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Biodegradation Effect of some Bacterial Isolates on some Endocrine Disruptors (EDCS)

Amna M.A. Al-Hashimi*
Department of Biology, College of Science, Mustansiriyah University, IRAQ.
**Author email: dr.amnaalahshimi@uomustansiriyah.edu.iq*

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Abstract

Endocrine disruptors [EDCs] raised a certain concern for living health began since last century, via interfere with natural hormone functions and produce reversible or irreversible biological effects. Bisphenol (BPA) is an organic compounds that causing human health risks. Different bacterial spp. has biodegradation ability for wide range of EDC. Twenty water samples were collected from different area around Baghdad city. Four bacterial isolates were isolated included [*Pseudomonas aeruginosa*, *Pseudomonas luteola*, *Proteus penneri*, and *Escherichia coli*]. All isolates were characterized morphologically and biochemically. The effect of substrate tolerate bisphenol (BPA) [5 mg/ ml] using well diffusion method were investigated. The biodegradation effect of bacterial isolates on breakdown BPA and its derivatives using UV vis spectrophotometer were studied and comparing in various incubation time and temperatures to assess the effect of physical conditions on bacterial ability of BPA degradation. *P. penneri* showed a significant ability to resist para-phenol and meta-phenol, while highly sensitive to ortho-aminophenol and paracresol. *P. aeroginosa* was sensitive to para and meta- aminophenol, while resist to degraded phenol compounds ortho aminophenol and para cresol]. *P. luteola* was resistance for all phenolic compounds, while E.coli showed sensitivity for para cresol only.

Biodegradation effect data showed a significant effect for *P. luteola* after 15 days of incubation followed by *P. penorri* and *E.coli.* to degrade phenolic compounds. Data demonstrated that *P. luteola* has an obvious degradation effect for BPA after 15 days of incubation. However, *P. aeruginosa* showed an absolutely different behavior toward BPA which showed an raising absorbance after 15 days of incubation. The aim of this study is to identify the ability of different local bacterial isolates to breakdown the phenol compounds and its derivatives in surface water. This has certain impact on the water purification and industry to provide safe water for consumers.

Keywords: Bisphenol, Bacterial isolates, EDC, Degradation.

الخلاصة

أثارت المواد الكيميائية المسببة اختلال الغدد الصماء مخاوف معينة بشأن صحه الانسان والتي بدأت منذ القرن الماضي،تأثر هذه المواد على وظائف الهرمون الطبيعي ومسببه آثار بيولوجية قابله للالنعكاس على صحه الإنسان والكائنات الحيه] . مركب البسفينول هو مركب عضوي يسبب مخاطر على صحة الإنسان. جمعت 20عينه من الماء من مناطق مختلفه من بغداد. تم اختياراربع عزلات بكتيريه لأجراء اختبارات االقدره على تحليل المركبات الفينوليه [*Pseudomonas aeruginosa*, *Pseudomonas luteola*, *Proteus penneri*, and *Escherichia coli*]. شخصت جميع العزلات البكتريه على الصفات المظهريه و الفحوصات البايو كيمائيه . تمت دراسة تأثير البسفينول [5 ملغم / مل] باستخدام طريقة النشر على العزلات البكتريه. كما وتم دراسة تأثير التحليل الحيوي للعزلات البكتيرية على البيسفينول ومشتقاته باستخدام جهاز المطياف الضوئي للأشعة فوق البنفسجية ومقارنت النتائج في ظروف مختلفه [مده الحضن ودرجة الحرارة] لتقييم تأثير الظروف الفيزيائية على قابليه العزلات البكتيريه المنتخبه على تحليل البيسفينول. أظهرت بكتريا *P. penneri* قدرة كبيرة على مقاومة para-phenol and meta-phenol، في حين كانت حساسه للغاية ل ortho- aminophenol and para- cresol. بينما كانت بكتريا *P. aeruginosa* حساسه لل و para aminophenol meta-phenol بينما كانت مقاومه لمركبات الفينول الاخرى ortho aminophenol, and para cresol. في حين اظهرت بكتريا *Ps. Luteola* مقاومه عاليه لكل مركبات الفينول. بينما اظهرت بكتريا *E.coli* حساسيه للpara- cresol فقط.

