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EFFECT OF U.V. LIGHT EXPOSURE ON DEGRADATION OF NAPHTHALENE BY FILAMENT FUNGI

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ABSTRACT

Aspergillus niger and Trichoderma viridi were tested for its ability to degrade naphthalene after exposing to U.V light (30, 45, 60 min) by using solid mineral salts medium (SMS) with different concentrations 100, 300, 500 ppm of naphthalene. Results showed that 100ppm was the best concentration consumed by the fungal test then 300ppm and 500ppm and the results for secondary test by using Liquid Mineral Salts Medium (LMSM) 95% of degradation for 100ppm after 30 min of U.V. light exposure for *Aspergillus niger* while the results for *Trichoderma viridi* the best ratio for degradation was 97% for 100ppm after 30 min of U.V. light exposure.

KEYWORDS: Biodegradation, Naphthalene, filament fungi, U.V. Light.

INTRODUCTION

Filamentous fungi play an important role in degradation and detoxification of polycyclic hydrocarbons, including condensed aromatic ring systems as well as other xenobiotic compounds, present in polluted environments. Some of these compounds are very harmful and carcinogenic.[1]

Filamentous fungi have been reported to colonize the solid substrate and tolerate high concentration of toxic compounds.[2] Species belonging to the genera *Trichoderma harizianum, Fusarium avaneceum, Penicillium chrysogenum, Aspergillus niger* and *Aspergillus flavus* are some examples of this kind of fungi that have been recently described as tolerant to pollutants such as B(a)Pyrene, anthracene, naphthalene.[3]

Naphthalene is a volatile organic compound (VOC) with $C_{10}H_8$ that is a widespread atmospheric contaminant. It is the simplest polycyclic aromatic hydrocarbon.

UV light has been shown to be lethal and mutagenic in a variety of organisms, including fungi. The correlation between the quantity of energy absorbed by DNA and the observed biological effects (survival and mutation frequency) are illustrated in the wavelength region between 254 and 320 nm. UV irradiation was found to be best for the improvement of strains like *Aspergillus niger* for maximum production of various enzymes.[4] In recent years, attempts have been made for the overproduction of microbial enzymes by induced mutagenesis. Suntornsuk and Hang[5] have reported that the strain improvement in *Rhizopus oryzae* by UV resulted in the production of more glucoamylase by a mutant than the parent strain. Mutational experiments were performed to produce morphological mutants from *Aspergillus wentii* by UV and X ray irradiation. The purpose of the present investigation is to know whether an selected fungal strain was still active in degradation after exposing it to UV light.

MATERIALS AND METHODS

Fungal inoculums preparation

Inoculums suspension of selected fungal isolate was prepared from periphery 7 days old culture grown on PDA slants, the colonies was covered with 5 ml of Tween 20 (5%). The inoculum were achieved by carefully rubbing the colonies with a sterile loop, the slants were then shaken vigorously for 15 min with a vortex and then transferred to a new sterile tube then the inoculum was transferred to a sterile syringe attached to a sterile filter Millipore 0.22µm. The suspension was filtered and collected in a sterile tube. This procedure removed the majority of the hyphae producing inoculums mainly composed of spores.[5]

UV Mutagenesis

The best natural selectant *Aspergillus niger and Trichoderma viridi* were grown on potato dextrose agar (PDA) medium for 7 days at 30°C.

Fungal spore suspensions were distributed into sterilized petri plates (2 ml in each plate).

They were exposure to UV light in a “Dispensing – Cabinet” fitted with TUP 40w Germicidal lamp which has about 90% of its radiation at 2540-2550. Optimum dose required to get maximum mutants was arrived by exposing the organism for different periods of time

(10 and 20min) at the distance of 20 cm from the UV source. Each UV exposed spore suspension was stored in dark overnight to avoid photo reactivation.

UV treated fungal spore suspensions of 0.1 ml was inoculated into petri plate containing potato dextrose agar medium. The plates were incubated for 7 days at 30°C. Then developed fungal strains whose survival rate was less than 1%. and tested for degradable enzymes production.[6]

Testing of the isolate for naphthalene biodegradation

Primary Test

Fungal isolate was tested for its ability to degrade naphthalene using solid mineral salts medium and 0.1 ml from naphthalene solution containing (100,300,500ppm) was spreader on solid medium agar plates then methanol evaporated by left plates for 1 hr inside sterile hood, white thin layer formed, then the plates were inoculated with fungal disk 7mm from7 days old culture of fungal isolate. The plates were incubated at 28C^{o} for 7 days to allow growth of test fungi. They were then examined for their growth formation around the fungal test. The growth diameter was measured in each case.[7]

Good: - Radial growth rate (50-80mm)

Moderat: Radial growth rate (20-49mm)

Weak: - Radial growth rate (1-19mm)

Secondary test

The fungal isolate was grown in 100 ml Erlenmeyer flasks containing 25 ml liquid mineral salts medium, pH was adjusted to 7, then autoclaved at 121^{0}C for 15 min, the sterilized media were inoculated with fungal disk (7mm) from7 days old culture of fungal isolate with 100,300,500ppm naphthalene separately. Duplicate for each concentrate of isolate and control, then flasks were incubated in shaker incubator 130rpm for 7days at 30^{0}C and naphthalene residue was determined.[8]

Determination of naphthalene concentration by HPLC

After incubation, the flasks were removed and the cultures centrifuged at 10000 rpm for 20 min, then filtered through whatman no.1, 10ml of hexane was added to 25 ml of mineral medium and was shaken for 30 min in separate funnel then 1ml of upper phase (hexane) was shifted to the sterile tubes and was used for HPLC analysis.[9] HPLC analyses were performed with reverse-phase column C18 (Syknm Chromatography Products, Germany).

