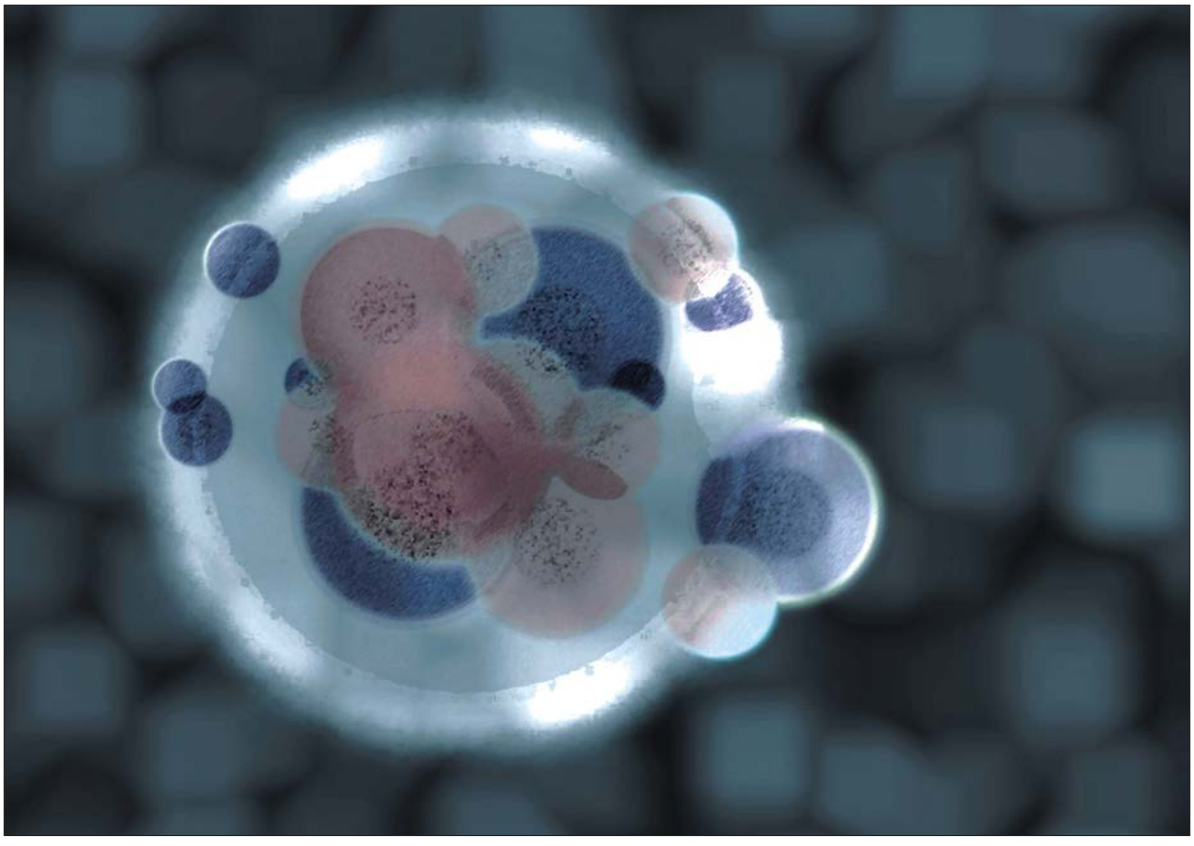

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Research Article
Biological Treatment of Carcinogenic Acrylonitrile Using *Pseudomonas aeruginosa* in Basra City

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Abstract

Background and Objective: Biological treatment has become a temptic alternative for expensive ways. However, the low costs and technology of bio-remediation means that it is possible to use for addressing ecological problems in many polluted locations around the world. The objective of this study was to determined the biodegradation ability of *Pseudomonas aeruginosa* to utilize carcinogenic acrylonitrile, via an environmentally-sound method. **Materials and Methods:** Comprised isolation of bacteria using suspended soil dilution method, where eight soil samples were collected from polluted sites in the Southern part of Basra city. In return, the best isolation was chosen in its growth on a media containing acrylonitrile as a sole-source of carbon and nitrogen, isolate was identified using biochemical tests that shows it belong to the species of *Pseudomonas aeruginosa*. However, substantial cultural processes were conducted at this isolate to reveal the preferable period of adaptation. Additionally, series of acrylonitrile (C_3H_3N) concentrations were prepared within the purity of 96%, a pure culture of bacteria was obtained to determine its efficiency for acrylonitrile degradation and the bacterial-culture was concentrated using enrichment media. Aforementioned concentrations were inoculated with 1.5×10^8 cell mL^{-1} of bacterial culture inferenced by McFarland standard (600 nm, OD = 0.5) and were incubated for 7 days at 37°C, then the concentration of acrylonitrile was measured before and after treatment using HPLC and FTIR spectrum, to set the efficiency of biodegradation confirmed by the production of ammonia, through decomposition processes. **Results:** The under study bacteria have the ability to degrade the carcinogenic material, the best adaptation period was for 7 days as well as the optimum concentration for treatment is 500 ppm, with a degradation efficiency of 92.7%. **Conclusion:** The extracted results revealed that the isolated bacteria demonstrated eminent quantities of acrylonitrile degradation. Thus, it could be used to bio-remediate contaminated soils in such environments.

