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Bioremediation of some types of aromatic compounds by *Candida* spp.

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ABSTRACT

It was isolated and diagnosis of 109 isolates (C. tropicalis) and 85 isolates (C. glabrata) , isolated from water and soil Basra/ Iraq . These isolates have shown its ability to get rid of the bioremediation of environmental pollutants such as hydrocarbons oil Aromatic compounds (indole , 2- methyl naphthalene, 1- methyl naphthalene, biphenyl and dibenzofuran) , as they have the ability to removal or bio – accumulation rates different from those compounds. And during the current study proved that the time has an important role increasing the bioremediation any rate the greater the bosom of microbiology period including yeasts in rural contaminated increased disposal of environmental pollutants and vice versa ratio. It excelled as isolates removing compound indole final 100% lap within 24 h. and removed three compounds final during 48 h. incubation.

INTRODUCTION

Petroleum - based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [1]. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution[2]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations[3]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutant including the products of petroleum industry[4]. In addition, bioremediation technology is believed to be noninvasive and relatively cost-effective[5]. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [6].and is cheaper than other remediation technologies [7].

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) [8]. Different factors influencing hydrocarbon degradation have been reported by Cooney et al. [9]. One of the important factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds bind to soil components, and they are difficult to be removed or degraded [10]. Hydrocarbons differ in their susceptibility to microbial attack. The susceptibility of

hydrocarbons to microbial degradation can be generally ranked as follows: linear alkanes >branched alkanes >small aromatics >cyclic alkanes [6; 11]. Some compounds, such as the high molecular weight polycyclic aromatic hydrocarbons(PAHs), may not be degraded at all [12].

In recent years, various technologies have emerged in order to manage oil residues and effluents contaminated with hydrocarbons. Bioremediation is one of the most extensively used because of its low cost and high efficiency [13;14]. Biodegradation of hydrocarbons by natural populations of microorganisms is the main process acting in the depuration of hydrocarbon-polluted environments. The mechanism has been extensively studied and reviewed [15;8;16;17;14;18]. The utilization of n-alkanes by yeast as a sole carbon and energy source has been reviewed by [19] and [20].

In this study different combinations of microorganisms were used to evaluate the best combination for efficient removal of aromatic compounds.

1- Species of isolates :
109 isolate to the yeast *Candida tropicalis* and 85 isolate to yeast *C. glabrata* from water and soil of environmental of Basrah / Iraq.

MATERIALS AND METHODS

Yeast Isolates: Two species of *Candida* viz *C. tropicalis* and *C. glabrata* previously isolated from water and sediments of Basrah water bodies (unpublished data) were used in this study. Yeast grown on PDA medium and store in refrigerator until used.

Preparation concentration of crude oil to the test by yeasts.
Use the crude oil from the rivers field life of a specific gravity for the purpose of removal biological experiments by yeasts were exposing crude oil for weathering snapped 30 g of it and put it in flask 100ml and then subjected to evaporation at a temperature of 40c. for a period of five days where measurement of weight every 12h. for the first day and during which then once every 24h. and be followed by the lack of difference in weight weathering process is complete guide [21], and then sterilizes crude oil [22;23;24], after used the concentration of 0.2g / 100ml of the metal media of yeasts.

Susceptibility testing of yeasts remove or the accumulation of aromatic compounds.
Preparation has been the concentration 0.2g/100ml from crude oil by 25 flasks ,one flask as control without adding yeast isolate , and the rest divided into two groups of 12 each isolate and for the isolation of time 24h. and 48h. where it was added 0.5g of yeast in each flask at room temperature on a rotary shaker at 120 rpm . At the end of incubation duration , the biomass was separated by centrifugation at 4000 rpm for 30 minutes and supernatants were separation of the into Alfa tic and aromatic compounds[25] , left to dry and then measured the concentration of compounds by GC device.

Equation calculating the proportion of compounds removed:
Where as: [26].

$$R\% = ((C_0 - C_1) / C_0) * 100$$

R= The percentage of removal.
C_0= The concentration of compounds in the primary solution.
C_1= The concentration of compounds in the final solution.

