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Biodegradation of Nonylphenol Ethoxylate in Wastewater by *Penicillium chrysogenum*

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Abstract

Current research aims to isolate fungal isolates capable of biodegrading Nonylphenol Ethoxylate (NPEOs) from secondary activated sludge. Samples were collected from Zenin wastewater treatment plant, El-Giza, Egypt. Physical and chemical analyses were performed. Screening of microbial isolates capable of degrading Nonylphenol Ethoxylate was performed on basal salts medium with Nonylephenol Ethoxylate as the only carbon source. Quantification of the residual amount of NPEOs was determined by using a liquid chromatography technique. Morphological identification of the most promising fungal isolates degrading NPEOs was performed based on current universal keys, Penicillium database management system and molecular technique 18S rDNA using ITS1 (forward) and ITS4 (reverse) primers. Cytotoxicity test was performed by using different concentrations of NPEOs. Results revealed that eight most promising fungal isolates capable of degrading NPEOs were obtained , which achieved the highest percentage of biodegradation (70 - 80 %).One novel species named *Penicillium chrysogenum* was able to biodegrade (NPEOs) (80% degradation). In case of the ***Penicillium chrysogenum*** cytotoxic activity against mammalian cells from African green monkey kidney cells was detected under these experimental conditions (CC_{50}) was 46.5 ± 4.8 µg/ml and the inhibitory cytotoxic activity against normal human lung fibroblast cells was detected under these experimental conditions with CC_{50} was 74.1 ± 6.1 µg/ml.

Key words: Biodegradation, activated sludge, Nonylphenol Ethoxylates (NPEOs), *Penicillium chrysogenum,* cytotoxicity

1. Introduction

Numerous chemical compounds have been synthesized in large quantities to sustain our luxurious life [1]. But a lot of them are proven to cause harmful side effects on both the health of humans and wildlife.stated that surfactants are commonly used in biotechnological processes but are also major pollutants of the novel world [2]. They are existing in cleaning agents, softeners, indigenous detergents and hygienic products such as shampoos, shower gels and toothpaste [3] [4]. Informed that many classes of surfactants including nonionic surfactants play a great role within the novel world surfactant market [5].Reported that Nonylephenol ethoxylates (NPEOs) recorded annual global production around 700,000 tons and most of these compounds including nonylphenol polyethoxylates (NPEOs) are incorporated to aqueous solutions, and after being used, they are discharged in industrial or municipal wastewater and eventually enter wastewater treatment plants [6].Nonylphenol ethoxylates (NPEOs) are an important group of nonionic surfactants including a wide range of applications such as detergents, emulsifiers, dispersants, antifoams and pesticides products [7].

Informed that nonylphenol ethoxylates manufacture was the origin of both releases of nonylphenol and nonylphenol ethoxylates; mostly through aqueous discharges besides nonylphenol and its ethoxylates gain a greatest current concern because of their toxic or inhibitory effect on living organisms by their presence or accumulation in environment such as water, sediments, soils and atmosphere [8]. As a result of widespread usage of these products its main percentage is discharged into the sewage [9]. Therefore, it is important to look for indigenous microorganisms able to degrade native NPEOs in

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addition to its derivatives.

Reported that sewage sludge is produced from wastewater treatment in sewage treatment plants. Mainly the sludge consists of two basic forms, sludge and secondary sludge, also known as activated sludge in the case of activated sludge process [10]. reported that various microorganisms are almost present in large numbers in sewage or soil amended with activated sludge especially fungi arise in abundance in sewage sludge and amended soils [11] [12] .

Bioremediation considered as a key for removing harmful contaminants from the environment but traditional bioremediation processes have a lot of limitations, so it is important to find a new bioremediation technologies for better results [13].Biodegradation can define as a process in which microorganisms by its enzymatic or metabolic processes can break down compounds into tiny constituents or completely broken down into carbon dioxide and minerals [14] [15] . Nonylphenol (NP) also can be decreased in the ecosystem by biodegradation in the water, sediment and soils through the action of microorganisms [16] . used Pseudomonas sp. strain TR01 for Nonylphenol ethoxylates degradation. Results showed that the isolated Pseudomonas bacterium has unique substrate assimilability: it metabolizes the EOs chain only when the chain linked to bulky hydrophobic groups [17] .

