Hindawi Publishing Corporation
ISRN Microbiology
Volume 2013, Article ID 284950, 9 pages
http://dx.doi.org/10.1155/2013/284950

Research Article

Biodegradation of Polyethoxylated Nonylphenols

Yassellis Ruiz,[1,2] Luis Medina,[1] Margarita Borusiak,[2] Nairalith Ramos,[1] Gilberto Pinto,[3] and Oscar Valbuena[1]

[1] *Centro de Investigaciones Microbiológicas Aplicadas (CIMA), Facultad de Ciencias de la Salud, Universidad de Carabobo, Valencia 2005, Venezuela*
[2] *Centro de Investigaciones Químicas (CIQ), Facultad de Ingeniería, Universidad de Carabobo, Valencia 2005, Venezuela*
[3] *Departamento de Química, Facultad de Ciencias y Tecnología (FACYT), Universidad de Carabobo, Bárbula 2001, Naguanagua 2005, Carabobo, Venezuela*

Correspondence should be addressed to Yassellis Ruiz; yjruiz1977@gmail.com

Received 14 May 2013; Accepted 4 June 2013

Academic Editors: P. Di Martino and T. P. West

Polyethoxylated nonylphenols, with different ethoxylation degrees ($NPEO_x$), are incorporated into many commercial and industrial products such as detergents, domestic disinfectants, emulsifiers, cosmetics, and pesticides. However, the toxic effects exerted by their degradation products, which are persistent in natural environments, have been demonstrated in several animal and invertebrate aquatic species. Therefore, it seems appropriate to look for indigenous bacteria capable of degrading native $NPEO_x$ and its derivatives. In this paper, the isolation of five bacterial strains, capable of using $NPEO_{15}$, as unique carbon source, is described. The most efficient $NPEO_{15}$ degrader bacterial strains were identified as *Pseudomonas fluorescens* (strain Yas2) and *Klebsiella pneumoniae* (strain Yas1). Maximal growth rates were reached at pH 8, 27°C in a 5% $NPEO_{15}$ medium. The $NPEO_{15}$ degradation extension, followed by viscometry assays, reached 65% after 54.5 h and 134 h incubation times, while the COD values decreased by 95% and 85% after 24 h for the Yas1 and Yas2 systems, respectively. The BOD was reduced by 99% and 99.9% levels in 24 h and 48 h incubations. The viscosity data indicated that the $NPEO_{15}$ biodegradation by Yas2 follows first-order kinetics. Kinetic rate constant (k) and half life time (τ) for this biotransformation were estimated to be 0.0072 h^{-1} and 96.3 h, respectively.

1. Introduction

Alkyl polyethoxylates (APEO), widely used as industrial and domestic surfactants, are added to a variety of products such as dispersants, emulsifiers, detergents, dyes, antioxidants, pesticides, spermicides, and cosmetics [1–4]. Most of these compounds including nonylphenol polyethoxylates ($NPEO_x$) are incorporated to aqueous solutions, and after being used, they are discharged in industrial or municipal water waste and eventually enter water treatment plants [5, 6]. Due to their persistency at low temperatures $NPEO_x$ remain in the environment and could be bioaccumulated, which is harmful to animals, humans, and other biological aquatic species [7–10], as its degradation products are more toxic than the original molecule [1, 3, 8, 11–13]. The toxic effects exerted by $NPEO_x$ and its degradation products include reduction of spermatozoid number, increase of testicular cancer, and feminism in aquatic male species [7, 8, 14, 15]. The highly ethoxylated $NPEO_x$ compounds lack estrogenic activity, whereas the low ethoxylated ones, included nonylphenol, which arise from nonylphenol ethoxylates by degradation in natural environments, do affect fishes, amphibians, birds, mammalians, invertebrates species such as crustaceans, mollusks, algae, yeast, and plants [3, 16–19]. These compounds also negatively affect microbial biomass growth by competitive inhibition mechanisms when the biomass is not acclimated [20]. Apparently, the estrogenic activity is induced because nonylphenol mimics the structure of the female sexual hormones, the estrogens [5, 21, 22]. Thus, nonylphenol and ethoxylated alkylphenols have been classified as pseudoestrogens and endocrine disrupters because of the harmful effects on the endocrine systems, the reproductive cycles, and other vital functions in humans and other animal species [4, 6, 13, 23, 24].