RESULTS AND DISCUSSION

The results showed the ability of some species of yeasts isolated(*C. tropicalis* & *C. glabrata*) on biological treatment to remove environmental pollutants or aromatic compounds(indole, 2- methyl naphthalene, 1- methyl naphthalene, biphenyl and dibenzofuran) that present in water and sediments. These species were involved in the process of bio – accumulation of these elements , and it was found through statistical analysis at the level of probability 0.05.

2- Susceptibility testing of yeasts remove or the accumulation of aromatic compounds.

As showed the decomposition of a standard container for the sample on the concentration of crude oil without the yeasts, by using aromatic compounds standard solution that contains five compounds (indole, 2-methyl naphthalene, 1- methyl naphthalene, biphenyl and dibenzofuran). As stated by table (1) and form (1).

The results showed that isolate *Candida tropicalis*, their ability to biodegradable compounds aromatic oil during 24h. as stated by table (2), form(2).

The results also showed increased susceptibility decomposition of petroleum compounds cuddling up period as stated by table (3, 5), form (3, 5).

The results showed that isolate *Candida glabrata* , her ability to biodegradable compounds aromatic oil during 24h. as stated by table (4), form(4).

The results showed that in the case of the integration of two types of isolates leads to increased susceptibility analytical oil for compounds as stated by table (6,7), form(6,7).

One major obstacle that has slowed the implementation of microbial enhanced oil recovery has been the difficulty in isolating and/or engineering microorganisms that can survive the harsh environment of the oil reservoir.

Crude petroleum oil and hydrocarbon degradation has been analyzed for several fungal and bacterial species, showing variable potentialities [27; 28; 29; 30].

However, only few works referred to the isolation and characterization of yeast strains able to utilize hydrocarbons [27].

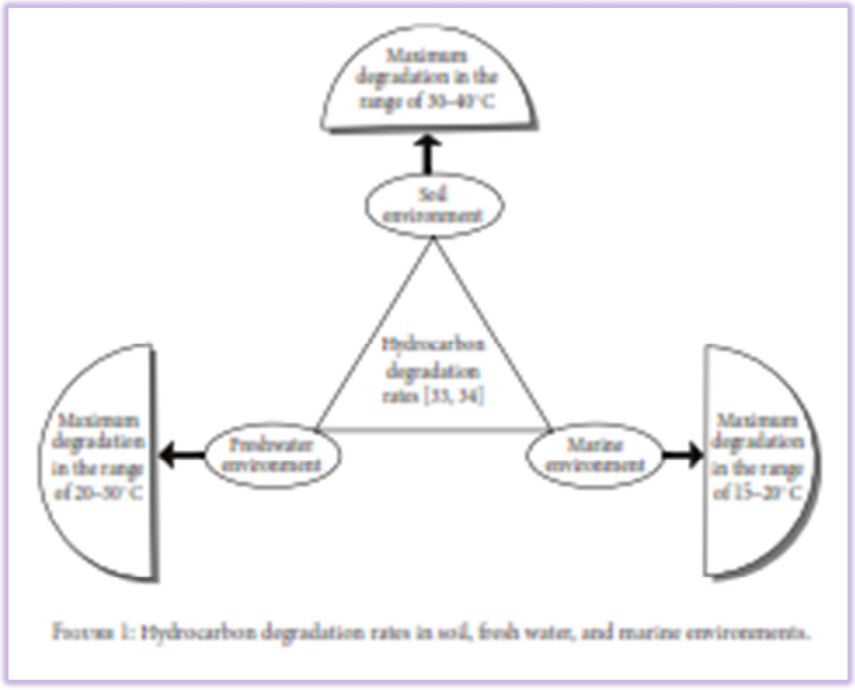


Figure 1: Hydrocarbon degradation rates in soil, fresh water, and marine environments.

Bioremediation in expected to play an important role as environmentally safe and cost-effective response to marine oil spills.

Microbial degradation is the major and ultimate natural mechanism by which one can clean up the petroleum hydrocarbon pollutants from the environment[31; 32; 33].

The recognition of biodegraded petroleum-derived aromatic hydrocarbons in marine sediments was reported by [34].They studied the extensive biodegradation of alkyl aromatics in marine sediments which occurred prior to detectable biodegradation of n-alkane profile of the crude oil and the microorganisms.

Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous Heme-thiolate Monooxygenases which play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds [35]. Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation (Table 1). Higher eukaryotes generally contain several different P450 families that consist of large number of individual P450 forms that may contribute as an ensemble of isoforms to the metabolic conversion of given substrate. In microorganisms such P450 multiplicity can only be found in few species [36].

Cytochrome P450 enzyme systems was found to be involved in biodegradation of petroleum hydrocarbons (Table 1). The capability of several yeast species to use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon and energy is mediated by the existence of multiple microsomal Cytochrome P450 forms. These cytochrome P450 enzymes had been isolated from yeast species such as *Candida maltosa*, *Candida tropicalis*, and *Candida apicola*[37]. The diversity of alkaneoxygenase systems in prokaryots and eukaryotes that are actively participating in the degradation of alkanes under aerobic conditions like Cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., *alk B*), soluble di-iron methane monooxygenases, and membrane-bound copper containing methane monooxygenases have been discussed by [38].

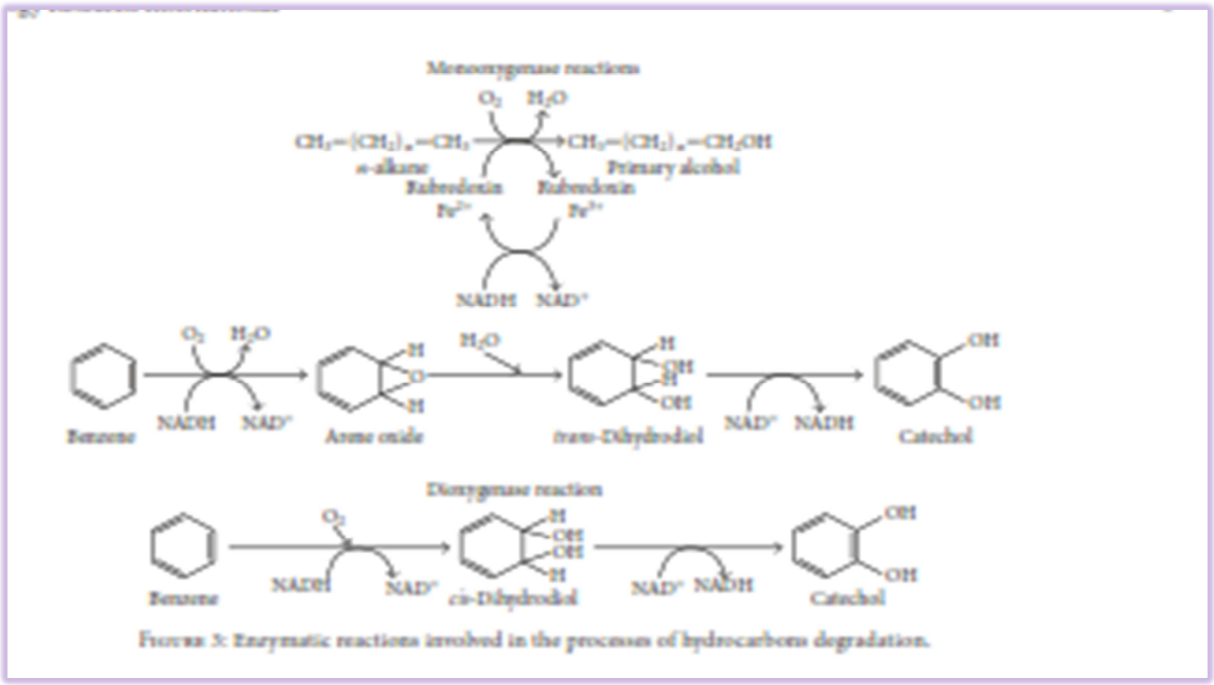


FIGURE 3: Enzymatic reactions involved in the processes of hydrocarbons degradation.