Fungi play a major role in all ecosystems as a decomposers and symbionts and in soil and aquatic habitats, as a result of their robust morphology and diverse metabolic capacity fungi are especially suited for the purpose of bioremediation. Mycoremediation is a method of bioremediation in which fungi are employed to decontaminate the contaminated areas [18] .

Fungi which belonged to genus Penicillium is common and isolated from all environments and it is mainly found in warm regions because of its ability to produce massive numbers of reproductive elements which scattered and diffused easily in air and soil [19] .Moreover the widespread occurrence of Penicillium species may be as a result of their various enzymatic capacities [20].

This work aims to screen and study the biodegradation activity of NPEOs using some microbial isolates from activated sludge. More consideration was given to one novel species named *Penicillium chrysogenum* which were able to highly biodegrade NPEOs. Also, the aim of current work extends to determine the cytotoxic activity of the NPEOs on *Penicillium chrysogenum* and determine the residual concentrations of NPEOs after biodegradation by liquid chromatography technique.

1.1. Collection of waste water samples

Samples of activated sludge's were collected from secondary activated sludge from Zenin wastewater treatment plant, El-Giza, Egypt. The collected samples were kept in refrigerator at 5 °C until analysis

2.2. Physicochemical parameters of secondary activated sludge samples

Physicochemical parameters such as color, viscosity, temperature, chemical oxygen demand (COD), Biochemical oxygen demand (BOD), Total dissolved solids (TDS), total volatile solids (TVS) , total solids (TS), volatile suspended solids (VSS), total suspended solids (TSS) , and pH were determined [21] .

2.3. Isolation of fungal isolates from secondary activated sludge

Serial dilutions of each sample were prepared using distilled water and then spread over minerals salts medium with NPEOs as the only carbon source (0.9 -7.2 ppm) [22] **.**The inoculated plates were incubated at 27 °C for 48 to 72 hrs. Colonies of fungi were picked from each plate and streaked on the minerals salts agar medium for further purification [23] .

2.4. Primary screening of fungal isolates capable of degrading Nonylphenol Ethoxylate on minerals salts medium

Fungal isolates were tested on minerals salts agar medium with Nonylphenol Ethoxylate (NPEOs) as the only carbon source (0.9 -7.2 ppm) for primary selection of isolates that were able to degrade Nonylphenol Ethoxylate . PH was adjusted to 7 with buffer solution. All inoculated plates were incubated at 30°C for 5-7 days [24] .Control was prepared simultaneously without carbon source. Three parallel tests for NPEOs and control plates were carried out.

2.5. Secondary sludge samples acclimation test

Before starting the biodegradation tests, acclimation of secondary sludge was requested. According to [24] flasks contained NPEOs of 0.9 -7.2 ppm in culture medium were also inoculated with 5 ml of 15 g/l suspended solids content aerobic activated sludge. Then, flasks were placed in a table shaker in a temperature-controlled cabinet at 25ºC and shaken continuously at 200 rpm for 27 days of acclimation time. Then 5 ml of the acclimated solution was added to another 500 ml of freshly sterilized basal salts medium with the concentration of NPEOs kept at 0.9-7.2 ppm for 27 days.

The acclimation was repeated. Then 5 ml of the twice acclimated solution were inoculated into the

NPEOs solutions with an initial concentration of 0.9-7.2 ppm in culture medium, and biodegradation tests were performed for a maximum of 27 days [25]

2.5. Biodegradation tests

Microbial isolates on minerals salts medium amended with NPEOs as the only carbon source (0.9-7.2 ppm) were grown on minerals salts broth medium with NPEOs (0.9-7.2 ppm) as the only carbon source. Inoculated broth medium incubated with shaking (200 rpm) at 27 °C for 48-72 hrs.

