Investigation Aromatic Compound of industrial wastewater by a Aquatic Fungus

Shahrouz Fazeli [1*] ,Akbar Esmaeili [2]

[*] Iran - Khozestan - Mahshahr - Bandar Imam Petrochemical. P.O.BOX 138 Bandar Imam - Iran

Email Address: shahrouzfazeli@yahoo.com

Abstract

The metabolism of α-naphthol by *Aspergillus niger*, a coelomycete isolated in Ardebil, Iran, from industrial wastewater, was studied. *Aspergillus niger* metabolized approximately 80% of α-naphthol within 5 days. The identification and quantification of degradation products using gas chromatography–mass spectrometry (GC-MS) demonstrated that approximately 41% of the parent compound was converted into 1-ethyl-2-methyl benzene, 7.43% was converted into acetonaphthone, 5.55% was transformed into 4-hydroxy-1-naphthyl sulfate, 3% into 1,4-naphthoquinone, and about 6.68% into 2-phenyl-1,2,3-tetrahydro-1-naphthol. These results support a role for *A. niger* in affecting the environmental fat of pollutants in ecosystems.

Keywords: Biotransformation, Bioconversion, Aspergillus niger, α-naphthol, Fungi

1- M.S, Applied chemistry from Department of chemistry, North Tehran Branch, Islamic Azad University, P. O. Box 19585-936, Tehran, Iran.

2- Dr, Organic chemistry from Department of Chemical Engineering, North Tehran Branch, Islamic Azad University, Tehran, Iran.

1- Introduction

The polycyclic aromatic hydrocarbon naphthalene is used in the production of phthalate plasticizers and resins, azo dyes, dispersants, and tanning agents in the rubber and leather industries [1-21]. Naphthol is a common bicyclic aromatic often released into the environment [2]. Naphthol is often liberated into the environment as a result of the oxidation of naphthalene by certain fungi and bacteria [3-4-5] and is particularly important both as a synthetic precursor of the insecticide Sevin (1-naphthyl-N-methylcarbamate) and as a degradation product of this compound via chemical and biological processes [6]. It has toxic effects in aquatic ecosystems, especially on marine invertebrates [7].

Due to its toxicity to marine life [6] and human beings [8-9], industrial wastewater containing α-naphthol must be treated before it is discharged into or reused in the environment [10].

Fungi play an important role in the metabolism of many chemicals, including aromatic hydrocarbons, in both aquatic and terrestrial environments [22-12-13-14-9]. There are studies attesting to the ability of strains of different bacteria, e.g., *Brevibacterium* spp. HL 4, *Pseudomonas* spp. DLC-P11, *Arthrobacter sulphureus* RKJ4, *Acidovorax delafieldii* P4-1 [15], *Halosphaeria mediosetigera*, *Culcitalna achraspora*, *Humicola alopallonella*, *Brevibacterium* spp., *Flavobacterium* spp., *Serratia marina*, *Spirillum* spp., *Candida parapsilosis*, *Rhodotorula glutinis*, *Trichosporon fermentans*, *Aspergillus fumigatus* [16], *Micrococcus* spp. [8], *Heliscus lugdunensis* [11], and *Rhodococcus* spp. [2], to disintegrate α-naphthol and reports about isolation and examination of these fungal strains with respect to their α-naphthol tolerance. However, we are not aware of studies describing biotransformation of α-naphthol by *Aspergillus niger* PTCC 5011. This paper describes the ability of *A. niger* PTCC 5011 to transform this compound and the identification of pathways involved in the biotransformation of the resultant metabolites.

2- Materials and methods

2-1- Chemicals

All chemicals were of analytical grade (gradient grade in the case of chromatography solvents). α-naphthol was purchased from Panreac Química S.A.U. (PS) of Barcelona, Spain (>99% purity). Tween 80 was provided by the Department of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran. Czapek-Dox broth (Czapek's medium) was purchased from Difco of Detroit Michigan, USA. All other chemicals were purchased from Merck (Darmstadt, Germany).

