International Journal of Biological, Physical and Chemical Studies
ISSN: 2709-1554
DOI: 10.32996/ijbpcs
Journal Homepage: www.al-kindipublisher.com/index.php/ijbpcs

IJBPCS
AL-KINDI CENTER FOR RESEARCH AND DEVELOPMENT

| RESEARCH ARTICLE

Isolation and Characterization of Phenol-degrading Bacteria from Wastewater

Abd El-Rady A. Mousa
Lecturer, Department of Botany, Faculty of Science Kufrah, Benghazi University, Libya
Corresponding Author: Abd El-Rady A. Mousa, **E-mail**: bdalradyMsy51@gmail.com

| ABSTRACT
Out of 30 bacterial isolates from wastewater were checked for growth on a minimal salt medium amended with different concentrations of phenol by flask culture technique. The eight most tolerant bacterial strains to the higher concentrations of phenol, designated as W2, W5, W9, W12, W14, W15, W19 and W29, were investigated for their ability to grow and degrade phenol. Among the eight higher phenol degrading isolates, W15 can tolerate up to 1000 ppm of phenol concentrations and grow and degrade 94% of phenol within 72 hrs. The optimum temperature and pH condition were 37C◦ and 7, respectively. The yeast extract is the best organic nitrogen source, while ammonium chloride is the best inorganic nitrogen source for the growth and degradation of phenol.

| KEYWORDS
Characterization; Isolation; Phenol-Bacteria; wastewater

| ARTICLE INFORMATION
ACCEPTED: 25 August 2023 **PUBLISHED:** 10 September 2023 **DOI:** 10.32996/ijbpcs.2023.5.2.3

1. Introduction

Phenol is highly toxic for a number of organisms, including microorganisms, plants, animals and humans. Nowadays, with increasing industrial growth, aromatic compounds are environmental pollutants that exist in different regions such as freshwater, sea and land (Deng *et al.*, 2018). Phenol and phenolic compounds are hazardous when present in low concentrations. The high and acute concentration of phenol can cause in humans' disorders in the central nervous system, myocardial depression, irritation of the eyes, swelling, corneal whitening and also blindness. Also, cardiovascular diseases and gastrointestinal damage (Govindarajalu, 2003; Naresh *et al.*, 2012). The production and application of phenol and phenolic compounds in industrial activities make them a major environmental pollutant in most wastewater, such as oil refineries, coking plants, pharmaceuticals and plastic industries (McCall *et al.*, 2009; Park *et al.,* 2012). Due to these adverse health effects of phenolics, as per the rules of the World Health Organization, the maximum permissible level for phenol in the environment is 0.1 mg/L (Nuhoglu and Yalcin, 2005; Saravanan *et al.*, 2008) and in tap water to below 1–2 µg/L (Gami *et al.* 2014).

A variety of treatment methods, such as adsorption (Carmona *et al.*, 2006), solvent extraction (La) zarova and Boyadzhieva, 2004), Wet oxidation, and hydrogen peroxide. Fenton's reagent has been employed to eliminate phenol in polluted samples (Lin and Chuang, 1994), chemical oxidation and incineration (Wu *et al.*, 2005). But these methods are complex, high cost and not environment friendly (Yan *et al.*, 2006; Zhai *et al.*, 2012). Thus, biodegradation is the best way to rid of the phenol since this process is cheap, environment friendly and easy to handle (Tay *et al.*, 2005; Basha *et al.*, 2010). Bacteria oxidize phenol into CO_2 and H_2O during metabolic processes (Loh and Chua, 2002). and can utilize phenol for their growth by utilizing phenol as the sole source of carbon and energy (Geng *et al.*, 2006; Tuah *et al.*, 2009). Such microorganisms have the potential to degrade phenol (Nair *et al.*, 2008). Several phenol degrading bacteria have been isolated like *Rhodococcus* (Rehfuss and Urban, 2005), *Gulosibacter* sp. (Zhai *et al.*, 2012), *Acinetobacter sp*. (Ahmad *et al.*, 2011), *Bacillus cereus* (Banerjee and Ghoshal, 2010), *Staphylococcus epidermis* (Mohite *et al.*, 2010), *Xanthobacter flavus* (Lowry *et al.*, 2009), *Burkholderia cepacia* (El-Sayed *et al.*, 2003), *Pseudomonas* (Mahiudddin *et al.*,

2012; Ahmad *et al*., 2014). Therefore, the aim of this work was to isolate and characterize phenol degrading bacteria from wastewater.

2. Materials and Methods

2.1. Chemicals and reagents

Phenol and chemicals used in the study were of analytical grade; glucose and inorganic salts, used in preparing microbial growth media, were purchased from El-Gomhoria company, Zagazig City, Egypt.

2.2 Sample collections

Ten samples were collected from the sewage water treatment plant (SWTP) at Zagazig City, Egypt. These samples were collected every month for ten months, and each sample was analyzed one hour after collection from SWTP.