2.5. Confirmatory analysis of microbes that able to biodegrade NPEOs

As it was reported 100 ml of biodegradation solution was transferred to a 125 ml separatory funnel, and 35.5 g of NaCl was added immediately [26] . Then the funnel was manually shaken for 1 min to dissolve completely after that the solution was extracted twice with 50 mL of chloroform then collected in a 100 ml vessel through defatted cotton. A rotary evaporator was used for dried the combined chloroform phase. The extract was then dissolved in 10 ml of chloroform, and the absorbance at 277 nm was measured with a 1 cm quartz cuvette using a UV-1600 spectrophotometer to quantify the degradation percentage of the benzene ring of the NPEOs molecule [27] . Then chloroform was removed again by gentle nitrogen gas blowing. The sample was kept in a refrigerator at 4ºC for HPLC (high performance liquid chromatography) analysis

2.6. Fungal isolates that have been confirmed for biodegradation of NPEOS

Eight fungal isolates (3A, 3H, 3C, 4O, 4F, 4A, 6D and 6B) were obtained from the biodegradation experiments which achieved the highest percentage of NPEOS biodegradation using spectrophotometer by determining of NPEOS residues [28] . The fungal isolates were identified by morphological examination and its characteristics. Morphological characteristics were examined under microscope [29] (Table.1). Identification of Penicillium chrysogenum was based on current universal keys as Penicillium database management system (table.2) and 18S rDNA . Table 1. Phenotypic characterization of 24 pectinolytic microbial isolates

2.7. Determination of residual NPEOs by HPLC analysis

Residual concentration NPEOs was also determined using a liquid chromatography technique [30] , which was performed by using a HPLC equipped with an automated gradient controller, two Model 510 pumps, an multifunctional sampler injector, a waters differential refractometer R 401, a C18 column (DiamonsilTM, 5 µm, 250 x 4.6 mm, Dikma Corporation, China). The mobile phase was 95% methanol/5% water, and the flow was kept at 1.0 ml/min. The sample extract was dissolved in 0.5 ml of methanol, and filtrated using a 0.22 µm membrane filter. The injection volume was 100 µl [31] .

2.8. Quantitative determination

Quantification of the residual amount of the NPEOs by fungal isolates in minerals broth medium with NPEOs) as the only carbon source (0.9 -7.2 ppm) at pH 7 was tested. The culture was incubated for 21 day at 27°C on shaking incubator at 200 rpm/min for measuring microbial activity then filtration of these flasks and separation of the formed biomass. The amount of substance remaining in the filtrate remaining after the filtration was estimated by HPLC

2.9. Molecular identification of the most promising fungal isolate (4A)

The fungal isolate was grown in sterile Petri plates containing autoclaved potato dextrose agar (PDA) medium and incubated for 7 days at 28°C [32] . The culture was sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. Fungal DNA samples were then sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) and 18S rDNA gene sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture. Primers have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC - 3'). The purified PCR product (amplicons) was sequenced with the same primers with the incorporation of ddNTPs in the reaction mixture [33] . PCR amplification was done according to the following temperature profile: an initial step of 2 min at 94 °C, 40 cycles of 60 s at 94 °C, 90 s at 52 °C and 2 min at 72 °C, and a final step of 7 min at 72 °C. The PCR products were analyzed by agarose gel electrophoresis and purified using kit (AccuPrep PCR DNA Purification Kit, K3034–1, Bioneer Corporation). The purified PCR products were sequenced with Macrogen Inc. (Seoul, South Korea), all inter transcribed spacer sequencing work was also done by Macrogen and was applied on both strands of the submitted DNA fragments. The sequences were assembled, edited and aligned by the DNA STAR SeqMan (DNASTAR Inc.) and the CLC sequence viewer.The obtained sequences were analyzed using Basic Local

Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

2.11. Evaluation of cytotoxic effects of APEOs using viability assay

cytotoxicity test for microbes was performed by using different concentrations of the NPEOs [34] . The cytotoxic activity against mammalian cells from African green monkey kidney (Vero) cells and against MRC-5 cell line under experimental conditions (CC50) for both Penicillium chrysogenum .Vero cells (African Green Monkey and Normal Kidney Cells) and MRC-5 cells (Normal human Lung fibroblast cells), were obtained from VACSERA Tissue Culture Unit. Fetal Bovine serum, DMEML-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza, Belgium. Crystal violet stain (1%) composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with double distilled H2O and filtered through a Whatmann No.1 filter paper. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L- glutamine, and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO2 and were subcultured two times a week. For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×104 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37ºC in a humidified incubator with 5% CO2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, medium was aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates was measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and NPEOs concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound .The Cytotoxic concentration (CC50), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each concentration using Graph pad Prism software (San Diego, CA. USA).