$NPEO_x$ degradation occurs through several pathways: (I) Cleavage of the ether bonds with the generation of alkyl-phenol derivatives with shorter ethoxylated chains, which yields mono- and diethylated compounds ($NPEO_1$ and $NPEO_2$) that could further be carboxylated to form ethoxyacetic and acetic nonylphenol acids [11, 25–27]. (II) ω-Carboxylation of the ethoxylated chain, yielding different polyethoxylated derivatives, being the most abundant the diethoxylated species ($NPEO_2C$); simultaneously, it is possible that the oxidation of the nonyl chain generates dicarboxylic compounds ($CNPEO_1C$), without production of nonylphenol [12]. (III) Simultaneous shortening of the ethoxylated and alkylic chains to produce propyl and heptyl diethoxylated compounds [2] and (IV) oxidation of the polyethoxylated chain, without any shortening, to yield the corresponding carboxylic acids [27, 28]. Besides, it has been reported that the initial step in the $NPEO_x$ degradation occurs on the ethoxylated moiety of the molecule, followed by further attack on the aromatic nonylphenol ring [29].

The degradation of the aromatic ring occurs under aerobic conditions yielding several soluble metabolites which are finally degraded to CO_2 [30]; the kinetic order of the reaction corresponds to a first-order process [31, 32]. In anaerobiosis, *Pseudomonas putida* degraded $NPEO_x$, with different length chains (x = 6, 9 and 20), yielding as a final product $NPEO_2$, no carboxylic acids were detected [8].

Sphingomonas xenophaga was able to cleave the aliphatic nonyl chain, bound to the phenol, depending on a specific branching pattern [5]; only the *para* isomer was degraded [23].

Finally, degradation of $NPEO_x$ is carried out by several bacterial genera: *Pseudomonas, Sphingomonas, Cupriavidus, Ralstonia, Achromobacter, Staphylococcus, Ochrobactrum, castellania, Variovorax, Bacillus,* and *Psychrobacter* [25, 27, 28, 33–35].

Due to the negative effects of $NPEO_x$ on animals and the natural environment, its use for manufacturing domestic detergents has been banned, and their industrial applications are seriously restricted in the European Community, United Kingdom, United States of America, New Zealand, and Japan [36]. However, in many countries, including Venezuela, China, and India [4], legislation to control the use of such surfactants is inexistent or is not realistic. Therefore, it seems necessary to carry out studies to search for indigenous bacterial strains able to degrade $NPEO_x$ to be used as possible bioremediation agents on contaminated natural environments.

This paper describes the isolation of five $NPEO_{15}$ degrading bacterial strains. Two of them, *Pseudomonas fluorescens* (strain Yas2) *and Klebsiella pneumoniae* (strain Yas1), showed high capacity for using $NPEO_{15}$ as sole carbon source. The degradation process was followed by viscometry of the previously used culturing media and measurement of the chemical and biological oxygen demands. To our knowledge, this is the first report of the $NPEO_x$ degrading activity by *Klebsiella* sp.

2. Materials and Methods

2.1. Biological Samples. The bacterial strains were isolated from soil samples collected in the gardens of the Centro de Investigaciones Microbiológicas Aplicadas (CIMA-UC), Campus Bárbula, Carabobo, Venezuela. To our knowledge these gardens are not contaminated with $NPEO_{15}$.