Separation was achieved by isocratic elution in acetonitrile: water (70:30) respectively, with a flow rate of 1.0 ml/min and UV absorbance detector set at 279 nm.

Biodegradation% = initial concentration of naphthalene - naphthalene concentration after incubation periods / initial concentration of naphthalene *100

RESULTS AND DISCUSSION

Testing of the isolate for naphthalene biodegradation

Primary Test

The disappearance of Naphthalene from the solid MSM, suggested being consumed by the fungal isolate, but no growth on this medium was observed by survival of a white thin layer of naphthalene on the surfaces of the dishes indicating the inability of the isolate to consume this compound. This may be attributed to the reason that fungal colony are not growing on this medium because they do not possess the ability to degrade this compound as a result of lack specialist enzymatic system, or due to the absence of metabolic capacity .Other reason could be the low solubility of this compound which reduce the availability to microorganism and then cannot be attacked.[10]

Identical study done by[11] found failure of fungal isolates to grow on solid MSM with anthracene which attributed to the degradation of compound containing more than one cycle and more resistant to oxidative enzymes figure (1).

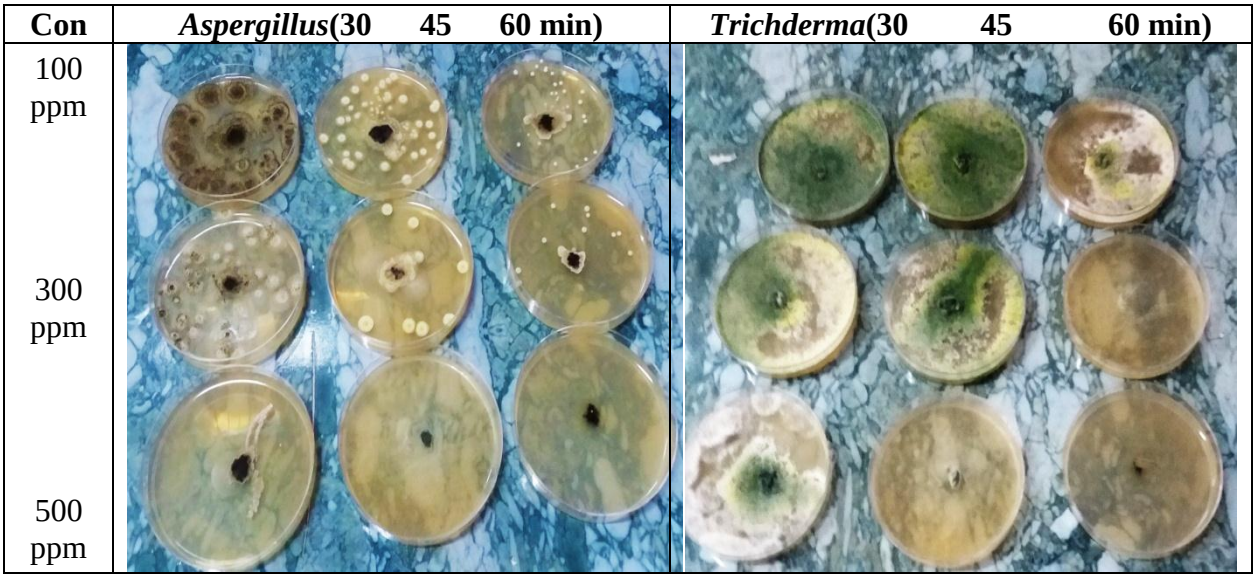

Figure (1): The ability of mutate fungal isolates growth on solid MSM, pH 7 containing pure naphthalene 100,300,500ppm after incubation period of 7 days at 30^{0}C.

Secondary Test

The results showed variation in the capacity of the fungal isolates in consumption of naphthalene in the liquid mineral salts media**.** When the fungal tests exposed to U.V. light the degradation occur well in 30 and 45 min. with 100 and 300 ppm in two tested fungi but in 500ppm results not well.

Earlier study with *A. niger* in which increased time of UV exposure (>55 minutes) resulted in decreased alpha amylase production.[12,13]

Other studied showed that *A. fumigatus* loose its ability to lip enzyme production after 30 min of U.V light exposure.[14]

Other studied revealed that *T.harizianum* lose its ability to Mnp and Lac enzyme production after 50 min of U.V light exposure.[15]

Table 1: Naphthalene biodegradation by *Aspergillus niger and Trichoderma viridi* in liquid MSM with 100,300,500ppm naphthalene, pH 7 and incubated for 7 days, 120 rpm at 30^0C.

S.no.	percentage of biodegradation %			
	concentration(ppm)	100	300	500
1	control	6.1	5.4	6.4
2	*Aspergillus niger* after 30 min of U.V light exposure	95	87	70
3	after 45 min of U.V light exposure	89	79	54
4	after 60 min of U.V light exposure	55	22	18
5	control	5.7	5.2	6.7
6	*Trichoderma viridi* after 30 min of U.V light exposure	97	92	57
7	after 45 min of U.V light exposure	85	78	31
8	after 60 min of U.V light exposure	57	24	21

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