Key words: Acrylonitrile, biological treatment, *Pseudomonas aeruginosa*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hazardous chemical compounds are the most dangerous environmental pollutants if managed incorrectly-that find their way to the ecosystems whether they were air, soil, water, or even in sediments that destroy the human life as well as other living organisms[1]. Acrylonitrile is a raw organic substance, which is widely used in the manufacturing of acrylic fibers, nylon, rubber, manufactured chemical solutions and dyes[2]. This substance is considered as a contaminant that has the priority to treatment processes of many countries due to its high toxicity and as a carcinogenic substance, in which it has been treated with many techniques such as activated sludge, oxidation processes and activated carbon processes[3]. The biological treatment for such substance considered as a hot spot of many current global researches, which is specialized in the biological treatment of organic pollutants because they are hazardous substances to humans and the environment[2].

The organic Acrylonitrile substance is organically decomposable by microorganisms which have the ability to metabolize it as a source of carbon and nitrogen. More than 200,000 t of this substance is processed annually in the world by bio-degradation[4].

Acrylonitrile is chemically or biologically decomposed by acid or alkaline[5], as it shown in Fig. 1a and b. When microorganisms are exposed to such compounds, they will develop special mechanisms to normalize on these conditions. The development of these techniques is necessary and essential in the process of bio-remediation, some enzymes are responsible for the breaking and treating the chemicals are used, such as nitrile hydratase, amidase and nitrilase. In some cases, enzyme induction time is needed and this time reflects the length of the lag period or the time of the microorganism adaptation[6].

Pseudomonas spp. emerged at the top of the environmental microbial population, used for the environmental pollution treatment of complex chemical compounds, especially *Pseudomonas aeruginosa*, due to its ability to use more than 90 complex organic materials as sources of carbon and energy. In addition to the lack of juxtaposition of degradation and/or demolition genes and instead of that it form a supra-operonic clustering of functionally related genes. It also contains special plasmids responsible for degradation processes such as; plasmid PWWO, Tol-plasmid and Oct-plasmid[7]. In this study of bioremediation, a pure culture of *Pseudomonas aeruginosa* was used, which is one of the most common Gram-negative bacteria in the soil that do not need special nutritional requirements for growth, because are characterized by their high susceptibility to normalize and consume a range of carbon and nitrogen compounds such as aromatic rings. Therefore, this bacterium playing an important role in destroying natural or industrial compounds that resist degradation by other microorganisms[8].

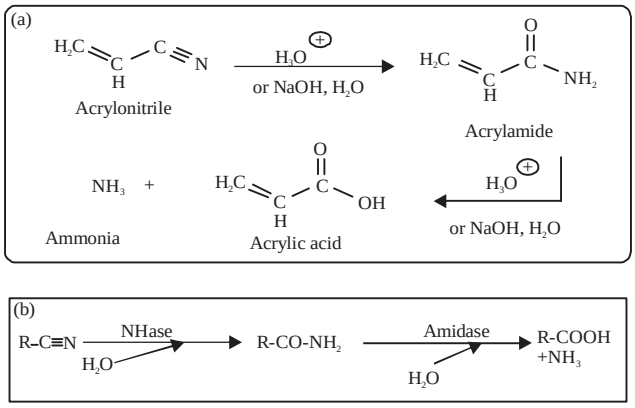


Fig. 1(a-b): (a) Reaction diagram of acrylonitrile chemical decomposition and (b) Reaction diagram of acrylonitrile biological decomposition

Due to the high cost of physio chemical remediation and the possibility of producing by-products substances that may pollute the environment, this research attempt was carried out to investigate and detect the possibility of biological treatment of this substance and to determine optimal conditions and to serve futuristic experiments in order to obtain the highest treatment ratio of acrylonitrile using *Pseudomonas aeruginosa* for their susceptibility of treatment.

MATERIALS AND METHODS

Samples collection: Soil and acrylonitrile substance samples were collected from 8 locations in the southern part of Basra city during the period from December, 2017 to January, 2018 in triplicates for each location till a depth of 15 cm, for the necessary bacteriological and chemical tests. The collected samples were kept in 500 mL sterilized glass bottles and then moved to the laboratory for immediate use.

Chemicals: All substrates were produced from Merck (USA), the culture media ingredients were from Oxoid (UK). All other chemicals were of analytical grade and purchased from different suppliers.

