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Research Article

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Biodegradation of sulfonated aromatic amine by *Pseudomonas desmolyticum* NCIM 2112

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ABSTRACT

Environmental pollutions caused by the release of wide range of compounds from various industries such as paper pulp and textiles have a serious effect on ecosystem and human health. Sulphonated azo dyes represent a large class of dyes used in textile industries. The precursors of these dyes are sulphonated aromatic amines. Release of such toxic compounds in the environment cause toxic effects on soil fertility and aquatic ecosystem. Limited attention has been paid towards the fate of sulphonated aromatic amines and substituted sulphonated aromatic amines in the environment previously. The present research work describes biodegradation of azo dye precursor 1- amino 2- napthol 4 - sulphonic acid by Pseudomonas desmolyticum NCIM 2112 which was studied by FTIR and GCMS analysis. The resulting metabolites of biodegradation include propylmethane thiosulfonate and aminobenzene and they were found to be nontoxic in the environment.

Keywords: 1- amino 2- napthol 4 - sulphonic acid, *Pseudomonas*, biodegradation

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INTRODUCTION

Azo dyes are frequently used in dying, textile, paper, printing, pharmaceutical, food and leather industries worldwide and they constitute a major class of environmental pollutants. Release of coloured compounds into the environment is undesirable not only because of their aesthetic appearance and colour but also because of their breakdown products which may be toxic or mutagenic to life [1]. Sulphonated azo dyes represent a large class of dyes used in textile industries. These dyes may form sulphonated aromatic amines which are recognized as possible human carcinogens [2]. Both sulfonated and unsulfoated aromatic amines formed during the reduction of sulfonated azo dyes are an important group of environmental pollutants that can potentially pass through biological treatment system [3]. These are easily discarded via waste water and therefore they are commonly found in surface as well as river water [4]. The discharge of these waste residues into the environment eventually poison, damage or affect one or more species in the environment with resultant changes in the ecological balance. They may present an ecotoxic hazard and introduce the potential danger of bioaccumulation that may affect man by transport through food chains [5]. Physicochemical methods like coagulation, flocculation, adsorption, chemical transformation etc. do not adequately eliminate dyes from effluent water [6]. Bioremediation constitutes best alternative to such methods. Biotransformation by microorganisms can be used for their proper degradation and safer disposal. Limited attention has previously been paid towards the fate of sulfonated aromatic amines in aquatic and soil environment [7]. Therefore this investigation deals with the study of biodegradation of sulphonated aromatic amine - 1 Amino 2 napthol 4 sulphonic acid by *Pseudomonas desmolyticum* NCIM 2112.

EXPERIMENTAL SECTION

Chemicals

1-Amino-2-napthol-4-sulphonic acid, nutrient media and all other chemicals were obtained from HiMedia Laboratories Pvt. Ltd. Mumbai.

Bacterial Culture

Pseudomonas desmolyticum NCIM 2112 was obtained from National Chemical Laboratory, Pune. It was maintained on nutrient agar containing (gm/l of) Beef extract 3.0, Bacteriological peptone 5.0 and NaCl 5.0, pH 7.0. The pure culture was preserved on the nutrient agar slant at 4^0C.

Medium for biodegradation

Synthetic medium containing (gm / L) NH_4Cl 1.00 , K_2HPO_4 0.38, KH_2PO_4 1.0, $MgSO_4.7H_2O$ 0.2, pH 7.0 [8] and supplemented with 20 mg of 1 amino 2 napthol 4 sulphonic acid as a sole source of carbon and nitrogen was used to study the degradation.

Biodegradation of 1 Amino 2 napthol 4 sulphonic acid

The culture of *Pseudomonas desmolyticum* NCIM 2112 was inoculated in an Erlenmeyer flasks containing synthetic medium of above composition and the flask was incubated at an ambient temperature of 30^0C at shaking (150 rpm in an orbital shaker) conditions for 6 days and growth of microorganism was monitored at 660nm by using spectrophotometer. The degradation of 1 Amino 2 napthol 4 sulphonic acid was determined after every two days by measuring decrease in λ_{max} of the compound at 217nm. For this, the samples were collected after every two days of incubation and centrifuged at 10000 rpm for 12 minutes in cooling centrifuge adjusted to 4^0C. The supernatant was taken, filtered through 0.2 µm membrane filter and then the filtrate was scanned in the UV- Vis Spectrophotometer (Cyberlab UV 100). The band width was set to 1 nm during scanning program.