وأظهرت بيانات تأثير التحلل البيولوجي تأثير كبير لبكتريا *Ps. luteola*بعد 15 يوما من الحضانة تليه ا *Proteus penneri* و *E.coli*. اهميه الدراسه هي امكانيه تطبيقها واستخدامها في مجالات صناعه وتنقيه المياه والتقنيات الحيويه

لتوفير حياه افضل للانسان. الهدف من هذه الدراسة هو التعرف على قدرة انواع مختلف من البكتيريا المعزولة محليا على تحليل مركبات الفينول ومشتقاته المثبطه لعمل الغدد الصماء.

Introduction

Endocrine distrptour (EDCS) include various domestic products and manufacturing crops which have amplified risks of environmental pollution such as bisphenols, alkylphenols, diadzein, genistein, lindane, paraquat, benzoic acid, dibutylphthalates, diethylhexylphthalates and diethylstilbesterol. Most used in pesticides, plastics, cosmetics, electrical transformers etc. That might interfere with the synthesis, storage, release, secretion, transport, elimination, binding of endogenous hormones. Consequently, effect on modify purpose of brain endocrine glands. Bisphenol (BPA) is an organic compound that contains two phenol groups and is synthesized through the condensation of one part acetone with two parts [1].

Recently , understanding types of chemicals which might interfere with the action of endocrine gland has exceeded because excessive exposure to chemicals from domestic use which are resulting from different sources such as pharmaceuticals, personal care products, electronics, food packaging, clothing, metals, and current-use pesticides, which increasingly raise a serious concern for human life [2].

It has reported that bio-accumulative effect for both human and wildlife exposures to EDCs consist of complex mixtures of chemicals appear for long time exposure. However, there is partial explanation mechanism of chemical compounds which seriously hurt the endocrine system, even though combined exposures can result into greater risk than exposure to single agent at a time [3].

Bisphenol A (BPA): is an estrogenic compound with (228 Da) it characterized as a monomer which can make a polymer to manufacture the plastic materials lining metal cans and pots. BPA is also added for many other domestic materials such as bottled water and water pipes [4]. Brominated BPA is one of the major flame retardants and is also a known endocrine-disrupting chemical (EDC). It has been reported BPA has reduce the role of steroid hormones in organs and BPA has lately been exposed to provoke thyroid glands hormones [4] and antagonize androgen action [5]. It has been reported that EDCs are environmental estrogens therefore, that feminization is often observed in the environment. In addition to estrogen-like compounds, some other endocrine disrupter is known to show anti-estrogenic activity, or thyroid hormone activity disruption of the endocrine system will lead to failure of reproduction and subsequently to loss of living forms [6]. Research illustrated that low concentrated of EDCs was found in rivers and surface water, whereas accumulative EDCs found in soils nearby industrial areas discharged directly to rivers.

Most of EDCs lyses in water, therefore, the concern of chemical contaminated drinking water are very low. However, still there is a good effort spent by many of big water industries plan to remove EDCS from sewage and waste water. The results are very encouraging and show that treatment is highly effective at removing EDCs [6].

EDC reached surface water through manufactured discharged from bad hygienic recycling process [7, 8, 9].

An ancient water treatment method was depending on chemical reactions to remove EDCs from water but, recently biodegradation has been extensively employed in water treatment plan. The allowance concentration of Phenol compounds in surface water is 1 mg/l. Currently, phenolic compounds extract using traditional precipitation/coagulation, osmosis, ion-exchange, ultra filtration, electro dialysis, electrochemical degradation, floatation, etc., which are expensive and incompetent methods. However, these methods might produce a poisoning by product which required a further processing to remove it from final product [10], microbial degradation has been studied as an alternative approach to remove EDCS from the environment because it's cheap and provide final extraction for long lasting [11]. Recently, bioremediation of microbial systems might be latent tool to deal with environmental pollutants [12].

Microbial degradation of phenol has been actively studied and these studies have shown that phenol can be aerobically degraded by

wide variety of fungi and bacteria cultures such as *Candida tropicalis* [13] *Acinetobacter calcoaceticus* [14], *Alcaligensmeutrophus* [15], *Pseudomonas putida* [16], [17].

Lately, researches concentrated on biodegradation throughout selective degrading bacteria isolated form environment. An increase interest has risen to process EDCs naturally using bacteria metabolic pathways. Researches argued that degradation effect of bacteria originated form sediment more than that isolated form surface water [17].

The aim of this study is to identify the ability of different local bacterial isolates to breakdown the phenol compounds and its derivatives from surface water. Which might become certain impact on water purification and industry to provide safe water for consumes.