2.3. Isolation and screening of phenol degrading bacteria

The isolated bacteria from sewage water were suspended in 100 ml of minimal salt medium containing (MSM) different concentrations of phenol as the sole source of carbon(0-1000ppm) and incubated at 37°C for a period of 48 hours.

2.4. Phenol degradation by the bacterial isolates

The best isolates showing growth in the higher concentrations (500 -1000 ppm) were selected for phenol removal assay was carried out by a modified colorimetric technique4-aminoantepyrene method as suggested by (Klibanov *et al*., 1980).

2.5. Estimation of Phenol

In this method: about 5 ml sample, 0.3 ml of 2% aqueous 4-amino antipyrine solution and 1 ml of 2N NH_4OH were added. After mixing the content thoroughly, 1 ml of 2% K_3FeCN_6 is added. Phenolic material reacts with 4-Amino Antipyrine in the presence of potassium ferricyanide at a pH of 10 to produce a purple-red colored end product. The absorbance at 520 nm was measured and compared with the phenol standards curve of phenol to determine phenol concentration. Phenol degradation was quantitatively analyzed by measuring the phenol concentration of the supernatant of respective samples, and degradation was calculated by using the equation: -

% Degradation = (A B)/ A×100; Where A is the initial concentration of phenol (Zero hrs. sample) and B is the concentration of phenol in a sample taken at various time intervals.

2.6. Optimization of environmental factors on growth and degradation.

To study the optimum functional pH, temperature., and nitrogen source for the growth and degradation of phenol at a constant concentration of phenol (1000 ppm) in incubation temperature (37°C) and neutral pH7 in the absence of carbon was carried out. Similarly, other parameters were kept constant, and pH was varied between 6 - 8. Assessment of growth was measured at 0, 24,48,72 and 96 hrs, and phenol degradation was made after an incubation period of 96 hrs. All the results were given as a mean with Standard Deviation (±SD). All experiments were carried out in triplicates, and the mean value was considered.

2.6.1. Effect of temperature on the growth and phenol degradation

The eight isolates were grown in MS medium with 1000 ppm of phenol at different temperature values (25°C, 30°C, 35°C and 40°C) at pH 7. This mixture was contained in 250 ml Erlenmeyer flasks. The cultures were placed on a shaker (120rpm) at the above temperatures. The growth was measured at 0, 24,48,72 and 96 hrs., and phenol degradation was measured at time 96 hrs.

2.6.2. Effect of pH on the growth and phenol degradation

The effect of pH (6-8) on growth and phenol degradation was tested. Cells were grown as shake cultures at 37°C in MS medium supplemented with 1000ppm phenol and inoculum size 5 %v/v in a 250 mL flask. The growth was measured at 0, 24,48,72 and 96 hrs., and phenol degradation was measured at time 96 hrs.

2.6.3. Effect of Nitrogen source on the growth and phenol degradation Effect of different nitrogen sources on growth and phenol degradation was determined in the previous medium in which nitrogen source was replaced with Peptone, Yeast, Urea extract, NaNO3, and KNo3 and NH4Cl at a concentration of 2 g/L. The positive control used was NH4Cl. The growth was measured at 0, 24,48,72 and 96 hrs., and phenol degradation was measured at time 96 hrs.

3. Results and Discussion

3.1. Screening of bacterial isolates from wastewater

The results presented in Table (1) show that out of 30 bacterial isolates were isolated from wastewater. The isolates were subjected to varied initial phenol concentrations of 50 - 1000 ppm phenol. The study was carried out at a temperature of 37°C and pH 7. Growth of bacterial isolates on treated media and control was taken and expressed by streaking growth degree as follows: no

growth (-), moderate growth (+), good growth (++) and very good growth (+++). Thirty bacterial isolates were isolated from wastewater. Data showed that only isolate W15 gave very good growth (+++) on all tested concentrations of phenol. While W2, W5, W12, W14, W19 and W29 exhibited good growth (++) at phenol concentration (500 ppm). However, W6, W9 and W10 isolates showed moderate growth (+) at a 500 ppm concentration of phenol. Data also showed that isolates W7, W8, W13, W23, W24, W25, W26 and W30 were the most sensitive since they gave no growth on the different concentrations of phenol. Data in Table (1) showed that W15 was found to have the highest isolates in growing on all tested conc. (+++).

3.2. Phenol degradation by selective isolates at higher conc. of phenol

In the present study, a total of 8 higher phenol degrading bacterial strains were isolated from wastewater samples (Table 2). Bacterial isolate W15 showed maximum degradation (95.0 %) of phenol within 96 hrs. of incubation (Table, 2) Followed by bacterial isolates W12, W9, W14, W5, W19, W2, and W29 shows 81 %, 71%,57%,57%,55%,55% and 49% phenol degradation, respectively. Thus, isolate W15 was selected for further study as it exhibits the highest degradation of phenol. Ghaima et al. (2017) isolated *P. aeruginosa* KBM13 from contaminated soil with diesel fuel was the most active in phenol degradation and efficient in removing 92 % of the initial concentration of 500 mg/L phenol within 48 hr and had a tolerance of phenol concentration as high as 1400 mg/L.