Control flask containing synthetic medium but without inoculum was run parallel along with the test flask. The degradation activity was expressed as percent degradation which was calculated by using formula,

Percent degradation = Ab – Aa /Ab X 100,

Where,
Ab is absorbance of compound at 217nm before degradation and
Aa is absorbance at same wavelength after degradation.

FTIR analysis

The biodegradation was also confirmed by Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum 65) analysis. For this, after 6 days of incubation, the culture broth was centrifuged at 6000 rpm for 10 min. and supernatant was separated. Equal volume of ethyl acetate was added to this supernatant and the organic phase containing extracted metabolites was collected. The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness in a rotary vacuum flash evaporator. It was then mixed with spectroscopically pure KBr in the ratio of 5:95 and pressed to obtain IR- transparent pellet. The pellet was placed in sample holder and the analysis was carried out in the mid IR region of 400 - 4800 cm^{-1} with 16 scan speed. FTIR analysis of 1 amino 2 napthol 4 sulphonic acid was also carried out by the same way.

GCMS analysis

For this, the dried metabolites obtained were dissolved in HPLC grade methanol and filtered through 0.2 µm membrane filters. The filtrate was then analyzed by Gas chromatography (Helwett Packard 984-BMS) engine with a Resteck column (0.25 mm × 30 mm; XTI-5) attached to mass spectrometry. The temperature programming mode was adjusted and samples were injected in splitless mode. During analysis the initial temperature of column was maintained at 80^0C for 2 minutes, increasing rate was by 10^0C and the final temperature was 290^0C holding for 5 minutes. Helium was used as carrier gas. The compounds were identified on the basis of mass spectra and were compared using National Institute of Standards and Technology (NIST) library.

Statistical analysis

All the experiments were carried out in triplicate. Analysis of the variants was carried out on all data at $P< 0.05$ using Graph Pad software. (Graph Pad Instat version 3.00, Graph Pad software, San Diego, CA, USA).

RESULTS

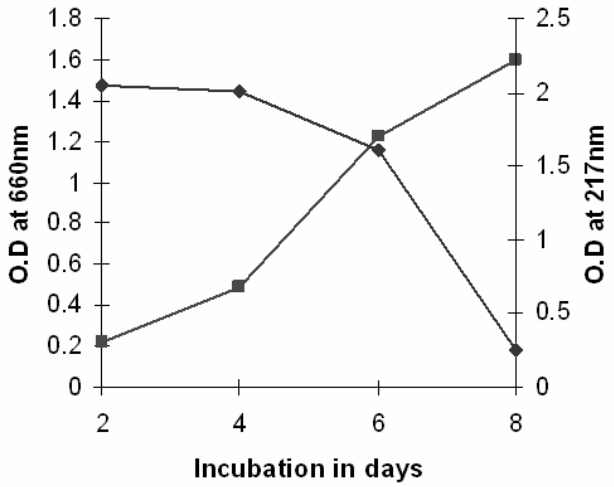
Biodegradation of 1 Amino 2 napthol 4 sulphonic acid

As shown in Table 1 and Figure 1, the percent degradation of the 1 Amino 2 napthol 4 sulphonic acid goes on increasing due to treatment with *P. desmolyticum* NCIM 2112 along with increase in growth rate of bacteria.

Table 1: Percent degradation of 1 Amino 2 napthol 4 sulphonic acid after every two days of incubation.

Degradation	After 2 days of incubation	After 4 days of incubation	After 6 days of incubation	After 8 days of incubation
Percent Degradation	52.39± 0.033	87.23± 0.033	92.28± 0.033	98.76± 0.033

Fig. 1 Degradation of the 1 Amino 2 napthol 4 sulphonic acid, measured at 217nm, and growth curve of bacteria, measured at 660nm up to 8 days.