2-2- Microorganisms

A strain of *Aspergillus niger* was isolated from the wastewater of an industrial plant in Ardebil, and was identified according to its physiological and morphological characteristics. *Aspergillus niger* (PTCC 5011) was identified according to the Persian Type Culture Collection, Iranian Research Organization for Science and Technology, Tehran, Iran.

2-3- Culture conditions

Spores and mycelia (2×10^8 conidia/mL medium) were aseptically inoculated into 250 mL Erlenmeyer flasks containing 100 mL of Czapek-Dox broth. The Czapek's

medium (mg/L distilled water) consisted of bacto saccharose, 30.0; sodium nitrate ($NaNO_3$), 3.0; dipotassium phosphate (K_2HPO_4), 1.0; magnesium sulfate ($MgSO_4$), 0.5; potassium chloride (KCl), 0.5; and ferrous sulfate ($FeSO_4$ $7H_2O$), 0.01. The flasks were incubated for 48 h at 30 ^{0}C on a rotary shaker at 150 rpm in the dark.

3- Analytical methods and degradation experiments

3-1- Isolation, detection, and identification of metabolite

To identify α-naphthol and metabolites, the fungus was cultivated in 250 mL Erlenmeyer flasks containing 100 mL of Czapek-Dox medium and inoculated with 2×10^8 conidia/mL medium. α-naphthol (75 mg/L), with Tween 80 (400 mg//L) added to one flask after 24 h of incubation in darkness. Five days after α-naphthol addition, the suspension was extracted three consecutive times with ethyl acetate. The organic extracts were combined after the separation of organic and water phases. Afterward, the organic extracts were concentrated at 50 ^{0}C by means of a rotary evaporator to about 5 mL [10]. Residues (5 mL) were examined for α-naphthol and acetate-extractable transformation products by GC-MS. Abiotic controls (without microorganism) were always included.

3-2- Analysis of the sample with GC-MS

The samples were assayed with GC-MS as follows. The samples were analyzed using a Hewlett-Packard 6890 with a DB-5 capillary column (30m × 0.25 mm; film thickness 0.25 μm) was programmed as follows: 60 ^{0}C for 5 min and 220 ^{0}C at a rate of 4 ^{0}C/min. The flow rate to helium as a carrier gas with (2 mL/min) MS was taken at 70 eV.

4-Results

4-1-Biotransformation of α-naphthol by *A. niger*

In this experiment, the biotransformation of α-naphthol by *A. niger* grown on Czapek's medium for only 5 days was performed. After incubation, Czapek's culture was extracted (see analytical methods and degradation experiments section). The suspension was extracted with ethyl acetate three consecutive times and directly analyzed by GC-MS (Figure 1).

