Degradation of Polycyclic Aromatic Hydrocarbons as Affected by some Lactic Acid Bacteria

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings that are formed from the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuel, garbage or other organic substances, such as tobacco, charbroiled meat and exhaust from automobile and trucks. They enter the environment and release to air, soil, water and food. Some PAHs have shown to have toxicological, carcinogenic and mutagenic effects on animals and humans. Biodegradation of PAHs in the presence of the three types of lactic acid bacteria (*Bifidobacterium bifidium, Streptococcus thermophilus* and *Lactobacillus bulgaricus*) were studied during the different incubation periods (2, 4, 6,8,10,12,24,48 and 72 h) at 37˚C. The reduction of PAHs concentration proved that these compounds were affected by the previous lactic acid bacteria. At the end of incubation period (72 h), the reduction percent were 46.6, 87.7 and 91.5% with *Bifidobacterium bifidium, Streptococcus thermophilus* and *Lactobacillus bulgaricus*, respectively. These results could be explained as the bacterial cell is a high proteinous material and so may adsorbs PAHs which could interfere with cellular metabolism. Also, the variation of pH values during the incubation periods may control in the adsorbed PAHs on the cells. The biodegradation of PAHs by yoghurt starter during yoghurt manufacture were studied. Slightly reduction was observed during the incubation periods (1, 2 and 3 h). The reduction percent was 3.46 at the final product. It could be revealing that the persistence of PAHs depend on a number of factors such as the type of microorganism, the interaction between microorganisms, the microbial concentration, the composition of the medium, and the microbial growth conditions of temperature and pH. The foregoing information reveal that extra care must be taken when comparing the results since in-vitro studies are not always relevant to real situation in food products.

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1. Introduction:

Polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals composed of two or more fused aromatic rings that are formed during the incomplete combustion or high-temperature pyrolysis of coal, oil, gas, wood, fossil fuels, garbage, or other substances, such as tobacco and charbroiled meat (*Mottier et al.,2000*). The quantity and composition of PAHs produced are closely related to the reaction conditions, temperature and amount of air and, therefore, may vary considerably (*Vaessen et al., 1988).* Over 100 PAHs have been identified and occur as complex mixtures, never as individual components.

PAHs comprise the largest class of known chemical carcinogens and have been detected in the environment especially in air, water, soil and foods. They enter the environment mostly as releases to air from volcanoes, forest fires, and residential wood burning, cigarette smoke, asphalt roads, coal, coal tar, agricultural burning, municipal, industrial waste incineration, hazardous waste sites and exhaust from automobiles and trucks.. They can also enter surface water through discharges from industrial plants and waste water treatment plants. These compounds can be released to soils at hazardous waste sites if they escape from storage containers (*ATSDR 1995)*. The populations may be exposed to PAHs in the soil at or near hazardous waste sites. The movement of PAHs in the environment depends on properties such as how easily they dissolve in water, and how easily they evaporate into the air. PAHs in general do not easily dissolve in water. They are present in air as vapors or stuck to the surfaces of small solid particles. They can travel long distances before they return to earth in rainfall or particle settling (*ATSDR 1995).* The PAHs content of plants and animals living on the land or in water can be many times higher than the content of PAHs in soil or water.

Polycyclic aromatic hydrocarbons (PAHs) are proven animal carcinogens; in humans they are suspected of causing cancer. Clinical studies have shown that exposure a mixture of highly concentrated PAHs may cause various cancers, in skin, lung, stomach and liver. It is generally convinced that PAHs are responsible for the increasing cancer risks as PAHs are capable of damaging genetic materials and thus initiating the development of cancers (*Schneider et al., 2000*). Some of PAHs compounds such as benzo(a) pyrene and dibenzo (a,h) anthracene were reported to be the most carcinogenic (*Schneider et al., 2000*). So, the presence of these compounds in food has received considerable attention over the past three decades (*Maga, 1988*). Food quality and safety is a pertinent issue, consumers are concerned that their food should be both of high nutritional value and free from chemical residues.

