Journal of Engineering and Development

www.jead.org
Vol. 20, No.02, March 2016
ISSN 1813-7822

NAPHTHALENE BIODEGRADATION USING ACCLIMATIZED MIXED CULTURE FROM SEWAGE WASTE SLUDGE

Dr. Yasmen Abdulaziz Mustafa[1], Dr. Mohanad Jasim Mohammed Ridha[2],*Zainab Abdul Razak Naser [3], Walla Agmei Abdul Hussain Ali[4], Mohammed Salih Mahdi Al-Azzawi[5]

1) Asst. Prof., Environment Engineering Department, Baghdad University, Baghdad, Iraq.
2) Lecturer, Environment Engineering Department, Baghdad University, Baghdad, Iraq.
3) Asst. lecturer. Environment Engineering Department, Al-Mustansiriayah University, Baghdad, Iraq
4) Biologist., Environment Engineering Department, Baghdad University, Baghdad, Iraq.
5) Environmental Engineer, Environment Engineering Department, Baghdad University, Baghdad, Iraq.

(Received:26/05/2015; Accepted:16/09/2015)

Abstract: Naphthalene biodegradation using acclimatized mixed culture from sewage waste sludge was studied. Different initial Naphthalene concentrations (30to1000 mg/L) were used to evaluate the biodegradation rate of naphthalene. When the initial concentration of naphthalene increased from 30 to 1000mg/L the time required to utilize naphthalene increases from 2 to 21 days. The specific growth rate increases from 0.0092 to 0.0346 h-1 when the initial naphthalene concentration increases from 100 to 700 mg/L. Then the specific growth decreases to 0.0201 h-1 for 1000mg/L naphthalene concentration, indicating that there is an inhibitory effect on the microbial growth at high concentration. Different kinetic models for microbial growth, Monod's, Haldane, Webb and Aiba were used. The experimental results confirm well with Haldane model as compared with the other models. Logistic models were used to describe the microorganism's growth and the naphthalene degradation.
A good fitting was obtained between the logistic models and the experimental data.

Keywords: *Biodegradation acclimatized mixed culture, kinetic models, and Logistic models.*

التحلل البايولوجي للنفثالين باستخدام عينة من الاحياء المجهرية الي تم اقلمتها من حماة الصرف الصحي

الخلاصة: تم دراسة التحلل البايولوجي للنفثالين باستخدام عينة من الاحياء المجهرية التي تم اقلمتها من حماة الصرف الصحي . استخدمت عدة تراكيز من النفثالين من 30-100 ملغرام/لتر لغرض تقييم التحلل البايولوجي للنفثالين.عند زيادة التراكيز من 30 الى 1000ملغرام / لتر,الزمن الازم لاستهلاك النفثالين ازداد من 2 الى 21 يوم. معدل النمو للاحياء المجهرية ازداد من0.0092 الى0.0346 ساعة-1 عند زيادة تركيز النفثالين من 100-700 ملغرام / لتر بينما معدل النمو للاحياء المجهرية انخفض الى 0.0201 ساعة-1 عند التركيز 1000 ملغرام / لتر, مما يدل ان هناك اعاقه لنمو الاحياء الدقيقة عند التراكيز العالية.عدة موديلات لدراسة حركية النمو البكتيري تم استخدامها(Monod's, Haldane, Webb and Aiba) , النتائج بينت ان موديل Haldane اعطى انطباق جيد للنتائج العمليه مقارنة مع الموديلات الاخرى. تم استخدام الLogistic models لغرضوصف نمو الاحياء الدقيقه وكذلك تحلل النفثالين, النتائج بينت ان هناك انطباق جيد بين النتائج العملية والنظرية.

*Corresponding Author zainabrazak6@gmail.com

1. Introduction

Petroleum refineries generate huge amounts of wastewater that usually go through a treatment plant to reduce the concentrations of the different contaminants to acceptable discharge levels. The main contaminants of refinery wastewater include phenols, polycyclic aromatic hydrocarbons (PAHs) as well as heavy metals. PAHs are considered to be the most hazardous, and they are the most difficult to remove. PAHs are chemical species of diverse class of organic compounds consisting of two or more benzene rings fused in linear, angular or cluster orientations [1, 2].

