IJBPAS, October, 2015, 4(10), Special Issue: 434-451

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ISOLATION AND IDENTIFICATION OF ANILINE DEGRADING BACTERIA FROM URBAN AND HOSPITAL WASTE OF JAHROM AND ANALYSIS OF ANTIBIOTIC RESISTANCE

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ABSTRACT

The aim of this study was to isolate and identify molecules of degrading aniline bacteria from urban and hospital waste in Peymanieh hospital of Jahrom and also the resistance of the endemic bacteria.In this study, during two consecutive seasons of the year (summer and autumn 2013) of six stations of urban and hospital waste in Peymanieh hospital of Jahrom were sampled.For enrichment and isolation of resistant bacteria from the PNR culture environment containing 100 ppm of aniline was used. Identification of isolated bacteria by conventional biochemical tests and PCR was carried out, and to determine the resistance of the bacteria through the MIC, MBC, growth kinetics at different concentrations of aniline and antibiogram tests were evaluated.By morphological and biochemical and molecular tests of Pseudomonas aeruginosa, Klebseilla pneumonia, Klebsiella rhinoscleromatis, Klebsiella oxytoca, Escherichia coli and Citrobacter bacteria separated and identified. All resistant bacteria isolated were gram-negative

Some bacteria are able to tolerate high concentrations of aniline in Klebseilla pneumonia was the most resistant to aniline at MIC 6400 ppm and MBC 6500 ppm concentration. Also, it has the highest growth in the presence of 5000 ppm of aniline. The isolated bacteria particularly Klebsiella pneumoniae bacteria is a good candidate for treatment and removal of aniline in contaminated wastewater.

Keywords: Aniline degrading bacteria, biological remove, waste, MIC, MBC, growth kinetics.

INTRODUCTION

Aniline and its derivatives are among the most important environmental pollutants aromatic amino in buildings medicines, paints, insecticides, herbicides, etc., are used.Population growth causes increase of industrial and economic activities that expand the range of contaminants in the environment and environmental cycles of living organisms, including human beings (Authman et al., 2009). Urban and hospital waste containing aniline and its derivatives are often used for urban and industrial wastetreatment moving bed biofilm reactors or Moving Bed Biofilm Reactor (MBBR), but this method is not efficient for waste containing aniline.However, the use of biological and chemical aniline treatment has been effective and more efficient.Because of costly chemical methods best method to remove organic (by microorganisms) is aniline (Mucha et al., 2010).

1.1. Aromatic Amines

Aromatic amines, aromatic loops contain amine in which NH2, NH or nitrogen groups attached to the aromatic hydrocarbon.Building these compounds typically include one or more benzene ring.The simplest aromatic amines are aniline and other members of the group are known as aniline derivatives (Avatefinejad, 2012).

1.2. Aniline

Aniline, phenyl amine or amino benzene is an organic compound with the formula of primary aromatic amines with the formula C6H7N and molecular weight of 93/13 g/mol. It combines for the first time in 1826 by Unverdoben indigo dye was produced from the distillation of alkali and then Fritsche in 1840, this substance called aniline. Building aniline was found in 1843 by Hofmann (Sihtmae., 2010). It consists of a phenyl group attached to the amino group and as a precursor to many industrial chemicals. Moreover, its main application is the production of polyurethane. (Northcott, 1978). Due to the short half-life (weeks) aniline in water, soil and air to survive the unexpected is the substance in the environment (Howard, 1989). Based on the relatively low vapor pressure and high water solubility prediction is an aniline in the environment is mainly water-soluble. Microbial degradation of aniline in water is the important process of continuing However Fvtvaksydasyvn (in the water) and biological degradation (in water) is also significant (Aelion, 1987; 1989).

1.3. Resources of Aniline

There are no known natural sources for aniline. However, sufficient information

was not available, but some said that the substance can be entered into the environment by human resources during the production, storage, transport and disposal of aniline containing compounds as well as other countries through climate change and the destruction of some chemical pesticides (Lyons et al., 1985a, b).

1.4. Effects of Aniline on Human

Aniline through steam inhalation, ingestion or absorption through the skin can be toxic. Human exposure to high concentrations of aniline leads to cyanosis, headache, nausea, vomiting, tachycardia, dizziness, loss of balance, weakness, dizziness, drowsiness, renal failure, hematotoxicity, atrophy of the liver and cirrhosis, epilepsy, cancer, allergic skin reactions and death. (Donovan., 1983; Scott et al., 1983) These compounds can also cause menstrual disorders, dysfunction of the ovaries and cause spontaneous abortions as well. Long-term exposure with a less aniline may cause similar symptoms (BASF, 2008). Presence of Matt hemoglobin in the blood and breakdown products in the body, such as the wake-amino-phenol aniline in the urine indicate toxic effects of aniline in the body that the amount can be measured.

Aniline may be toxic for spleen, subsequently splenomegaly increase in red blood cells, hyperplasia, fibrosis and primary tumors on the spleen.Alanine inhalation 7-22 ppm for several hours causes little damage, but exposure to 100-160 ppm for 1 hour resulting disorder is unpredictable (khezri, 2013). The International Agency for Cancer (IARC) does not know aniline as a carcinogen to humans, but the Environmental Protection Agency (EPA) has introduced aniline as carcinogenic agent.