2.2. Culturing Broth. The bacteria were grown in a minimal mineral medium (MM) containing the followings salts (p/v): 1% $CuSO_4$, 0.1 mL; 0.5% $FeSO_4$, 2 mL; 1% $MgSO_4$, 0.2 mL; 1% $ZnSO_4$, 0.5 mL; 0.7% NaCl, 0.05 mL; 0.1% NH_4Cl, 1.0 mL; 0.8% NH_4NO_3, 0.125 mL and 1% $CaCO_3$, 0.5 mL, volume was adjusted to 1.0 L with 0.1 M phosphate buffer at appropriated pH values, as it will be indicated further (MM medium). All salts were proanalysis quality and the ethoxylated nonylphenol ($NPEO_{15}$, MW 880 g mol^{-1}) was kindly donated by Palma Products, CA Valencia, Venezuela.

2.3. Culture of Bacteria (MM/$NPEO_{15}$ Medium). Soil samples (100 g) were suspended in MM medium (250 mL) in 500 mL flasks, at pH 7.0, in aerobiosis, and appropriate volumes of $NPEO_{15}$ were added to reach final concentrations of 3% v/v and 30% v/v (0.0365 and 0.365 M, resp.). The systems were incubated at room temperature (22–25°C) under constant shaking during 45 days. Aliquots (3 mL), taken at different cultivation times, seeded in nutritive broth tubes and the bacterial growth was evaluated, after 24 h at 37°C, by single visual inspection of turbidity. After streaking of the total bacterial population on nutritive agar plates, after 24–48 h at 37°C, colonies were selected and assessed for growth in McConkey, Kliger's iron and oxidase media and finally the isolated colonies were stored in nutritive broth at 4°C until further use. The taxonomic identification was carried out by using the Analytical Profile Index (API) 20E and 20NE systems (bioMérieux sa, France).

2.4. Optimization of Bacterial Growth. The selected bacterial colonies were grown in 250 mL flasks containing MM/3% $NPEO_{15}$ medium (50 mL), pH 7, in aerobiosis for 12 h at 27°C, and then aliquots were submitted to different experimental protocols.

(I) To assess the purity and viability of the colonies, aliquots (1 mL) were added to the same medium (150 mL) at 27°C and the bacterial growth was assessed, at intervals of 3 h, over a period of 9 h, by measuring the absorption at 660 nm on a Spectronic Genesys II spectrophotometer. Simultaneously, aliquots (1 mL) were used, to prepare solutions by successive dilution that allowed determining the number of colony forming units per milliliter (CFU mL^{-1}).

(II) The MM/3% $NPEO_{15}$ medium was adjusted to different pH values (7, 8, 8.5 and 9) and the bacterial growth was followed as indicated in protocol I.

(III) The MM medium was adjusted to 1, 3, and 5% $NPEO_{15}$ concentrations (0.0121, 0.0365, and 0.0605 M, resp.). The bacterial growth was followed as indicated in protocol I.

2.5. Chemical Oxygen Demand (COD). The assays were executed according to the open reflux method 5220B [37].

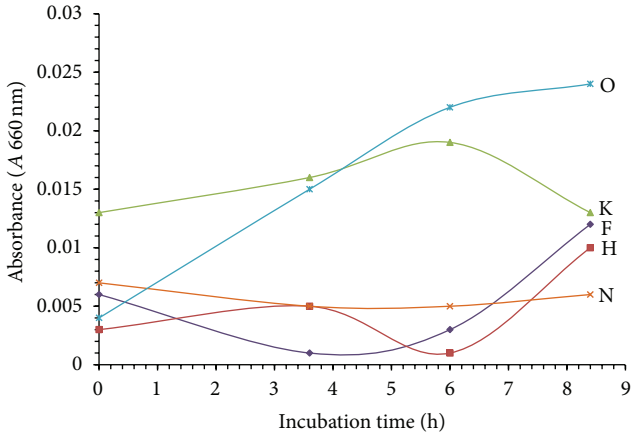

Figure 1: Growth of bacterial strains isolated from soil.

Bacterial strains were incubated for 24 h in MM/1% $NPEO_{15}$ medium, 27°C, and pH 8, and bacterial growth was followed every 3 h, at 660 nm absorbance. Aliquots were withdrawn after 0, 6, and 24 h incubations, and the samples were filtered through Millipore membranes (0.45 μm pore size) to obtain free bacterial filtrates (FBF) and submitted to analysis. A blank system (without bacterial inoculums) was also assayed.

