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Modeling Biodegradation of Nonylphenol

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Abstract Nonylphenol is the primary breakdown product of nonylphenol ethoxylates, a certain class of nonionic surfactants. Nonylphenol has been found to be toxic to aquatic organisms and has been suspected of being harmful to humans due to its xenoestrogenic properties. Although there are known releases of nonylphenol to the environment, there is a lack of data describing the extent of biodegradation. This study thus focuses on much needed information on the biodegradation kinetics of nonylphenol. Oxygen uptake, cell growth and nonylphenol removal data were collected using batch reactors in an electrolytic respirometer. Nonylphenol removal, cell growth and substrate removal rates were modeled by the Monod, Haldane, Aiba, Webb, and Yano equations. The differential equations were solved by numerical integration to simulate cell growth, substrate removal, and oxygen uptake as a function of time. All models provided similar results with the Haldane model providing the best fit. The values of the kinetic parameters and the activation energy for nonylphenol were determined. These values can be used for predicting fate and transport of nonylphenol in the environment. The validity of applying each model to the biodegradation of nonylphenol was analyzed by computing the R^2 values of each equation.

Keywords Biodegradation · Kinetics · Nonylphenol · Electrolytic respirometry · Nonionic surfactants

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Nomenclature

A	Arrhenius Frequency Factor (s^{-1})
E_a	Activation Energy (kJ/mol)
k_d	decay coefficient (h^{-1})
k_T	reaction rate at temperature T, °C
k_{20}	reaction rate at temperature 20°C
K_s	half-velocity constant (mg/L)
K_i	Haldane inhibition coefficient (mg/L)
OX	Cumulative Oxygen Uptake (mg/L)
R	Universal Gas Constant (kJ/mole. °K)
S	substrate concentration (mg/L)
t	time
T	temperature °C
X	cell concentration (mg/L)
Y	cell yield
Y_{OX}	oxygen consumption coefficient
$Y_{OX,d}$	oxygen consumption coefficient for endogenous respiration

Greek Symbols

μ	Specific growth rate (h^{-1})
μ_m	Maximum specific growth rate (h^{-1})
Θ	Temperature activity coefficient

1 Introduction

Alkylphenol ethoxylates (APEs) are nonionic surfactants made up of a branched chain ethylene oxide to produce an ethoxylate chain (Ahel et al. 1994a; 1994b). The main alkylphenol ethoxylates used in industry are nonylphenol (NPE) and octylphenol (OPE). Nonionic surfactants, such as APEs, have been shown to increase solubility and dispersion of poorly soluble hydrocarbons and oils thereby enhancing desorption and bioavailability. They function as emulsifiers, wetting agents and dispersants. Nonylphenol ethoxylates comprise about 80% of the worldwide surfactant market volume (Giger et al. 1984). Formulating products that commonly contain NPEs include those used for fiber sizing, spinning, weaving, fabric dyeing, herbicides, and cosmetics, scouring and washing. Materials also likely to contain NPEs are water based paints, inks and adhesives (Corvini et al. 2006). In the household market, APEs are used mainly in laundry detergents and hard-surface cleaners. There are many other significant sources of NPEs including textile manufacturing, pulp and paper production and recycling, and some pesticide formulations (Talmage 1994).

There is an abundance of literature that indicates that NPEs can be biologically degraded in sewage treatment plants and in natural environments (Brunner et al. 1988; Ahel et al. 1996; Naylor 1995; Yuan et al. 2004; Johnson et al. 2005; Cheng et al. 2006; Hseu 2006). Most of these articles have focused on the fate of NPEs and their metabolites. Researchers have indicated that the major biodegradation product of NPEs is nonylphenol (NP). They have further identified that NP is much more persistent than the parent compound and can mimic estrogenic properties. NP has a phenolic ring and an attached lipophilic linear nonyl chain or, more usually, branched nonyl group. The structure of NP is presented in Fig. 1.

NP has been identified to be approximately ten times more toxic than its ethoxylate precursor (Brunner et al. 1988). Brunner et al. (1988) indicated that biodegradation of NPEs is accomplished by stepwise shortening of the ethoxylate chain. This produces a complex mixture of compounds that can be divided into three main groups: short-chain ethoxylates, nonylphenoxy carboxylic acids, and nonylphenol. As the chain gets shorter, the molecule becomes less soluble. The nonylphenoxy carboxylic acids and longer chain NPEs are soluble in water; the shorter chain NPEs are insoluble in water; particularly NP.