1.5. Decomposition of Aniline

1.5.1. Biological Methods of Aniline Decomposition

Several bacteria are known to aniline under aerobic conditions through intermediate catechol to decomposition. Aniline by these microorganisms through hydroxylation and deamination converted to catechol.Catechol Krebs cycle or cycle through the Meta cleavage converted to organic compounds. By microorganisms metabolize aniline through Meta cycle in a number of material can be seen.In addition, genes aniline dioxygenase (AD), the material converted to catechol.This enzyme (dioxygenase) is madeof 5 polypeptides and coded by genes tdnA1, tdnA2, tdnB, tdnQ and tdnT.

tdnA1, tdnA2 and tdnB are the large and small subunit of dioxygenase terminal and encode protein of electron transfer.TdnQ and TdnT are related to transfer amino

group of aniline and ammonia emissions.This enzyme in Pseudomonas putida and Acinetobacter have been shown that accelerate the metabolism of aniline by Meta cleavage (Talaei, 2010). Metabolizing aniline in microorganisms are seen by the Orto cleavage in sex Frateuria, and Acinetobacter and Rhodococcus.(Murakumi et al., 1999). Denitrifying Paracoccus sp. In anaerobic conditions, they do aniline incomplete destruction.Aniline degrading bacteria was first recognized in full and sulfate-reducing Desulfobacterium aniline is in aerobic conditions, microorganisms metabolize aniline in anaerobic conditions and the presence of with the goal of providing energy and carbon source for growth.In these circumstances aniline converted to 4-amino benzoic acid via carboxylation reaction and finally CO2 and acetyl coenzyme A are produced (Sehnell., 1991).

1.5.2. Traditional Methods of Aniline Decomposition

Widespread use of aniline in various industries have resulted in a significant amount of the substance in the effluent to enter the industry. Aniline and its derivatives cannot be analyzed for biological or that are difficult to decomposition. Aniline in the traditional way through electrolysis, ozone oxidation and adsorption to the resin decomposed. In this method, secondary metabolites such as carboxyl acids and Para Methyl phenol may be produced that have harmful effects on the environment and (Qi et al., 2002; Furumoi et al, 1997).

1.6. The definition of Waste

The collection used Water for different purposes is called waste or sewage.In other words, it's a collection of waste water that after collection and treatment may have the ability to reuse.The combination 9/99 sewage water and about 1% / 0% of impurities and contaminants in wastewater, including biodegradable organic matter, suspended solids, nutrients, pathogens, heavy metals, organic materials resistant to biodegradation and dissolved solids, which is associated with each of these pollutants and their concentration depends on the type and nature of the waste water.

1.7. Segmentation of Sewage

The most common source of production wastewater is divided on the basis of domestic sewage effluent, sewage commercial, industrial wastewater, sewage and wastewater rehabilitation centers and offices are public.

1.7.1. Hospital Sewage: Characteristics and Risks

1.7.1.1. Pathogenic Microbial Agents

The main concern associated with hospital waste, which has enteric pathogens, bacteria, viruses and parasites are that these pathogens are easily transmitted through water. Contaminated wastewater generated from the section that treats intestinal disease during outbreaks of epidemic diarrhea is one of the most important environmental health problems (Chu et al., 1982). Some pathogens in hospital sewage are drug-resistant that's why they are as a serious threat to the health of society. Moreover, some of these microorganisms may transfer antibiotic resistance to other pathogens. For this reason, in case of outbreaks of infectious agents in society, the treatment can be difficult.

1.7.1.2. Hazardous Chemicals

Small amounts of chemicals for disinfection and cleaning up wastewater collection network. But if management does not apply to large amounts of chemicals may enter the wastewater collection network.

1.7.1.3. Medical Waste

Often small amounts of pharmaceutical wastes by different parts of the hospital and also by the wastewater collection network pharmacy within discharged.If management does not apply to pharmaceutical waste which may contain higher amounts of aromatics amines (aniline), antibiotics and genotoxic drugs will be discharged in wastewater collection network.

1.7.1.4. RadioactiveIsotopes

Minor amounts of radioactive isotopes by the Department of Oncology at the discharge of wastewater collection networks will not be a threat to the health of the environment provided that proper management is applied.

1.7.2. Sewage Treatment with Aniline

Moving bed biofilm reactors or Moving Bed Biofilm Reactor (MBBR) are used for urban and industrial wastewater treatment, but this method is not efficient for treating wastewater containing aniline. However, the use of biological and chemical aniline treatment has been effective and more efficient.The biological methods of filamentous bacteria, Pseudomonas, Rhodococcus and Acinetobacter used and the efficiency of this method is over 93%.In addition, you can use other methods such as the use of aqueous emulsion membranes, ultra-water heat regenerative adsorption method and biological Alktrvfntvn wastewater containing aniline used (Datta, 2003; Wang, 2007). Similar research has been done in this field that can be pointed to some of them,Wang and his colleagues in 2011 AN1 bacteria strain Candida tropicalis as aniline degrading bacteria

were isolated from treated wastewater (Wang et al., 2011). Kafilzadeh et al. (2013), enables the isolation and identification of aniline degrading bacteria deposits Kharg Island in the Persian Gulf and the growth rate and resistance tested (Kafilzadeh et al, 2013). Because so far on aniline degrading bacteria in urban and hospital waste no investigation has been arranged for the purpose of this study was to isolate and identify aniline degrading bacteria in urban and hospital waste in Peymanieh hospital arranged in two seasons of 2013 (summer and fall) and determine the whole resistance of these bacteria by measuring the minimum inhibitory concentration of growth (MIC), minimum bactericidal concentration (MBC), the kinetics of growth and antibiotic test and finding bacteria with high resistance.

