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Stimulation of the hydrocarbon compounds degrading *Saccharomyces rosinii* by low power laser radiation

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ABSTRACT

Saccharomyces rosinii which was isolated from River Nile water samples (Embaba) was selected as the most potent hydrocarbon degrading yeast isolate. The cell suspension (10^4 CFU/ml) was subjected to different irradiation doses using low power He-Ne laser (λ=632.8nm) with varying exposure durations (1, 3 and 6 minutes). Complete degradation of the normal octane, ethyl benzene and their mixture as representative components of their families was achieved after 3 minutes (equivalent dose = 99.6 J/ cm^2) of laser irradiation. The bioremediation of paraffinic and polynuclear aromatic hydrocarbon mixtures was studied using irradiated and non irradiated cell suspensions. Gas chromatographic analysis of the residual substrates indicates the degradation of 98.31% of the paraffinic hydrocarbons and 98.09 % of the polynuclear aromatic hydrocarbons after 30 hrs of incubation. These results demonstrates the superiority of the irradiated yeast isolate and supports its future use in the bioremediation programs of industrial waste water.

Key words: *Saccharomyces rosinii*, He-Ne laser irradiation, paraffinic hydrocarbons, polynuclear aromatic hydrocarbons (PAHs), gas chromatographic analysis.

INTRODUCTION

Environmental pollution with hazardous wastes containing recalcitrant chemicals has become one of the major ecological problems [1]. By the end of the last century a noticeable increase in the pollutants' level was recorded in the aquatic environment of the River Nile [2]. This was attributed to the increased industrial activities around the Nile bank and the continuous discharge of wastes without adequate treatment [3]. Most of these wastes contain hazardous components in small amounts which might show adverse effects on the ecosystem[4] Aliphatic and polynuclear aromatic hydrocarbons (PAHs) form an important class of contaminants due to their toxic, mutagenic and carcinogenic effects [5]and[6] Bioremediation is a technology that offers great promise in converting toxic compounds to non toxic products[7]. Bioremediation of contaminated sites relies on the presence of indigenous degrading microorganisms, the capabilities of which might be stimulated, or on the inoculation of selected microorganisms with desired catabolic traits as in the bioaugmentation techniques [8].

A vast array of microbial species (bacteria, yeast and fungi) can utilize paraffinic and PAHs as a sole carbon and energy sources [9] Temperature plays a significant role in controlling the nature and the extent of microbial metabolism of hydrocarbons [10] .Also the pH of the environment affects the solubility and availability of many toxic and nutritive chemicals [11].

The activation of microorganisms by laser radiation "Biostimultion" is of most interest. The most commonly used sources of laser radiation are the helium-neon laser (He-Ne,λ=632.8nm), the gallium-aluminum laser (Ga-Al ,λ=630-685nm), the helium-neon-arsenate laser (He-Ne-As , λ=780-870nm) and the gallium arsenate laser (Ga-As, λ=904nm) [12]. It was found that the dynamics of cell bioenergetic processes are known to increase after the exposure of the microbial population to laser light of specific wave length [13] **.**Also it was reported that low power laser radiation can brought about acceleration in the cell division and enhanced the protein synthesis in various microorganisms. However higher doses are inhibitory to the microbial growth [14].

In this study our objective aim was to isolate ,identify the most potent hydrocarbon degrading yeast isolate and to investigate the effect of He- Ne laser radiation on stimulating its ability to degrade the hydrocarbon contaminants, so it can be used in future in the bioremediation programs of contaminated water.

MATERIALS AND METHODS

Sampling sites:
Water samples were collected from five locations {Kafr Elelw (site I), El Maasara (site II), El Giza (site III), Embaba (site IV) and El Galatma (site V)} extending for about 60 Km along the main stream of the River Nile in greater Cairo.

2.1 Water samples for chemical assessment:
Water samples for chemical assessment were collected in duplicates in 1 liter dark glass bottles and acidified to pH 2 by using HCl in order to preserve the samples against the microbial action. The samples were then transferred to the laboratory for hydrocarbon extraction and chromatographic analysis [15].

2.2 Water samples for microbiological assessment:
Water samples were collected in duplicates in a screw capped sterile glass bottles, and transferred to the laboratory within 1-2 hours where the isolation step was carried out immediately [16].

2.3 Isolation of the hydrocarbon degrading yeast strains:
The hydrocarbon degrading yeast cultures were isolated by plating 0.1 ml of the water samples on the surface of basal mineral salts (BMS) agar plates [composition (g/L) $NaNO_3$, 3; KH_2PO_4, 1; $MgSO_4$. $7H_2O$, 0.5; KCl, 0.5; yeast extract, 1 and agar, 20 (pH 4.5)] supplemented with 0.5% V/V paraffinic oil as a sole carbon source. After 48 hrs of incubation at 30°C all the hydrocarbon degrading yeast isolates were purified [17]**.**

2.4 Screening of the most potent hydrocarbon degrading yeast isolate:
The screening of the most potent yeast isolate was monitored by recultivation of all the purified isolates (10^4 CFU/ml) on BMS agar media seeded with normal octane (n C_8) and ethyl benzene (EB) (0.5% V/V) individually as carbon sources. After 24 hrs of incubation at 30°C the isolate with the maximum count percentage on both substrates was selected for further studies.