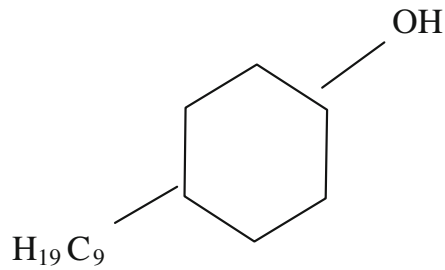

Fig. 1 Structure of nonylphenol

1.1 Nonylphenol

NP is a lipophilic organic micropollutant and is a problem in wastewater treatment, sewage sludge, surface waters and sediments (Chang et al. 2007). Nonylphenol has a log K_{ow} value of 4.48 which contributes to its tendency to bioaccumulate. It is suspected of having estrogenic effects, including contributing to the feminization of male fish in sewage outflows (Tanghe et al. 1998; Topp and Starratt 2000). This suspicion is based in part on evidence that nonylphenol can bind to human estrogen receptors, stimulate human breast cancer cell growth, and induce the expression of the egg protein vitellogenin in fish (Naylor 1995). The evidence of estrogenic effects has intensified concern over environmental and human health effects. Throughout northern Europe a voluntary ban on APE use in household cleaning products began in 1995 and restrictions on industrial applications followed by the year 2000 (Ahel et al. 1994a).

Many researchers over the years have investigated the biodegradation of NP under various conditions. Banat et al. (2000) reported results of aerobic treatment under thermophilic conditions of sewage sludge artificially contaminated with 4-NP. Experiments were carried out using three parallel laboratory-scale batch reactors operating with blank, 50 and 100 mg/l of 4-NP concentration. For the two studied concentrations, up to 66% NP reduction was achieved at a specific air flow rate of 16 L/h and a thermophilic temperature of 60°C, within 10 days of operation. The presence of 4-NP has minor effect on the rate of sludge oxidation and the nitrogen and phosphorous content in the sludge. Chang et al. (2007) investigated the effects of various factors on the aerobic degrada-

tion of NP in sewage sludge. NP (5 mg/kg) degradation rate constants calculated were 0.148 and 0.224 day^{-1} and half-lives were 4.7 and 3.1 days, respectively. The optimal pH value for NP degradation in sludge was 7.0 and the degradation rate was enhanced when the temperature was increased Cheng et al., (2006) investigated the biodegradation of nonylphenol monoethoxylate (NP1EO) and nonylphenol (NP) by aerobic microbes in sediment samples collected at four sites along the Erren River in southern Taiwan. Aerobic degradation rate constants and half-lives for NP (2 μg g^{-1}) ranged from 0.007 to 0.051 day^{-1} and 13.6 to 99.0 days, respectively; for NP1EO (2 μg g^{-1}) the ranges were 0.006–0.010 day^{-1} and 69.3–115.5 days. Aerobic degradation rates for NP and NP1EO were enhanced by shaking and increased temperature.

Levels of nonylphenol in the environment, as reported by Tanghe et al. (1998), are presented in Table 1.

While there is an abundance of literature focusing on the fate of NPEs and NP, very few studies have focused on the biodegradation kinetics of NP. Staples et al. (1999) reported that 62% of NP was biodegraded in 28 days using the OECD 301F method. However NP required more than 10 days to undergo 10–60% biodegradation. The researchers reported a half life of 20 days for NP at 22°C. However this was not a detailed study of biodegradation kinetics. As such a study of the biodegradation kinetics of NP was undertaken. The research objectives for this study were to

- Develop a mixed culture capable of biodegrading nonylphenol;
- Conduct batch biodegradation experiments under varying temperatures and concentrations of nonylphenol using respirometry;
- Model experimental data to determine the biodegradation kinetics; and
- Determine the activation energy for nonylphenol.