2.6. Biological Oxygen Demand (BOD5). The assays were carried out according to the electrode membrane method 5210B [37]. After 24 h at 27°C, pH 8 in MM/1% $NPEO_{15}$ medium, inoculums (1 Ml) were incubated in the same medium and conditions. Aliquots were withdrawn after 6, 24, and 48 h incubation times and dilutions prepared in water (300 mL) were incubated for 5 days at 20°C in a dark and dry chamber. The oxygen was determined by an OAKTON DO 100.A electrode system. The value at zero time was also assayed.

2.7. Viscosity Studies. Volumes (5 mL) of bacterial cultures grown in MM/5% $NPEO_{15}$ medium, pH 8, at 27°C for 12 h, were transferred to the same medium (300 mL) and aliquots were withdrawn at different times for one week at 25°C. Immediately after the removal, the samples were filtered through Millipore membranes (0.45 μm pore size) to obtain the FBF and stored at −20°C until further use. The viscosity changes were determined in a Cannon 50W404 Ostwalt viscometer at 25°C and densities in a 10 mL pycnometer. A calibration curve relating flow times (seconds) in the viscometer to $NPEO_{15}$ concentrations (mM) was obtained in order to determine the remaining $NPEO_{15}$ concentrations. Values, at 25°C, for water density (0.99704 g mL^{-1}) and viscosity (0.8904 g cm^{-1} s^{-1}, centipoises) were taken from Weast [38]. Relative viscosity values were calculated by the expression $\eta_s = \eta_o t_s \rho_s / t_o \rho_o$, where zero subscript refers to water and s to $NPEO_{15}$ aqueous solutions [39, 40].

3. Results and Discussion

3.1. Bacterial Colony Isolation and Taxonomic Identification. From the soil samples five indigenous bacterial colonies were isolated, of which strain H grew on MM/3% $NPEO_{15}$ medium and the other four (strains F, K, N, and O) on MM/30% $NPEO_{15}$. Table 1 shows the morphological and biochemical characteristics of the isolated strains.

The bacterial growth on MM/3% $NPEO_{15}$ medium, pH 7, 27°C is shown in Figure 1. Strains K and O showed the highest growth rates; both grew without lag phase and the logarithmic phase was observed until 6 h incubation time. Because of this behavior both strains were chosen to perform subsequent experiments. According to the API identification system strains K and O were identified as *Pseudomonas fluorescens* and *Klebsiella pneumonia* and were named Yas2 and Yas1, respectively.

3.2. Bacterial Growth Conditions. The behavior of the bacterial strains at different pH values is described in Figure 2. Maximal growth rates were achieved at pH 8 (0.04 and 0.033 absorbance units), whereas the minimal growth was observed at pH 9 (0.008 and 0.01 au) after 6 h culturing time. These data correlated well with the CFU mL^{-1} numbers at 6 h culture: 10^6–10^7 and 10^3–10^4 CFU mL^{-1} at pH 8 and 9, respectively (data not shown).

In Figure 3 the bacterial growth patterns with respect to the $NPEO_{15}$ concentration in the media are shown. Maximal (0.09 and 0.062 au) and minimal (0.019 and 0.012 au) growth values were achieved at 5% and 1% $NPEO_{15}$, respectively, at 9 h incubation times.

Usually, in the culture media of 1% and 3% the maximal growth was observed at 6 h incubation, and then it reached the stationary growth phase at 9 h. The logarithmic growth phase persisted until 9 h for the 5% $NPEO_{15}$ system, and then the stationary phase disappeared. Finally, a mixture culture of Yas1/Yas2 in 1% $NPEO_{15}$ medium, pH 8, showed a synergistic effect on the bacterial growth, reaching maximal values (0.095 au) at 9 h incubations and 27°C, which represents a stimulatory average factor close to 5.7 with respect to the individual bacterial cultures at 1% $NPEO_{15}$ medium (0.014 and 0.02 au); additionally, the lag phase was absent (see Figure 4). This stimulatory effect is probably due to $NPEO_x$ cometabolism by the Yas1/Yas2 system, as it has been described for other bacterial consortiums [41].