2. MATERIALS AND METHODS

2.1. Measuring Aniline of Samples

The analysis was carried out to measure aniline in samples of HP's model 1090 HPLC system equipped with a 3-solver in Dr. Siroeinejad laboratory in Shiraz. It includes:

1.The c18 column and detector DAD were used.

- chem. Station was used as software

The mobile phase consisted of acetonitrile - water in the stream 20/80 ml / min 1 and the 1 micro liter and detection at 230 nm wavelength is done (Dastgheib et al., 2012).

2.2. Detection and Identification of Bacteria

Identification of isolated bacteria using, conventional biochemical according to the book Bergey's Manual of Systematic Bacteriology and by Gram stain, check morphological tests for catalase, oxidase, KOH, Simon citrate, urease and other diagnostic tests to discriminate between the were identified.

2.2.1. Gram Stain

The Pap smear and fixed with heat and color crystal violet flame for 1 minute and then paint was poured on the smear was washed with water. Logol solution is poured on the smear for 1 minute and after 1 minute, the color was washed with water and then go for color, use of alcohol, acetone for 6 seconds and re-washing was done. Finally, Safranin solution for 30 seconds was poured on the smear, and then smears were washed with water and allow to air dry, smear after drying it under light microscopy as a result of gram-positive bacteria, gram-negative bacteria, purple and red were seen.

2.2.2. Catalase Test

Catalase reagent was prepared so that ml3 of 2 O2H in ml100 water was diluted to 3% hydrogen peroxide solution is

achieved. For catalase test using a loop of the colony was removed and it was put on clean microbial slide and a drop of three percent hydrogen peroxide was added. Oxygen bubbles a few seconds because the reaction is positive. In this test, catalase converts hydrogen peroxide into water and oxygen.

2.2.3. Oxidase Test

Oxidase reagent is prepared so that gr o/1 of the tetra-methyl ammonium phenylene dichloride was poured into water ml 10 and after 10 minutes the purple color of this reagent was used for oxidase test. Oxidase test filter paper into a square shape was cut and placed on glass slides, then on filter paper using a sterile loop, part of the net to put a colony and it was poured a drop of reagent oxidaseand after 30 seconds if the colony was purple, positive oxidase were considered.

2.2.4Test KOH

To perform this test using loop, a single colony was placed on a slide and a drop of KOH is poured on the colony and with the help of a sterile loop, the colony KOH were mixed, the colonies which are gram-negative cache were observed (Moqtaderi, 2013).

2.3. Kinetics Growth of Resistant Bacteria in the Two Consecutive Seasons

After all the procedures and identification of aniline degrading bacteria, to assess the ability of various bacteria, bacterial growth kinetics of aniline was evaluated in the four different concentrations. Optional concentrations are as follows [100] 150 [200] 250 2000 3000 4000 5000ppm concentration of aniline.

Table 1: The amount and concentration of aniline in the medium of growth kinetics

Concentration	Aniline amount
→ **ppm**2000	λ 100
→ **ppm**3000	λ 150
→ **ppm**4000	λ 200
→ **ppm**5000	λ 250

2.4. Preparation of the Medium and Inoculation

For every bacteria, five flask with a capacity ml100, each containing Luria Bertani broth medium was autoclaved ml 50, and then McFarland bacterial suspension was prepared in accordance with the standard half. For every five flask, an environment Luria Bertani broth without bacteria (sterile) was intended to be used to zero the spectrophotometer. Immediately after the addition of bacteria, absorbance at 600 nm was recorded for each environment (khezri, 2013).

2.5. Measurement of Aniline in the Medium of Bacteria (hplc)

In this study, to measure changes in the concentration of aniline in the bacteria HPLC, model Shimatozo Japan in the 4 days in the laboratory of the Food and Drug Department of Medical Sciences has been arranged. The method to do this research on the device has been set as follows:

1. Isocratic method
2. Type column C18 (750 * 4.6 * 5μg), Babar column temperature to the ambient temperature
3. Wavelength detector nm254
4. The flow rate of 8.0 ml solution per minute
5. The injection volume of 20 ml
6. The mixture of deionized water and acetonitrile mobile phase at a ratio of 22 to 78
7. Running time 10 minutes

2.6. Preparation of standard solutions

To work with standard HPLC system must first concentration of a sample that is prepared to identify and measure it. To determine the concentration of aniline in vitro bactericidal concentrations 5-10-15-20-25-30 ppm standard preparation of aniline and aniline injection machine and the standard curve was plotted.