Preparation of solutions: A standard solution with a concentration of 10000 ppm of standard acrylonitrile of 96% purity has been prepared by applying the following equation:

1% = 10000 ppm

96% = 960000 ppm

From the standard solution, a series of dilutions was prepared:

- 50, 100, 150, 200, 500 and 1000 ppm to use them in the next experiments. The potential of hydrogen was measured by using pH-meter and adjusted to 7 using (1 N) of NaOH and HCl[9]

Screening of acrylonitrile biodegrading bacteria: Acrylonitrile biodegrading bacteria were screened through the application of enrichment culture of brain heart infusion (BHI) and indicator method. The microbial community was obtained from acrylonitrile-contaminated soil from their location of storage in Basra City, by using biochemical tests approved by the APHA[10] and by the use of the Bergey's Manual[11] for isolating and diagnosing microbiology and assuring the result of biological testing by using VITEK 2 compact system (Biomerieux, France). Bacterial culture was grown in the mineral salts medium (MSM), which was lacking for carbon and nitrogen source, containing the following components; $MgSO_4.7H_2O$, 0.4 g, K_2SO_4, 0.4 g, $FeCl_3$, 0.01g, NaCl 0.1 g, K_2HPO_4, 0.1 g and KH_2PO_4 1 g). pH of the medium was adjusted to 7.0, then autoclaved at 121°C for 15 min. Next, the bacteria were propagated by adding filtered acrylonitrile to the (MSM) as the sole source of nitrogen, carbon for energy. The bacteria were incubated in a 37°C shaker incubator (Lab Tech, Korea) at 135 rpm for 24 h and the optimal growth period of the microscopic organisms were observed using a Visible Spectrophotometer (PG instrument T80, UK) at a wavelength of 600 nm. The optimum duration of primary growth was 10 days. Bacterial cells were diagnosed using light microscopy.

Bacterial growth with acrylonitrile: In the aforementioned bacterial culture, the bacteria were activated using brain heart infusion (BHI) media and incubated at 37°C for 24 h. After this time, the enrichment media was removed, thereafter the well adapted activated bacteria were collected using cooling centrifuge (Sigma 2-16 K, Germany) at 4000 cycles min^{-1}. Then another mineral salt media have been prepared consisting of acrylonitrile and was inoculated with 10% of the adapted and activated bacterial solution in the BHI media with 1.5×10^8 cell mL^{-1} according to McFarland tube. The incubation was at 37°C and the optimum growth period of the normalized was 7 days via monitoring the viable bacterial growth curve.

HPLC reversed phase: The acrylonitrile was detected by high performance liquid chromatography-HPLC (Shimadzu LC20, Japan) using a reversed phase HPLC column C18 (250×4.6×5 mm) with UV detector, a mobile phase consisting of acetonitrile: Water (70:30 v/v) with a flow rate of 1.0 mL min^{-1}, runtime of 10 min and detection was at $\lambda = 254$ nm.

FTIR test: Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the acrylonitrile spectra in the polymer solution. The spectra were recorded using FTIR system (Shimadzu 8400 S, Japan) in the range from 500-4000 cm^{-1} with the resolution of 4 cm^{-1}.

Batch experiment: A series of acrylonitrile concentrations mentioned previously in the preparation of the solutions have been prepared and placed in 100 mL volumetric flasks and inoculated with 10% of the bacterial suspension (after isolating it from the culture media by centrifuge device). The volumetric flasks containing bacteria and the salt media that containing acrylonitrile with different concentrations have been incubated for 7 days at 37°C with pH 7.0. The bacteria were then isolated from the supernatant in the culture media by using centrifuge and then filtered with a filtration paper of 45 μm. Afterwards, the supernatant liquid was taken to estimate the concentration of acrylonitrile substance and to determine the change in the composition of the substance before and after treatment by using HPLC and FTIRdevices[4,12].

Ammonia concentration assay: Method was used in order to determine the concentration of produced ammonia during the process of acrylonitrile biodegradation. About 0.99 mL of (0.1 N) NaOH and 20 mL of Nessler reagent were mixed with 10 mL of the growth solution, then incubated for 20 min at 30°C and measured then by spectrophotometer at $\lambda = 420$ nm[13].

SEM test: The superficies morphology of the degraded acrylonitrile were studied using Scanning Electron Microscope (SEM-Philips XL, Netherland) with magnification of 1000x before and after treatment, in the Ministry of Science and Technology.

Statistical analysis: The data was analyzed using t-test and Pearson correlation test (2-tailed), by Statistical Package for the Social Science (SPSS) program (Version 21.0), in order to find out if there are significant differences ($\alpha = 0.05$) among the means of concentrations and according to Sandar and Richard[14].

RESULTS

Isolation and Identification of bacteria: Results of microbial growth showed the presence of *Pseudomonas aeruginosa* in all eight soil samples contaminated with acrylonitrile.

Colonies were diagnosed by their morphological properties. The isolates appeared as short rod, Gram-negative cells, non-spore forming and forming large pallid colonies when they growing on MacConkey agar, because they do not ferment lactose sugar and excrete a bluish green dye when growing on nutrient agar and on King Media and have the potential to grow in different temperatures[11]. However, biochemical tests showed negative results for the analysis of indole, voges proskauer, methyl red, starch and citrates. Whilst, positive results for tests of urea, gelatin liquefaction, oxidation, catalase, motility and none spore former, in which the results were confirmed by VITEK 2 tests (Fig. 2).

Growth assay of bacteria: The reading results of the Visible Spectrophotometer showed that the growth period of the bacteria continued for 10 days after incubated at 37°C in salt media solution (MSM) indicated by the total bacterial count which prepared for adapting the bacteria in the primary culturing process. After isolating the adapted bacteria in the salt media, these bacteria were activated and enriched by using the BHI media[9] for further tests. The test results were shown that the best period for growing are 7 days in which it reached the optimum bacterial count. This means that the period was decreased because of the bacterial normalizing or adaptation on growing in media containing acrylonitrile and thus its resistance to the different conditions of growth.