As environment pollution in different countries is becoming a serious problem, it is possible that PAHs may be widely distributed in the environment and thus contaminates food. The occurrence of PAHs in food may result from their sorption from a contaminated environment or from food preparation. The variation in PAHs profile in food products also depends on the source of the contamination (*Vaessen et al., 1988*). PAHs have been detected in fresh vegetables, fruits, and cereals as a result of the deposition of airborne PAHs, particularly near industrial sources or in areas with high traffic (*Dennis, 1991)*. They have also been found in mussels, snails, and fish from contaminated waters (*Speer et al., 1990*). *Kan et al.* (2003) reviewed the occurrence of PAHs in animal products. In France, PAHs have been found in milk at total levels of 37 and 27 ng/g fat (*Grova et al*., 2001). Concentrations up to 70 µg/kg were found in meat (SCF, 2002). PAHs are also present at elevated levels in some vegetable oils and margarine (*Thomson et al., 1996*), probably formed during processing. They are also formed during some methods of food preparation, such as char-broiling, grilling, roasting, frying, or baking (*Yabiku et al., 1993*).

PAHs can breakdown to longer-lasting products by reacting with sunlight and other chemicals in the air, generally over a period of days to weeks. Breakdown in soil and water generally takes weeks to months and is caused primarily by microorganisms (*ATSDR 1995).* Biodegradation of chemicals by living organisms is one of the most important mechanisms for the breakdown of organic compounds and the microorganisms are the most important agents for such degradation. However, degradation is a very specific process and the growth of some microorganisms can even be inhibited by a xenobiotic. If degradation does occur, it is likely to result from enzymatic activity and may either occur immediately or only after a period of adaptation to the chemicals (*Boethling*, 1993).

The study of degradation of such residues in these foodstuffs is very important because of their increasing rate of consumption world-wide. Therefore, technological procedures in food production should be developed to reduce the content pollutants hazardous to public health in food products. Nowadays, in the food industry it is very common to use starter cultures to improve the characteristics of the food products, and the possibility that these microorganisms would degrade these contaminants is of great interest because the de-chlorinated products are generally less toxic to animals, less likely to bio-accumulate, and more susceptible to further microbial attack (*Bayarri et al*., 1997).

Report on microbial degradation of PAHs appear increasing numbers, but such investigation tend to be focused on soil or aquatic microorganisms (*Luning and Pritchard, 2002 and Story et al., 2004),* while the activity of microorganisms associated with food fermentation has been less will investigated.

With this in view, the present work was conducted to unveil and throw more light on the biodegradation of the target PAHs as affected by some types of lactic acid bacteria (dairy and fermented foods starter) in different media.

2. Materials and methods

1. Polycyclic aromatic hydrocarbons (PAHs) reference standards

A mixture (16 compounds) of PAHs reference standards containing acetaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3,-cd)pyrene, naphthalene, phenanthrene, pyrene and 2-bromonaphthalene was purchased from Supelco company (Supleco Park, Bellefonte, PA, U.S.A.).

2. Bacterial Strains

Strains of *Bifidobacterium bifidium* (*B. bifidium*), *Streptococcus thermophilus* (*S. thermophilus*) and *Lactobacillus bulgaricus* (*L. bulgaricus*) were obtained from Cairo Microbiological Research Center, Cairo MIRCEN, Faculty of Agriculture, Ain-Shams University, Egypt. The strains were stored at -18˚C until utilized .

3. Degradation of polycyclic aromatic hydrocarbons (PAHs) by lactic acid bacteria

Sterilize liquid medium De Man-Rogosa-Sharpe

(MRS) was prepared (300ml) according to *Man et al.* (1960) and spiked by PAHs mixture containing 16 compounds of acetaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3,-cd)pyrene, naphthalene, phenanthrene, pyrene and 2-bromonaphthalene 0.25µg of each/ml medium). The medium was divided into three portions. The first, second and third portions were inoculated by 1% *B. bifidium*, S. *thermophilus* and *L. bulgaricus*, respectively. All flasks incubated at 37˚C for 2, 4, 6, 8, 10, 12, 24, 48 and 72 h .The collected samples (10 ml of each) were extracted according to the method of *Hodgeson (1990)* for PAHs residues. The extraction method was validated. The limit of detection (LOD) is equal to 3 times the standard deviation (SD) of the lowest standard concentration used for the calibration curve (*Chantra* and *Sangchan, 2009*). Minimum detectable concentrations of PAHs in the present investigation were ranged between 0.007 to 0.020 µg/ml (Table 1). Recovery results refer to complete method with concentration of 0.25 µg/ml of each compound PAHs (total compounds of 16 was 4µg/ml) used in this study ranged from 88 to 96 % (Table 1). The experiment was repeated by injecting a mixture of 16 PAHs standards 6 times.