2. Results and discussion

Physicochemical parameters of secondary activated sludge samples

As shown in (Table 3) the physicochemical parameters were measured and it was found that sample color was light green and it was liquid in texture, also temperature was 26.7°C, COD = 295 mg/l, BOD = 130 mg/l, TDS = 363mg/l, TVS = 270 mg/l, TS = 670 mg/l, VSS = 104 mg/l, TSS =170 mg/l, TEMP 29.4 and pH was 7.72. As it was in wastewater, temperature was basically important for its effect on other physical and chemicals factors, and the rate of some reactions could be increased by the discharge of this wastewater into streams [35] . So wastewater can reduce the solubility of oxygen and amplified odour due to anaerobic reaction. In parallel study of physicochemical properties of secondary activated sludge samples collected from different sewage sources revealed that the color of the collected sewage sludge was pale yellow to black and was turbid in some selected stations [36] . Unpleasant odor was noted in all selected stations. The pH of the wastewater varied from 6.3 to 7.3, while the water conductivity ranges from 650 to 2390 µScm/l. The maximum total dissolved solid was 1495 ppm, and the maximum biological oxygen demand was 569.5 mg/l. The chemical oxygen demand of the selected sites varied widely (507.1–602.9 mg/l), and the dissolved oxygen content varied from 0.01 to 0.242 mg/l.

Table 1. Phenotypic characterization of 24 pectinolytic microbial isolates.

MORPHOLOGY	Isolate

Septate hyaline hyphae, conidiophores, phialides, and conidia are observed.	1 (3A)
Conidiophores are hyaline, branched, and may occasionally display a pyramidal arrangement	2 (6B)
The fungus produce Macro- and microconidia.	3
Hyphae are septate and hyaline	4
The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex.	5
Long rod, gram positive motile spore forming bacteria	6
Short rods, gram negative motile non-spore forming bacteria	7
The fungus produce Macro- and microconidia.	8
Hyphae are septate and hyaline	9 (4A)
Hyphae are septate and hyaline	10
The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex.	11 (6D)
Long rod, gram positive motile spore forming bacteria	12
Short rods, gram negative motile non-spore forming bacteria	13
Conidiophores are hyaline, branched, and may occasionally display a pyramidal arrangement	14
Long rod, gram positive motile spore forming bacteria	15
Septate hyaline hyphae, conidiophores, phialides, and conidia are observed.	16 (3H)
Septate hyaline hyphae, conidiophores, phialides, and conidia are observed.	17 (4O)
Short rods, gram negative motile non-spore forming bacteria	18
Long rod, gram positive motile spore forming bacteria	19

Table 2. *Penicillium* database management system of *Penicillium chrysogenum* (4A).

Characteristics	**Examinassions**
Growth characteristics	Colonies on minerals agar at 25 □C attaining a diameter of 3.0-3.5 cm within 7 days gives buff to yellow brown reverse.
Conidial heads	Columnar , 60.5 µm
Vesicle diam.	Sub-globose 15.4 µm in diameter.
Primary sterigmata	6.4 x 2.3 µm
Secondary Stigmata	6.0 x1.2 µm.
Conidiophore diam	5.2 µm in diameter.
Conidia	Globose, smooth, 2.1 µm in diameter.

Table 3. Physicochemical parameters of secondary activated sludge samples collected from secondary activated sludge from Zenin wastewater treatment plant.

Parameter	**Result**
Color	Light green
Viscosity	Liquid
Temp.	26.7 ℃
COD (mg/l)	295
BOD (mg/l	130
TDS (mg/l)	363
TVS (mg/l)	270
TS (mg/l)	670
VSS (mg/l)	104
TSS (mg/l)	170
TEMP (°C)	29.4
Ph	7.72

Effect of concentration of NPEOs on degradation rate

Microbial degradation is an important process in many environments controlling for instance the cycling of nutrients or the biodegradation of contaminants [37] . At high substrate concentrations toxic effects may inhibit the degradation process. Bioavailability limitations of a degradable substrate can therefore either improve the overall dynamics of degradation by softening the contaminant toxicity effects to microorganisms, or slow down the biodegradation by reducing the microbial access to the substrate. As shown in figure 1a and figure 1b degradation (ppm) was varied by using different substrate concentration of NPES and the highest degradation(ppm) was observed at substrate concentration equal 1.8 ppm and the lowest degradation (ppm) was observed at substrate concentration equal 0.9 ppm.