Analytical tests: Acrylonitrile was diagnosed before and after bio remediation by FT-IR spectrum, which gave an initial indicator of the transformation of nitrile group (-CN) into carboxyl group (Fig. 3), according to the total disappearance of absorption band of nitrile group -CN at 2227.78 cm^{-1}, with the formation of new absorption bands at three levels; the first at 1799.59 cm^{-1} which returns to the bond pattern of -C=O, the second at 3383.14 cm^{-1} belong to the absorption band of -C-OH and the third at the range of 1321.24 cm^{-1} returns to the absorption band of -C-O, which are all within the -COOH group in the product. Additionally, the FT-IR infrared spectra showed absorption bands belong to the patterns of (C-H) bond within aliphatic -CH_2- group for both symmetrical and asymmetrical types in the range of (2850.79-2920.23 cm^{-1})[15].

The optimal conditions for the HPLC examination were determined like temperature and column type. The samples were divided into two parts: a pre-treatment sample and a post-treatment sample to determine the concentration of acrylonitrile to demonstrate the efficiency of bacteria used in the bio-treatment of organic substance and according to HPLC results as it shown in Table 1, which reveal that the highest percentage of biological treatment of (C_3H_3N) was 92.7%,

bioMerieux Customer:
System #:

Laboratory Report

Printed Mar 25, 2017 12:50 CDT
Printed by: labadmin

Patient Name:
Isolate Group: 5-5

Patient ID:

Card Type: GN Testing Instrument: 000012C574F6 (6567)

Bionumber: 0003453203500000
Organism Quantity:

Comments:	

Identification Information	**Card:** GN		**Lot Number:** 2410015103	**Expires:**	Dec 11, 2017 12:00 CST
	Completed: Oct 26, 2017 16:44 CDT		**Status:** Final	**Analysis Time:**	5.00 hours
Selected Organism	98% Probability	**Pseudomonas aeruginosa**			
	Bionumber: 0003453203500000			**Confidence:**	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s) Pseudomonas aeruginosa					

مختبر الطائف التخصصي للتحليلات المرضية والمناعية والهرمونات
Al-taif Specialist Laboratories