3.3. Optimization of environmental factors on growth and degradation of W15 isolate.

3.3.1. Effect of temperature on growth and degradation of W15 isolate

Temperature exerts an important regulatory influence on the rate of growth. The results in Fig. 1 and Fig.2 showed that the bacterial growth and degradation of phenol of strain W15 is the maximal value at about 35 °C (90.33%)., On the contrary, the phenol degradation declined sharply when the temperature reached 25°C and above 35 °C. Therefore, the optimal temperature for the growth of W15 was 35 °C. It is believed that sudden exposure to temperatures higher than 35 °C may have a detrimental effect on the bacteria enzymes that are usually responsible for the benzene ring cleavage, which is the main step in the biological degradation process. On the other hand, exposure to lower temperatures is expected to slow down bacterial activity. Also, one of the studies demonstrated that the strain *Pseudomonas aeuriginosa* MTCC 4997 isolated from effluents collected from petrochemical industries exhibited a complete degradation of phenol at a wide temperature from 15°C to 45°C with an optimum of 37°C (Kotresha and Vidyasagar, 2014). Ghaima et al. (2017) found that the maximum phenol degradation of *P. aeruginosa* KBM13 was observed at 40˚C.

3.3.2. Effect of pH on growth and phenol degradation of W15 isolate

The effect of pH value on the growth of W15 was investigated. The bacterial isolate W15 grew within a range of pH 6–8 (Fig. 3). The results showed that the optimum growth of the isolate was observed at pH 7. The growth decreased as the pH decreased. At pH 8, the growth is less. The degradation rate of W15 at different pH values is shown in Fig. (4), and the degradation rate of phenol was the highest at 91.2% at only pH 7.0 when cultured for 72hrs., and the degradation of phenol at pH 6.0 was the least (33.0%). From the obtained result, it was observed that the pH of the medium has a significant effect on the growth of the W15 isolate and, ultimately, the degradation of the phenol. Also, the optimum degradation rate was at pH 7.0. Many previous studies indicated that the optimum pH for the growth and phenol degradation of *Pseudomonas sp*. was in the range of 7-10, and it's dependent on the bacterial origin (Sarnaik and Kanekar, 1995, Shahriari et al. 2016). Ghaima et al. (2017) showed that the maximum rate of phenol degradation by *P. aeruginos*a was at pH 8. Studies have shown each strain shows the best phenol biodegradation at certain pH. For instance, the best pH range for the biodegradation of phenol by *Klebsiella oxytoca* was 6.8 (Khleifat, 2006). The effect of pH in phenol degradation may be because of its effects on transportation, stimulating the enzymatic activities and nutrient solubility (Lin *et al*. 2010). The pH significantly affects the biochemical reactions required for phenol degradation. Reports indicate that the pH affects the surface charge of the cells of the activated sludge biomass (Aksu and Gonen, 2004).

3.3.3. Effect of nitrogen sources on growth and degradation by W15 isolate

The effect of three organic nitrogen sources, peptone, yeast extract and urea and three inorganic nitrogen sources, namely, sodium nitrate, potassium nitrate, and ammonium chloride, on the growth and degradation of phenol by W15 isolate. The nitrogen sources were supplemented to MS medium containing 1000 ppm phenol and incubated at 37°C in a temperature and pH 7 for 96 hrs. in triplicates, and the results were shown in Fig. (5 and 6). It was observed that the growth of W15 isolates and phenol degradation were affected by the type of nitrogen source clearly. Hence, among the organic nitrogen sources tested, yeast extract was the best source for maximum growth and phenol degradation. The percentage of phenol degradation with the addition of yeast extract was 91.2. %. While urea gave the lowest growth and phenol degradation (70.47 %). The inorganic nitrogen source ammonium chloride recorded the best source for maximum growth and phenol degradation (83.2%), while potassium nitrate gave the lowest growth and phenol degradation (46. 1%). In general, the nitrogen sources, peptone extract, yeast and NH_4Cl exhibited high rates of growth and phenol degradation (more than 70%). The current study demonstrated that the addition of yeast extract in the medium showed the highest phenol removal efficiency of 91.2%, and it also enhanced the growth of strain W15. This might be due to the structure of yeast extract, as it is readily available as amino acids in the mineral salt's medium, which is essential to the phenol degradation process by bacterial cells. (Ochieng *et al.* 2003; Walecka and Walworth, 2006). Ghaima et al. (2017) showed that among all nitrogen sources, yeast extract was found to be the best for growth and phenol degradation.