2.5 Characterization of the most potent yeast isolate:
The traditional laboratory were used for the identification of the selected yeast isolate [18] and [19].

2.6 Stimulation of the most potent yeast isolate using He-Ne laser radiation:
The laser used was He-Ne Laser (NEC, Japan) with an output power of 7.3 mW, λ=632.8nm and beam diameter of 1.3mm. The yeast suspension was prepared and subjected to different radiation doses by varying the exposure durations for 1, 3 and 6 mins. Non irradiated suspensions were used as control [12]. Inocula of cell density 10^4 CFU/ml were seeded in 250ml screw capped bottles each containing 100ml BMS broth media adjusted at pH 5.5 and supplemented with n C_8 and EB individually and in combination as sole carbon sources. The residual substrates were extracted and chromatographically analyzed after 4, 8 and 12 hrs of incubation at 35°C under shaking conditions (150 rpm).

2.7 Degradation of paraffinic and polynuclear aromatic hydrocarbons:
The paraffinic and the polynuclear aromatic fractions used were obtained from the distillation cuts of crude oil using the proper solvents (Central Analytical Lab, Egyptian Petroleum Research Institute, EPRI). They were added to the BMS broth individually at the level of 0.5% V/V; the media were inoculated with the selected isolate before and after its exposure to He-Ne laser for 3 mins. After incubation for 12 hrs and 30 hrs under shaking conditions (150 rpm) the residual substrates were extracted and chromatographically analyzed.

2.8 Extraction of residual substrates:
Extraction was done by the addition of chloroform to the culture media in the ratio of 1:3 and shaking vigorously in a separating funnel, the contents were allowed to settle in order to separate the different phases. The organic phase was drawn off and quantified via capillary gas chromatography (CGC) and high performance liquid chromatography (HPLC). The same was done for the control flasks [20].

2.9 Chromatographic analysis:
Capillary gas chromatography (CGC)
The CGC analysis was performed using Agilent 6890 plus gas chromatograph equipped with flame ionization detector (FID) and fused silica capillary column Hp-5 of 30m length, 0.35 mm internal diameter and 0.5um film thickness. The column was heated isothermally at 100 °C for separation of the individual components, while for the paraffinic hydrocarbons it was heated at temperature programming started from 80°C and ended at 250°C with a rate of 5°C min^{-1} and final time 20 min till the end of the program. The injector and detector temperatures are 250°C and 300°C respectively. Nitrogen was used as a carrier gas at a flow rate 2 ml min^{-1}. Degradation was estimated by the integration of the area under the resolved chromatographic profile [21].

High performance liquid chromatography (HPLC)
The Polynuclear aromatic hydrocarbon (PAH) fraction was analyzed using HPLC model Waters 600E equipped with auto sampler Waters 717 plus and dual wave length absorbance detector Waters 2487 set at 254 nm. The condition of operation is as follows: column: LC-PAH of 15cm length, 4.6mm internal diameter, 5mm particle size. The mobile phase is acetonitrile (Water HPLC grades), gradient from 50:50 to 100% acetonitrile. Flow rate: gradient program, 0-2 min., 0.2ml min^{-1}. The concentration of each PAH compound was calculated from the PAHs standard curve [22].

RESULTS AND DISCUSSION

3.1 Screening of the hydrocarbon pollutants in the collected water samples:
The hydrocarbon content in each collected water sample was extracted and then gravimetrically estimated (Table, 1). It was found that the concentration of the hydrocarbon pollutants was ranging between 34.7mg/l in Embaba and 41.5mg/l in El-Galatma. These values gave an indication that the studied sites at the time of samples collection were highly polluted according to law 4/1994, which illustrates the characteristics of fresh water bodies that should remain within certain standards and specifications after the discharge of treated industrial effluents [23].

Table (1): Concentrations of the hydrocarbon pollutants in the collected water samples from the five studied locations along the River Nile

Sampling sites	Concentration of hydrocarbon pollutants (mg/l)
Kafr Elelw	40.3
El-Maasara	38.3
El-Giza	37.5
Embaba	34.7
El-Galatma	41.5

River Nile receives huge amounts of industrial, domestic, and agricultural wastes [3] and [24] . About 350 industries are discharging their sewage water either directly into the Nile or through the municipal system. Also, it was found that the River Nile receives 3.8 billion m^3/year of industrial waste water; wastes from Greater Cairo represent 23% of the total industrial waste water in Egypt [25].

Gas chromatographic profiles for all the collected samples (Fig. 1) revealed that, the pollutants are of petrogenic origin, the symmetric peaks represent the normal and isoparaffinic hydrocarbons ranging nearly from heptadecane C_{17} to pentatetracontane C_{45}. There is only one maxima at the retention time of pentacosane C_{25} which represented some heavy isoprenoids eluted at its same retention time.

The hump under the observed peaks represents the unresolved complex mixture (UCM) which includes polynaphthenic hydrocarbons, polynucleur aromatic hydrocarbons, resins and some polymeric compounds. The chromatograms reflected a significant microbial effect not only due to the presence of isoprenoids which were overlapping with pentacosane C_{25}, but also due to the biodegradation of the paraffinic and aromatic hydrocarbons within the five collected water samples.