Table 1. Validation (detection limits and recovery) of PAHs

Compound	LOD (µg/ml)	Recovery (%)
1-Naphthalene	0.010	88.0
2-Acenaphthylene	0.020	89.2
3-2.Bromonaphthalene	0.010	94.0
4-Acenaphthene	0.008	94.0
5-Fluorene	0.010	94.0
6-Anthracene	0.009	93.0
7-Phenanthrene	0.008	90.0
8-Pyrene	0.020	92.0
9-Fluoranthene	0.008	90.0
10-Chrysene	0.008	96.0
11-Benzo(a)anthracene	0.007	94.0
12-Benzo(k)fluoranthene	0.007	92.0
13-Benzo(a)pyrene	0.008	96.0
14-Benzo(ghi)perylene	0.009	94.0
15-Dibenz(a,h)anthracene	0.008	94.0
16-Indeno(1,2.3cd)pyrene	0.010	89.0

Measured volumes of the medium were serially extracted with dichloromethane. Sixty ml of dichloromethane was added to the sample in separating funnel with shaking for two minutes with periodic venting to release excess pressure. Then, the organic layer separated from the liquid phase and the dichloromethane extract collected in 250 ml Erlenmeyer flask. The extraction steps repeated by adding another 60 ml dichloromethane. A third extraction in the same manner was performed. The combined extracts of dichloromethane was dried through column containing about 10 cm of anhydrous sodium sulphate and the extracts were collected in Kuderna- Danish (K-D) concentrator and the K-D placed on a hot water bath (60-65˚C), so that the concentrator tube is partially immersed in hot water, and the entire lower rounded surface of the flask was bathed with hot vapor. When the apparatus volume of liquid reaches 0.5 ml, the K-D was removed and allowed it to drain and cool for at least 10 min. Then the synder column was removed. The flask and its lower joint into the concentrator tube were rinsed with 1-2 ml dichloromethane. The extract was evaporated with a gentle steam of N_2 flow to defined volume.

One micro-liter of each sample extract was injected into a Hewlett Packard 5890 gas chromatograph fitted with a HP-5 fused silica capillary column (50m x 0.2mm x 0.33µm film thickness) and connected to Hewlett Packard 5970 series mass selective detector. The carrier gas was helium, maintained at a flow rate of 1.0 ml/min. The injection port temperature was 275˚C with electron energy of 70 eV. The quadrupole temperature was 280˚C. The oven programmed was as follows: 70˚C for 5 min, 3˚C/min to 290˚C for 30 min. The mass spectrometer is tuned by letting in a small amount of perfluorotributylamine ($C_{12}F_{27}N$) gas as a reference. The fragments of peak for m/z, 69, 219 and 502 were observed and tune results were recorded and the

masses are calibrated. The mass spectrum for each of the peaks from the resulting chromatogram from analyzed samples was observed by the total ion count (TIC) mode. Calibration was carried out by external standards, mixture of 16 compounds (Fig.1). The mass spectrometer was operated in selective ion monitoring mode using separate ions to identify and confirm compounds. Acquired mass spectrum in samples was compared with the standard and library spectra for identification.