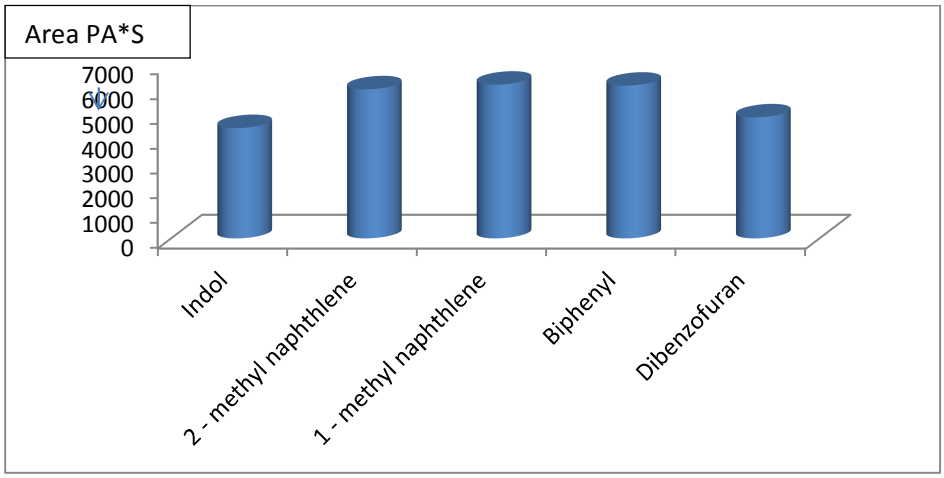
This is what was agreed with the current study on the viability of some species of yeasts isolated from Basra, Iraq on environment degradation or bio – accumulation of petroleum compounds.

Table (1) shows concentration Aromatic compounds in the standard solution (control)

compounds	readings
Indole	4450
2 - methyl naphthalene	5992
1 - methyl naphthalene	6176
Biphenyl	6136.5
Dibenzofuran	4875

Table 1: Enzymes involved in biodegradation of petroleum hydrocarbons.

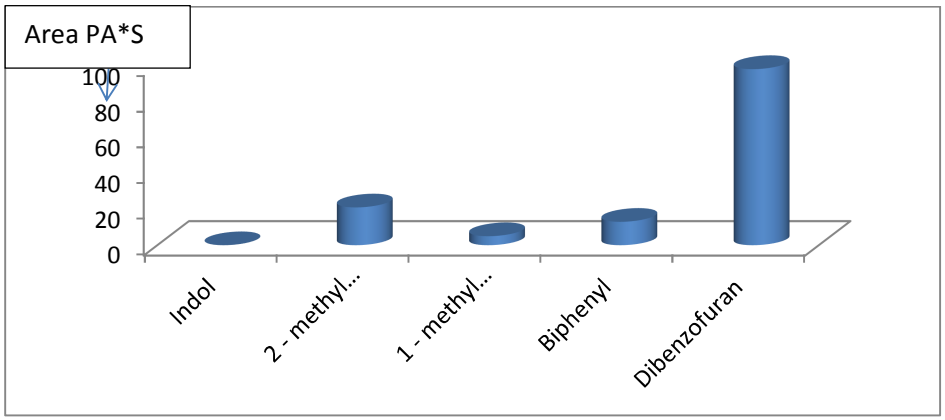
Enzymes	Substrates	Microorganisms	References
Soluble Methane Monooxygenases	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus Methylosinus Methylocystis Methylomonas Methylocella*	McDonald et al. [46]
Particulate Methane Monooxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter Methylococcus, Methylocystis*	McDonald et al. [46]
AlkB related Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	*Pseudomonas Burkholderia Rhodococcus, Mycobacterium*	Jan et al. [47]
Eukaryotic P450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltosa Candida tropicalis Yarrowia lipolytica*	Iida et al. [48]
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter Caulobacter Mycobacterium*	Van Beilen et al. [49]
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*	Maeng et al. [50]



Form (1) shows concentration Aromatic compounds in the standard solution (control)

Table (2) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* after 24h

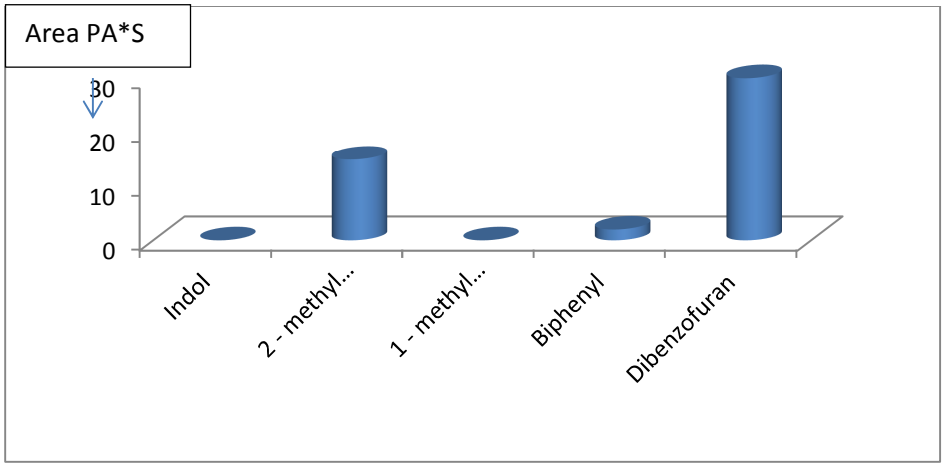
compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	21	99.6
1 - methyl naphthalene	5	99.9
Biphenyl	13	99.7
Dibenzofuran	98	97.9