2.7. Continuous recording OD600 to Determine the Kinetics of Bacterial Growth

After the initial registration of amount of light absorption, cotton was placed to the flask and was placed in a shaking incubator at 30 ° C for one week every 12 hours (6 am and 6 pm), OD600 readings were recorded (khezri, 2013).

2.8. Antibiogram Test

In this test for determining the resistance of bacteria to antibiotics based on Kirby-Bauer disk diffusion method, eight types of antibiotics were used. For this purpose, a transparent medium was shown giving the medium Muller Hinton Agar is the perfect environment for this test. 24hours before the test, in accordance with the standard half McFarland bacterial suspension of isolated bacteria and cultured in Luria Bertani broth cultures were incubated,in addition to plenty of culture and sterile Muller Hinton Agar plates poured to cool, then using a sterile swab, bacterial suspension on Muller Hinton Agar culture medium and then insert the discs antibiotic medium, to the plates were incubated for 24 hours. Finally the zone diameter obtained around each of the drives antibiotic resistance or sensitivity which indicates the pretext of antibiotics are bacteria were measured by a ruler(Raja et al, 2009).

2.9. Discs Used in Antibiogram

Suitable discs should be used for the isolated bacteria. Selecting the type of

drive is very important. Because drug resistance should be considered and on the other hand the person's health should be considered. Disc according to hospital type and the type of test can be changed. Antibiotic discs vials used in this project is 60 × 4 that were prepared from Padtan Teb Company, including Gentamicin, Nalidixic acid, Nitrofurantoin Sulfamethoxazole trimethoprim, Ciprofloxacin, Amikacin, Ceftriaxone Tetracycline, and Imipenem.

3. RESULTS

3.1. Identification of Bacteria

44 colonies were evaluated by Gram stain test of conventional biochemical 6 bacteria resistant to aniline was isolated and identified in Tables 1 and 2 shows the name of bacteria andthe presence or absence of bacteria at various stations during the summer season and fall.

Table 2: Isolation and identification of bacteria and the presence or absence of the stations surveyed in the summer

Station6	Station5	Station4	Station3	Station2	Station 1	Bacteria
+	+	+	+	+	+	Escherichia coli
+	-	-	+	+	+	Citrobacter
-	-	+	-	+	+	Pseudomonas aeruginosa
-	-	-	+	+	+	Klebseilla pneumonia
-	-	-	-	+	+	Klebsiella rhinoscleromatis
-	-	-	-	+	-	Klebsiella oxytoca

a) Positive signs mean the presence of bacteria, b) negative signs mean the absence of bacteria

Table 3: Isolation and identification of bacteria and the presence or absence of the stations surveyed in fall

Station6	Station5	Station4	Station3	Station2	Station 1	Bacteria
+	+	+	+	+	+	Escherichia coli
-	-	-	+	+	+	Citrobacter
-	-	+	-	+	-	Pseudomonas aeruginosa
-	-	-	+	+	+	Klebseilla pneumonia
-	-	-	-	+	+	Klebsiella rhinoscleromatis
-	-	-	-	-	-	Klebsiella oxytoca

a) Positive signs mean the presence of bacteria, b) negative signs mean the absence of bacteria

Percentage frequency of identified Gram-negative bacteria was more than Gram-positive bacteria, so 100% of resistant isolated bacteria were gram-negative. The highest percentage of detected bacteria E.coli with many stations and different seasons of 100% and the lowest percentage

was Klebseilla oxytoca 8/33 with an abundance of summer and was isolated in only 2 stations. E.coli bacteria were isolated in all seasons and sampling stations (Figure 1). The percentage of isolated bacteria in summer was more than in autumn (Figure 2).