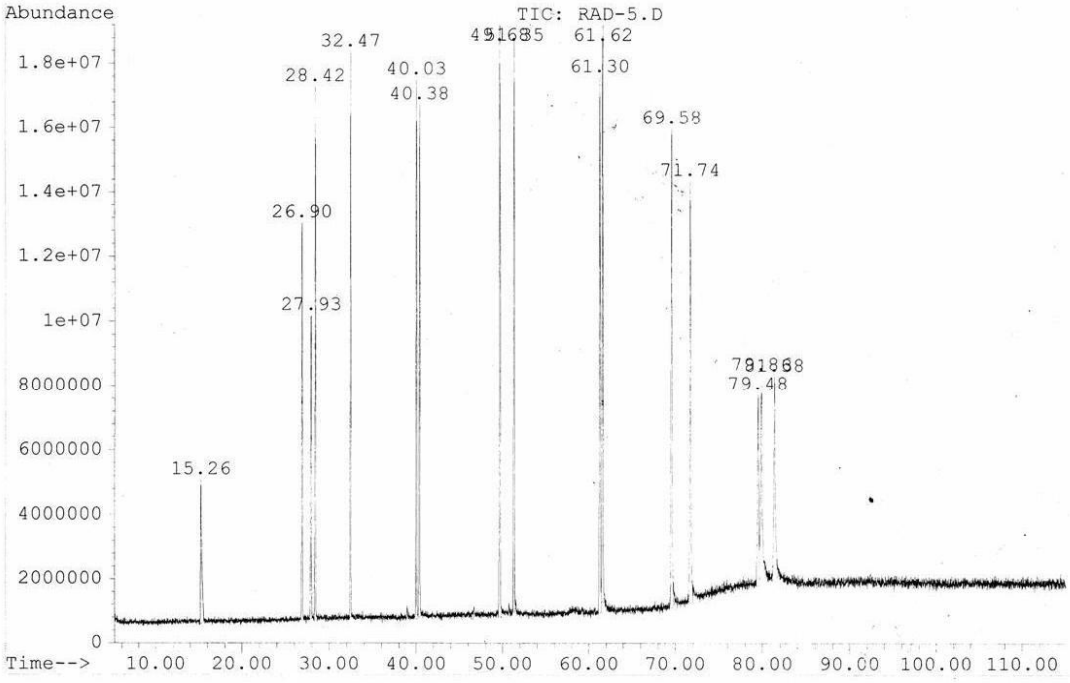

Fig .6. Mixture of PAHs separated by GC/MS.

Fig.1. Mixture of polycyclic aromatic hydrocarbons (PAHs) analyzed by GC/MS

Degradation of PAHs by yoghurt starter

Mixture of buffalo's and cow's milk was heated at 80-82˚C for 20 min and cooled to 40˚C. The milk was polluted by polycyclic aromatic hydrocarbons (PAHs) mixture (16 compounds) to give concentration around 0. 02 µg/ml of each compound in the mixture and the PAHs concentration of the polluted mixture was determined.

Polluted milk was inoculated with 2% yoghurt starter (mixture of *S. thermophilus and L. bulgaricus*) and incubated at 40°C for 3.0 h as described by the Egyptian Organization for Standardization, *EOS* (1970). The samples were analyzed at zero time and after 1, 2 and 3 h (yoghurt product) intervals. The extracted samples for PAHs were applied and determined by GC/MS according to the method of *Hodgeson (1990)* as described before.

Statistical analysis

The data were statistically analyzed by analysis of variance and least significant difference (L.S.D) at 0.05 levels according to the method described by *Snedecor and Cochran* (1980).

3. Results and Discussion:

Degradation of polycyclic aromatic hydrocarbons (PAHs) by lactic acid bacteria:

MRS media broth contaminated by PAHs (16 compounds, 0.25 µg of each/ml media) and inoculated with *B. bifidium*, *S. thermophilus* and *L. bulgaricus* and incubated at 37°C for 72 h, critical and significant role of lactic acid bacteria (LAB) in uptake and/or degrade PAHs was observed. It could be revealing that the persistence of PAHs depends on bacterial species and incubation period.

The obtained results revealed that PAHs was affected by B. *bifidium* strain during the incubation period (Table2). After 2 to 48 h of incubation, naphthalene, acenaphthylene, 2-bromonaphthalene and acenaphthene weren't detected in the various samples. However, 2-bromonaphthalene and acenaphthene were appeared after 72 h of incubation and the reduction (%) was 74.8 and 87.6, in this order. Regarding to the other compounds in different samples, they were detected at fluctuation levels and

the sum of total mixture compounds was decreased during the incubation periods.

The same pattern was detected in case of *S. thermophilus* (Table 3) except, the presence of residues of acenaphthylene, 2-bromonaphthalene and acenaphthene after 2 h incubation, beside presence of 0.017 µg/ml of 2-bromonaphthalene when the incubation period was 72 h. Also, fluctuation levels were observed during the different period of incubation.