The presence of PAHs in the environment has been monitored since the 1970s by the US Environmental Protection Agency (USEPA) and16 PAHs have been defined as priority pollutants. They form a subset of ‘‘toxic pollutants’’ including, for example, naphthalene, phenanthrene and anthracene [3]. These types of compounds are persisten tin the environment due to their physicochemical properties, which include very low water solubility and vapor pressure, high molecular weight , melting and boiling points, strong hydrophobicity and high thermodynamic stability of the aromatic ring that makes them to be weakly bio available and cannot degrade easily under natural conditions[4].

Naphthalene is the simplest and most biodegradable PAH compound consisting of two fused aromatic rings. Being the simplest PAH compound, it is also abundantly found due to the natural degradation processes of larger PAH molecules found in crude oils, tars and coke. Naphthalene has relatively high water solubility (31 mg/L at $25C^{o}$) and can be found adsorbed to soils and in solution in wetlands contaminated by industrial or transportation spills [5] where it has been observed to persist for decades. It is a priority pollutant CEPA [6] because of its known toxicity to higher forms of life NIOSH [7]. Due to PAHs persistence in the environment different technologies have been explored during the last decades. These methods have been classified into three main categories, physical (volatilization, photolysis, adsorption, electro remediation and filtration), chemical (chemical oxidation, photo catalysis, and coagulation-flocculation) and biological (biosorption or biodegradation) [8].

Biodegradation is a promising option for the complete removal and destruction of contaminants. Biodegradation is the use of living organisms, primarily microorganisms, to degrade or detoxify hazardous wastes. The PAH-degrading microorganism could be algae, bacteria, and fungi. It involves the breakdown of organic compounds through biotransformation into less complex metabolites, and through mineralization into inorganic minerals, H_2O, CO_2 (aerobic) or CH_4 (anaerobic) [9].

Biodegradation of organic chemicals by microbes using pure cultures can produce toxic intermediates. This problem may be overcome by the use of mixed cultures which have a wider spectrum of metabolic properties, and it is recommended to prevent the production of toxic intermediates during biological degradation [10]. The metabolic cooperation by several microorganisms may result in enhanced PAHs utilization, since metabolic intermediates produced by some organisms may serve as substrates for the growth of others [2].

Sewage waste sludge is one example of mixed cultures it is a biomass-containing waste generated from regular biological activities of municipal wastewater treatment

plants. The biomass microorganism content is heterogeneous including bacteria, fungi, yeast and protozoa. One typical drawback of bioremediation methods is the longtime of treatment, due to the low PAHs solubility in aqueous media, to overcome this problem stirred tank bioreactors offer an advantage in this case since the dissolution rate can be increased with agitation.

In this work, the dissolution of naphthalene from pure naphthalene particles and the subsequent biodegradation in the aqueous phase by utilizing sewage waste sludge as mixed cultures, using shake flask bioreactor have been investigated to determine the underlying mechanisms controlling the removal of this simple PAH molecule. Modeling of biodegradation kinetics has been also carried out using different kinetic models and compared with experimental results.

2. Materials and methods

2.1. *Materials*

Naphthalene was used in the present study; it is of analytical grade and is purchased from CDH India. The various nutrient media used is of analytical grade and is purchased from CDH and Merck, India.

2.2 Nutrients

Modified McKinney's medium was used as mineral salt medium (MSM) for the bacterial growth. The composition of the minerals in one liter of growth media is shown in Table 1 and the composition of trace elements is shown in Table 2. The medium was prepared by mixing the appropriate inorganic chemicals with one liter of distilled water that resulted in a buffered medium with a pH of 6.5-6.7.