FTIR analysis

The difference in FTIR spectrum of 1 Amino 2 napthol 4 sulphonic acid (Fig.2A) and metabolites obtained after its degradation (Fig.2B) confirms biodegradation.

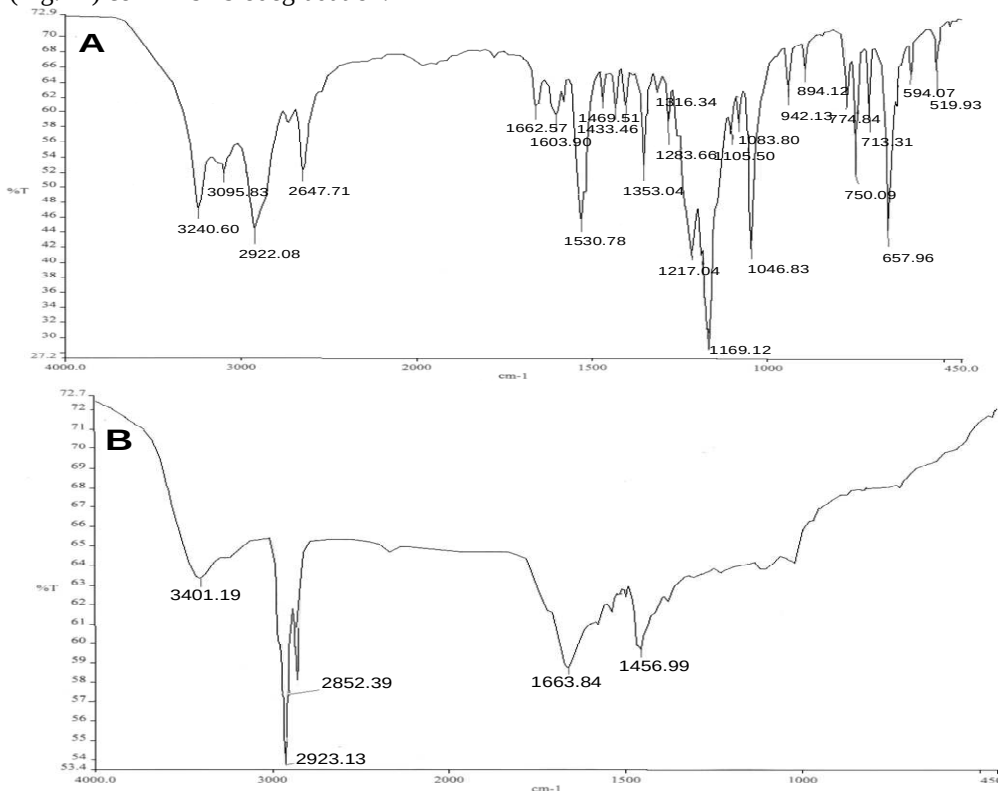


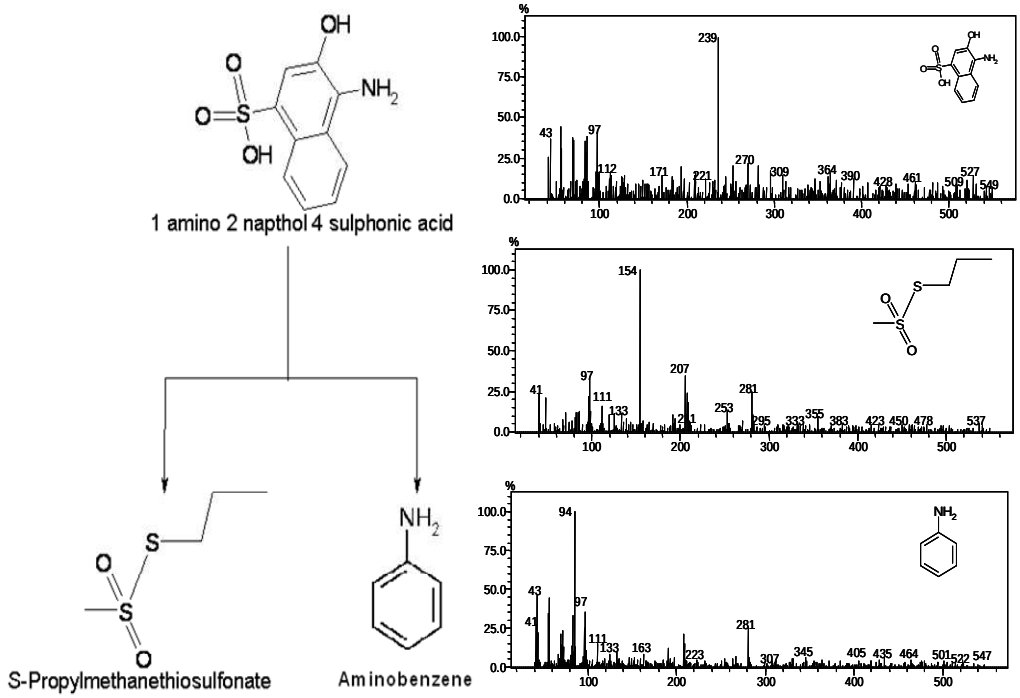
Fig.2A. FTIR spectrum of control 1 Amino 2 napthol 4 sulphonic acid, 2B. FTIR spectrum of metabolites obtained after degradation of 1 Amino 2 napthol 4 sulphonic acid.

The FTIR spectrum of 1 Amino 2 napthol 4 sulphonic acid showed stretching vibrations. 1353.04 cm^{-1} for S = O stretching vibrations of sulfonic acid, 3240.60 cm^{-1} for N-H symmetric stretching of NH_2 group and 1353.04 to 1283.66 cm^{-1} for C-N stretching which represent the nature of aromatic amine group. The peak at 1530 cm^{-1} represents stretching vibrations for C−C aromatic compound. The FTIR spectrum of metabolites obtained showed the absence of peak at 3240.60 cm^{-1} for N-H because of the cleavage of bond. Similarly, the absence of peak at 1046.83cm^{-1} indicates cleavage of $–SO_3H$ group. The metabolites formed after degradation of 1 Amino 2 napthol 4 sulphonic acid was further identified by means of GCMS analysis.

Proposed degradation pathway

The results of GCMS analysis clearly showed the formation of propylmethane thiosulfonate and aminobenzene from 1 Amino 2 napthol 4 sulphonic acid by *Pseudomonas desmolyticum* NCIM 2112 (Fig.3). The aminobenzene was further found to be converted into benzene by deamination.