Table 2. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Bifidobacterium bifidium.*

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
2.Bromonaphthalene	nd	nd	nd	nd	nd	nd	nd	nd	0.063
Acenaphthene	nd	nd	nd	nd	nd	nd	nd	nd	0.031
Fluorene	0.009	nd	0.017	nd	0.005	0.010	0.017	nd	0.110
Anthracene	0.076	0.248	0.059	0.024	0.038	0.012	0.204	0.071	0.182
Phenanthrene	0.151	0.056	0.037	0.022	0.007	0.099	0.053	0.013	0.023
Pyrene	0.089	0.143	0.068	0.102	0.057	0.121	0.103	0.203	0.220
Fluoranthene	0.080	0.140	0.083	0.106	0.054	0.121	0.097	0.196	0.211
Chrysene	0.008	0.155	0.061	0.085	0.012	0.101	0.059	0.112	0.185
Benzo(a)anthracene	0.083	0.196	0.099	0.118	0.052	0.153	0.109	0.201	0.250
Benzo(k)fluoranthene	0.084	0.163	0.095	0.028	0.045	0.105	0.078	0.127	0.128
Benzo(a)pyrene	nd	0.093	0.015	0.012	0.003	0.009	nd	0.019	0.117
Benzo(ghi)perylene	0.043	0.068	0.143	0.044	0.006	0.042	0.087	0.128	0.111
Dibenz(a,h)anthracene	0.053	0.128	0.144	0.057	0.007	0.094	0.078	0.173	0.229
Indeno(1,2.3cd)pyrene	0.080	0.239	0.186	0.188	0.010	0.090	0.108	0.173	0.250
Total (sum)	0.756	1.629	1.007	0.786	0.296	0.957	0.993	1.428	2.138

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds).
-nd: not detectable.

Table 3. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Streptococcus thermophilus* .

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthylene	0.023	nd	nd	nd	nd	nd	nd	nd	nd
2-Bromonaphthalene	0.045	nd	nd	nd	nd	nd	nd	nd	0.017
Acenaphthene	0.046	nd	nd	nd	nd	nd	nd	nd	nd
Fluorene	0.051	nd	nd	0.015	0.005	0.003	0.037	0.008	0.017
Anthracene	0.060	0.049	0.202	0.065	0.008	0.004	0.249	0.078	0.072
Phenanthrene	0.046	0.045	0.037	0.017	0.004	0.003	0.056	0.041	0.010
Pyrene	0.066	0.048	0.152	0.071	0.037	0.028	0.241	0.135	0.068
Fluoranthene	0.060	0.047	0.150	0.067	0.031	0.019	0.221	0.135	0.070
Chrysene	0.011	0.024	0.129	0.052	0.008	0.003	0.187	0.071	0.046
Benzo(a)anthracene	0.042	0.047	0.150	0.066	0.035	0.033	0.210	0.103	0.056
Benzo(k)fluoranthene	0.023	0.058	0.167	0.070	0.037	0.002	0.180	0.080	0.015
Benzo(a)pyrene	0.020	0.012	0.056	0.020	0.003	0.003	0.042	0.011	0.034
Benzo(ghi)perylene	nd	0.056	0.147	0.063	0.004	0.005	0.168	0.079	0.025
Dibenz(a,h)anthracene	nd	0.003	0.003	0.034	0.009	0.018	0.088	0.098	0.045
Indeno(1,2,3cd)pyrene	nd	0.071	0.193	0.048	0.019	0.028	0.248	0.150	0.019
Total (sum)	0.493	0.460	1.386	0.588	0.205	0.159	1.927	0.989	0.494

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds).
-nd : not detectable.

The effect of *L. bulgaricus* (Table 4) on PAHs was similar to that detected with *B. bifidium.* It was observed that naphthalene, acenaphthylene, 2-bromonaphthalene and acenaphthene were disappeared during the incubation for 48 h. However, traces of either naphthalene (0.013 µg/ml) and acenaphthylene (0.003 µg/ml) were detected after 72 h and 48 h incubations, respectively. With the other compounds, the same sequence was detected as in the other two strains.

Table 4. Persistence of PAHs in MRS media broth during incubation at 37°C as affected by *Lactobacillus bulgaricus* .