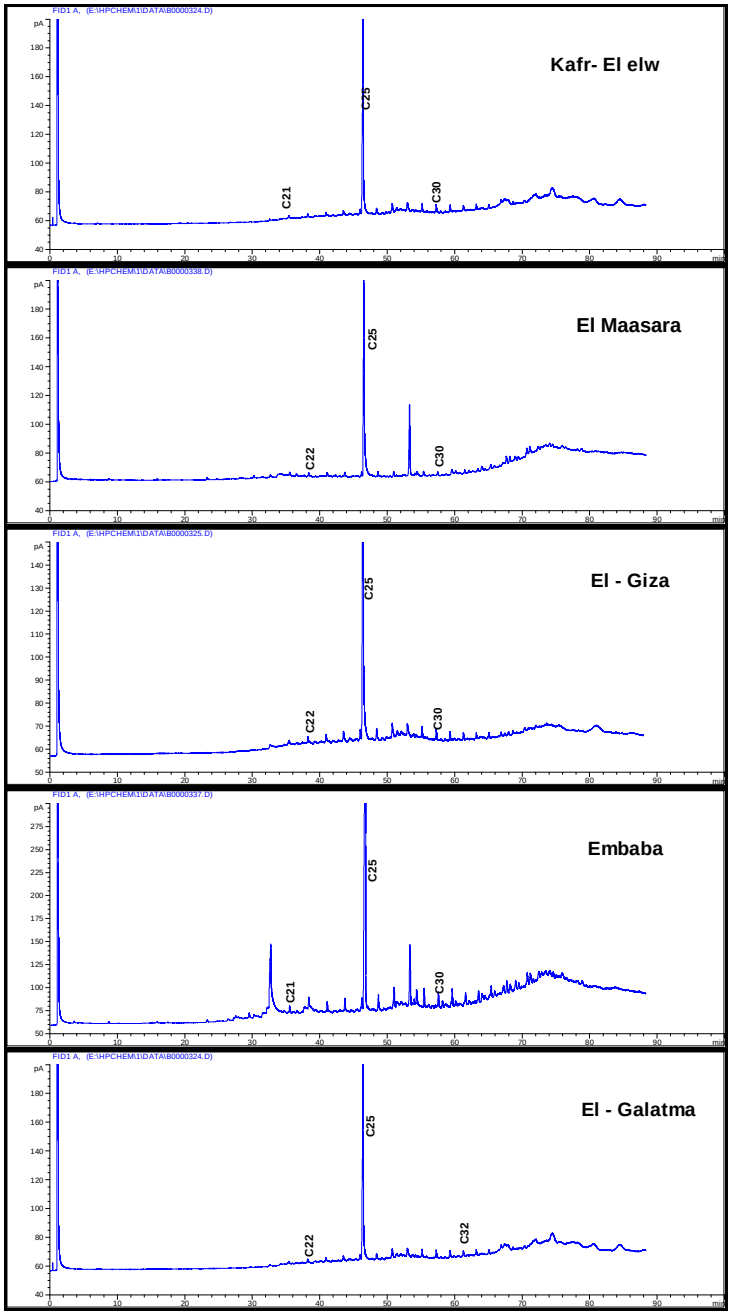

Fig. (1): Gas chromatograms of the extracted hydrocarbons in the collected water samples from the five studied sites along the River Nile.

From The obtained results it was found that, the analysis of the collected water samples from different sites along the River Nile stream in Cairo segment showed high concentration of the pollutant levels. These pollutants exerted negative effects on the water quality in these sites [3] and [26].

3.2 Isolation, purification and screening of the most potent hydrocarbon degrading yeast isolate:

Sixteen paraffinic oil degrading yeast isolates were isolated and purified from the studied five locations. All the isolates were recultivated on BMS agar media supplemented with nC_8 and EB. The isolate No 3 from Embaba site was the most efficient one, as the results in Table (2) showed that it has the ability to grow on the two studied substrates with the highest CFU count percentage and this was an indication of its ability to utilize the paraffinic and aromatic hydrocarbon pollutants present in the River Nile water [27].

Table (2): Count Percentage of nC_8 and EB degrading yeast isolates:

Isolate No.	Site I		Site II		Site III		Site IV		Site V	
	Count percentage (%)									
	n C_8	EB	n C_8	EB	n C_8	EB	n C_8	EB	n C_8	EB
1	88.9	84.9	88.9	00.0	83.1	80.1	77.0	75.0	87.3	62.6
2	86.2	60.4	88.3	87.3	55.8	00.0	69.5	00.0	67.5	00.0
3			58.1	60.4	88.3	82.0	93.6	91.0	89.5	83.6
4							89.7	87.0	88.6	82.5

3.3 Identification of the most potent yeast isolate:

The colonies are white to cream, filaments are absent. The spore stain revealed the presence of asci containing up to four smooth round ascospors. Vegetative reproduction by budding (Photo. 1). The yeast isolate was identified as *Saccharomyces rosinii* on the basis of the data concerning its morphological and physiological characteristics (Table, 3) [18] and [19].