Materials and Methodologies

Samples collection

Twenty water samples were collected in sterile plastic containers (500 ml) from Tigris River at different regions (Shorjah, Diyala bridge, Latefiah, Radwaniah, Dewaneah and Aljazeerha). Samples were incubated in refrigerator at (4°C) until day of experiments.

Bacterial isolates and Identification

Three L of base mineral medium (BMM) were prepared [1] 5ml water sample were added to 5 ml of [BMM] media [25.17 g K2HPO4, 1.70 g KH2PO4, 1.63 g NH4Cl, and 10 ml of a salt solution. One liter of salt solution contained 8.5 g MgSO4, 5g MnSO4, 5g FeSO4, and 0.3g CaCl2. The initial pH value of media was 7.2 [18] to prepare stock solution, media incubated for 24h at 37° C, 120 rpm. One hundred Ml of raw samples water were streaked on nutrient agar plates to identify bacterial isolates.

Fifty ml from each water samples were added to 750 ml of BMM media then the volume raised up to one liter, inoculated flasks were agitated by orbital shaker (120 rpm at 30°C for 72 h). Two ml culture medium was transferred to another 50 ml of fresh culture medium, and cultivation was carried out on the same condition for 2 to 3 times. One hundred µl of obtained batches were streaked on nutrient agar plates. All plates were stored for same conditions; bacterial isolates were preserved on nutrient agar slant at 4°C [18]. The purified isolated bacteria used to undergoes biochemical and Api 20 for further identification [19] [20].

Toxigenicity of phenolic compounds against bacterial isolates

Bacterial isolates re-cultured on nutrient agar using well diffusion method to determine the toxicity of phenol compounds on bacterial viability. One microliter of [1.para aminophenol, 2.ortho aminophenol, 3.meta aminophenol, 4.para-crysol] [5 mg/ml] inoculated in each well and incubated for 24h at 37 $_{o}$C to identify effect of these compounds on bacterial viability [19,20].

Biodegradation of phenolic compounds by bacterial isolates

Five ml of bacterial isolates suspension mixed with 5 ml of raw water samples [spiked with 1.para aminophenol, 2.ortho aminophenol, 3.meta aminophenol, 4.para-crysol] [5 mg/ml] separately to investigate the activity of different bacterial isolates on degradation phenolic compounds and its derivatives using the UV-vis spectrophotometer at different incubation conditions [15days time and 5 and 45 C temperatures] [21].

Results and Discussion

Bacterial Isolation & Identification

A total of 20 bacterial isolates were obtained from raw water samples. Only 4 isolates: *Proteus penneri*, *Pseudomonas aeruginosa*, *Escherishia coli*, and *Pseudomonas luteola* [4] were selected for further examination and tested their degrading efficiency under different cultivation conditions.

Toxigenicity of phenolic compounds against bacterial isolates

Four isolates *Proteus penneri, Pseudomonas luteola, Psedomonas aeuroginosa, and Escherishia coli* were investigated for ability to

grow in the presence of phenol compounds in culture medium. Results showed that most of bacterial isolates were highly resistance to grow in the presence of phenol chemicals as showed in Figure 1B. *E.coli* was highly resisted for ortho-aminophenol and paracresol Figure [1C].

P. penneri showed a significant sensitivity to para-aminophenol and para- cresol, while highly resistance to ortho-aminophenol and meta-phenol [1A], that might belong to bacterial isolates does not have enzymes which necessary for degraded these compounds. *E.coli* showed little sensitivity to only orhto aminophenol [1D], while, it could degraded three phenol compounds [para, meta aminophenol, and para-cresol]. *P. luteola* showed ability of breakdown degradation for para-cresol, but it appeared sensitivity to other phenol compounds para, and aminophenol Figure [1C]