PAHs compounds	Residues of PAHs (µg/ml) during the incubation period (hr)								
	2	4	6	8	10	12	24	48	72
Naphthalene	nd	nd	nd	nd	nd	nd	nd	nd	0.013
Acenaphthylene	nd	nd	nd	nd	nd	nd	nd	nd	nd
2.Bromonaphthalene	nd	nd	nd	nd	nd	nd	nd	nd	nd
Acenaphthene	nd	nd	nd	nd	nd	nd	nd	0.003	nd
Fluorene	nd	nd	0.013	0.027	nd	nd	nd	nd	0.003
Anthracene	0.041	0.068	0.013	0.029	0.025	0.019	0.034	0.016	0.032
Phenanthrene	nd	0.011	nd	0.007	0.016	0.019	0.033	nd	0.009
Pyrene	0.035	0.125	0.115	0.084	0.061	0.073	0.029	0.047	0.037
Fluoranthene	0.024	0.115	0.133	0.080	0.060	0.074	0.010	0.003	0.038
Chrysene	nd	0.047	0.053	0.057	0.051	0.037	0.090	0.072	0.039
Benzo(a)anthracene	0.034	0.111	0.126	0.096	0.073	0.083	0.151	0.002	0.049
Benzo(k)fluoranthene	0.092	0.051	0.094	0.069	0.065	0.087	0.031	0.049	0.033
Benzo(a)pyrene	nd	0.009	0.028	0.019	0.016	0.031	0.035	0.004	0.026
Benzo(ghi)perylene	0.087	0.089	0.029	0.039	0.049	0.032	0.140	0.021	0.006
Dibenz(a,h)anthracene	0.081	0.095	0.045	0.036	0.043	0.073	0.217	0.027	0.024
Indeno(1,2,3cd)pyrene	0.140	0.047	0.172	0.023	0.102	0.071	0.121	0.062	0.032
Total (sum)	0.534	0.768	0.821	0.566	0.561	0.599	0.891	0.306	0.341

-Total of mixture compounds (4.0 µg/ml, 0.25 µg of each 16 compounds). -nd : not detectable.

Data presented in Table 5 proved the critical and significant role of LAB in uptake and/or degrade PAHs. During the incubation periods (2, 4, 6, 8, 10, 12, 24, 48 and 72 h), the reduction (%) relative to the initial concentration of PAHs (4 µg/ml) ranged from (46.6 to 92.9), (51.8 to 94.9) and (77.7 to 92.4), by *B. bifidium, S. thermophilus and L. bulgaricus,* respectively. It is worthy to mention that the highest reduction of PAHs by *B. bifidium and S. thermophilus* was observed after incubation for 10 and 12 h, and was found to be 92.6 and 96.0 %, respectively. However, the highest reduction by *L. bulgaricus* was recorded after 48 h and was found to be 92.4%. In a descending order, the strains tested could be arranged according to their ability to assimilate the PAHs at the end of incubation (72 h), to be as follows: *L. bulgaricus* (91.5%), *S. thermophilus* (87.7%) and *B. bifidium* (46.6%) as shown in Table 5.

Table 5. Persistence of polycyclic aromatic hydrocarbons (PAHs) in MRS media broth during incubation at 37°C as affected by lactic acid bacteria.(LAB).

Incubation periods/ hr	*Bifidobacterium bifidium.*		*Streptococcus thermophilus* .		*Lactobacillus bulgaricus*	
	Residue (µg/ml)	Reduction (%)	Residue (µg/ml)	Reduction (%)	Residue (µg/ml)	Reduction (%)
2	0.756	81.1	0.493	87.7	0.534	86.7
4	1.629	59.3	0.460	88.5	0.768	80.8
6	1.007	74.8	1.386	65.4	0.821	79.5
8	0.786	80.4	0.588	85.3	0.566	85.9
10	0.296	92.6	0.205	94.9	0.561	86.0
12	0.957	76.1	0.159	96.0	0.599	85.0
24	0.993	75.2	1.927	51.8	0.891	77.7
48	1.428	64.3	0.989	75.3	0.306	92.4
72	2.138	46.6	0.494	87.7	0.341	91.5

-Zero time: 4.0 µg/ml of sum total mixture (16 compounds) of PAHs (0.25 µg of each).

Degradation of polycyclic aromatic hydrocarbons (PAHs) by yoghurt starter

The purpose of this item, is to determine the role of yoghurt starter (*S. thermophilus* and *L .bulgaricus*) in degradation of PAHs compounds by in milk as complex medium . During the manufacture of yoghurt, data in Table 6 proved slightly significant role of yoghurt starter in degradation of PAHs (0.4044 µg/ml). The mean reduction (%) after 1 h of incubation at 40°C was 1.11%. However, after 2 h and 3 h, the reduction (%) increased to 2.15 and 3.46 % of sum PAHs compounds, respectively. These results indicate that the level of PAHs compounds were variable. The highest reduction were recorded with the compounds of indeno(1,2,3-cd)pyrene (5.81%), benzo (ghi) perylene (5.16%) followed by dibenz(a,h)anthracene (4.17%) at the end of incubation period (3 h). However, these reductions were slightly significant.