1.2. Microbes that have been confirmed for biodegradation of NPEOS

As shown in (Figure 2 &3) eight fungal isolates were obtained, which achieved the highest percentage of this biodegradation, by a spectrophotometer measurement. By increasing time from day 3 to day 21 the biodegradation rate increased. Bodegradation of Nonylphenol Ethoxylates (NPEOs) by Bacillus sp . Results showed that NPEOs were readily degraded by Bacillus sp. Around 80% of the total NPEOs being removed after 7 days [38] . A lot of microorganisms have the ability to assimilate NPEOs contaminants as the sole carbon and energy source have been also reported [40] [41] [42]. Studies revealed that the primary degradation of a technical nonylphenol ethoxylate surfactant with an average chain length of 10 ethoxylate units (NPEO-10) was studied in a flow-through system by means of miniaturized biofilm reactors (mBFR) with bacteria from an activated sludge plant. 5 mg/L of the test compound (total EO concentration) were spiked in synthetic wastewater (SWW) and fed to the reactors continuously for 64 days.

4.3. Morphological identification of the most promising fungal isolate capable of biodegrading NPEOs

the identification of *Penicillium* based on morphological methods requires adequate growth for evaluation of colony characteristics and microscopic features [43] . A culture time of 5 days or more is generally required for identification of anamorphic forms of *Penicillium*. There are more than 180 species in the *Penicillium* genus. As shown in table 1 and figure 4 the isolates codes: 3A, 3H, 3C, 4O, 4F, 4A,6D and 6B identification was based on current universal keys *Penicillium* Database Management System and using an image analysis system, accordingly the specimen is: *Penicillium chrysogenum* as shown in. The fungus was identified as *Penicillium chrysogenum* (Fig. 4) based on morphological, microscopic features

Figure (4). Microscopic examination of *Penicillium chrysogenum*.

Identification of the most active isolates based on molecular phylogenetic analysis

Based on 18S rDNA sequence (ITS phylogenetic analysis). The sequence data have been deposited at GenBank as following sequence and phylogenetic tree (Figure 5).

TGCGGAAGGATCATTACCGAGTGAGGG
CCCTCTGGGTCCAACCTCCCACCCG
TGTTTATTTTACCTTGTTGCTTCGGCGGGC
CCGCCTTAACTGGCCGCCGGGGG
GCTTACGCCCCCGGGCCCGCGCCCGCCGA
AGACACCCTCGAACTCTGTCCGA
AGATTGTAGTCTGAGTGAAAATATAAATT
ATTTAAAACTTTCAACAACGGAT
CTCTTGGTTCCGGCATCGATGAAGAACGC
AGCGAAATGCGATACGTAATGTG
AATTGCAAATTCAGTGAATCATCGAGTCT
TTGAACGCACATTGCGCCCCCTGG
TATTCCGGGGGGCATGCCTGTCCGAGCGT
CATTGCTGCCCTCAAGCACGGCTT
GTGTGTTGGGCCCCGTCCTCCGATCCCGG
GGGACGGGCCCGAAAGGCAGCGG
CGGCACCGCGTCCGGTCCTCGAGCGTATG
GGGCTTTGTCACCCGCTCTGTAGG
CCCGGCCGGCGCTTGCCGATCAACCCAAA
TTTTTATCCAGGTTGACCTCGGAT
CAGGTAGGGATACCCGCTGAACTTAAGCA
TATCAATAAGCGGAGGAA

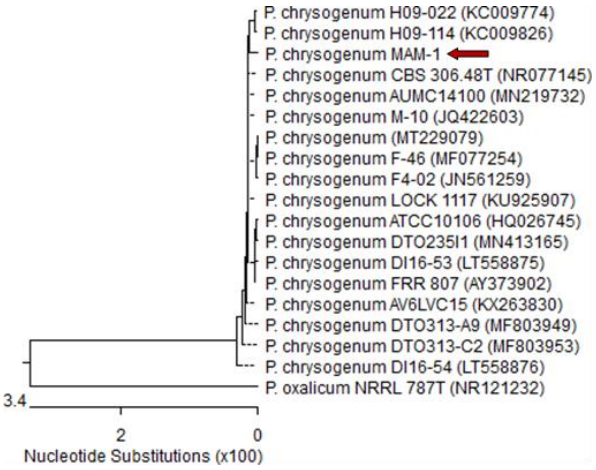

Figure (5). Phylogenetic tree based on ITS sequences of 18S rDNA of the fungal strain isolated in the present study (MAM-1, arrowed) aligned with closely related sequences accessed from the GenBank, (P. = Penicillium). Sample MAM-1 showed 99.82% and 99.83% identity with several strains of P. chrysogenum including the type strain CBS 306.48 (NR077145). P. oxalicum is included in the tree as an out group strain.