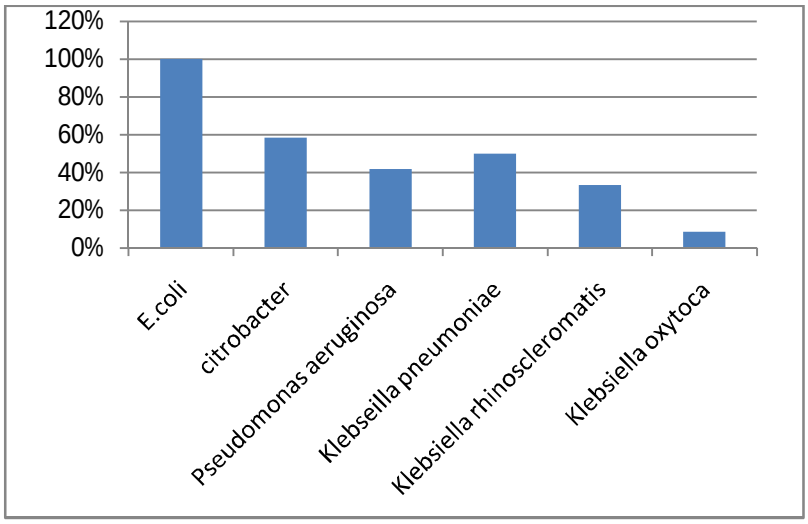

Figure 1: Frequency of sex bacteria isolated in different seasons and stations

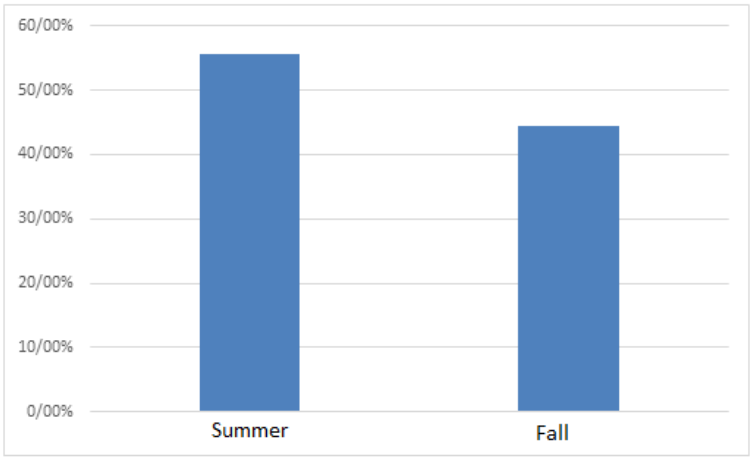

Figure 2:Comparison of seasons in the percentage of isolated bacteria

2.3. The Removal of Aniline in the Medium

After identifying the isolated bacteria, the removal of aniline in culture by bacteria Citrobacter, Klebseilla pneumonia and Escherichia coli were measured by HPLC, and the results are shown in Tables 3 to 6

Table 4: the removal of aniline on the first day by the isolated bacteria

Concentration	Area under the curve	Percent	Bacteria
10/693 12/654 11/771	376279 441372 412059	100 100 100	Citrobacter Klebseilla pneumoniae Escherichia coli
20/557 25/148 21/563	703758 856201 737149	100 100 100	Citrobacter Klebseilla pneumoniae Escherichia coli

27/667 26/236 28/380	943130 892316 963503	100 100 100	Citrobacter Klebseilla pneumoniae Escherichia coli

Table 5: The removal of aniline on the second day by the isolated bacteria

Concentration	Area under the curve	Percent	Bacteria
4/256 10/126 8/304	162562 357471 296975	60/2 20 29/4	Citrobacter Klebseilla pneumoniae Escherichia coli
14/358 19/723 16/827	497952 676088 579920	30/1 21/6 22	Citrobacter Klebseilla pneumoniae Escherichia coli
19/326 23/568 21/311	662907 803727 728793	30/4 10/2 %25	Citrobacter Klebseilla pneumoniae Escherichia coli

Table 6: The removal of aniline on the third day by the isolated bacteria

Concentration	Area under the curve	Percent	Bacteria
8/101 8/742 8/665	290227 311498 308937	24/2 30/9 26/4	Citrobacter Klebseilla pneumoniae Escherichia coli
16/947 18/945 15/416	583898 650257 533074	17/6 24/7 28/5	Citrobacter Klebseilla pneumoniae Escherichia coli
22/545 23/807 20/119	769767 811653 689225	18/8 9/3 29/1	Citrobacter Klebseilla pneumoniae Escherichia coli

Table 7: The removal of aniline on the fourth day by the isolated bacteria

Concentration	Area under the curve	Percent	Bacteria
7/720 6/750 6/664	277577 245366 245846	27/8 46/7 43/3	Citrobacter Klebseilla pneumoniae Escherichia coli
15/291 13/750 13/306	528951 477780 463034	25/6 45/3 38/3	Citrobacter Klebseilla pneumoniae Escherichia coli
17/042 19/636 15/097	587067 673180 522488	38/6 25/2 46/8	Citrobacter Klebseilla pneumoniae Escherichia coli

3. Results of Growth Kinetics of Resistant Bacteria to Aniline

Figure shows the growth of resistant bacteria aniline in five states. Medium with different concentrations of aniline (concentration of 5000 ppm, 4000 ppm, 3000 ppm, 2000 pmm) and medium without aniline (control) (Figure 3 to 8).