Fig. 2: VITEK 2 system testing results

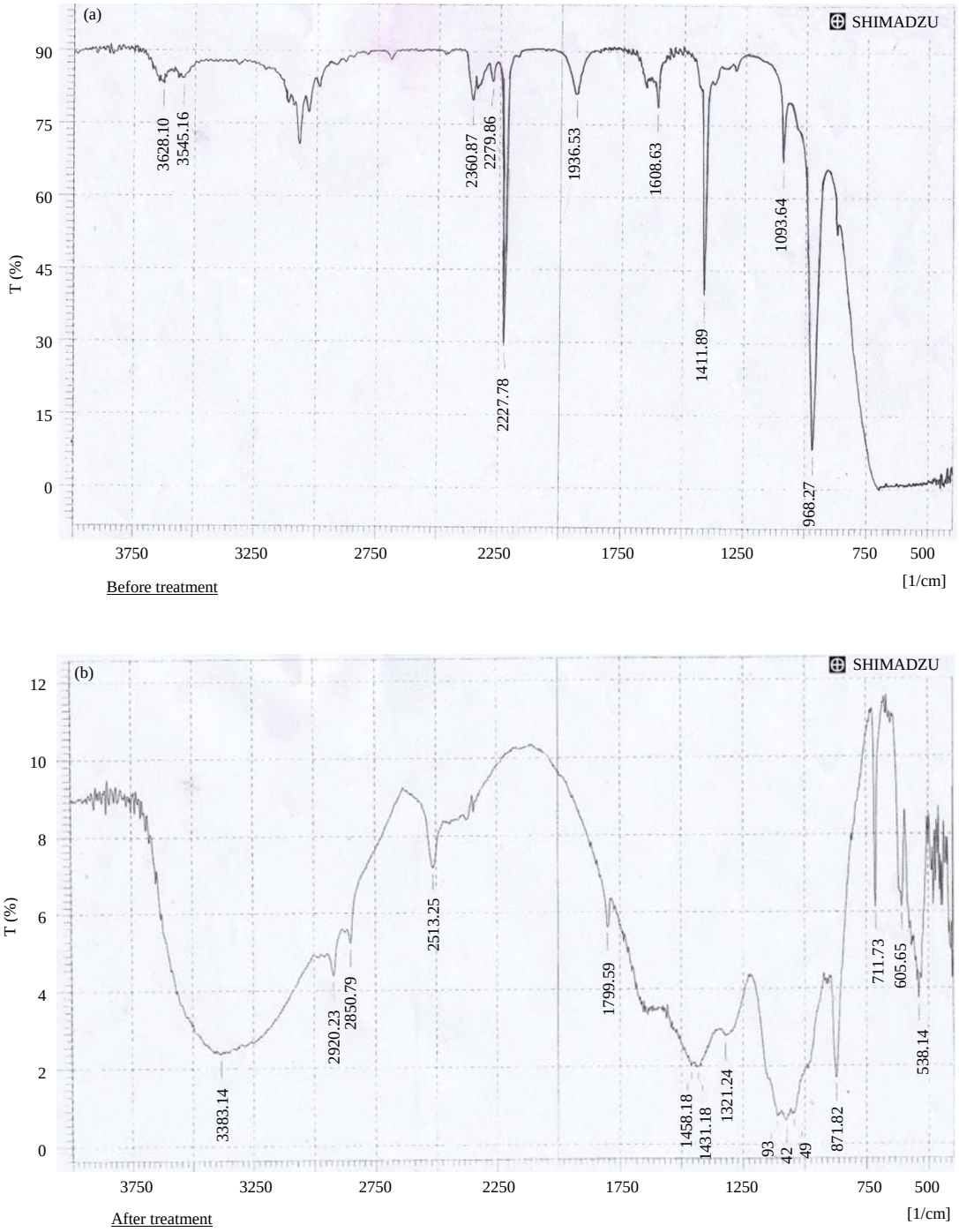
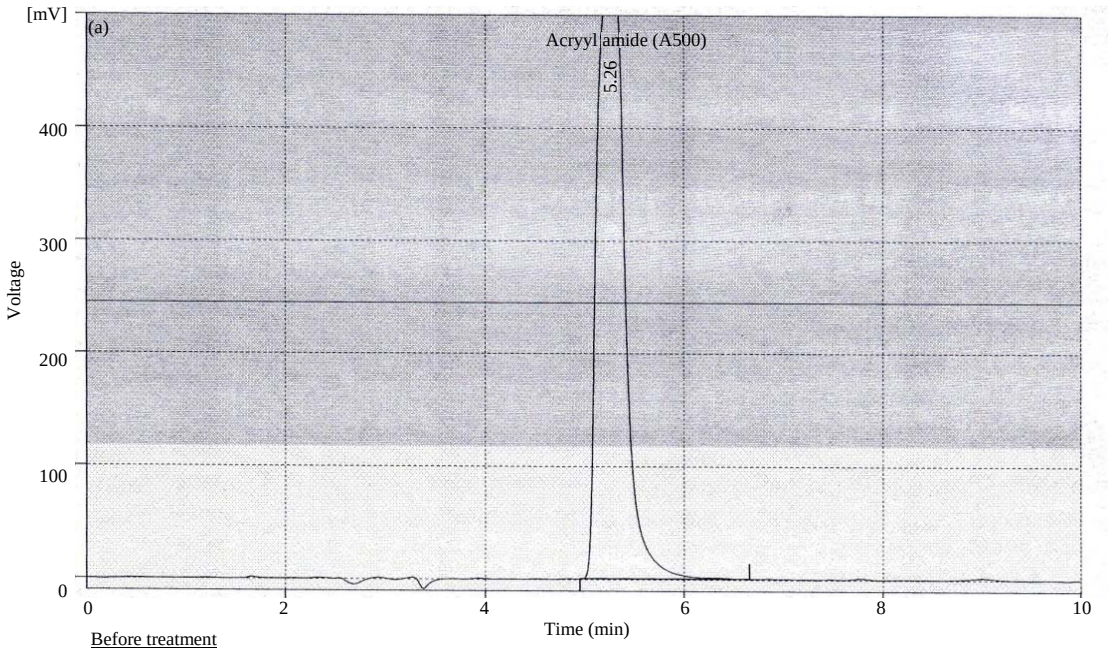


Fig. 3(a-b): FTIR spectra of acrylonitrile (a) Before and (b) After treatment of 7 days

Table 1: Concentration of acrylonitrile before and after treatment measured by HPLC