Table 1 Reported nonylphenol levels in the environment

Surface water	Ahel et al. (1996)	<0.1 to 180 μg NP/L
Sewage sludge	Brunner et al. (1988) and Giger et al. (1984)	1 g NP/kg dry matter in sewage sludge
Secondary effluents	Ahel et al. (1994a)	2.2–44 μg NP/L
	Blackburn et al. (1999)	Up to 330 μg NP/L
Groundwater	Barber et al. (1988)	± 1 μg NP/L
	Ahel et al. (1996)	7.2 μg NP/L

2 Materials and Methods

2.1 Respirometric Studies

An N-CON electrolytic aerobic respirometer (NCON Systems Co. Inc., Crawford, GA) with adjustable temperature controlled water baths was used for this study. Acclimated cultures capable of degrading nonylphenol were developed using wastewater from the Mullica Hill wastewater treatment plant located in New Jersey. The acclimated cultures were grown at the temperature the experiments were to be carried out. Enrichment of bacteria capable of degrading nonylphenol was accomplished in batch reactors containing nonylphenol as the sole carbon source. The culture was acclimated to nonylphenol through repeated transfers into fresh autoclaved nutrient buffer media, thereby ensuring that at least 99% of the bacteria used in the experiments were involved in the biodegradation of nonylphenol.

Nonylphenol with 98.0% purity was obtained from Fluka (Fluka, Milwaukee, WI). The analytical standard of NP with 98.0% purity was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other solvents were purchased from Sigma Chemical Co. (St. Louis, MO). Nonylphenol concentrations of 30, 90, and 150 mg/L were used for this study. HACH BOD nutrient buffer pillows (HACH Chemical Company, Loveland, CO) were used for the growth media. The respirometer was set at a constant temperature of 20°C for conducting experiments with various NP concentrations. Separate trials were conducted at 15, 20, and 30°C to determine the effects of temperature on the biodegradation kinetics of nonylphenol at a concentration of 30 mg/L. NP and cell concentrations were measured at certain intervals by extracting samples from the reactors that were equipped with side arms with septa for sample collection. NP was measured using a Hewlett Packard 1100 HPLC according to the method outlined by Giger et al. (1984). Cell mass was measured as protein by the method of Lowry et al. (1951). Literature indicates that dry cell mass is twice the protein concentration. As such cell mass was calculated by doubling the protein concentrations. Controls without NP and cells only were maintained to determine oxygen uptake due to endogenous cell growth. Abiotic degradation of NP was also assessed by maintaining controls with NP only. All experiments were conducted in duplicates.

2.2 Modeling NP Biodegradation

Biodegradation kinetics play an important role in the fate of synthetic organic chemicals in both natural and laboratory environments. As a consequence, prediction of the fate of such chemicals requires quantification of their biodegradation rates (Brown et al. 1990). Determination of the parameters describing biodegradation was once a tedious and labor-intensive undertaking. However, it is now possible to determine biodegradation parameters by oxygen uptake data collected automatically from batch reactors using electrolytic respirometry. The principles of aerobic respirometry have been described in details by Grady and his coworkers (Dang et al. 1989; Gejlsbjerg et al. 2003 ; Grady et al. 1989; Smets et al. 1996). The key advantage of using respirometric data for estimation of substrate biodegradation kinetics is the fact that substrate removal, cell growth and oxygen consumption are all stoichiometrically linked.

The most widely used expressions for biodegradation kinetics is the Monod equation which is applicable to non-inhibitory substrates (Monod 1949). This model has been further modified for inhibition and a number of models have been proposed for substrate inhibition. Inhibition is defined as the interference of enzyme activity that affects reaction rates. In biological treatment biodegradation rates can be impacted tremendously due to the inhibitory nature of external compounds. Many of the toxic compounds in the environment are in fact enzyme inhibitors (Bailey and Ollis 1986). Depending on the impact of the inhibitor on the biochemical reaction, inhibition can be classified as competitive, un- or non competitive, feedback or as substrate inhibition. Competitive inhibition can be overcome by increasing substrate concentration. Non competitive inhibition is irreversible. Substrate inhibition occurs when concentrations of the substrate itself become inhibitory to the biochemical reaction.

The Monod model and the modified model for inhibition by various researchers are presented in Table 2.