Figure (1.a). Effect of concentration of NPEOs (0.9-7.2 ppm) on degradation.

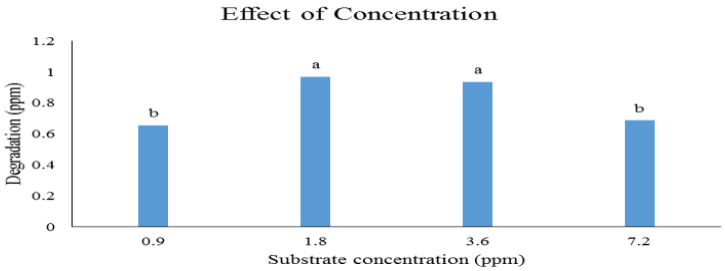

Table 4*. Biodegradation rate (%) of NPEOs by the most promising isolates from day 3 to day 21.*

Isolate code	**Days**				
	3	**5**	**7**	**15**	**21**
3A	25%	40%	55%	75%	75%
3H	20%	40%	65%	80%	80%
3C	25%	40%	55%	75%	**75%**
4O	20%	40%	65%	80%	**80%**
4F	30%	40%	60%	80%	**80%**
4A	20%	35%	50%	75%	**80%**
6D	25%	40%	50%	70%	70%
6B	25%	40%	55%	65%	**70%**

Figure)2(. Biodegradation rate of NPEOs by isolates 3C, 4O, 4A and 6B.

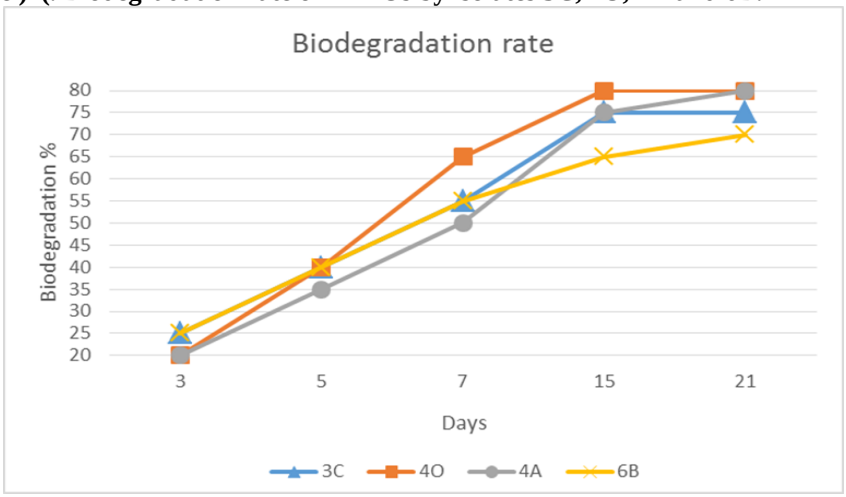

Figure (3). Biodegradation rate of NPEOs by isolates 3A, 3H, 4F and 6D.