Table (1): Capability of bacterial isolates from wastewater to grow on different concentrations of phenol

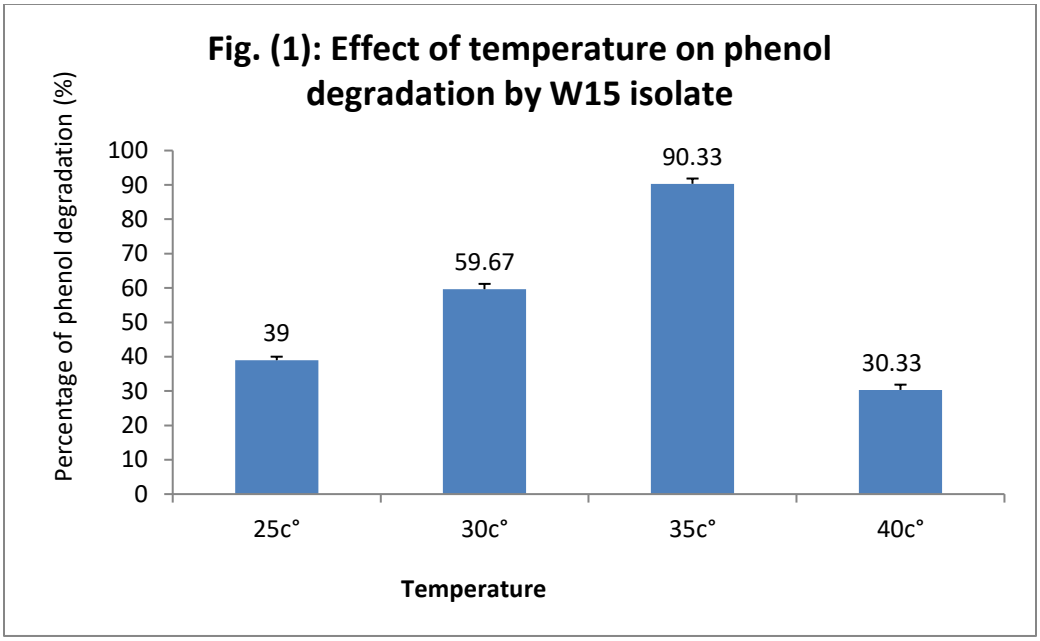
Isolates	Concentrations of phenol ppm						
	Control	**100**	**200**	**300**	**400**	**500**	**1000**
W1	+++	++	+	+	+	-	-
W2	+++	+++	+++	+++	+++	++	-
W3	+++	+	-	-	-	-	-
W4	+++	++	+	-	-	-	-
W5	+++	+++	+++	+++	+++	++	-
W6	+++	+++	+++	+++	++	+	-
W7	+++	-	-	-	-	-	-
W8	+++	-	-	-	-	-	-
W9	+++	+++	+++	+++	++	+	-
W10	+++	+++	+++	+++	++	+	-
W11	+++	++	+	+	+	-	-
W12	+++	+++	+++	+++	+++	++	-
W13	+++	-	-	-	-	-	-
W14	+++	+++	+++	+++	+++	++	-
W15	+++	+++	+++	+++	+++	+++	+++
W16	+++	++	+	-	-	-	-
W17	+++	+	-	-	-	-	-
W18	+++	++	-	-	-	-	-
W19	+++	+++	+++	+++	++	++	-
W20	+++	+	-	-	-	-	-
W21	+++	+	-	-	-	-	-
W22	+++	+	-	-	-	-	-
W23	+++	-	-	-	-	-	-
W24	+++	-	-	-	-	-	-
W25	+++	-	-	-	-	-	-
W26	+++	-	-	-	-	-	-
W27	+++	++	-	-	-	-	-
W28	+++	+	-	-	-	-	-
W29	+++	+++	+++	+++	+++	++	-
W30	+++	-	-	-	-	-	-