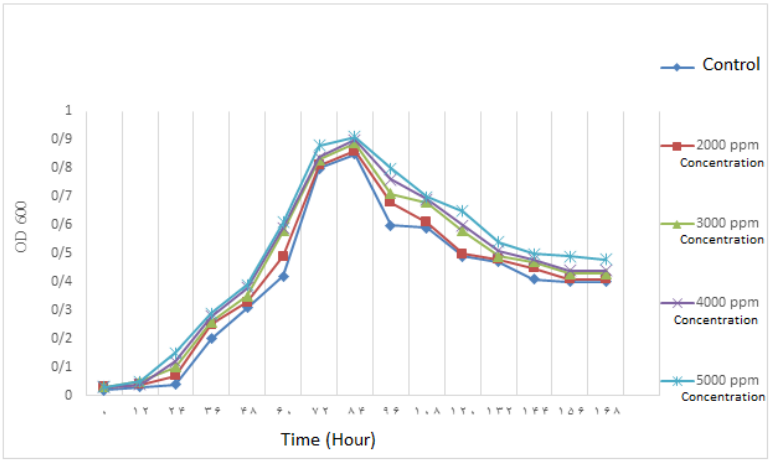

Figure 3: The results of the growth kinetics of *Escherichia coli*

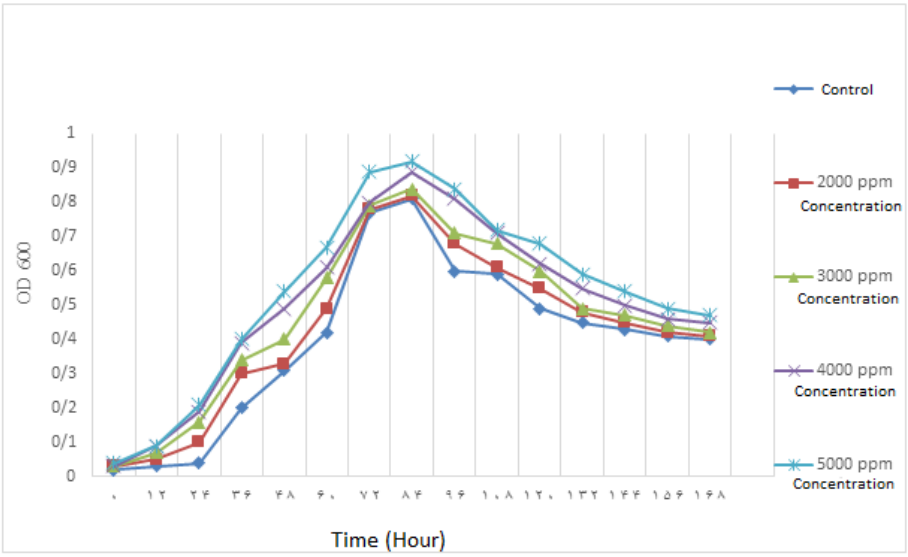

Figure 4: The results of the growth kinetics of *Citrobacter*

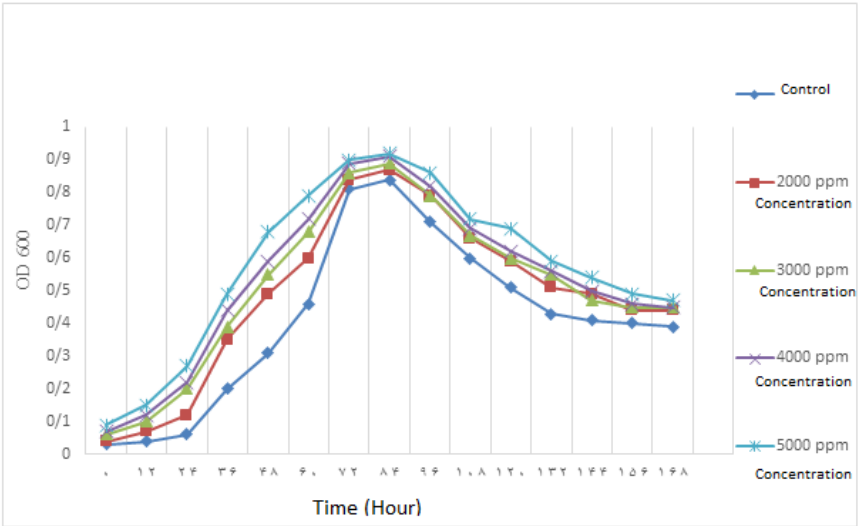

Figure 5: The results of the growth kinetics of Pseudomonas aeruginosa

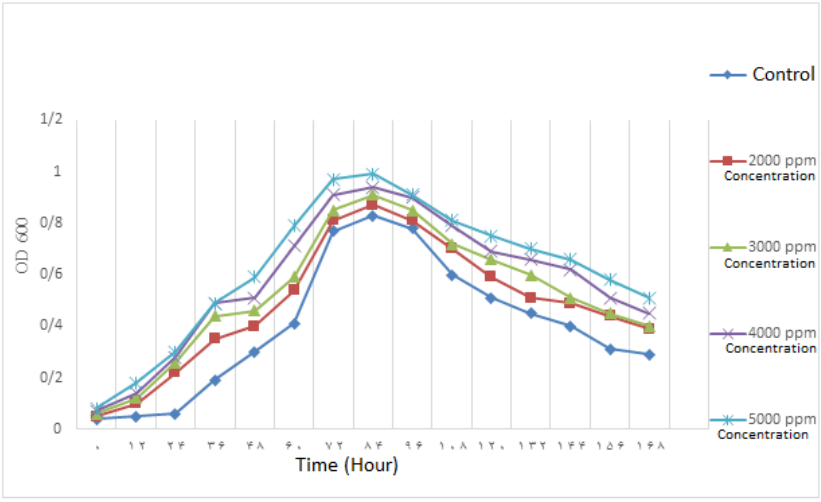

Figure 6: Results of the growth kinetics of *Klebseilla pneumonia*

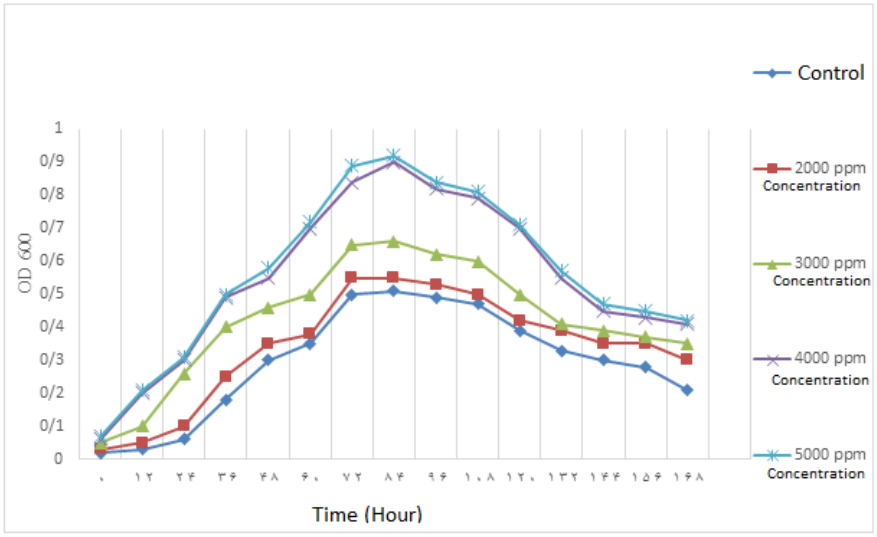

Diagram 7: Results of the growth kinetics of *Klebsiella rhinoscleromatis*

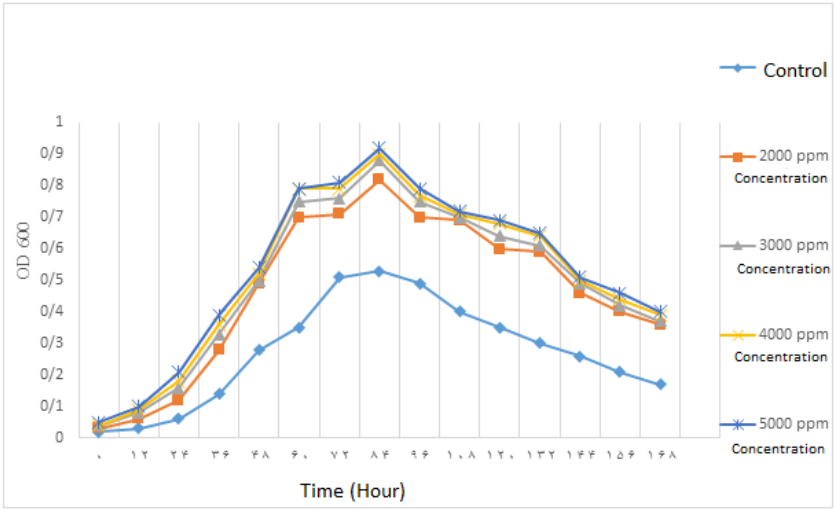

Figure 8: Results of the growth kinetics of *Klebsiella oxytoca*

4. Antibiogram Test of the Resistant Isolated Bacteria

The growth of bacteria in the presence of different antibiotics due to the zone diameter created around antimicrobial discs is measured and thus resistant or susceptibility of bacteria to various antibiotics was determined (Table 8).

Table 8: Antibiogram Test of resistant isolated bacteria

Klebsiella oxytoca	*Klebsiella rhinoscleromatis*	*Klebseilla pneumoniae*	*Pseudomonas aeruginosa*	*Citrobacter*	*Escherichia coli*	Bacteria / Antibiotics
I	I	I	S	S	I	Gentamicin
R	R	R	R	S	R	Nalidiic acid
R	R	R	S	S	I	Nitrofurantoin
S	S	S	I	S	S	Sulfamethoxazole
S	S	R	R	R	R	Ciprofloxacin
I	I	I	S	R	I	Amikacin
S	S	S	S	S	S	Ceftriaxone
S	S	S	S	I	S	Imipenem
R	R	R	R	R	R	Tetracycline

R: Resistant; I: Intermediate; S: Sensitive

3. Argument and Conclusion