Table 1. Modified McKinney's medium in 1 litter of distilled water

Substance	Mass or Volume
KH_2PO_4	420 mg
K_2HPO_4	375 mg
$(NH_4)_2SO_4$	237 mg
NaCl	30 mg
$CaCl_2$	30 mg
$MgSO_4$	30 mg
$Fe(NH_4)_2SO_4$	10 mg
Trace element	1mL

Table 2. Trace elements composition in 1 litter of distilled water

Substance	Mass(mg)
H3BO3	300
CoCl3	200
ZnSO4.7H2O	100
MnCl2	30
Na2MoO4	30
NiCl2	20
CuCl2	10

2.3. Microorganisms Culture

The microbial mixed culture from sewage waste sludge was used in the present study as naphthalene degrading microorganisms' source. The sewage waste sludge was collected from the sludge drying beds in Al-Rustamiyah Sewage Treatment Plant, Baghdad, Iraq.

The isolation of the microbial mixed culture from the sewage waste sludge was done by taking 10ml of sewage sludge and mixing it with 250 ml of nutrient broth. The solution is then kept in a rotary shaker at 37^{o}C for around one week. This procedure was repeated five times to insure isolation of the microbial mixed culture from the sewage waste sludge. The acclimation of the microbial mixed culture with naphthalene as the carbon source was done by taking 10ml of the isolated microbial mixed culture and mixed with 250ml nutrient broth. 2grams of glucose (as simple carbon source compound) and 0.1gram of naphthalene (as complex carbon source compound) were added to solution. The solution was then kept in a rotary shaker at 37^{o}C for one week. This procedure was repeated by slowly increasing the naphthalene concentration and decreasing the concentration of glucose in the nutrient broth solution. This is carried out by a series of transfers at one weak interval for a period of more than 8 weeks to obtain a final well acclimatized mixed culture grown in naphthalene.

2.4 Biodegradation Study

The biodegradation of naphthalene in this work is studied for a concentration range of 30-1000 mg/L individually in 500 mL Erlenmeyer flasks. In these experiments, 400 mL of MSM is autoclaved and mixed with 10mL of acclimatized mixed culture and a fixed amount of naphthalene to maintain the required concentration. Then it is kept in a rotary shaker at 150 rpm. The temperature is maintained at 37^{o}C throughout the inoculation process. Flasks are sealed with cotton stoppers to minimize VOCs loss and insure aeration. Samples are collected at regular intervals and are analyzed for residual naphthalene concentration and biomass.

2.5 Naphthalene Concentration Measurements

To determine the residual concentration of naphthalene during the biodegradation experiments, the flask contents vigorously shaken and 5 ml of a well-mixed sample was taken every day and dissolve in 10ml of ethanol. The functions of ethanol were to dissolve naphthalene and to suppress bacteria. After being shaken on a vortex mixer for 1 minute, the sample was then centrifuged and filtered through a 0.22 µm nylon micro filter to eliminate any particles including biomass. The sample was then injected into High Performance Liquid Chromatography (HPLC), PerkinElmer series 200, USA. The stationary phase is C18 column (25cm × 4.6mm, 5µm particle size) Discovery, from Supelco. The mobile phase used was a mixture of acetonitrile and distilled water (65:35). The mobile phase was pumped at a flow rate of 0.9 mL/min. Peaks were detected with a UV detector at 275nm. The HPLC reading was multiplied by 3 to obtain the naphthalene concentration before dilution. The analyses were performed in the laboratories of the Environmental Engineering Department/ University of Baghdad.

2.6 Biomass Concentration Measurements

5 ml of a well-mixed sample was taken every day to measure the biomass concentration during biodegradation experiments. The optical density (OD) was measured using UV-Vis spectrophotometer (Model T80 from PG Instrument Ltd, England).To exclude the effect of bubbles and naphthalene particles on the OD value of sample, the sample was filtered through coarse paper (What man Grade 41) into a curette. The absorbance of the supernatant was then measured at 600 nm. The obtained values were converted to grams of cell dry weight per liter using an experimental calibration curve (Biomass concentration (g/L) = 0.9* optical density)[1-5, 15].