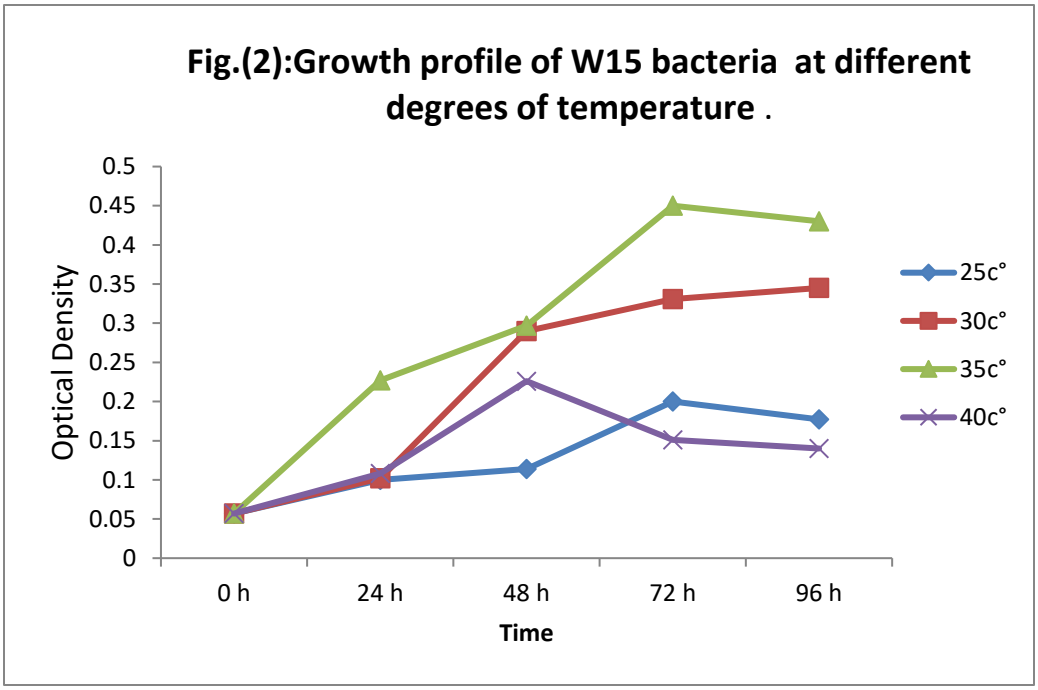
Very good (+++) Good growth (++) Moderate (+) No growth (-)

Table (2): Screening of phenol degrading isolates

Isolates	% Degradation of phenol			
	After 24 hrs.	**After 48 hrs.**	**After 72 hrs.**	**After 96 hrs.**
W2	25	34	45	55
W5	34	45	56	57

W9	33	39	59	71
W12	35	43	77	81
W14	32	39	47	57
W15	56	65	93	95
W19	24	32	34	55
W29	27	33	45	49





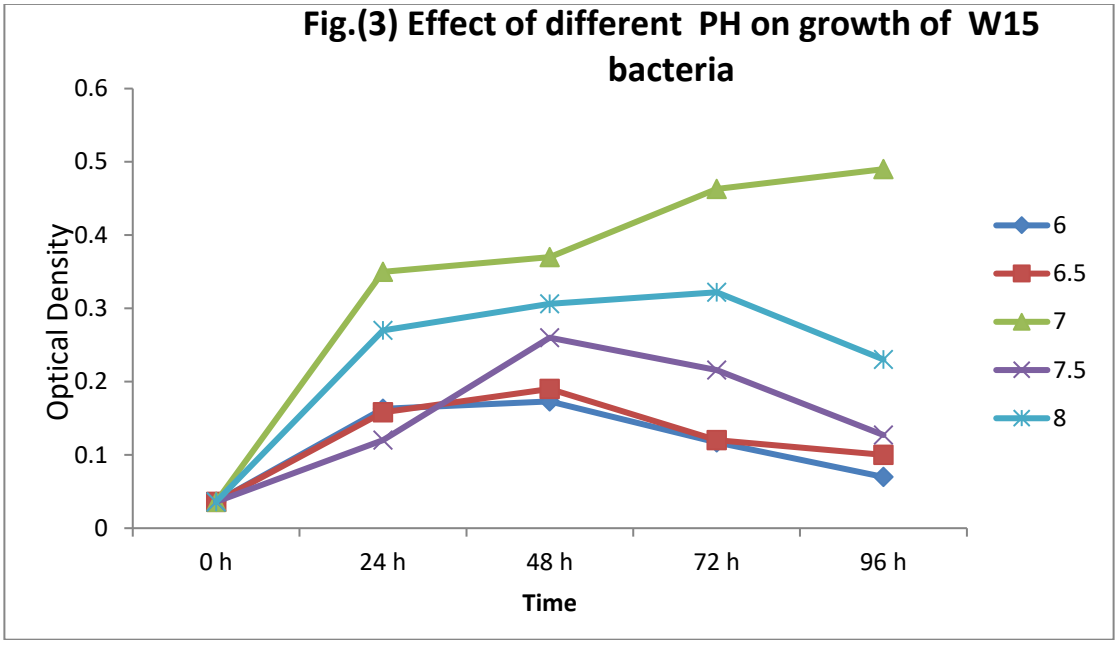


Fig.(3) Effect of different PH on growth of W15 bacteria
Optical Density
0.6
0.5
0.4
0.3
0.2
0.1
0
0 h
24 h
48 h
72 h
96 h
Time
6
6.5
7
7.5
8