In this study, sampling accomplished from six stations in two consecutive seasons of summer and autumn of 2013(three samples of Peymanieh hospital waste in Jahrom, three samples of treatment plant waste) that according to the results, no significant difference was observed in the temperature and pH stations, as well as the alanine concentration was high at all stations .In the current study, the number of bacteria in the medium control (no aniline) was more of a medium containing aniline.In fact, aniline causes stunted growth, death and reducing the number of bacteria in the environment has been aniline. This was the same in Udeani and colleagues (Udeani et al., 2009). Also,Farrukh nisar and colleagues in 2010 used PNR medium for the isolation of the aniline degrading bacteria which contained 100ppm aniline (Farrukh nisar et al., 2010).On the other hand, Chengbin and colleagues in 2009 after separating the bacterial decomposition of Delftia sp XYJ6 studied aniline by the new bacteria (Chengbin et al., 2009). In this study, the largest number of resistant aniline bacteria found in summer, which is likely due to weather conditions in autumn and biofilm formation to create more resistance than the aniline is destroyed because the biofilm formation to deal with the bacteria toxic substances such as aniline in the environment and thus less bacterial diversity (Harrison et al., 2007; Warren., 2005). Kafilzadeh and colleagues in 2013, after the separation of aniline degrading bacteria in the spring and summer they looked at the average bacteria count was higher in the summer because of the weather conditions. (Kafilzadeh et al., 2013) In this study, all isolated identified bacteria are gram-negative that shows the