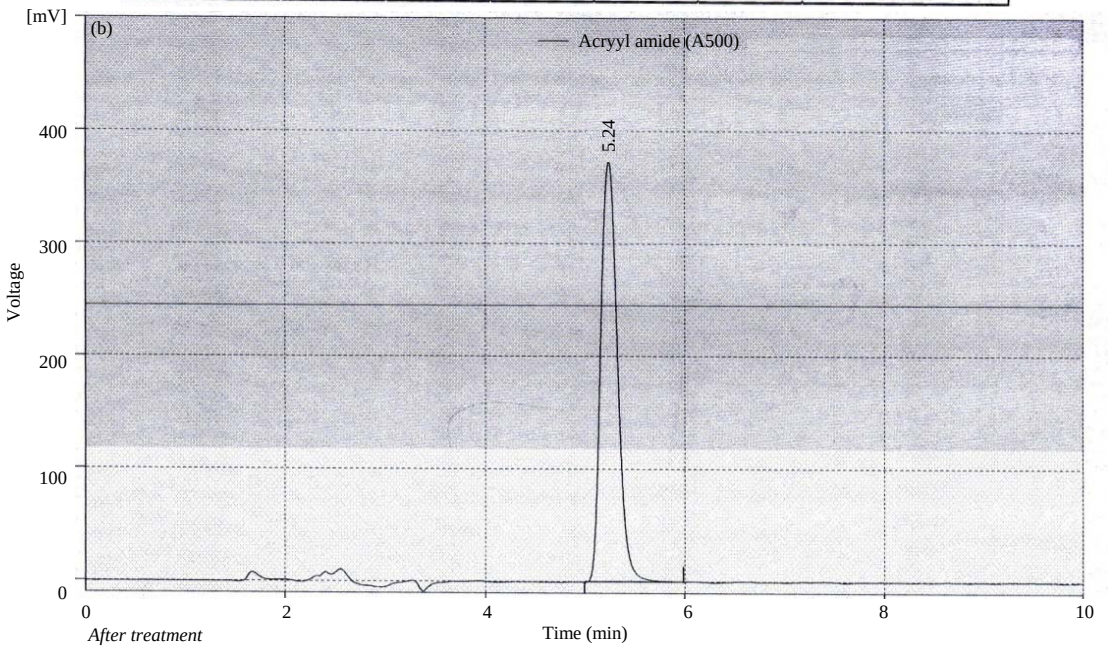
Dilution	Concentration of substance before treatment (ppm)	Mean concentration of substance after treatment (ppm±SD)	Treatment (%)
1	50	5.80±0.36	88.4
2	100	11.10±0.20	88.9
3	150	15.24±0.50	89.8
4	200	18.70±0.20	90.6
5	500	36.10±0.32	92.7
6	1000	194.70±1.70	80.5
	Mean = 333.33	Mean = 46.94	



Before treatment

Result Table (Uncal-ACRYYL AMIDE (A500)

	Reten. time (min)	Area [mAU.s]	Height [mAU]	Area (%)	Height (%)	W05 [min]	Compound name
1	5.263	25960.495	1328.823	100.0	100.0	0.31	
	Total	25960.495	1328.823	100.0	100.0		



After treatment

Result Table (Uncal-ACRYYL AMIDE (A500)

	Reten. time (min)	Area [mAU.s]	Height [mAU]	Area (%)	Height (%)	W05 [min]	Compound name
1	5.240	1875.721	934.244	100.0	100.0	0.18	
	Total	1875.721	934.244	100.0	100.0		

Fig. 4: HPLC testing results of acrylonitrile before and after treatment of 7 days

within 500 ppm. However, concentrations after 500 ppm were dropped due to the decreasing of the treatment (%) values. The concentrations of the organic substance (acrylonitrile) were calculated based on the peaks area obtained from the HPLC graph (Fig. 4) by applying the following Equation:

Table 2: Statistical analysis of correlation coefficient test

Before treatment	Pearson correlation	1	0.952
	Sig. (2-tailed)		0.003
	Sum of squares and cross-products	658333.333	126296
	Covariance	131666.667	25259.2
	N	6	6
After treatment	Pearson correlation	0.952**	1
	Sig. (2-tailed)	0.003	
	Sum of squares and cross-products	126296	26729.916
	Covariance	25259.2	5345.983
	N	6	6

**Correlation is significant at the 0.01 level (2-tailed)

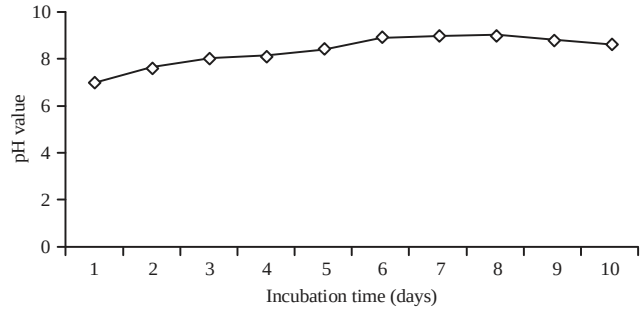


Fig. 5: pH values of bacterial growth within 10 days

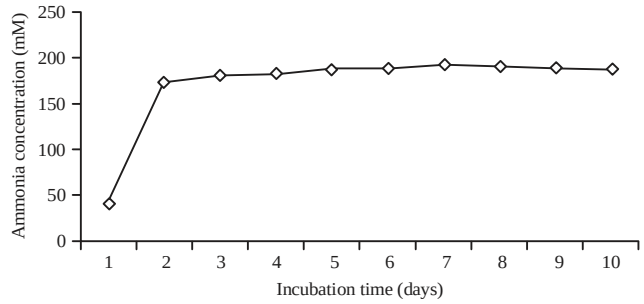


Fig. 6: Ammonia concentrations (mM) of bacterial growth within 10 days

$$C_{sample} = \frac{C_{st} \times A_{sample}}{A_{st}}$$

Where:

C_{sample} = Sample concentration
C_{st} = Primary sample concentration
A_{sample} = Peak area after treatment
A_{st} = Peak area before treatment

Bio-removal assay of acrylonitrile: The experiment was under aerobic conditions, a dilution series of acrylonitrile were prepared as aforementioned, the best bio-removal percentage of acrylonitrile was 92.7% at 500 ppm within the 7th day of incubation (Table 1). During the bacterial growth, pH values were ranged from 7-9 (Fig. 5). The concentration of ammonia increased and reached to the maximum value of 192.1 mM at the 7th day due to the biodegradation of acrylonitrile (Fig. 6). The essential concentration and the incubation period were fixed to do the experiment. The composite description was examined before and after treatment with the FTIR device[5,16], as shown in Fig. 3. However, the statistical analysis results refer to significant differences ($p<0.05$) before and after treatment samples using t-test, in which were confirmed by the results of correlation coefficient test, through the highly significant correlation levels ($p<0.01$), as it reached to 95% (Table 2). This indicated that the increase in the concentrations of acrylonitrile will increase the effectiveness of the microbial activity of degradation.