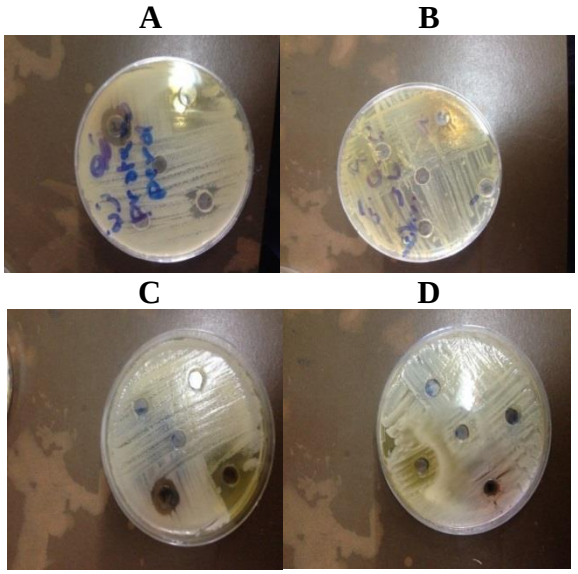


Figure 1: Toxigenicity of phenol compounds (1.para aminophenol, 2.ortho aminophenol, 3.meta aminophenol, 4.para-cresol] against bacterial isolates *P. penneri, B. Pseudomonas luteola C. Escherishia coli and D. Proteus penneri* .

Degradation of Phenolic compounds

Data showed a significant effect for bacterial isolates on degradation of phenolic compounds (para aminophenol, ortho-aminophenol, Meta aminophenol, and Paracrysol 5 mg/ml) as shown in Figure 2. Microbial degradation of phenol has been comprehensively studied by [14,15,16] and these studies have come compatible with results obtained which shows 50 % decrease in phenol compounds after 15 days of incubation time. Lately, researches concentrated on efforts on biodegradation throughout selective degrading bacteria isolated form environment. An urgent interest required to process EDCs naturally using bacteria metabolic pathways which degraded EDCs in water, bacterial isolate require 10 to 20 days to reduce the EDCs concentration biologically [17].

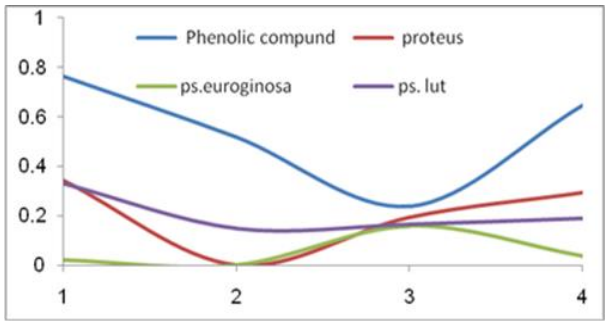
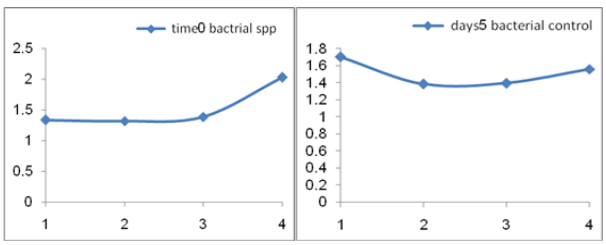


Figure 2: Biodegradation of bacterial isolates *Pseudomonas aeuroginosa, Pseudomonas luteola and Proteus penneri* suspected with (X-axis) water mixed with of (1.para aminophenol, 2.ortho aminophenol, 3.meta aminophenol, 4.para-crysol) separately incubated for 12 days, Y-axis (O.D.).

Effect of incubation time on degradation of BPA

Results reveled there was a significant degradation after incubate the bacterial isolates with phenolic compound. *P. aeurginosa* has a significant reduction in para aminophenol and ortho aminophenol, while there was a less effect on meta aminophenol. *P. penneri* showed a significant 50 % reduction in BPA conc. within 15 days of incubation; however *Pseudomonas aeruginosa* revealed breakdown of BPA to its derivatives after 15 days of incubation at 37 $_{o}$C

In comparison to *P.luteola* which illustrated a constant reduction for the phenolic compounds Figure 3. It has been reported that bacteria can breakdown the BPA available in surface water with average of 20 to 30 days [23].



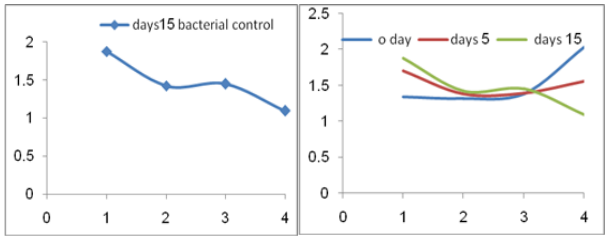


Figure [3] Biodegradation effect of bacterial isolates (X-axis) incubated with BPA at 37 ₀C for (0, 5, 15 days) (Y-axis (O.D.)).