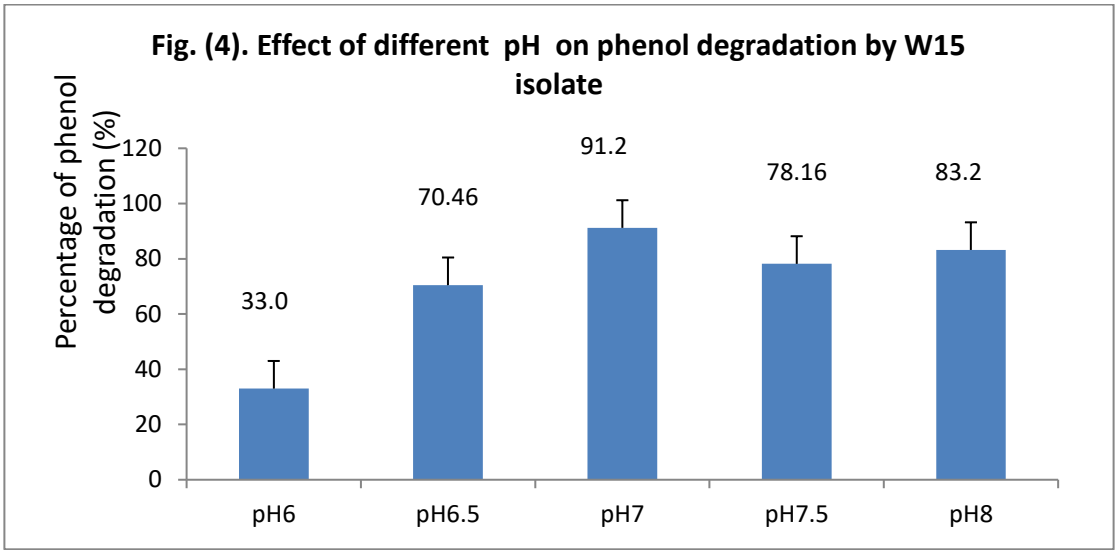


Fig. (4). Effect of different pH on phenol degradation by W15 isolate
Percentage of phenol degradation (%)
120
100
80
60
40
20
0
33.0
70.46
91.2
78.16
83.2
pH6
pH6.5
pH7
pH7.5
pH8

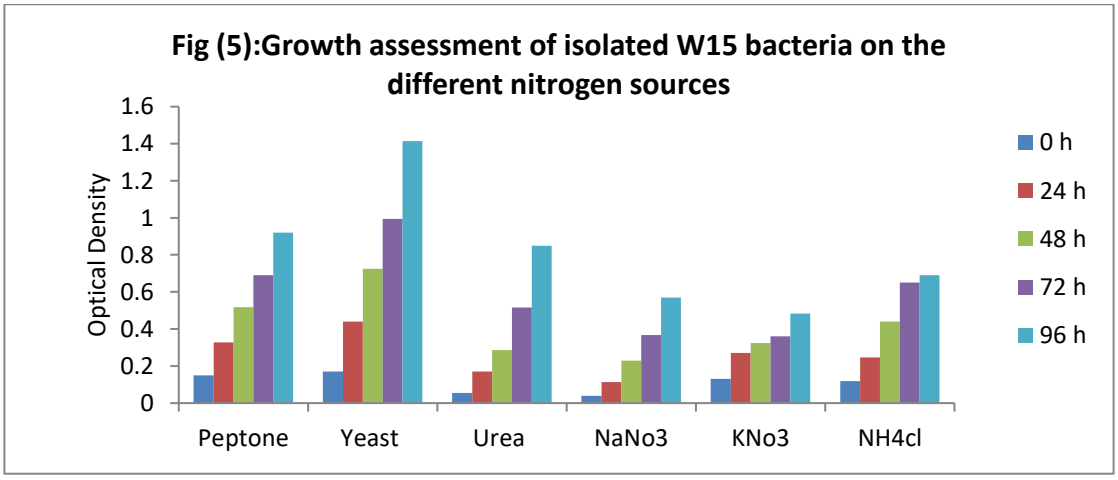
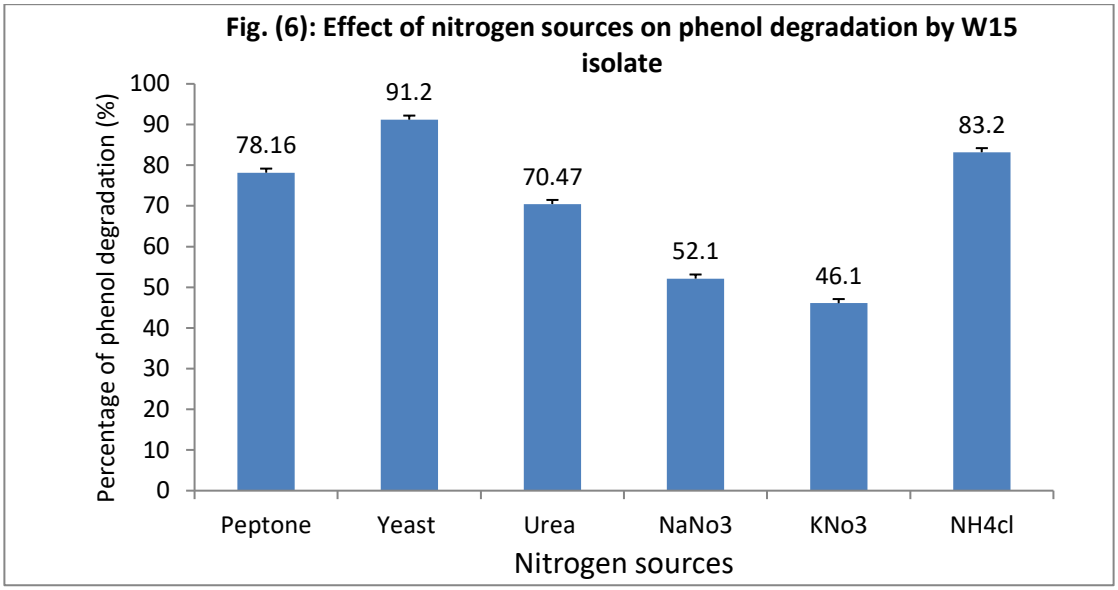