DISCUSSION

The obtained data indicated that all experimental concentrations of acrylonitrile after treatment were decreased from their basic values at stable conditions. The highest percentage of acrylonitrile biological treatment was 92.7%, obtained from the 500 ppm concentration, while the lowest percentage of treatment was 80.5% obtained from the concentration of 1000 ppm, where the 500 ppm concentration represents the critical point of the biological treatment process, because the ability of bacteria has decreased to treat existing acrylonitrile as indicated in Table 1. This means that the ability of bacteria had decreased to degrade the acrylonitrile when the concentration of this substance increase more than 500 ppm through breaking the triple bond between carbon and nitrogen because the bacteria have the ability to produce nitrile hydratase enzyme (NHase), which breaks the bond and releases nitrogen and carbon as a source for the organism growth[16,17]. In other words, the biological treatment potential for the bacteria used in this study is due to having the special enzymes that breakdown the triple bond between the nitrogen and the carbon in the chemical composition of the substance[18]. This converts acrylonitrile into

simpler compounds that can be used and adopted as sources of carbon and nitrogen[19]. These results were obtained after 7 days incubation period and then the concentrations have been measured after the bio-remediation process and compared with the primary concentration using HPLC device depending on the peak area obtained from the examination (Fig. 4). The low bioremediation of the substance in the low concentrations is due to insufficient nutrient availability for growth and reproduction, therefore organisms die before the process is totally completed[8,12].

Nitrile hydratase are mononuclear iron or non-corrinoid cobalt enzymes that catalyse the hydration of divers nitriles to their corresponding amides[20]. It can say that the enzyme reactions of nitrilase, nitrile hydratase and amidase are complex and intertwined with each other. Consequently, it can sometimes be difficult to distinguish nitrilase activity from nitrile hydratase plus amidase activity under aerobic conditions[16]. Thus, nitrilase should not be confused with nitrile hydratase. In general, screening of microbial producing NHase from environment is difficult because of gene regulation of NHase is heavily influenced by amide compounds and few species of bacteria are able to produce such enzyme using selective media[18,20]. The most complex compounds destruction paths are loaded on Oct-plasmid within *Pseudomonas* bacteria, which utilize the organic complex compounds to alcohols, then to aldehydes followed by organic acids, down to the β-oxidation phase and entering Crips cycle. However, this process is conducted via components linked to the membrane, such as; mono-oxygenase and others in the cytoplasm[7].

The ammonia concentration increased with the increasing of acrylonitrile decomposition (Fig. 6), so as pH values within the progress of the reaction (Fig. 5). However, to examine the ability of *Pseudomonas aeruginosa* of using acrylonitrile for growth, 500 ppm of the substance were mixed with the (MSM). Thus, the concentration of acrylonitrile was decreased during the 7 days of the experiment reaching its minimum concentration at the 7th day (Table 1). Such results were confirmed by the increasing concentration of ammonia and pH at the end of the reaction. Hence, the aforementioned observations show that *Pseudomonas aeruginosa* have the ability to decompose acrylonitrile under aerobic conditions. However, in this experiment, acrylonitrile was not directly used as a source of carbon and nitrogen, but its intermediate by-products, such as acrylic acid and ammonia, in which they used as a carbon and nitrogen sources by the under study bacteria, according to the decomposition diagram.

During the bacterial growth, pH values changed from neutral to alkaline, this was due to the production of ammonia in the medium. It was noted that the presence of extracellular enzyme activities by microbes during growth process often yields by-products that may change the medium pH and that gives an evidence of the ability of *Pseudomonas aeruginosa* to grew up in an alkaline pH[21]. Thus, it is possible to be as alkali tolerant bacteria, because the pH increasing did not inhibit the bacterial growth, as well as did not inhibit the activity of acrylonitrile degrading enzymes. Duthaler[22] stated that the enzymatic hydrolysis reaction of nitrile compounds in alkaline conditions has its own advantages, especially when cyanide compounds contribute in the enzymatic reaction. Through the biotransformation of nitrile compounds, acrylonitrile was the only carbon and nitrogen source for the bacterial growth. Hence, this event caused a decrease in the acrylonitrile concentration in the medium because the bacteria will produce metabolizing enzymes that use the substrate through the process of degradation[23,24]. In other word, the acrylonitrile biotransformation process, amide compounds were produced in the form of carboxylic acid and ammonia in which these processes involving the NHase and amidase enzymes.