3. Results and Discussion

3.1 Effect of naphthalene concentration on biomass

In the present study, biodegradation of naphthalene is being studied for the initial concentration range of 30-1000 mg/L. Fig.1 shows the change in naphthalene concentration with time. It is clear from the figure that the time taken by mixed culture to degrade naphthalene is dependent upon the initial concentration. When the initial concentration of naphthalene is increased from 30 to 1000mg/L the time increases from 2 to 21days. The naphthalene concentration was decreasing with time which shows that naphthalene is being consumed by the microbes as the utilize the naphthalene as a carbon source.

Fig.2 shows the growth curve which represents the change in optical density and the biomass concentration with time. The biomass concentration (OD*O.9) is increasing with time which shows that naphthalene is degraded by microbes. The growth curve can be categorized in phases namely, lag, log, stationary and death phase. Initially, there is no increment in the biomass concentration with time giving the lag phase. In log phase, the biomass concentration increases exponentially and after some time there is no further increment in biomass concentration which indicates the stationary phase. An exponential decrease in biomass concentration is observed during death phase. The maximum biomass concentrations that obtained in these experiments are 0.135, 0.157, 0.392, and 0.315 g/L for initial naphthalene concentrations100, 300, 700, and 1000mg/L respectively.

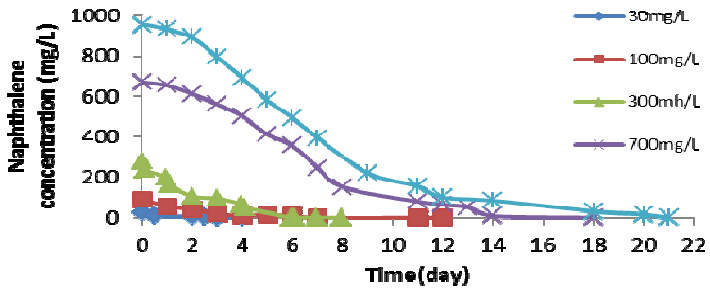

Figure 1. Residual concentrations against time at different initial naphthalene concentration

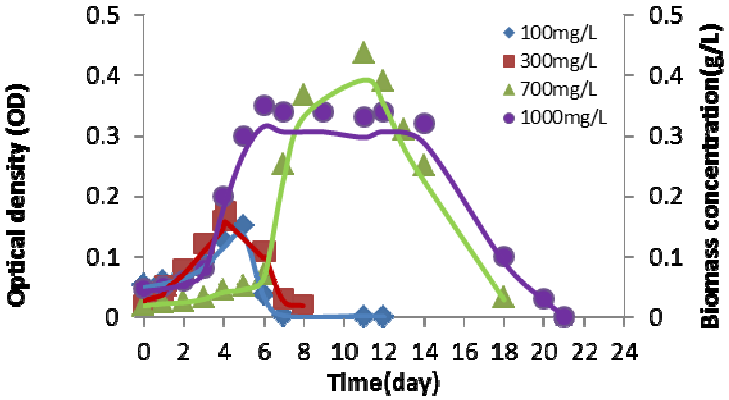

Figure 2. Change in optical density and the biomass concentration with time

3.2. Growth and biodegradation assays

Cell growth kinetics in a batch reactor can be demonstrated by Malthus law, as stated below:

$$\frac{dX}{dt} = \mu X \quad (1)$$

Where X is biomass concentration (g/L) at time t, μ is the specific growth rate (h^{-1}) and t is the incubation time (h).

The specific growth rate, μ for each value of the initial naphthalene concentration, was determined in the exponential growth phase. μ was determined from the slope of $Ln(X/X_0)$ against time, during the exponential growth phase as shown in Fig.3.X_o represent the initial biomass concentration.

The plot between specific growth rates versus initial naphthalene concentration is given by Fig.4. It is clearly observed that the specific growth rate increases from 0.0092 to 0.0346 $h^{-1}$when the initial naphthalene concentration increases from 100to 700 mg/L.T hen the specific growth decreases to 0.0201 h^{-1} for 1000mg/L naphthalene concentration. The decrease in specific growth rate shows that there is an inhibitory effect on microbial growth at high concentration. This result was observed by many authors using mixed cultures [12, 13 and 14].