Biodegradation rates are also impacted by temperature. The Arrhenius model has been used extensively in biological and engineering literature over limited temperature ranges (Onysko et al. 2000) in quantifying the effect of temperature on reaction rates. The

Table 2 Substrate-inhibition models

Source	Model	Equation
Monod (1949)	$\mu = \frac{\mu_{max} S}{S+K_s}$	(1)
Haldane (1930)	$\mu = \frac{\mu_{max} S}{S+K_s+\frac{S^2}{K_i}}$	(2)
Webb (1963)	$\mu = \frac{\mu_{max} S\left(1+\frac{S}{K}\right)}{S+K_s+\frac{S^2}{K_i}}$	(3)
Yano et al. (1966)	$\mu = \frac{\mu_{max} S}{S+K_s+\left(\frac{S^2}{K_i}\right)\left(1+\frac{S}{K}\right)}$	(4)
Aiba et al. (1968)	$\mu = \frac{\mu_{max} S_e^{-\frac{S}{K_i}}}{S+K_s}$	(5)

Arrhenius equation for cell growth rate μ_m is presented below:

$$\mu_m = Ae^{-\frac{E_a}{RT}}$$

The above mentioned models were used to analyze the experimental data obtained for NP removal, cell growth and oxygen consumption. Equations used to describe the removal of nonylphenol (S), cell growth (X) and oxygen uptake (OX) with time are presented below:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} \tag{7}$$

$$\frac{dX}{dt} = \mu X - k_d X \tag{8}$$

$$\frac{dOX}{dt} = Y_{ox}\mu X + Y_{ox,D}k_d X \tag{9}$$

where: $\mu = \frac{\mu_m S}{S+K_s+\frac{S^2}{K_i}}$ and the inhibition factor is $\frac{S^2}{K_i}$

These modeling equations have been used by other researchers for modeling pentacholophenol and phenanthrene biodegradation kinetics (Klecka and Maier 1985; Jahan et al. 1998). Kinetic coefficients related to cell growth (endogenous decay coefficient k_d, half-velocity constant K_s, inhibition constant K_i, maximum specific growth rate μ_m, and maximum yield coefficient Y) were determined by developing and implementing a MATLAB™ mathematical model (Shah et al. 2003). The MATLAB™ function *ode15s*, which implements a variation of the Runge-Kutta numerical method, was used to solve the differential equations. The MATLAB™ function *lsqnonlin* was used to perform nonlinear optimization to determine the parameters in the kinetic model that best match the experimental data sets. The model predictions were compared to the experimental oxygen uptake data, NP

removal and cell growth and the process was repeated until the best agreement was obtained between the simulated and observed values. The routine minimized the residual sum of the squares error (RSSE) between the experimental and observed values.

2.3 Statistical Analyses

Statistical data analyses were conducted to determine if the data for oxygen uptake in the presence of nonylphenol were statistically different from the control data. Data for the 1–10 day duration was fitted by linear regression for each curve to obtain the slopes of the regression lines. The significance of the difference of the slopes between the control and the NP reactors was tested using a paired *t*-test (Crow et al. 1960). A null hypothesis that states that the difference between the slopes of the regression lines is zero was tested by comparing the "t" value obtained to the critical "t" value selected at a significance level of 2%. If "t" experimental exceeded the critical "t" value, the null hypothesis is rejected and the difference between the data is significant at the 2% significance level.

3 Results and Discussion

No oxygen uptake and cell growth were observed in the control reactors that received cells only. Abiotic degradation of NP was also not observed. All NP reactor data were statistically different when compared to the control results. Moreover there was no lag or acclimation period observed in all experiments. This was due to the use of acclimated mixed cultures. Initial cell mass added in all reactors was 5.0 mg/L. The Monod model did not represent any of the experimental data adequately. As such the four different inhibition models were used to model the experimental data.

Initial estimates of cell growth rate were obtained from separate batch studies conducted at 20°C. These were conducted to determine cell growth rate μ and cell yield Y from the slope of the straight line plots of ln X versus time and X versus S, after the acclimation period. Initial values of μ_m, K_s and K_i were determined using nonlinear regression on S and μ_m using MATLAB™. The values obtained for μ_m, K_s and K_i were 0.14 h^{-1}, 387 mg/L and 290 mg/L respectively. A cell yield value Y of 0.8 was obtained and used in the modeling studies. Y_{OX} values were estimated to be around 2.5 from total oxygen uptake and total NP concentrations. Model sensitivity analyses were conducted to see which kinetic parameters impacted the shape of the oxygen uptake, cell growth and substrate removal curves drastically. The parameters that appeared significant included μ_m, K_s, Y_{OX}, Y and K_i. The endogenous decay coefficients k_d and $Y_{OX,d}$ did not impact the curves significantly and were assumed to be 0.005 and 0.008 h^{-1}.