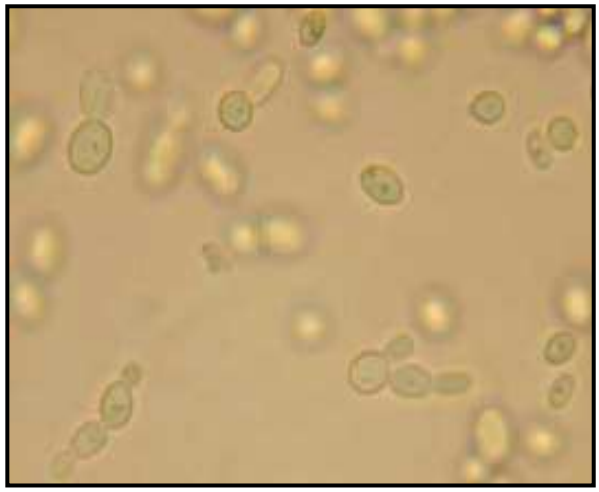

Photo.1 Microphotograph of the most potent hydrocarbon degrading yeast isolate *Saccharomyces rosinii* after the growth on corn meal agar medium supplemented with tween 80 (x1000).

3.4 Stimulation of the most potent yeast isolate using He-Ne laser radiation:

In a trial to enhance the potentiality of biodegradation of the studied yeast isolate, it was subjected to different doses of low intensity He-Ne laser. From Table (4) it was found that the exposure of the cell suspensions of the yeast isolate to He-Ne laser for 1 min (33.2 J/cm^2) had induced a significant enhancement of the bioenergetic processes of the microbial cells resulting in an increase in the percentage of degradation of the studied substrates so after 12 hrs of incubation complete degradation was achieved. Extending of the exposure time to 3 mins (99.6 J/cm^2) resulted in an increase in the degradation potentiality .Complete degradation of the studied substrates was achieved only after 8 hrs of incubation. Prolongation of the exposure duration to 6 mins (199.3 J/cm^2) led to decrease in the microbial growth accompanied by suppression of the vital activities of the culture cells. So that the biodegradation activity of the studied isolate had decreased as compared to the non-irradiated one.

It has been reported that, irradiation with He-Ne laser (λ=632.8nm) in a strictly definite doses causes a substantial shortening of the generation time occurred in the lag phase of growth of yeast cultures, which in turn leads to the speeding of the cells for division or budding and the intensification of protein synthesis [14], [28] and [29] Several reports indicated that different yeast cultures irradiated with low power He-Ne laser showed an increased activity of production of extracellular enzymes associated with the biodegradation processes [30] and [31]. On the other hand large doses of laser can evoke damage in cell structures and morphological deformations associated with enzymatic changes [29].

Table (3): Physiological characteristics of the most potent hydrocarbon degrading yeast isolate

Physiological test	Result	Physiological test	Result
Carbon source assimilation		***Nitrogen source utilization***	
D-glucose	+	Nitrate	-
D-galactose	+	Nitrite	-
L-sorbose	-	Ethylamine	-
D-ribose	-	Creatine	-
D-glucosamine	-	Creatinine	-
D-xylose	-	Cadaverine	+
L-Arabinose	-	***Cycloheximide sensitivity***	
D-Arabinose	-	0.01% cycloheximide	+
L-Rhamnose	-	0.1% cycloheximide	+/D
Sucrose	-	***Vitamins***	
Maltose	-	W/O Vitamins	-
Salicine	-	W/O Pantothenate	+/D
Lactose	-	W/O Biotine	-
Melibiose	-	W/O Thiamine	-
Raffinose	-	W/O Pyridoxine	+/D
Inuline	-	W/O Niacin	-
Starch	-	***Urea hydrolysis***	-
Methanol	-	***Starch formation***	-
Glycerol	-		
Ribitol	-		
Xylitol	-		
D-mannitol	-		
2-keto-D-gluconate	-		
Succinate	-		
Citrate	-		
Carbon sources fermentation			
D-glucose	+		
D-galactose	+		
Maltose	-		
Sucrose	-		
Lactose	-		
Melibiose	-		
Cellobiose	-		
Raffinose	-		
Inuline	-		
D-xylose	-		
Starch	-		

+: Positive; –: Negative; D; Delay. W/O: without

Table (4): Weight percentage of degraded substrates after exposure to different doses of He-Ne laser radiation

	Weight Percentage of degraded substrate (%)											
	n-C_8	**n-C_8 in mix**	**EB**	**EB in mix**	**n-C_8**	**n-C_8 in mix**	**EB**	**EB in mix**	**n-C_8**	**n-C_8 in mix**	**EB**	**EB in mix**
Incubation time Exposure time (mins)	**4 hrs**				**8 hrs**				**12 hrs**			
Zero	37.32	29.19	17.06	22.31	47.25	32.05	21.74	27.27	55.06	43.19	26.04	30.62
1 33.2 J/cm^2	75.64	67.96	62.19	66.19	86.58	78.70	72.86	75.39	100	100	100	100
3 99.6 J/cm^2	85.10	77.10	76.90	85.39	100	100	100	100				
6 199.3 J/cm^2	32.90	27.62	9.55	13.31	38.50	30.48	12.55	16.53	42.33	35.52	21.03	29.75

3.5 Degradation of paraffinic hydrocarbons:

Paraffinic hydrocarbons represent the straight chain normal paraffins and the side chain iso paraffins. They are considered the most wide spread contaminants related to soil and water [7]. The CGC profile of the paraffinic mixture before treatment exhibits iso and normal paraffins which ranged from undecane C_{11} to octatricontane C_{38}. After inoculation and incubation for 12 hrs, there was a reduction in the weight percentage of all iso and normal paraffinic hydrocarbons (72.46 %). The total n- paraffins exhibit high reduction in their weight percentage (47.32%) compared with their corresponding iso- paraffins which were attributed to the increasing ability of their utilization by the studied isolate. The relative weight percentage of the corresponding iso components was increased to be 25.14 % in order to maintain the weight percentage of total paraffins at 72.46%.