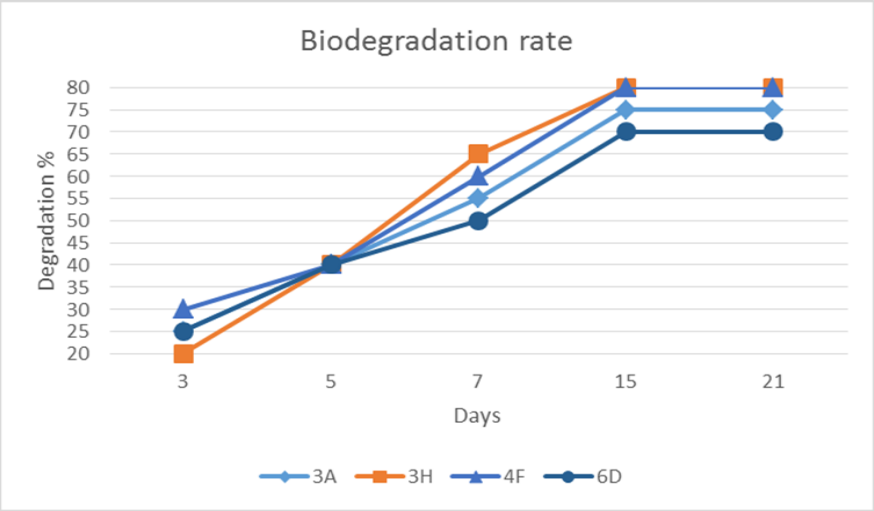

Figure (5). Phylogenetic tree based on ITS sequences of 18S rDNA of the fungal strain isolated in the present study (MAM-1, arrowed) aligned with closely related sequences accessed from the GenBank, (P. = *Penicillium*). Sample MAM-1 showed 99.82% and 99.83% identity with several strains of *P. chrysogenum* including the type strain CBS 306.48 (NR077145). P. oxalicum is included in the tree as an out group strain.

4.6. Evaluation of cytotoxic effects of NPEOs

4.6.1. *Evaluation of cytotoxicity against African green monkey kidney (VERO) cell line.*

That mammalian cell lines such as VERO cells (African Green Monkey Normal Kidney cells) and MRC-5 cells (Normal human Lung fibroblast cells), were obtained from VACSERA Tissue Culture Unit [44]. As shown in Table 4 and figure 6 in case of *Penicillium chrysogenum* the cytotoxic activity against mammalian cells from African green monkey kidney (Vero) cells was detected under these experimental conditions with 50 % cell cytotoxic concentration (CC50)= 14.2 ± 1.1 µg/ml. cytotoxicity is one of the most important indicators for biological evaluation in vitro studies chemicals such as drugs and pesticides have different cytotoxicity mechanisms such as destruction of cell membranes, prevention of protein synthesis, irreversible binding to receptors etc [45] .

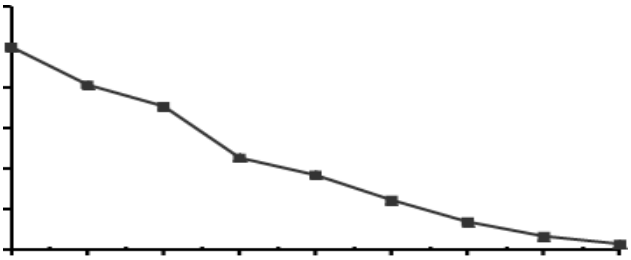

Figure (6). VERO cell line of Penicillium chrysogenum

As shown in Table 5 and figure 6 in case of Penicillium chrysogenum the cytotoxic activity against mammalian cells from African green monkey kidney (Vero) cells was detected under these experimental conditions with 50 % cell cytotoxic concentration(CC50)= 46.5 ± 4.8 µg/ml

4.7. Evaluation of cytotoxicity against MRC-5 cell line

As shown in Table (6) and figure (7) in case of *Penicillium chrysogenum* the Inhibitory cytotoxic activity against Normal human lung fibroblast cells was detected under these experimental conditions with CC50 = 28.6 ± 2.7 µg/ml. In this accordance demonstrated that nonylphenol isomers had differential effects on the release of hormones in Leydig cells [46] . Also treated rat Leydig cells with different concentrations (42.5; 127.5 and 425.0 µM) of 4-nonylphenol [47] . This highest dose exhibited a cytotoxic effect. examined the effect of nonylphenol (0.1; 1.0; 10.0; 20.0 and 30.0 µM) during time periods 6h, 12h and 24h on viability of rat testicular Sertoli cells [48] [49] . As shown in Table (7) and figure (8) in case of *Penicillium chrysogenum* the Inhibitory cytotoxic activity against normal human lung fibroblast cells was detected under these experimental conditions with CC50 = 74.1 ± 6.1 µg/ml.