Form (2) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* after 24h

Table (3) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* after 48h

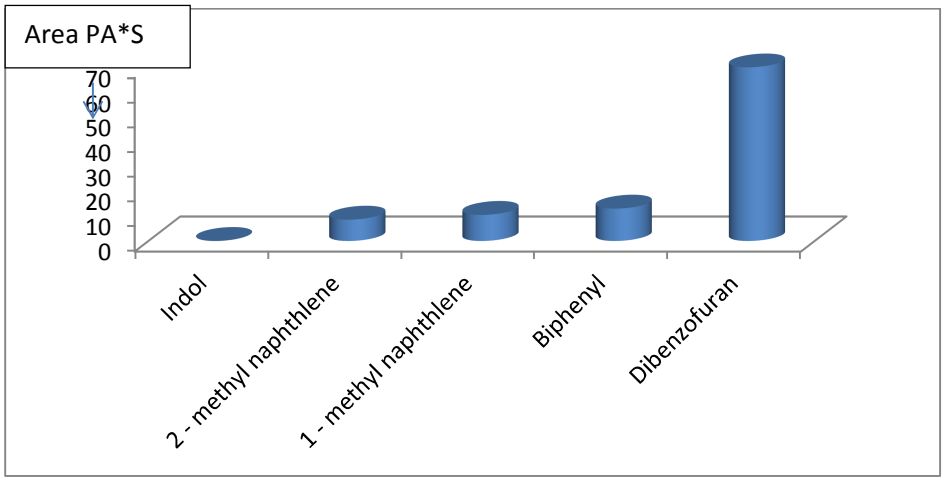
compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	15	99.7
1 - methyl naphthalene	0	100
Biphenyl	2	99.9
Dibenzofuran	30	99.3



Form (3) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* after 48h

Table (4) shows concentration aromatic compounds in the solution with yeast *Candida glabrata* after 24h

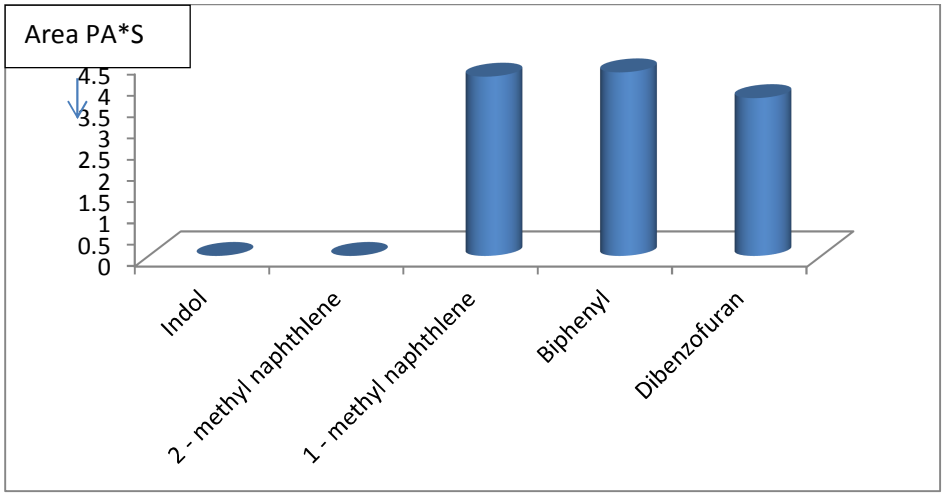
compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	8.5	99.8
1 - methyl naphthalene	10.4	99.8
Biphenyl	12.9	99.7
Dibenzofuran	70	98.5