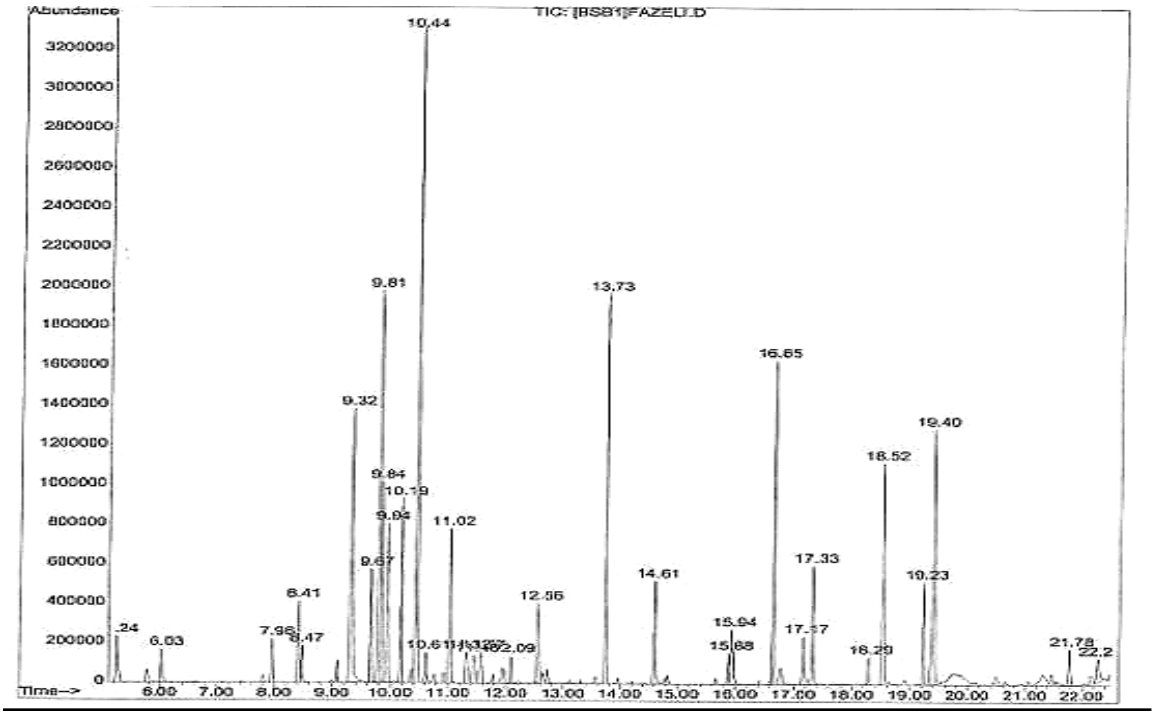

Figure 1 . GC-Mass spectrum of biotransformation of α-naphthol by *A. niger*

In these analyses, various chemicals were obtained (Table 1).The main products obtained in the bioconversion of *A. niger* of α-naphthol were 1-ethyl-2-methyl benzene (41%) and acetonaphthone (7.43%), respectively.

Table 1. Mass spectral data, retention times, and proposed identities of metabolites derived from α-naphthol after 5 days of incubation

Proposed compound	Retention time (min)	Mass spectal ions, *m/z*(% relative intensity)
1-Eethyl-2-methyl benzene	10.45	105(100), 120(53.58), 119(13.51), 77(10.63), 91(9.27)
Acetonaphthone	13.73	57.1(100), 43.1(72.28), 71.1(59.42), 85.1(35.96), 41.1(34.23)
1,4-Naphthoquinone	17.33	158(100), 102(45.43), 104(43.56), 130(33.49), 76(33.46)
4-Hydroxy-1-naphthyl sulfate	18.52	144(100), 115(80.27), 116(37.31), 145(10.85), 89(8.79)
2-Phenyl-1,2,3-tetrahydro-1-naphthol	19.40	105(100), 134(86.38), 77(31.34), 133(27.28), 120(23.89)

In a previous study of biotransformation of menthol by sporulated surface cultures of *A. niger* and *Penicillium* spp. the main bioconversion product obtained from menthol of *A. niger* was *cis*-*p*-menthan-7-ol, and the main products obtained by sporulated surface cultures of *Penicillium* spp. were limonene, *p*-cymene, and γ-terpinene [10]. Leuenberger [15] reported that product yields could be effectively increased by solubilizing/emulsifying immiscible substrates. However, careful selection of the nature and concentration of the solvent is necessary, because many miscible solvents are

cytotoxic at lower concentrations [15]. Comparing the above investigation with the present study showed oxygenated monoterpenes to be the main compounds, with more monoterpenes yielded in the biotransformation. Figure 2 shows that transformation of α-naphthol by *A. niger* PTCC 5011 produces more oxidation products.