Fig (5):Growth assessment of isolated W15 bacteria on the different nitrogen sources
Optical Density
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Peptone
Yeast
Urea
NaNo3
KNo3
NH4cl
0 h
24 h
48 h
72 h
96 h



4. Conclusion

The aim of this study was to isolate phenol degrading bacteria from wastewater. The results indicated that from 30 bacterial isolates were appeared eight tolerant bacterial isolates that have ability to grow and degrade phenol at higher concentrations of phenol. Among the eight higher phenol degrading bacteria, only one isolate (W15) could be tolerated up to 100 ppm of phenol concentrations grow and degrade 94% of phenol within 72 hrs.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers

References

[1] Ahmad SA, Syed MA, Arif NM, Abdul Shukor MY, Shamaan NA (2011).Isolation, identification and characterization of elevated phenol degrading Acinetobacter sp. strain AQ5NOL.

[2] Ahmad, N., I. Ahmed, A. Shahzad, N. Khalid, F. Mehboob, K. Ahad and G.M. Ali. (2014). Molecular identification and characterization of *Pseudomonas sp*. NCCP-407 for phenol degradation isolated from industrial waste. J. Korean Soc. Appl. Biol. Chem.

[3] Aksu Z and Gonen F. (2004). Biosorption of phenol by immobilized activated sludge in a continuous packed bed: prediction of breakthrough curves, Process Biochem, 39: 599–613.

[4] Basha KM, Rajendran A, Thangavelu V (2010). Recent Advances Bioresource Technology 99, 205–209.

[5] Carmona, M., A.D. Lucas, J.L. Valverde, B. Velasco and J.F. Rodríguez. (2006). Combined adsorption and ion exchange equilibrium of phenol on Amberlite IRA-420. *Chemical Engineering Journal, 117*, (2), 155-160.

[6] Deng ,T Hongyu W and Kai Y(2018). Phenol biodegradation by isolated Citrobacter strain under hypersaline conditions.Water Sci. Technol;77(1-2):504-510.

[7] El-Sayed, W.A., M.K. Ibrahim, M. Abu-Shady, F. El-Beih, N. Ohmura, H. Saiki and A. Ando (2003). Isolation and characterization of phenol-catabolizing bacteria from a coking plant. Biosci. Biotechnol. Biochem., 67 (9): 2062-2029.

[8] Gami, A.A., M.Y. Shukor, K.A. Khalil, F.A. Dahalan, A. Khalid and S.A. Ahmad (2014). Phenol and its toxicity. J. Environ. Microbiol. Toxicol., 2: 11–24.

[9] Geng, A., Soh, A., Lim, C. and Loke, L. (2006). Isolation and characterization of a phenol degrading bacterium from an industrial activated sludge. Appl. Microbiol. Biotechnol., 71, 728–735.

[10] Ghaima K. K.; Raha ,B. S. and Mohamed, M. M. (2017). Biodegradation of phenol by *Pseudomonas aeruginosa* isolated from soil contaminated with diesel fuel. Global J. Environ. Sci. Manage., 2 (3):249-256.

[11] Govindarajalu, K. (2003). Industrial effluent and health status a case study of noyyal river basin. Proc. 3rd Ed. Int. Conf. Environ. And Health, Chennai, India, 150-157.

[12] Khleifat, K.M. (2006). Biodegradation of phenol by Ewingella Americana: Effect of carbon starvation and some growth conditions. Process Bioch., 41(9):2010-2016.

[13] Klibanov AM, Alberti BN, Morris ED, Felsin LM (1980). Enzymatic removal of toxic phenols and anilines from wastewaters. J. Appl. Biochem. 2: 414-421

[14] Kotreshha, D. and G.M. Vidyasaga (2014). Degradation of phenol by novel strain Pseudomonas aeruginosa MTCC4997 isolated from petrochemicalindustrial effluent. Int. J Microb. Res. Technol., 2 (3): 7-15.