Effect of incubation temperature on degradation of BPA

It is clear that temperature had a significant influence on reduction of BPA in bacterial suspensions *P. luteola* identified a 90% reduction in the BPA concentration after 15 days of incubation at 5°C. Experiments has conducted with same condition but incubated at 45°C to assess the effect of incubation temperature on ability of bacteria to degradation BPA in Figure 4.

Data showed that *P. luteola* had a significant impact on reduction of BPA conc. reached to 95% after 15 days of incubation at 45°C as illustrated in Figure 5, [24] found that 20% of the 0.04 BPA concentration were reduced after incubated at 30°C for 20 days.

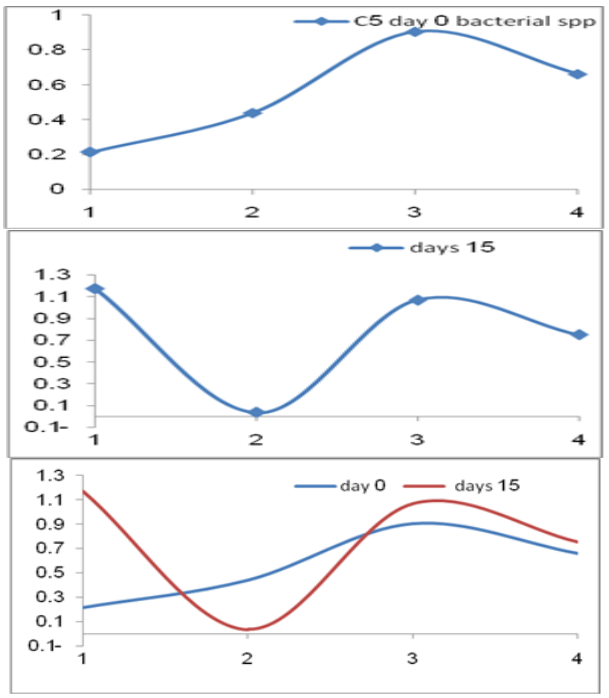


Figure 4: Bacterial isolates (X-axis) mixed with BPA [5 mg/ml] for 15 days incubated at 5 °C Y axis [O.D.].

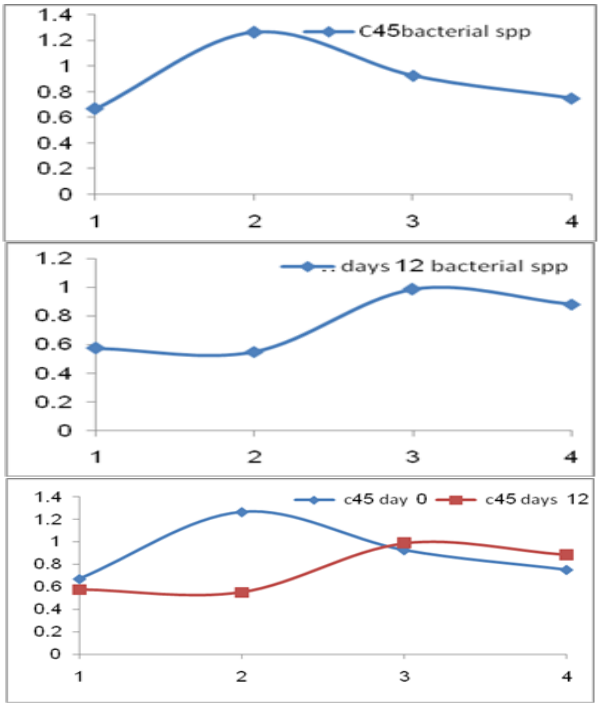


Figure 5: Bacterial isolates (X-axis) mixed with BPA [5 mg/ml] for 12 days incubated at 45 ₀C Y axis (O.D.)

Conclusion

Contamination of environment with hazardous and toxic chemicals is major issues faced by industrialized nations today. This research spotted the light on bioremediation of industrial wastes via using the locally bacterial isolates. This study conducted that *Pseudomonas spp*. and other isolates can be a promising phenol compounds degraders. Hence, bacterial degradation of BPA has remarkable potential for application in the bioremediation and wastewater treatment, especially in detoxification of phenol wastes. The present study mainly focused on bacterial isolates for its dynamics on phenol degradation as a part of developing an innovative microbial technology for cheaper and effective treatment of phenol degradation.

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