The average results of NP removal, cell growth and oxygen uptake for 30 mg/L NP at 20°C is presented

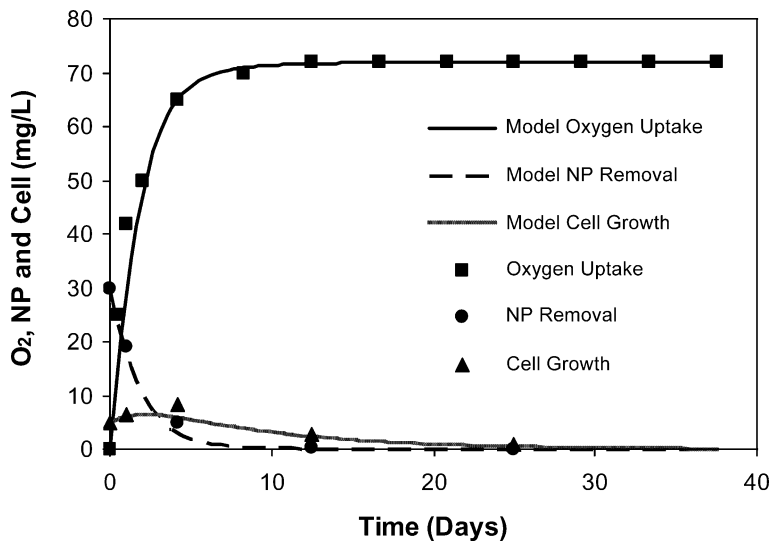

Fig. 2 Oxygen uptake, cell growth and NP removal for 30 mg/L nonylphenol at 20°C

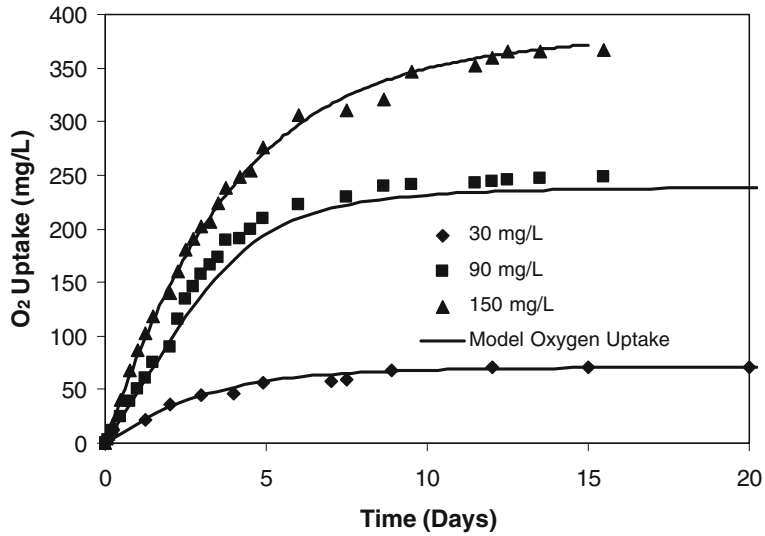

Fig. 3 Oxygen uptake at various NP concentrations at 20°C

in Fig. 2 while the results of reactors containing NP at a concentration of 30, 90, and 150 mg/L at 20°C are shown in Fig. 3.

The lines in Figs. 2 and 3 represent the Haldane model predications for the experimental data. The Haldane model adequately represented the experimental data at 20°C.

It is evident from Fig. 3 that NP biodegradation was still evident at high concentrations and toxic effects were not noted for the concentrations of NP used in this study. Similar plots were obtained for all data sets and each set was modeled with the four select models.

The kinetic parameters obtained for the selected inhibition models are presented in Table 3.

It is evident from Table 3 that the modeling results do not vary significantly from each other. Since the Haldane model yielded an R^2 value of 0.9744 it was used for further data analyses. The optimum Haldane model kinetic coefficients at 20°C were close to the values reported earlier from the batch studies. The Haldane model (Bailey and Ollis 1986) is a special case of uncompetitive inhibition and is termed as *substrate inhibition* which occurs at high substrate concentrations. It is primarily caused by more than one substrate molecule binding to an active site meant for just one, often by different parts of the substrate molecules binding to different sub-sites within the substrate binding site. If the resultant complex is inactive this type of inhibition causes a reduction in the rate of reaction, at high substrate concentrations.