On increasing the incubation time to 30 hrs the total weight percentage of the residual iso and normal paraffins were decreased to 11.44% and 26.77% respectively accompanied with complete disappearance of the carbons from C_{35} to C_{38}. Treatment of the mixture by the irradiated yeast suspension at dose 99.6 J/cm^2 led to an additional reduction of the total paraffinic fraction by 91.53%after 12 hrs of incubation. On increasing the incubation time to 30 hrs, about 98.31% of the paraffinic mixture was degraded leaving some carbons in trace concentrations (Table 5 and Fig. 2).

Table (5): Gas chromatographic analysis of the undegraded (control) and degraded paraffinic hydrocarbons using *S.rosinii* (before and after irradiation).

Compound name		*Weight percentage of residual components (%)*									
		Control		**12hrs**				**30hrs**			
				Before irradiation		*After irradiation*		*Before irradiation*		*After irradiation*	
		Iso-	Normal	Iso-	Normal	Iso-	Normal	Iso-	Normal	Iso-	Normal
Decane	C_{10}	0.00	0.00	0.04	0.24	0.00	0.02	0.00	0.00	0.00	0.00
Undecane	C_{11}	0.00	0.26	0.61	1.50	0.04	0.15	0.00	0.15	0.00	0.00
Dodecane	C_{12}	0.36	1.16	0.74	2.98	0.09	0.34	0.13	0.70	0.00	0.01
Tridecane	C_{13}	1.22	4.69	2.83	3.79	0.35	0.46	0.90	1.53	0.02	0.04
Tetradecane	C_{14}	1.18	5.88	3.12	4.28	0.36	0.56	0.87	2.21	0.04	0.08
Pentadecane	C_{15}	4.24	7.36	3.82	4.41	0.51	0.57	1.89	2.85	0.07	0.10
Hexadecane	C_{16}	2.68	7.01	1.35	3.84	0.23	0.50	0.64	2.62	0.04	0.10
Heptadecane	C_{17}	3.32	10.22	1.75	3.73	0.19	0.48	1.03	2.43	0.03	0.11
Pristane (pr)		3.61		4.38		0.64		2.85		0.18	
Octadecane	C_{18}	1.80	5.83	0.36	3.13	0.04	0.40	0.04	2.10	0.01	0.11
Phytane (ph)		1.28		1.22		0.17		1.02		0.06	
Nonadecane	C_{19}	1.09	5.69	1.45	3.15	0.01	0.38	0.34	2.11	0.02	0.10
Eicosane	C_{20}	1.00	4.59	0.77	2.56	0.03	0.31	0.30	1.76	0.01	0.08
Heneicosane	C_{21}	0.34	3.46	0.37	2.37	0.01	0.28	0.30	1.58	0.01	0.07
Docosane	C_{22}	0.43	3.17	0.37	1.96	0.02	0.24	0.22	1.27	0.01	0.07
Tricosane	C_{23}	0.36	2.99	0.25	1.78	0.02	0.22	0.11	1.14	0.00	0.07
Tetracosane	C_{24}	0.25	2.44	0.29	1.45	0.01	0.17	0.11	0.90	0.00	0.06
Pentacosane	C_{25}	0.30	2.18	0.13	1.32	0.01	0.15	0.12	0.79	0.00	0.04
Hexacosane	C_{26}	0.24	1.69	0.16	1.03	0.01	0.11	0.10	0.60	0.00	0.04
Heptacosane	C_{27}	0.16	1.53	0.15	0.95	0.01	0.10	0.14	0.53	0.00	0.03
Octacosane	C_{28}	0.22	1.06	0.17	0.67	0.00	0.07	0.07	0.37	0.00	0.02
Nonacosane	C_{29}	0.18	0.90	0.14	0.58	0.00	0.06	0.02	0.31	0.00	0.02
Triacontane	C_{30}	0.16	0.63	0.14	0.38	0.00	0.03	0.08	0.21	0.00	0.02
Entriacontane	C_{31}	0.06	0.98	0.01	0.63	0.00	0.05	0.01	0.30	0.00	0.02
Dotriacotane	C_{32}	0.08	0.61	0.10	0.19	0.00	0.02	0.05	0.11	0.00	0.00
Tritriacontane	C_{33}	0.09	0.44	0.14	0.25	0.00	0.03	0.07	0.15	0.00	0.00
Tetratriacontane	C_{34}	0.15	0.16	0.07	0.08	0.00	0.01	0.03	0.05	0.00	0.00
Pentatriacontane	C_{35}	0.04	0.12	0.21	0.05	0.00	0.01	0.00	0.00	0.00	0.00
Hexatriacontane	C_{36}	0.01	0.05	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Heptatriacotane	C_{37}	0.01	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Octatriacotane	C_{38}	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total		**24.86**	**75.14**	**25.14**	**47.32**	**2.75**	**5.72**	**11.44**	**26.77**	**0.50**	**1.19**
n-C_{17}/pr ratio		**2.83**		**0.85**		**0.75**		**0.85**		**0.61**	
n-C_{18}/ph ratio		**4.55**		**2.57**		**2.35**		**2.06**		**1.83**	