Form (4) shows concentration aromatic compounds in the solution with yeast *Candida glabrata* after 24h

Table (5) shows concentration aromatic compounds in the solution with yeast *Candida glabrata* after 48h

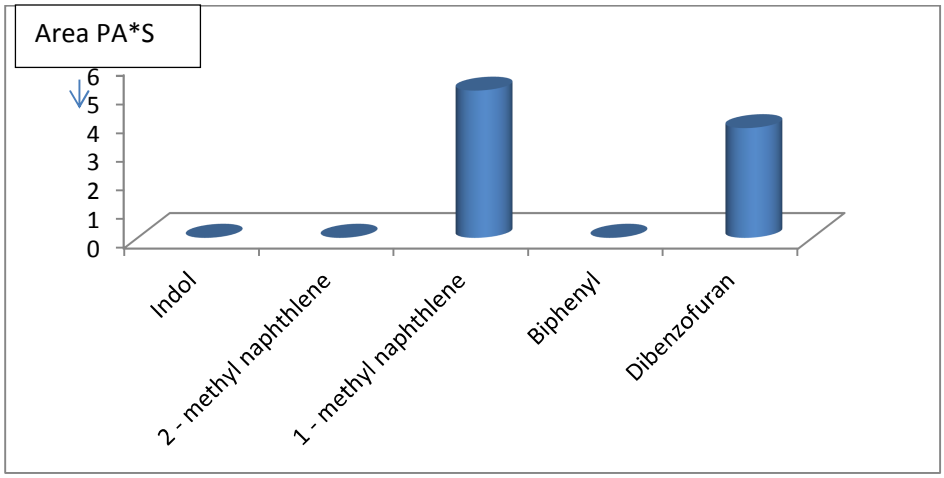
compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	0	100
1 - methyl naphthalene	4.2	99.9
Biphenyl	4.3	99.9
Dibenzofuran	3.7	99.9



Form (5) shows concentration aromatic compounds in the solution with yeast *Candida glabrata* after 48h

Table (6) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* and *C. glabrata* after 24h

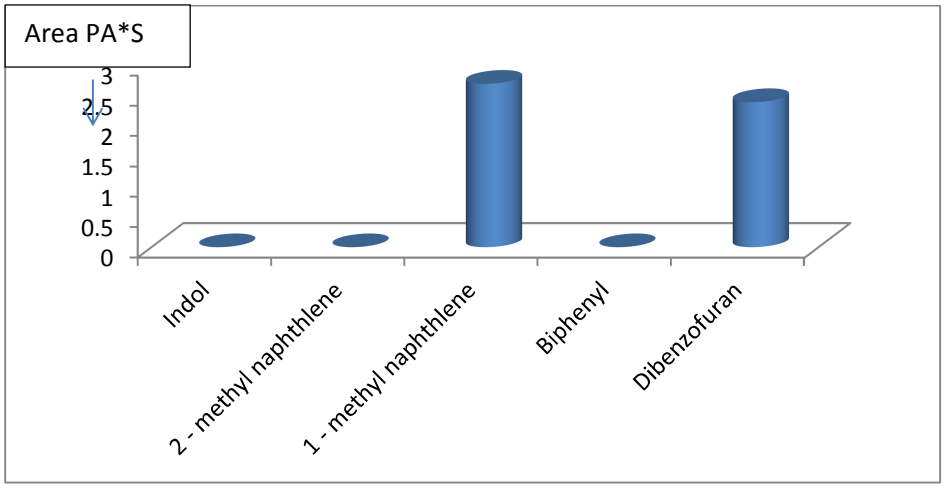
compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	0	100
1 - methyl naphthalene	5.1	99.9
Biphenyl	0	100
Dibenzofuran	3.8	99.9



Form (6) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* and *C. glabrata* after 24h

Table (7) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* and *C.glabrata* after 48h

compounds	readings	Percentages removing
Indole	0	100
2 - methyl naphthalene	0	100
1 - methyl naphthalene	2.7	99.9
Biphenyl	0	100
Dibenzofuran	2.4	99.9



Form (7) shows concentration aromatic compounds in the solution with yeast *Candida tropicalis* and *C. glabrata* after 48h

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