Table 6. Concentrations (µg/g) of PAHs during incubation at 40°C as affected by yoghurt starter

PAHs	Zero time	1 hour		2 hour		3 hour	
	concentration µg/g	Concentration µg/g	Reduction (%)	Concentration µg/g	Reduction (%)	Concentration µg/g	Reduction (%)
Naphthalene	0.0261	0.0259	0.77	0.0257	1.53	0.0251	3.83
Acenaphthylene	0.0228	0.0226	0.88	0.0224	1.60	0.0223	2.19
2-Bromonaphthalene	0.0295	0.0293	0.84	0.0291	1.36	0.0288	2.35
Acenaphthene	0.0258	0.0256	0.92	0.0253	1.92	0.025	3.10
Fluorene	0.0299	0.0296	1.00	0.0294	1.67	0.0289	3.34
Anthracene	0.0321	0.0317	1.25	0.0314	2.18	0.0311	3.12
Phenanthrene	0.0291	0.0288	1.03	0.0285	2.06	0.0281	3.44
Pyrene	0.0248	0.0245	1.21	0.0244	1.61	0.0239	3.63
Fluoranthene	0.0268	0.0265	1.12	0.0261	2.61	0.0259	3.36
Chrysene	0.0264	0.0259	1.89	0.0256	3.03	0.0253	4.17
benzo(a)anthracene	0.0212	0.0209	1.42	0.0207	2.36	0.0206	2.83
Benzo(k)fluoranthene	0.0220	0.0217	1.36	0.0215	2.27	0.0212	3.64
Benzo(a)pyrene	0.0212	0.0210	0.94	0.0207	2.36	0.0204	3.77
Benzo(ghi)perylene	0.0252	0.0248	1.59	0.0245	2.78	0.0239	5.16
Dibenz(a,h)anthracene	0.0215	0.0213	0.93	0.0209	2.87	0.0207	3.72
Indeno(1,2.3-d)pyrene	0.0200	0.0198	1.00	0.0195	2.50	0.1920	5.81
Total (sum)	0.4044	0.3999	1.11	0.3957	2.15	0.3904	3.46

-The intial pH of whole milk (O.T) was 6.8 -After 1 hr, pH was 6.1
-After 2 hr, pH was 5.9 -After 3 hr, pH was 4.8

4. Discussion:

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (*Wilson* and *Jones, 1992).* It is based on two processes: growth and co- metabolism. In the case of growth, organic pollutants are used as a sole source of carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants. Co-metabolism is defined as the metabolism of an organic compound in the presence of a growth substrate which is used as the primary carbon and energy source.

Enzymatic key reactions of aerobic biodegradation are oxidations catalyzed by oxygenases and peroxidases. Oxygenases are oxidoreductases that used O_2 to incorporate oxygen into the substrate. Degradative organisms need oxygen at two metabolic sites, at the initial attack of the substrate and at the end of the respiratory chain. Although the presence of PAHs in some types of food due to different treatments (*FSA, 2002* and *Falco el al., 2003*), no information available on the degradation of PAHs by LAB or by pure cultures of microorganisms isolated from food and dairy products. Most investigations studied the microbial degradation of PAHs in soil.

Factors that affect biodegradation include pollutant concentration and pure-exposure time. Microbial communities present in contaminated soil can metabolize PAHs at greater rates than soil microbial communities found in uncontaminated soils (*Rathbone et al., 1998*). Greater population density and diversity of microorganisms often result in increased degradation rates of PAHs in soil

(*Rathbone et al., 1998*). However, organic matter did not appear to increase the population of known PAH-degrading microorganisms as much as general heterotrophic microorganisms *(Carmichael and Pfaender, 1997).* PAH degradation capabilities are associated with members of certain taxa such as *Pseudomonas, Sphingomonas*, and *Burkholderia,* independent of origin of the soil from which bacteria isolated (*Mueller et al., 1997*). Moreover, genes responsible for PAH degradation are homologous and ordered (*Dagher et al., 1997*). These genetic characteristics restrict enzymes diversity in microbial communities of pyrene and phenanthrene contaminated soils.