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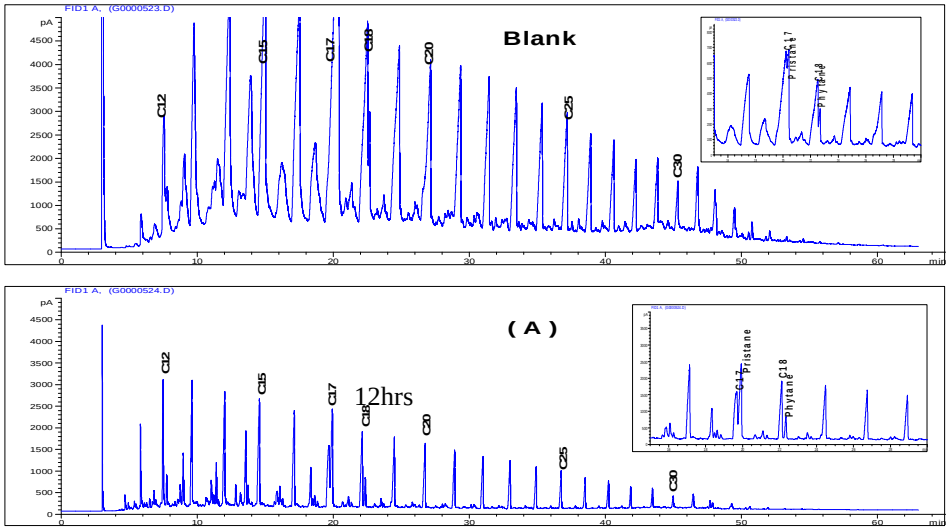


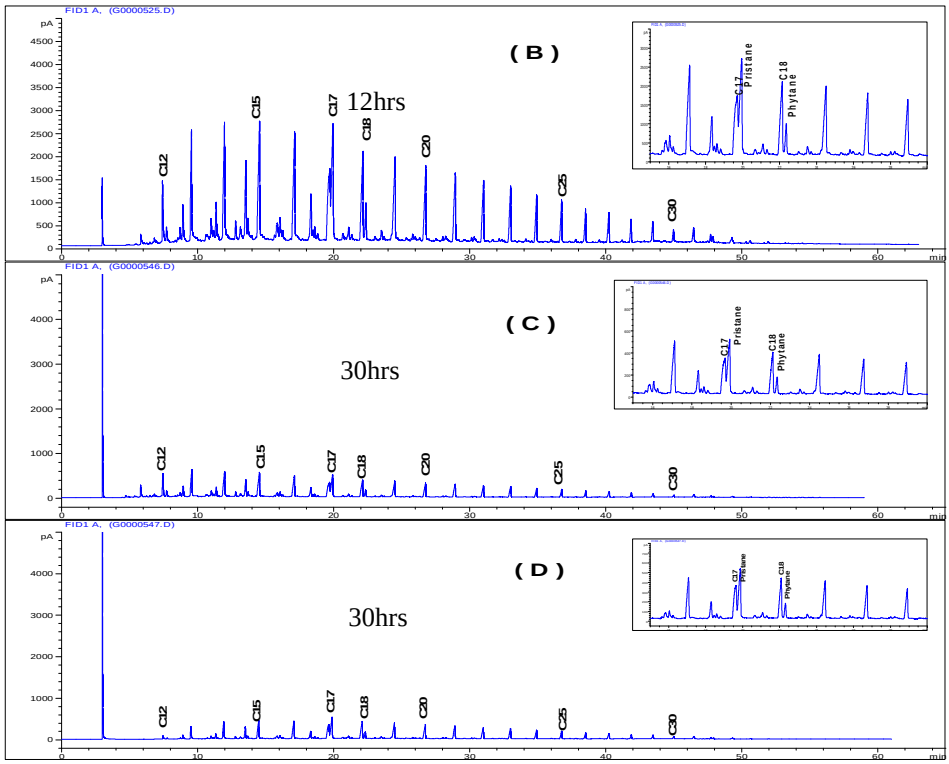
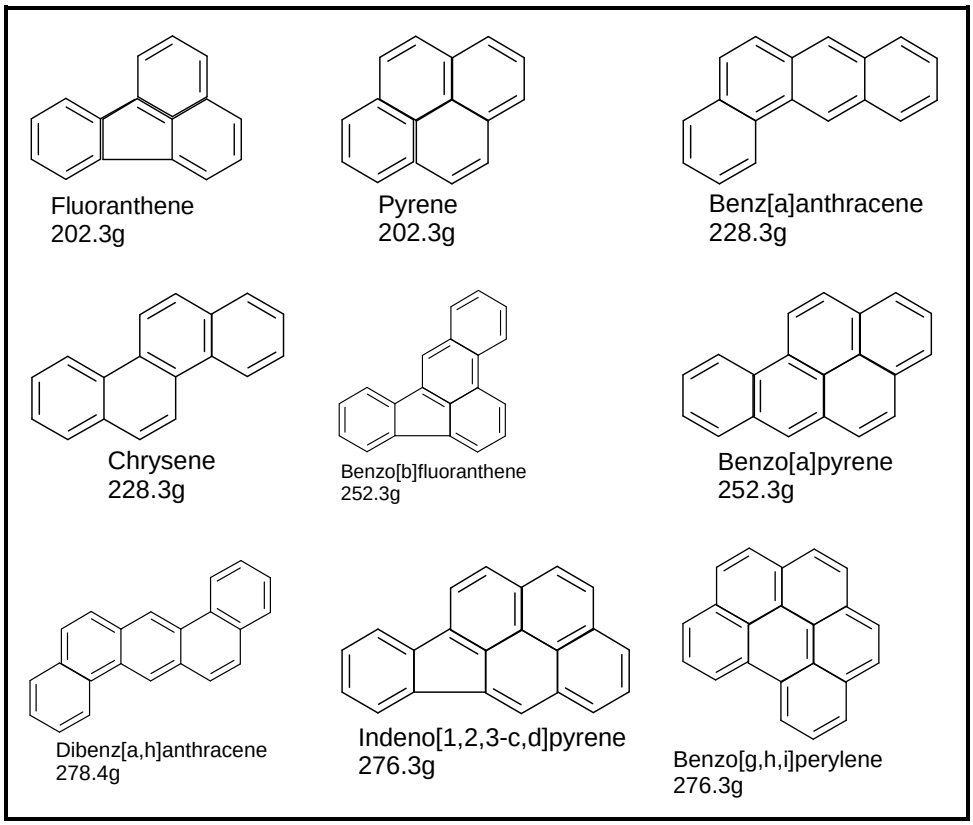