The kinetic behavior which is obtained by carrying out above analysis can be modeled using various kinetic models such as Monod's, Haldane, Webb and Aiba [15and 16]as described below:

$$\text{Monodmodel: } \mu = \frac{\mu_{max}.S}{K_s+S} \quad (2)$$

$$\text{Haldanemodel: } \mu = \frac{\mu_{max}.S}{K_s+S+S^2/K_i} \quad (3)$$

$$\text{Webbmodel: } \mu = \frac{\mu_{max}.S.(1+S/K)}{K_s+S+S^2/K_i} \quad (4)$$

$$\text{Aibamodel: } \mu = \frac{\mu_{max}.S.e^{(-S/K_i)}}{K_s+S} \qquad (5)$$

Where μ is the specific growth rate (h^{-1}), μ_{max} is the maximum specific growth rate (h^{-1}),$S$is the substrate concentration(mg/L),K_s is the half saturation constant (mg/L) indicates the affinity of biomass to substrate,$K_i$is the substrate inhibition constant (mg/L) and K is the Webb constant(mg/L).

The Monod's model only describes the dependence of biodegradation rate on the biomass concentration**.** When a substrate biodegradation exhibits self inhibition, the Monod model fails. In such cases substrate inhibition is considered by incorporating the substrate inhibition constant in Monod's model. Among the various substrate inhibition models, Haldane's model is widely used [18].The values of the parameters of every model are listed in Table 3, and graphically illustrated in Fig.4. The model equations (2,3and 4) is non linear and it is solved by using the non linear regression method using Statistic software12**.** Fig.4 shows the fit of Monod's, Haldane, Webb and Aiba models with the experimental results. The value of correlation coefficient (R^2 = 0.9261) showed that the present data confirm well to the Haldane model as compared with other models.

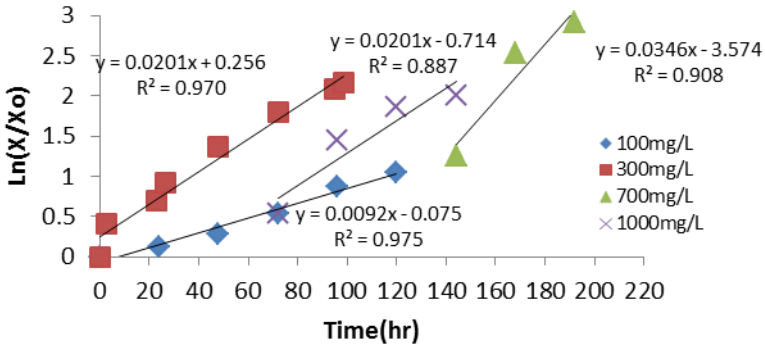

Figure 3.Rate of cell growth versus incubation time

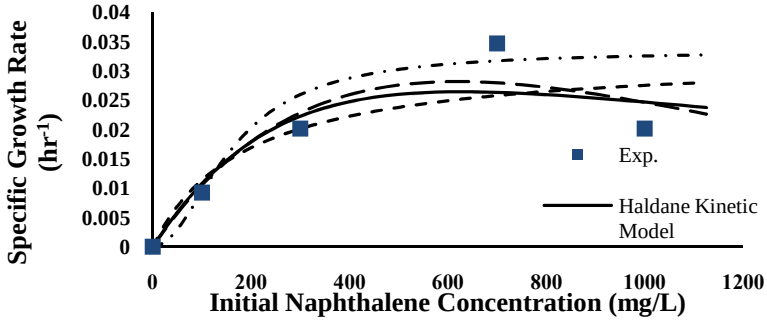

Figure 4.Specific growth rate for different initial naphthalene concentration

Table3. Growth kinetic parameter for naphthalene degradation for different models

Model	Parameters	Value
Monod	μ_m (hr^{-1})	0.0326
	K_s (mg/l)	187.14
	R^2	0.8928
Haldane	μ_m (hr^{-1})	0.0762
	K_s (mg/l)	594
	K_i (mg/l)	666.1
	R^2	0.9261
Webb	μ_m (hr^{-1})	0.1
	K_s (mg/l)	258417.9
	K_i (mg/l)	0.1
	K (mg/l)	0.3
	R^2	0.9078
Aiba	μ_m (hr^{-1})	82.1
	K_s (mg/l)	664551
	K_i (mg/l)	620
	R^2	0.914

However, the models showed a deviation in the values of biogenetic constants, such as μ_{max}, $K_s$and K_i, probably due to their differences in origin of development.