[15] Lazarova, Z. and S. Boyadzhieva. (2004).Treatment of phenol-containing aqueous solutions by membranebased solvent extraction in coupled ultrafiltration modules. Chem. Eng. J. 100:129-138.
[16] Lin, L.X., H. Liu and N.Y. Zhou (2010). MhbR, a LysR-type regulator involved in 3- hydroxybenzoate catabolism via gentisate in Klebsiella pneumoniae M5a1 Microbiol. Res., 165: 66-74.
[17] Lin, S.H.; Chuang, T.S. (1994). Combined treatment of phenolic wastewater by wet air oxidation and activated sludge. Toxicol. Environ. Chem., 44, 243–258.
[18] Loh, K.-C. and S.-S. Chua. (2002). Ortho pathway of benzoate degradation in Pseudomonas putida: induction of meta pathway at high substrate concentrations. Enz. Microbial Technol. 30:620-626.
[19] Lowry, M., A. Nagamani, K. Sreenivasulu and R. Soligalla. (2009): solation and characterization of phenoldegrading soil bacterium Xanthobacter flavus. Bioremed. J. 13:1-6
[20] Mahiudddin, M., A.N.M. Fakhruddin and Abdullah-AlMahin. (2012). Degradation of phenol via meta cleavage pathway by *Pseudomonas fluorescens* PU1. ISRN Microbiol.:1-6.
[21] McCall IC, Betanzos A, Weber DA, Nava P, Miller GW, Parkos CA. (2009). Effects of phenol on barrier function of a human intestinal epithelial cell line correlate with altered tight junction protein localization. Toxicol Appl Pharmacol. 15;241(1):61-70.
[22] Mohamed AD, Mohamed O, Abdel R and Osama AA (2003). 4- Aminoantipyrine as a chromogenic agent for aromatic amine determination in natural water. Water Pollution Control, National Research Centre; Dokki, Cairo, Egypt.
[23] Mohite, B., R. Jalgaonwala, S. Pawar and A. Morankar. (2010). Isolation and characterization of phenol degrading bacteria from oil-contaminated soil. Inn Roman Food Biotechnol. 7:61-65.
[24] Nair, C. I., Jayachandran, K. and Shashidhar, S., (2008). Biodegradation of phenol. African Journal of Biotechnology. 7 (25):4951-4958.
[25] Nuhoglu, N. and Yalcin, B., (2005). Modeling of phenol removal in a batch reactor. Process Biochemistry 40 (3-4): 1233-1239.
[26] Ochieng, A. Odiyo, J.O. and Mutsago, M. (2003). Biological treatment of mixed industrial wastewater in a fluidized bed reactor. J. Hazard. Mater., B96, 79-90.
[27] Park JS, Brown MT, Han T. (2012). Phenol toxicity to the aquatic macrophyte Lemna paucicostata. Aquat Toxicol. 15;106-107:182-188.
[28] Rehfuss, M. and J. Urban. (2005). *Rhodococcus phenolicus sp*. nov., a novel bioprocessor isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. Syst. Appl. Microbiol. 28:695-701.
[29] Saravanan, P., Pakshirajan, K., Saha, P., (2008). Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor. Bioresource Technology, 99, (1), 205-209.
[30] Sarnaik, S. and Kanekar, P. (1995). Bioremediation of color of methyl violet and phenol from dyeindustry waste effluent using *Pseudomonas spp*. Isolated from factory soil. Appl. Bacteriol., 79: 459-469.
[31] Shahriari M. M., Safaei, N. and Ebrahimipour, G.H. (2016). Optimization of phenol biodegradation by efficient bacteria isolated from petrochemical effluents. Global J. Environ. Sci. Manage., 2(3): 249-256.
[32] Tay ST, Moy BY, Maszenan AM, Tay JH. (2005). Comparing activated sludge and aerobic granules as microbial inocula for phenol biodegradation. Appl Microbiol Biotechnol. 67(5):708-713.
[33] Tuah, P.M., N.A.A. Rashid and M.M. Salleh. (2009). Degradation pathway of phenol through ortho-cleavage by Candida tropicalis Retl-Cr1. Borneo Sci. 24:1-8.
[34] Walecka, H, C.M. and Walworth, J.L. (2006). Assessment C:N ratios and water potential for nitrogen optimization in diesel bioremediation. Bioremediation J., 10, 25-35
[35] Wu, Z. G., Wang, Y. M., Xing, Z. H., Wu, X. W., and Song, S. S. (2005). Study on degrading phenol by immobilized *Ralstonia metallidurans* CH34. Microbiology 32, 31–36.
[36] Yan, J., Jianping, W., Jing, B., Daoquan, W., and Zongding, H. (2006). Phenol biodegradation by the yeast Candida tropicalis in the presence of m-cresol. Biochem. Eng. J. 29, 227–234.
[37] Zhai, Z., H. Wang, S. Yan and J. Yao. (2012). Biodegradation of phenol at high concentration by a novel bacterium: Gulosibacter sp. YZ4. J. Chem. Technol. Biotechnol. 87:105-111.