Biodegradation of PAHs in the present study by LAB, *B. bifidium, S. thermophilus* and *L. bulgaricus* were similarly to that recorded with PAHs degradation in soils. The reduction of PAHs concentration in this investigation proved that the studied microorganisms degraded the PAHs at different levels. The obtained results could be explained as the bacterial cell is a high proteinous material and so may adsorbs PAHs which could interfere with cellular metabolism. Also, the variations of PAHs levels detected during the incubation periods may be due to the lowering of PAHs values of the medium by the fermentation of their lactose contents. The variations of pH values during the incubation periods may determine whether PAHs could be adsorbed on the cells or became free in the MRS medium. The results of this study indicated that microbial communities exposed to PAHs contaminated media produced distinctive patterns of substrate utilization. The pattern indicated differences in community structure which resulted in a change in decomposition ability by the microorganisms. The PAHs have induced changes in type and amount of enzymes/or composition of the microbial population. The contaminants induced enzyme response from the microorganisms under their influence. The production of aromatic ring deoxygenase one of the PAH-degrading enzymes, was induced by the presence of PAH (*Dagher et al., 1997*). However, organic matter did not appear to increase the population of known PAH-degrading microorganism's as much as general heterotrophic microorganisms (*Carmichael* and *Pfaender, 1997*).

The slightly reduction of PAHs by yoghurt starter may be related to the pH effects of the culture medium after or during the incubation period. In this sense, several authors labeled the pH as a factor that influences the microbial degradation process (*Furukawa, 1982* and *Fewson, 1988*). On the other hand, the reduction of PAHs may be due to the protein affinity and/or adsorption ability of these compounds on the fat globule. Besides, the bacterial cell is high proteinous material and so may adsorb PAHs which could interfere with cellular metabolism.

The activity of microorganisms associated with food fermentation on the contaminants especially PAHs has been less will investigated. However, similar finding with pesticides (which represent the same group of PAHs, i.e. persistent organic pollutants) was recorded by *Hantke* and *Bradley* (*1972*) who found that adsorption of organochlorine pesticide residues was related to the interference with the cellular metabolism of organisms. Moreover, *Chacko* and *Lockwood* (1967) reported that bacterial cells can accumulate pesticide molecules. On the other hand, *Kim* and *Harmon* (1970) observed that amounts of dieldrin as pesticide are adsorbed or incorporated by the cells. In addition *Abou-Arab* (1996, 1999 and 2002) confirmed that the fermentation process in milk to produce dairy products (cheese) and meat products (fermented sausage) reduced pesticide residues and these reductions were due to the activity of milk or meat starter. Besides, the author reported that lactic acid bacteria decreased some types of pesticides (DDT, malathion and fenvalerate) during the incubation periods ranged from 2 to 12o h and the reduction (%) increase as incubation increased.

Slight reduction of PAHs during yoghurt manufacturing was observed.. This result coincides with those reported by *Montoure and Muldon* (*1968*), which explained the reduction in DDT content due to adsorption preferabilitiy by the milk protein, likewise, *Hugunin and Bradley* (*1971*) reported that significant amounts of dieldrin insecticide were associated with serum protein fraction in skimed milk . On the other hand, *Abou-Arab* (1987, 1991and 2002) reported significant role of lactic acid bacteria in degradation of some types of pesticides. The author reported that yoghurt starter reduced lindane, β.BHC and DDT by 77.3, 9.0 and 2.0 %, respectively. On the other hand, the reduction of DDT and lindane was (24.1-32.5) and (27.9-40.0%), respectively with *Micrococcus varians* as meat starter. Moreover, *Chacko et al.* (1966) reported that bacterial cells can accumulate pesticide molecules. However, *Kim and Harmon* (1970) observed that amount of dieldrin are adsorbed by the cells.

It could be concluded that, LAB may affected by PAHs during the first time of incubation. Nevertheless, the microorganisms rapidly adapted with presence of such PAHs and grow fast. Then critical and significant role of these strains in uptake and/or degrade PAHs was observed. But extra care must be taken when comparing the results since *invitro* studies are not always relevant to real situation in food products. This is due to the fact that

the biodegradation process may be affected by a number of factors such as the type of microorganism (even the type of strain), the interaction between microorganisms, the microbial concentration, the composition of the medium, whether the medium is liquid or solid, and the microbial growth conditions of temperature and pH. However, more studies must be done on the biodegradation of PAHs in food media.

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