Fig. (2): Gas chromatograms of the Control (undegraded) and degraded samples using *S.rosinii* before irradiation (A and C) and after irradiation (B and D) at two incubation times.

Fig. (3): Chemical structure and molecular weights of the studied nine polynuclear aromatic hydrocarbons.

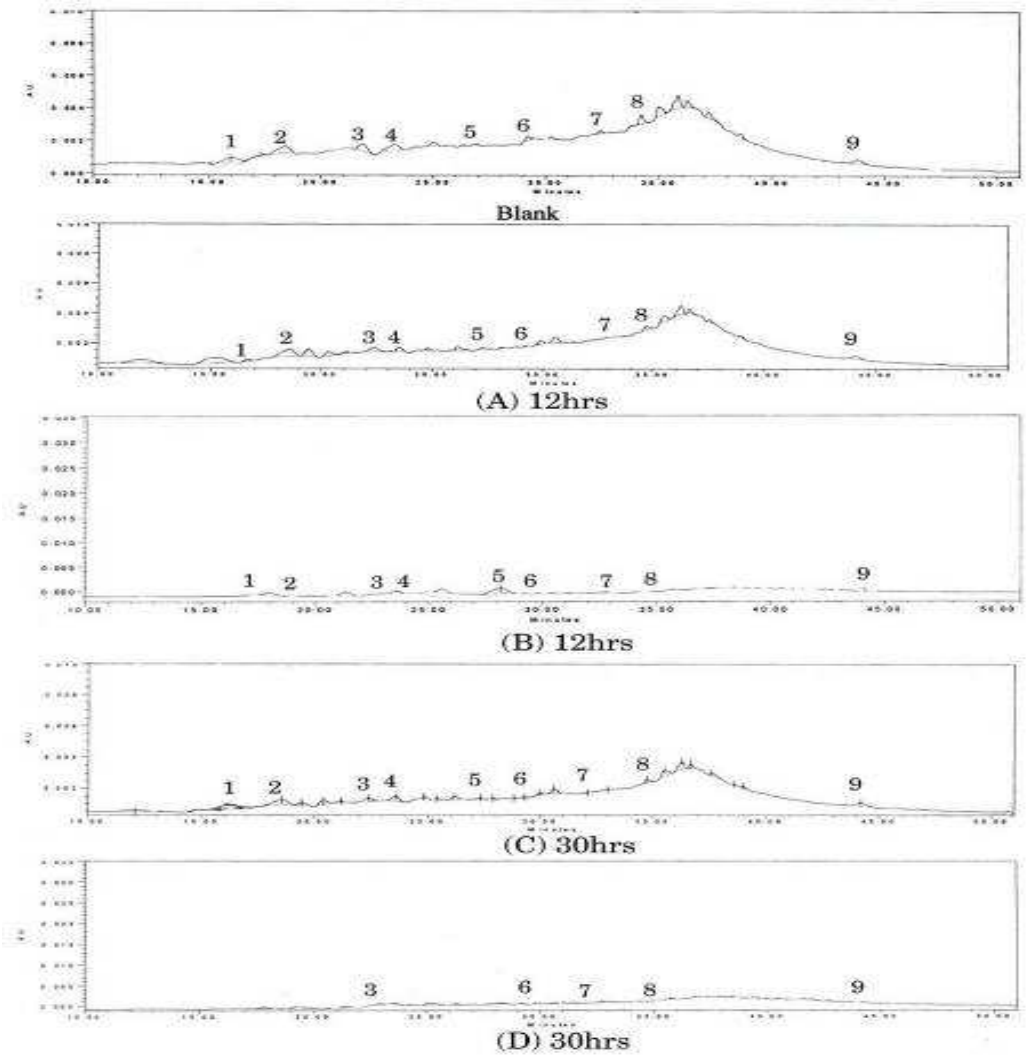


Pristane (2,6,10,14tetramethylpentadecane) and Phytane (2,6,10,14 tetramethyle octadecane) are two isoprenoids adjacent to normal heptadecane (n-C_{17}) and normal octadecane (n-C_{18}) peaks respectively in the chromatographic