The results of NP biodegradation at various temperatures are presented in Fig. 4. It is evident that the biodegradation was much slower at lower temperatures. NP biodegradation has also been shown to be temperature dependent in other studies (Tanghe et al. 1998; Banat et al. 2000; Manzano et al. 1999). Tanghe et al. (1998) indicated that NP added at concentrations of 8.33 mg/L was almost totally removed and biodegraded when the reactors were operated at 28°C. By lowering the temperature from 28°C to 10–15°C, elimination capacities decreased drastically. Chang et al. (2007) also indicated that the NP degradation rate was enhanced when temperature was increased.

The biodegradation kinetic parameters for temperature studies for 30 mg/L of NP were also modeled by

Table 3 Regression statistics and parameter estimates for various inhibition models

Temp (°C)	Model	μ_m (h^{-1})	K_s (mg/L)	K_i (mg/L)	K (mg/L)	R^2 Value
20	Aiba et al.	0.0800	345.00	349.09		0.9403
20	Haldane	0.1291	340.10	260.27		0.9744
20	Webb	0.0700	349.39	348.92	562.11	0.9720
20	Yano et al.	0.0755	348.58	347.47	515.92	0.9719

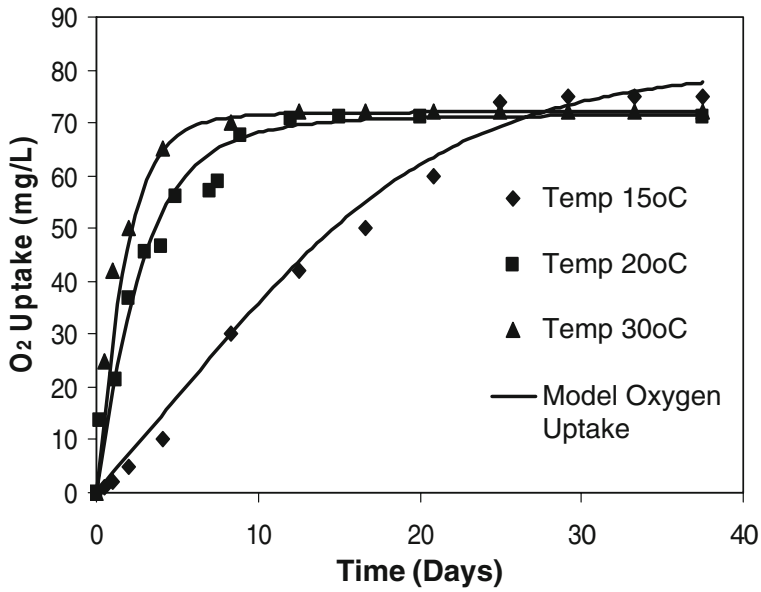

Fig. 4 Oxygen uptake at various temperatures for 30 mg/L NP

the Haldane model and the biodegradation kinetic parameters were determined. These values are presented in Table 4.

The table clearly indicates that the growth rate μ_m is a function of temperature and NP concentration. Increase in NP concentration led to decrease in μ_m. This implies that nonylphenol is inhibitory and that a higher concentration of the compound is a greater hindrance to growth.

Figure 5 shows the graph of the natural log of the growth rates for nonylphenol concentrations of 30 mg/L plotted as a function of the difference between the temperature at which the experiment was conducted and 20°C.

By substituting the values on this graph into the Q10 rule equation for reaction rates

$$k_T = k_{20}\Theta^{(T-20)} \tag{10}$$

a Θ (theta) value of 1.06 was calculated. This is within the accepted range of 1.01–1.07, with 1.04 being a typical value for biological activity (Metcalf and Eddy 1991). Although Onysko et al. (2000) had reported a similar temperature dependency for both K_i and K_s for phenol, this was not evident for our nonylphenol studies. More experiments on temperature effects may have to be conducted to obtain the impact of temperature on K_i and K_s.

Attempts were also made to determine the activation energy for nonylphenol biodegradation. Activation energy is defined as the energy, in excess over the ground state, which must be added to an atomic or molecular system to allow a particular process to take place (Bailey and Ollis 1986). It is typically denoted as E_a and has units of kJ (kilojoules). "Fast" reactions usually have a small E_a; those with a large E_a proceed slowly. E_a is independent of temperature and concentrations. Arrhenius proposed the following relationship between reaction rate (k), temperature (T in Kelvin) and activation energy E_a:

$$k = Ae^{-E_a/RT}$$

A is a pre-exponential factor or simply the *prefactor* and R is the universal gas constant. The units of the pre-exponential factor A are identical to those of the rate constant and will vary depending on the order of the reaction.