The maximum specific growth rate (μ_{max}) predicted by Haldane model equal to 0.0762h^{-1}. This value is low compared with the value reported by Jegan et al[16], which is equal to 0.318and 0.337 $h^{-1}$when using the surfactants Triton X-100 and Tween-80 respectively. The high μ_{max} may be due to the use of surfactants in addition to glucose with the specific in ocular, Micrococcus sp. which isolated from the effluent of the activated sludge plant.

The value of K_s, which indicate the affinity of biomass to substrate from Haldane model equal to 594 mg/L, indicate a good infinity of biomass toward the naphthalene.

The magnitude of $K_i$which indicate the inhibition tendency of naphthalene, equal to 666.1 mg/L. Larger K_i value indicates that biomass has a higher resistanceto substrate inhibition. This implies that at high value of K_i, inhibitory effect of substrate is low and substrate is less toxic toward microbial growth [16].

Since microbial biomass is sometimes used as a valuable tool for studying the dynamics of pollutant biodegradation, modeling the experimental growth of microorganisms and the degradation of pollutants can be relevant to achieve a better knowledge on the bioremediation process.

According to this, a logistic model (Equation 6 and 7) was used to fit the experiment a biomass and biodegradation data, which was previously described for other biodegradation processes [2, 15 and 17].

$$X = \frac{X_{max}}{1+e^{\left[\ln\left(\frac{X_{max}}{X_0}-1\right)-\mu t\right]}} \quad (6)$$

$$D = \frac{D_{max}}{1+e^{\left[\ln\left(\frac{D_{max}}{D_0}-1\right)-\mu_D t\right]}} \quad (7)$$

Where X is the biomass concentration (g/L) at time t(h), X_0 and X_{max} are the initial and maximum biomass concentrations(g/L),and μ is the specific growth rate (h^{-1}). $D$is the naphthalene removal (%) at a time t, D_0 and $D_{max}$are the initial and maximum naphthalene removal (%), and $\mu_D$is the specific degradation rate(h^{-1}).

The parameters defining the models are presented in Table 4. Fig.5 shows the comparison between the experimental the logistic model results for biomass concentration at naphthalene concentration of 100 and 300mg/L. It can be observed good fitting between the experimental data and the model. Fig.6 represents a comparison between the experimental and the logistic model results for removal efficiency% at different naphthalene concentration. A very good fitting was obtained. It can be concluded that the logistic equations (6 and 7) serve the goal of suitably model the biological process.

Table 4. Growth and naphthalene biodegradation kinetic parameters

Naphthalene concentration	Biomass parameters				Degradation parameters			
	X_0 (g/L)	X_{max} (g/L)	μ (h^{-1})	R^2	D_{max}	D_{max}	μ_D (h^{-1})	R^2
100	0.047	0.136	0.03	0.93	28.947	95.177	0.048	0.98
300	0.018	0.126	0.098	0.90	32.128	97.458	0.038	0.97
700	0.018	0.517	0.043	0.98	16.470	92.369	0.026	0.99
1000	0.042	0.344	0.048	0.98	16.573	85.974	0.025	0.99