profile, they are slowly assimilated as the presence of branching prevent the β cleavage reactions . So, the ratios n C_{17} / pr and n C_{18}/ ph are used as indices to follow the rate of biodegradation [32] and [33]. These ratios had decreased in all samples in comparison with that of the control which indicated the ability of *S. rosinii* before and after irradiation to degrade n-paraffins more than iso-paraffins.

Fig. (4): PAHs chromatograms of the Control (undegraded) and degraded samples using *S.rosinii* before irradiation (A and C) and after irradiation (B and D) at two incubation times.

1= *Fluoranthene;* ***2***= *Pyrene;* ***3***= *Benzo (a) anthracene;* ***4***= *Chrysene;* ***5***= *Benzo (b) fluoranthene;* ***6***= *Benzo (a) pyrene;* ***7***= *Benzo (g, h, i) perylene;* ***8***= *Dibenzo (a,h) anthracene;* ***9***= *Indeno (1, 2, 3-c, d) pyrene*



3.6 Degradation of polynuclear aromatic hydrocarbons (PAHs):

PAHs are ubiquitous environmental pollutants with low bioavailability, high persistence and potential deleterious effects on human health [34]. The environmental protection agency (EPA) has listed PAHs among the priority pollutants to be monitored in aquatic and terrestrial ecosystems, due to their potential toxicity, mutagencity and carcinogenicity [6]. A diverse group of microorganisms were identified as they partially degrade or mineralize some high molecular weight PAHs [35].

Nine PAH compounds were selected (Fig. 3) for this study from Table (6) and Figure (4) it was found that *S. rosinii* was able to degrade the selected nine PAHs after 12 hrs of incubation by 34.23%. Increasing the incubation time to 30 hrs was accompanied with the increase in the degradation percentage to 56.41%. Irradiation of the yeast suspension led to an enhancement in its degradation potential. So, after 30 hrs of incubation the degradation reached 98.09%. Complete degradation of fluoranthene, pyrene, chrysene and benzo(b) fluoranthene was achieved.

Table (6): HPLC analysis of the control (undegraded) and degraded polynuclear aromatic hydrocarbons using *S.rosinii* (before and after irradiation).

Polynuclear aromatic compounds	***Concentration of residual polynuclear aromatic compounds (μg/ml)***				
	Control	*Incubation time 12hrs*		*Incubation time 30hrs*	
		Before irradiation	After irradiation	Before irradiation	After irradiation
Fluoranthene	0.76	0.50	0.35	0.41	Nil
Pyrene	0.16	0.09	0.06	0.06	Nil
Benzo (a) anthracene	1.36	0.75	0.40	0.40	0.02
Chrysene	0.99	0.59	0.20	0.40	Nil
Benzo (b) fluoranthene	0.17	0.13	0.08	0.09	Nil
Benzo (a) pyrene	0.19	0.16	0.06	0.09	0.01
Dibenzo (a,h) anthracene	0.67	0.48	0.30	0.33	0.03
Benzo (g, h, i) perylene	0.27	0.16	0.07	0.10	0.02
Indeno (1, 2, 3-c, d) pyrene	0.66	0.58	0.32	0.40	0.02
Weight percentage of degraded polynuclear aromatic mixture		34.23	64.82	56.41	98.09

From the previous results it was found that the degradation of paraffins was more profound than that for the aromatic hydrocarbons [27]. It was reported that *Saccharomyces sp*. isolated from petroleum polluted environment had the ability to degrade linear and branched paraffins with great efficiency which revealed that it is effective in the treatment of pollution especially under favorable conditions [36] and [37] . Also PAHs were found to be degraded by pure yeast cultures [38]. *Saccharomyces sp*. was found to be one of the superior strains in the degradation of the tri, tetra, and penta cyclic aromatic compounds [39].

irradiation of the yeast isolate using low intensity He-Ne laser promotes the cell proliferation, activates the oxygen consumption and increase the activity of NADH dehydrogenase and cytochrome c oxidase [40] and [41] .This reveals the superiority of the irradiated yeast isolate and supports the application of the He-Ne laser induced technique on the environmental microorganisms

CONCLUSION

From the previous results it was concluded that:

- *S. rosinii* was selected as the most potent hydrocarbon degrading yeast isolate.
- The degradation potentiality was increased by irradiation with low power He-Ne laser (λ=632.8 nm) by a dose equivalent to 99.6 J/cm^2.
- The irradiated isolate causes degradation of 98.31% of the paraffinic fraction and 98.09% of the polynuclear aromatic fraction only after 30 hrs of incubation. So *S.rosinii* can be considered an effective isolate in the bioremediation strategy.

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