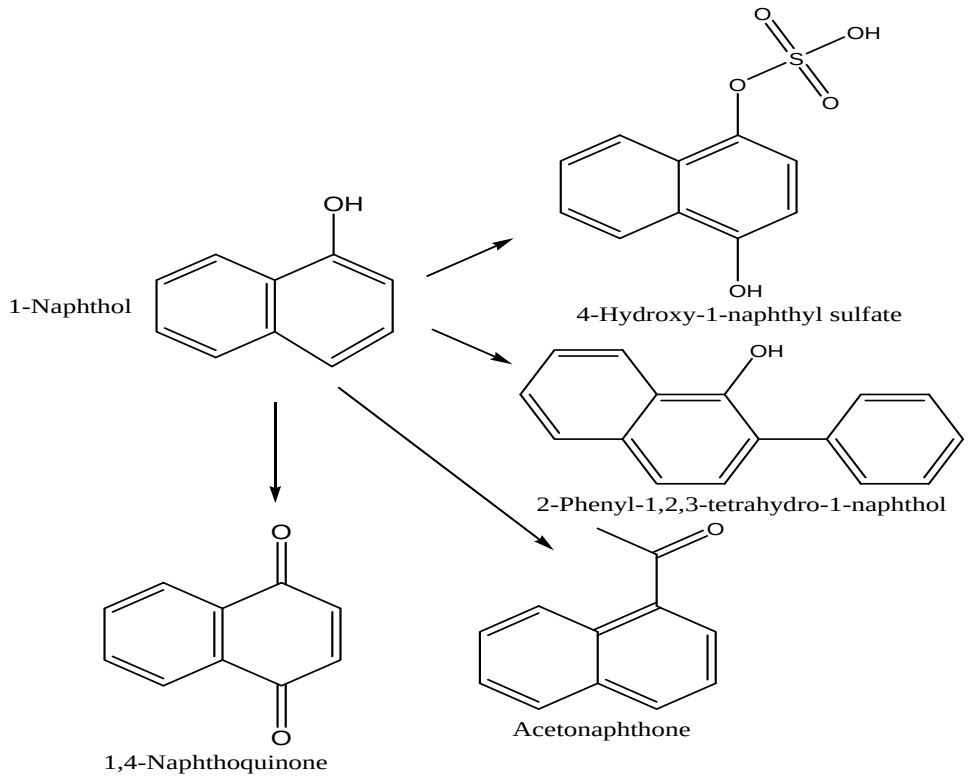

Figure 2. Transformation of 1-naphthol by *A. niger*. 4-hydroxy-1-naphthylsulfate, 2-phenyl-1,2,3-tetrahydro-1-naphthol, 1,4-naphthoquinone, and acetonaphthone were deduced from mass spectral data of 1-naphthol transformation products found in *A. niger*

5-Discussion

We compare this data(degradation extent of 1-naphthol was 80%) with result of Biotransformation of 1-naphthol by a Strictly Aquatic Fungus (degradation extent of 1-naphthol was 74%) [17], and this compare shown: degradation of 1-naphthol in this condition about 6% was enhanced.

The study of microbial transformation of one monoterpene by sporulated surface cultures of *A. niger* and *Penicillium* spp. produced *cis*-*p*-menthan-7-ol from *A. niger*; the main products obtained were limonene, *p*-cymene, and γ-terpinene [20]. The two main products of microbial transformation of citral were similar to those obtained in former work. The main bioconversion products of (-)-menthol by *Mucor ramannianus* using the sporulated surface cultures method were *trans*-*p*-menthan-8-ol, *trans*-menth-2-en-1-ol, sabinane, *p*-menthane-3,8-diol, isomenthol, and 1,8-cineole [18].

The experimental work (the two latest articles) suggested that microbial transformation of monoterpenes with different genus *Penicillium* and *Aspergillus* caused an oxidation reaction and resulted in a more stable product. But bioconversion by using *Aspergillus niger* PTCC 5011 showed that it was possible to obtain two or three main products with high percentage and selectivity. In conclusion, reported literature and this research show bioconversion of *Pseudomonas*, *Aspergillus*, and *Penicillium* have been similarly effective

for biotransformation. Oxidation and sulphation are two common characteristics in this investigation of bioconversion products [19].

Photocatalysis/chemical oxidation combined with biodegradation would be a more potential method for 1-naphthol degradation in theory based on its chemical structure.

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