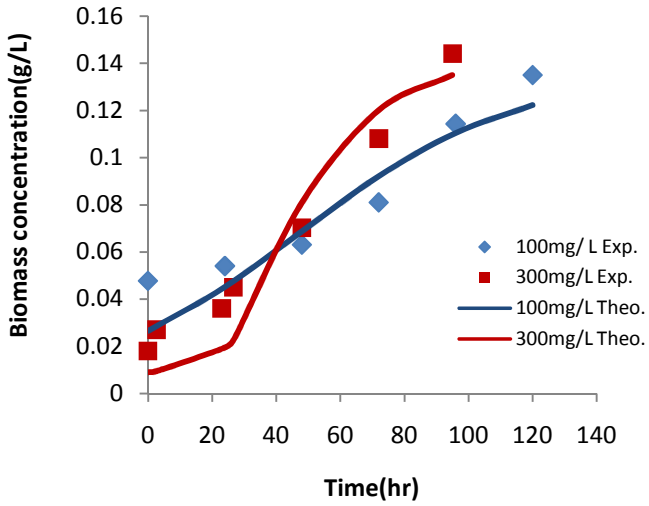

Figure 5. A comparison between the experimental and the logistic model results for biomass concentration at different naphthalene concentration

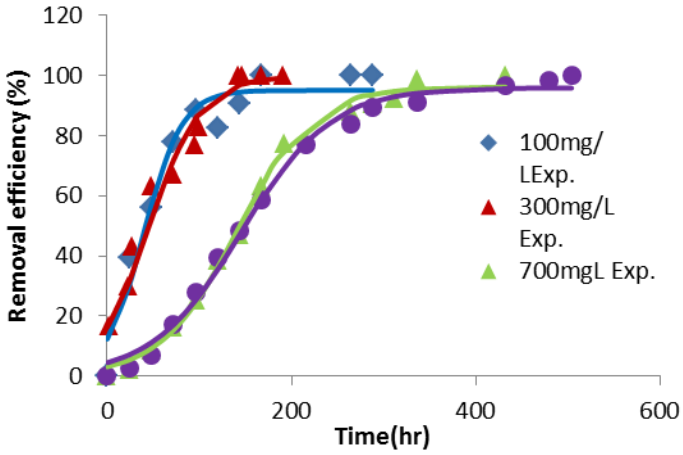

Figure 6.comparison between the experimental and the logistic model results for removal efficiency% at different naphthalene concentration

5. Conclusions

The biodegradation of naphthalene for concentrations ranging from 30 to 1000 mg/L was studied in the present work using acclimatized mixed culture from sewage waste sludge. The results show that the biodegradation of naphthalene occurred at all concentrations. The time required for utilizing naphthalene varies between 2-21 days depending on the initial concentration of naphthalene. The time of utilization increases as the initial concentration of naphthalene increase. The maximum specific growth rate was observed to be 0.0346 h^{-1}which obtained at 700 mg/L initial naphthalene concentration. While for an initial naphthalene concentration of 1000 mg/L, the specific growth rate was 0.0201 h^{-1}, indicating that there is an inhibition effect on microorganism growth. Various kinetic models for microbial growth, Monod's, Haldane, Webb and Aiba were used .The experimental results confirm well with Haldane model as compared with other models. Logistic models were used to describe the microorganism growth and the naphthalene degradation. A good fitting was obtained between the logistic models and the experimental data.

Acknowledgements

Financial support from Ministry of Higher Education and Scientific Research is gratefully acknowledged.

6. Reference

1. H.N. Muftah, A.M. Shaheen and M. Souzan,.(1009)."*Biodegradation of Phenol by Pseudomonas Putida Immobilized in Polyvinyl Alcohol (PVA) Gel*", Journal of Hazardous Materials, Vol.164, pp.720–725.