The Arrhenius plot, as shown in Fig. 6, provides values for the activation energy (E_a) and the Arrhenius

Table 4 Kinetic parameter values at varying temperatures using the Haldane model

Temperature (°C)	NP (mg/L)	μ_m (h^{-1})	K_s (mg/L)	K_i (mg/L)
15	30	0.080	1,000	2
20	30	0.129	340	260
30	30	0.200	340	260
20	90	0.104	340	260
20	150	0.075	340	260

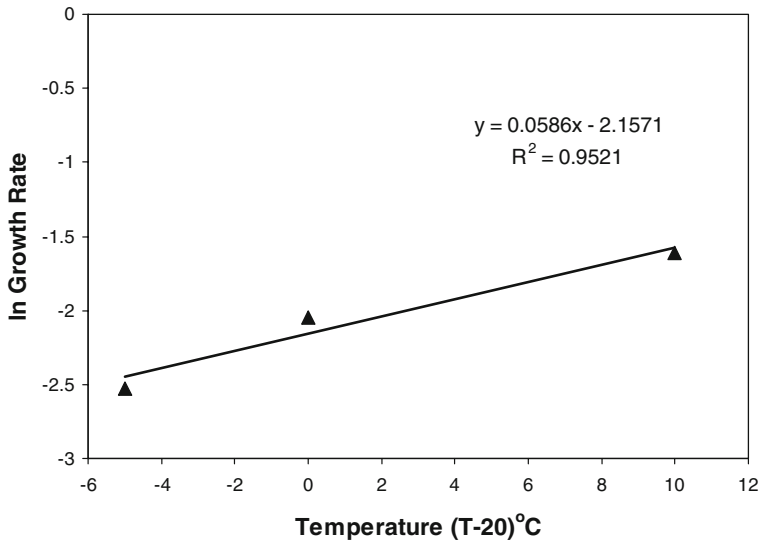

Fig. 5 Growth rate versus temperature for NP at 30 mg/L

frequency factor (A). These were determined to be A= $4.7x10^6$ h^{-1} and E_a=42.7 kJ/mole.

Since there are no existing values of E_a and A reported for nonylphenol, the nonylphenol activation energy was compared to values reported for phenol. Values of E_a for phenol were reported as 39.0, 28.4 and 61.6 kJ/mole (Onysko et al. 2000).

4 Conclusions

This study indicates that nonylphenol is biodegradable and its biodegradation is temperature dependant. The cell growth rate decreased with an increase in nonylphenol concentration. The Monod model did not adequately describe the biodegradation of nonylphenol. The Haldane, Webb and Yano inhibition models were found to adequately describe the experimental data at different nonylphenol concentrations and different temperatures. The Aiba model also described the kinetics well, but with more deviation from the actual data values. Values of activation energy of nonylphenol are similar to the ones reported for phenol in literature. Results of this study provide much needed insight into the values of the biodegradation kinetic parameters that can be used for predicting fate and transport of nonylphenol in the environment.

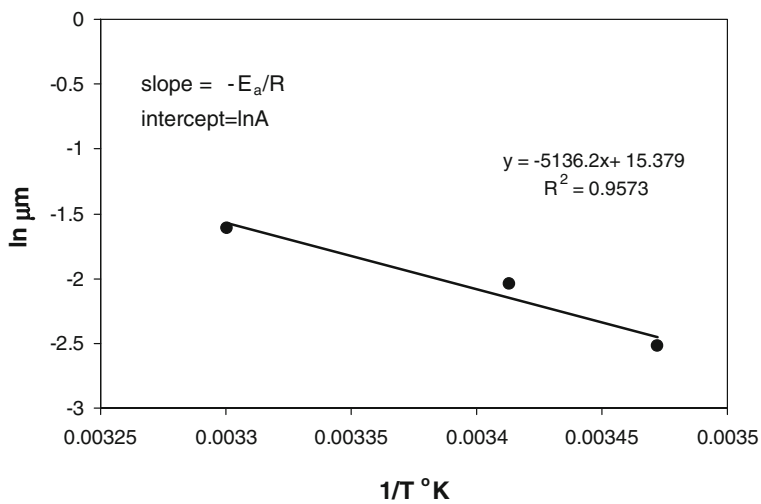

Fig. 6 Arrhenius plot for nonylphenol

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