Table (5). Different concentration of NPEOs tested in VERO cell line of *Penicillium chrysogenum*

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (□)
500	4.31	95.69	1.27
250	11.78	88.22	0.84
125	20.43	79.57	1.99
62.5	37.52	62.48	2.74
31.25	61.84	38.16	3.92
15.6	89.41	10.59	0.98
7.8	97.28	2.72	0.74
3.9	100	0	-
0	100	0	-

Table (6).different concentration of NPEOs used in MRC-5 cell line of *Penicillium chrysogenum*

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (□)
500	**4.59**	**95.41**	**0.35**
250	**9.87**	**90.13**	**0.67**
125	**20.68**	**79.32**	**0.94**
62.5	**32.73**	**67.27**	**1.85**
31.25	**46.02**	**53.98**	**3.49**
15.6	**69.56**	**30.44**	**2.82**
7.8	**83.21**	**16.79**	**1.37**
3.9	**90.67**	**9.33**	**0.95**
0	**100**	**0**	**0**

Table (7). Different concentration of NPEOs used in MRC-5 cell line of *Penicillium chrysogenum*

Sample conc. (µg/ml)	Viability %	Inhibitory %	S.D. (□)
500	7.62	92.38	0.84
250	19.25	80.75	1.31
125	32.84	67.16	2.37
62.5	53.90	46.1	2.89
31.25	79.06	20.94	1.42
15.6	93.67	6.33	0.95
7.8	99.85	0.15	0.17
3.9	100	0	0
0	100	0	0

Figure (7). Results of MRC-5 cell line of *Penicillium chrysogenum*

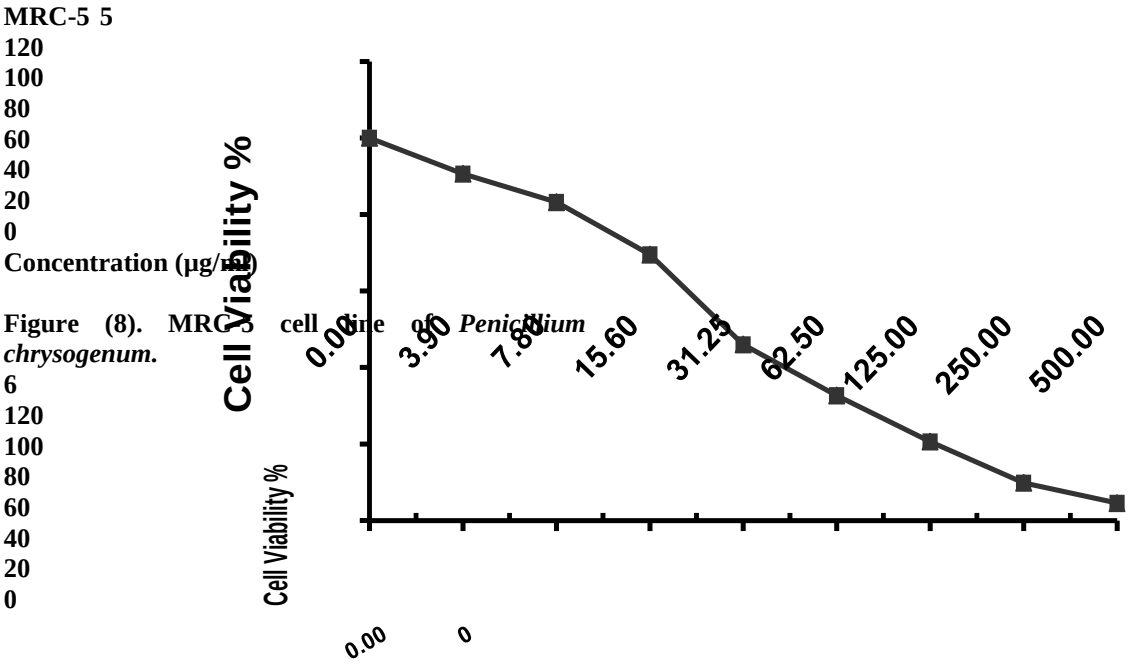

Figure (8). MRC-5 cell line of *Penicillium chrysogenum.*

4. Conclusions

Municipality sludge is very rich source of microorganism so our study uses it for the isolation of resistant microorganisms able to biodegrade recalcitrant nonionic detergent (NPEOs). In our work one novel species morphologically and molecularly identified as *Penicillium chrysogenum* is employed to the biodegradation process of NPEOs with 80 % high degradation rate within 21 days at concentration 1.8 ppm [50] .

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