outer membrane of Gram-negative bacteria due to the negatively charged surface of LPS in Gram-positive bacteria are less affected compared to research aniline Urata et al. in 2004 matched that after the aniline degrading bacteria isolated bacteria were identified in Japan 8 groups that 7 of them were gram-negative and 1 of them was gram-positive (Urata 2004). In the study, MIC and MBC were used, the most resistant bacteria accordingly found that Klebseilla pneumoniae that can tolerate concentrations of MIC 6400 ppm and MBC 6500 ppm of aniline was then the bacteria Escherichia coli, Citrobacter, Klebsiellarhinoscleromatis, Klebsiella oxytoca with MIC 6300 ppm and MBC 6400 ppm of aniline were the most resistant, Pseudomonas aeruginosa with MIC 6200 ppm and MBC 6300 ppm showed the least resistance. In this study, MIC and MBC was used, on this basis it was determined that the most resistant bacteria was Klebseilla pneumonia with the ability to tolerate concentrations of MIC 6400 ppm and MBC 6500 ppm of aniline, then the bacteria Escherichia coli, Citrobacter, Klebsiellarhinoscleromatis, Klebsiella oxytoca with MIC 6300 ppm and MBC 6400 ppm of aniline had the greatest resistance, Pseudomonas aeruginosa with MIC 6200 ppm and MBC 6300 ppm showed the least resistance. The study of khezri on the aniline degrading bacteria was around Shiraz Refinery MIC and MBC resistant bacteria isolated in different concentrations of aniline examined. The resistant bacteria with MIC and MBC was 0.4 ml and the bacteria was Entrobacter ludwigii and Delftia acisovorans (khezri, 2013). In this study, growth charts (kinetics of growth) aniline degrading bacteria in different concentrations of aniline was evaluated in 7 days, as Wang and colleagues in 2011, growth kinetics of aniline degrading bacteria in different concentrations of aniline tested within 7 days (Wang et al., 2011).Growth kinetics of aniline degrading bacteria in different concentrations showed that in the presence of 5000 ppm of aniline Klebseilla pneumoniae had the highest growth.As El-Rab and colleagues in 2006 showed that concentrations of toxic pollutants impact on growth charts resistant bacteria, and this effect was more in sensitive bacteria. In this study, the isolated bacteria resistant to aniline had no effect on reducing bacterial growth (El-Rab et al., 2006). The research of khezri (2013) the antibiotics ciprofloxacin, kanamycin, fluoroquinolones, novobiocin, streptomycin, penicillin and ampicillin were used and the results showed that Delftia acidovorans with a halo of inhibition with a diameter of 1.4 to

ciprofloxacin and Entrobacter ludwigii with halo 1.6 diameter growth than the growth in the presence of streptomycin and other antibiotics in the category of aniline degrading bacteria were resistant (khezri, 2013). In the present study Escherichia coli bacteria was resistant to antibiotics Nalidic Acid, Tetracycline, Ciprofloxacin Citrobacter was resistant to antibiotic Ciprofloxacin, Amikacin, bacteria Pseudomonas was resistant to Tetracycline aeruginosa was resistant to to antibiotics Nalidic Acid, Sulfamethoxazole trimetoprim ciprofloxacin, Tetracycline, and Klebseilla pneumoniae was resistant to antibiotics Nalidic Acid Nitrofurantoin , Ciprofloxacin and Tetracycline that was the most resistance among the isolated bacteria. In the current study, a direct relationship between the MIC and MBC with antibiogram test bacteria was obtained. Looking at Table Antibiogram and comparison with the MIC and MBC tables will realize that bacteria with the highest MIC and MBC with the highest resistance to antibiotics, which was consistent with Moqtaderi's research (2013) and suggests that antibiotic resistance is associated with the resistance to the bacteria aniline (Moqtaderi, 2013). In the present study, all resistant bacteria aniline were resistant to several antibiotics, the most sensitivity to antibiotics-resistant bacteria aniline were Sulfamethoxazole trimethoprim, Ceftriaxone and Imipenem (Ranbanshi, 2008), and bacteria that has the high growth in the presence of aniline such as Klebseilla pneumoniae with resistance to antibiotics are more and show that antibiotic resistance and resistance to aniline linked the bacteria resistance genes aniline together with genes for resistance to antibiotics Drugs. In a study of Raja et al. (2009) resistant of bacteria to the MIC and the antibiotic Ampicillin, Tetracycline, ChloramphenicolKanamycin,

Erythromycin, Streptomycin, was also reported and concluded that resistant to MIC and antibiotics related to each other because of resistance genes aniline and antibiotics often work together on a plasmid or operon (Raja et al., 2009; Lawrence, 2000). In general it can be concluded that bacteria grow in the presence of aniline has the high resistance to antibiotics more different. Also aniline has no effect on reducing the growth of bacteria that have a high resistance which has a higher concentration of MIC and MBC like Klebseilla pneumoniae bacteria and the bacteria is a good candidate for decomposition and removal of aniline from the contaminated areas.

Suggestions:

Growth kinetics test by the synergistic effect of aniline in the presence of bacteria

that have the most growth can be measured. The synergistic effect of the removerbacteria of isolated aniline from water or sediment can be achieved.

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