2. F. Moscoso, I. Teijiz, F.J. Deive and M.A. Sanromán, (2012)."*Efficient PAHs biodegradation by a bacterial consortium at flask and bioreactor scale*", Biore source Technology, Vol.119, pp. 270–276.
3. L.F.Bautista, R.Sanz, M.C. Molina ,N.González and D. Sánchez, (2009)."*Effect of Different Non-Ionic Surfactants on the Biodegradation of PAHs by Diverse Aerobic Bacteria*". International Biodeterioration and Biodegradation, Vol. 63,pp. 913–922.
4. B.Cao, K. Nagarajan, and K.C. Loh, (2009). "*Biodegradation of Aromatic Compounds: Current Status and Opportunities for Biomolecular Approaches*" Applied Microbial Biotechnology, Vol.85, pp. 207–228.
5. C.M. Reddy, T.I. Eglinton, A. Hounshell, H.K. Xu,L,White and R.B. Gaines, G.S. Frysinger, (2002). "*The West Falmouth Oil Spill After Thirty Years: The Persistence of Petroleum Hydrocarbons in Mash Sediments*" Environ Science Technology, Vol.36, pp. 4754–60.
6. CEPA (Canadian Environmental Protection Act, 1994). Priority substance list: polycyclic aromatic compounds. Ottawa, Canada: Minister of Supply and Services Publication.
7. NIOSH (National Institute of Occupational Safety and Health, 2003), www.cdc.gov/niosh.
8. A. Janbandhu and M.H. Fulekar, (2011)."*Biodegradation of Phenanthrene Using Adapted Microbial Consortium Isolated From Petrochemical Contaminated Environment*", Journal of Hazardous. Materrial, Vol. 187, pp. 333–340.
9. C.E. Cerniglia, (1992)."*Biodegradation of Polycyclic Aromatic Hydrocarbons*", Biodegradation, pp. 351–368.
10. A.M.G.Á. Monteiro, R. A. R. Boaventura, A. E. Rodrigues,.(2000)."*Phenol Biodegradation by Pseudomonas Putida DSM 548 in a batch reactor*", Biochemical Engineering Journal,Vol.6, pp. 45–49.
11. H.M..(2012)." *Flayeh, Bioremedation of Oily Sludge*" [Case Study in Al-Dora Refinery], PhD thesis, Baghdad University, Iraq.
12. P. Saravanan, K. Pakshirajan, P. Saha, (2006). "*Modleing the Degradation of Phenol by a Mixed Microbial Culture in a Batch Reactor*". Proceedings of International Symposium & 59th Annual Session of IIChE in association with International Partners (CHEMCON-2006), GNFC Complex, Bharuch, December 27-30.
13. S. E. Agarry, B. O. Solomon and S. K. Layokun,(2008) "*Kinetics of batch microbial degradation of phenols by indigenous binary mixed culture of Pseudomonas Aeruginosa and Pseudomonas Fluorescence*", African Journal of Biotechnology, Vol., pp. 2417-2423.
14. S. Raghuvanshi and B.V. Babu, (2009). "*Experimental Studies and Kinetic Modeling for Removal of Methyl Ethyl Ketone using Biofiltration*". Bioresource Technology, Vol.100, pp.3855 – 3861.
15. F. Moscoso, F.J. Deive, M.A. Longo and M.A. Sanromán,(2012). "*Technoeconomic Assessment of Phenanthrene Degradation by Pseudomonas Stutzeri CECT 930 in a Batch Bioreactor*",Bioresource Technology, Vol.104, pp. 81–89.

16. J. Jegan, K. Vijayaraghavan, R. Senthilkumar and M. Velan, (2010). "*Naphthalene Degradation Kinetics of Micrococcus sp.*", Isolated from Activated Sludge, Clean – Soil, Air, Water, Vol. 38, pp. 837–842.
17. Z.Bakhshia, G.Najafpourb, E.Kariminezhada, R.Pishgara, N.Mousaviaand T. Taghizadea, (2011). "*Growth kinetic models for phenol biodegradation in a batch culture of Pseudomonas putida*", Environmental Technology, Vol. 32, pp., 1835–1841.
16. J. Jegan, K. Vijayaraghavan, R. Senthilkumar and M. Velan, (2010). "*Naphthalene Degradation Kinetics of Micrococcus sp.*", Isolated from Activated Sludge, Clean – Soil, Air, Water, Vol. 38, pp. 837–842.
17.Z.Bakhshia, G.Najafpourb, E.Kariminezhada, R.Pishgara, N.Mousaviaand T. Taghizadea (2011). "*Growth kinetic models for phenol biodegradation in a batch culture of Pseudomonas putida*", Environmental Technology,Vol. 32, pp., 1835–1841.