In general, the acrylonitrile was bio-transformed by NHase into amide compound in the form of carboxylic acid and ammonia. Nevertheless, the biotransformation of nitrile compounds by bacteria usually takes place in two steps; the first reaction involves NHase and the second involves amidase or nitrilase[25] and that was corroborated by the formation of ammonia during the process as previously reported by Asano *et al.*[26] and Kim *et al.*[25]. Basically, the activity of nitrile compounds bio-transformation characterized by the formation of ammonia[27]. However, the results of this study were in agreement with previous studies[21,23,28].

The SEM micrographs of the acrylonitrile (AN) or poly acrylonitrile (PAN) were depicted in Fig. 7a and b before and after treatment by adding the bacteria of *Pseudomonas aeruginosa* and showed that the surface layer of the acrylonitrile (AN) is a homogeneous morphology and this is due to unavailability of cross linking agent and the network chains have arrange themselves. While Fig. 7b showed there was a clear change after the addition of bacteria, the first layer became semi-decomposed, with amounts of agglomerated on the surface due to chain fragmentation has occurred and expanded over time and irregularities are observed along the surface. The SEM observations were confirmed by the FTIR and HPLC results.

Finally, it is possible to say that this study can be developed based on the preliminary results to design a bioreactor system to treat large quantities and higher concentrations of this organic substance and complete the results obtained from this study to determine the quality of

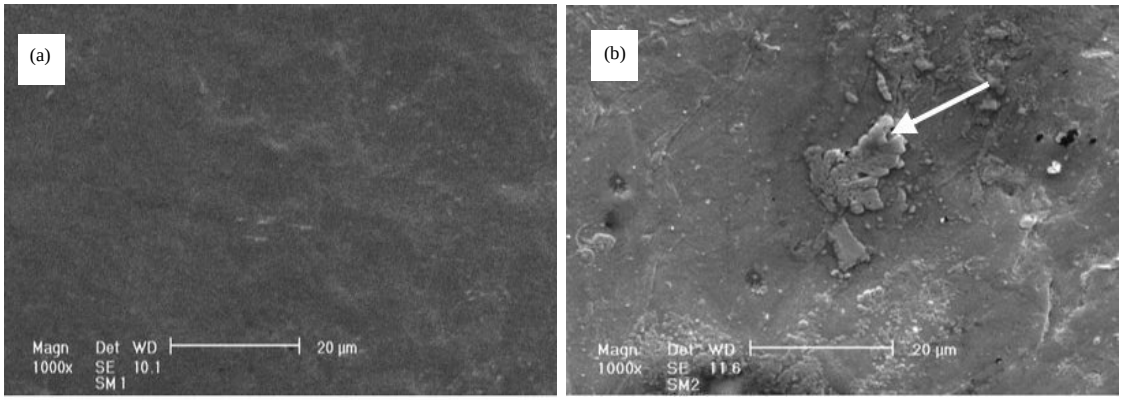


Fig. 7(a-b): SEM images showing the effect of adding bacteria to the acrylonitrile (AN) or poly acrylonitrile (PAN), (a) Before adding bacteria and (b) after adding bacteria

organic substance produced after the treatment process (stage II). However, through the results of this experiment, it can recommend the following; (1) Test more than one microorganism for comparative purposes to identify the largest proportion of bioremediation, (2) Working with more laboratory variables to determine the optimal conditions for treatment such as temperature, pH, retention time, primary concentration, biomass of the microorganism and others, (3) Using several modifications in more than one culture media to normalize the organism used in the treatment process, (4) Using adjuvants and stimulants for the treated organic substance, (5) Studying the possibility of designing bioreactor systems for treatment purposes and (6) Conducting other experiments to examine the resulting compounds after treatment and determine their toxicity.

CONCLUSION

The bacterial isolate from soil samples of southern part of Basra city was identified as *Pseudomonas aeruginosa* through the selective culture enrichment method, morphological and biochemical analysis as well as VITEK 2 instrument. The optimum environmental conditions for growth and degradation of acrylonitrile were determined in appropriate conditions and recorded as the inoculum density of (0.5.OD), pH (7.0), incubation period of (7 days) and 37-39°C temperature, which shows the ability of *Pseudomonas aeruginosa* to degrade all the acrylonitrile in a concentration of (500 ppm). HPLC analysis indicated the degradation of substance in the pathway of acrylonitrile degradation by *Pseudomonas aeruginosa* as well as the result of FTIR spectrum. From this research, the isolated *Pseudomonas aeruginosa* demonstrated eminent qualities of acrylonitrile degradation. Thus, it could be concluded that this bacterium can be used to bio-remediate acrylonitrile polluted soils in the environment.

SIGNIFICANCE STATEMENT

This study discovers the ability of *Pseudomonas aeruginosa* to degrade carcinogenic acrylonitrile that can be beneficial for avoiding the chemical treatment, which may affect the balance of the ecosystem, as well as the high costs. However, the under study treatment was conducted for the first time in Iraq, especially if we know that this huge amount of acrylonitrile was never found in the country before. The present study will help related researchers to uncover the critical areas for the biological breakdown of nitrogen triple bond within acrylonitrile that many researchers were not able to explore, especially if we know that all related studies were performed on prepared pellets of acrylonitrile and not on acrylonitrile wastes as hazardous material. Thus, a new theory on the biodegradation processes may be arrived at.

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