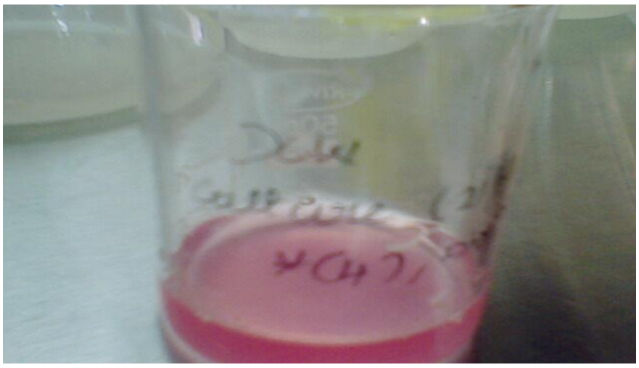
Table - 1.4. Test with synthetic wastewater.

Isolates	DN3	DN5	CONTROL
Nessler reagent	-	-	+
Tromsdorff reagent	-	-	-
Diphenylamine reagent	-	-	-
Sulphanlic acid and alpha naphthylamine	+	+	-

(a) DN3



(b) Control



(c) DN5

Fig. 1.8 Test with synthetic wastewater.

Addition of sulphanilic acid and alpha naphthylamine in the synthetic wastewater, the presence of cherry red color indicates the presence of nitrite i.e. nitrate is converted to nitrite.

CONCLUSION

About Five isolates were isolated from the deliming water and air. Five isolates were capable of performing denitrifying activity. Two efficient isolates (DN3, DN5) were selected and inoculated in the deliming water. The isolates were capable of reducing the nitrogen in the deliming water. Hence the developed methodology of reducing nitrogen by microbial mediated nitrification and denitrification is a viable method for reducing the nitrogen in tannery effluent. The DN5 strain had the additional ability to degrade the PAHs which are common environmental pollutants with toxic, genotoxic, mutagenic and/or carcinogenic properties. The strain DN5 showed growth over Naphthalene, Phenanthrene, Anthracene, Fluorene, as the sole carbon source with 1 mM concentration in MSM agar plates after 24 h. *Pseudomonas* sp. DN5 showed greater degradation capability nearly around 100% against the PAHs tested. To conclude, this isolate may be further exploited in the PAHs contaminated areas after careful investigations.

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