Fig. 3 Proposed pathway of 1 Amino 2 napthol 4 sulphonic acid by *Pseudomonas desmolyticum* NCIM 2112.



DISCUSSION

Poor degradation of sulfonated aromatic amines in the environment by both aerobic and anaerobic mechanism has been reported [9]. Biodegradation of sulfonated aromatic amines by pathways involving oxygenases has been reported [10]. The degradative pathways of sulfonated aromatic amines by means of various microorganisms were also reported [11]. *Sphingomonas* species strain BN6 is known to degrade various substituted naphthalene sulphonic acid aerobically which are building blocks of different azo dye [12]. It was previously reported that, the aromatic compounds are recalcitrant in nature and if get mixed in the soil atmosphere they inhibit the soil microflora as well as responsible for decreasing the soil fertility level and shows phytoxicity [13, 14].Toxicity of xenobiotic and recalcitrant compounds was also get studied [15,16]. So far various methods to control this pollution have been reported [17, 18, 19].

In the present investigation, it has been observed that *P. desmolyticum* NCIM 2112 degrade the 1 Amino 2 napthol 4 sulphonic acid into propylmethane thiosulfonate and aminobenzene. Aminobenzene is further converted into benzene. However, it does not open the aromatic ring of the formed metabolites and therefore may be funneled into the metabolic pathways.

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