3.3. Degradation of $NPEO_{15}$ by Bacteria. The $NPEO_{15}$ biodegradation was determined in FBF by following the flow time in a viscometer and by the chemical oxygen demand, also the biological oxygen demand was evaluated. Figure 5 shows the calibration curve that relates the molarity of $NPEO_{15}$ solutions and their flow times in a viscometer. The data fit the equation $Y = 226.9 + 0.9418X$ ($R^2 = 0.94$) calculated by linear regression.

Tables 2 and 3 show the viscosity and density changes, flow times (s), molarity (M) of remaining $NPEO_{15}$, and density (g L^{-1}) and viscosity (g cm^{-1} s^{-1}) of the FBF corresponding to different incubation times for both bacterial strains. A decrease in the FBF viscosity and density correlated well to the observed decrease of the determined flow times. From an initial value of 1.150 g cm^{-1} s^{-1} the viscosity was reduced to 0.8959 and 0.8490 for Yas1 and Yas2 systems, respectively.

Table 1: Morphological and biochemical characteristics of bacterial strains isolated from soil.

Strain	Morphology	Gram	MacConkey	Kliger iron's	Oxidase
F	Red colonies Large and thin bacilli	Negative	Lactose+	Glucose+	−
H	Light yellow colonies Large and thin bacilli	Negative	Lactose−	Glucose−	+
K Yas1	Dark yellow, mucous colonies Small bacilli	Negative	Lactose+	Glucose+	−
N	Brown colonies Small and thin bacilli	Negative	Lactose−	Glucose−	+
O Yas2	Ligth yellow colonies Small bacilli	Negative	Lactose−	Glucose−	+

Signs indicate positive/negative fermentation or enzymatic activity.

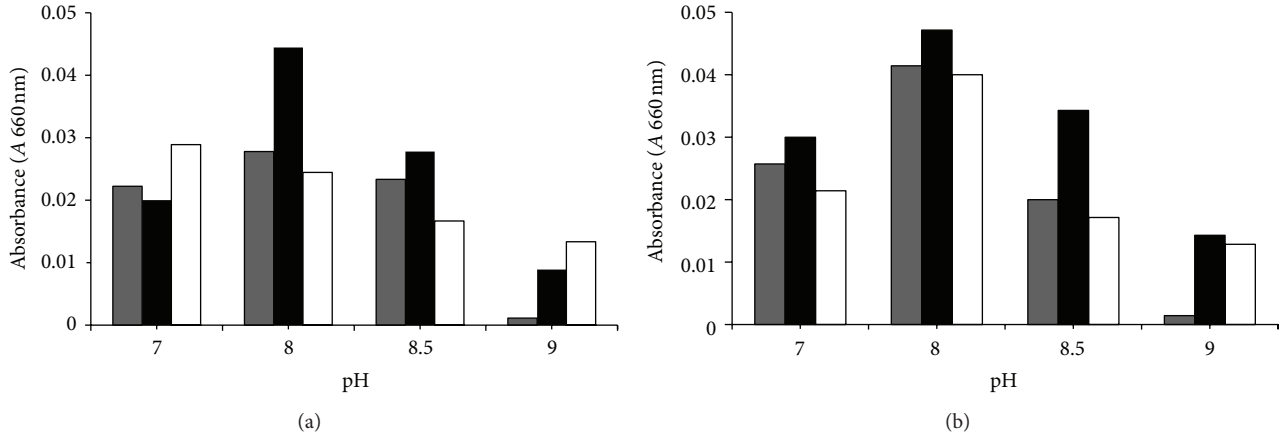

Figure 2: Bacterial growth on 3% $NPEO_{15}$ at different pH values. Gray, black, and white bars indicate bacterial growth at 3, 6, and 9 h, respectively. (a) *Klebsiella pneumoniae* strain Yas1; (b) *Pseudomonas fluorescens* strain Yas2.

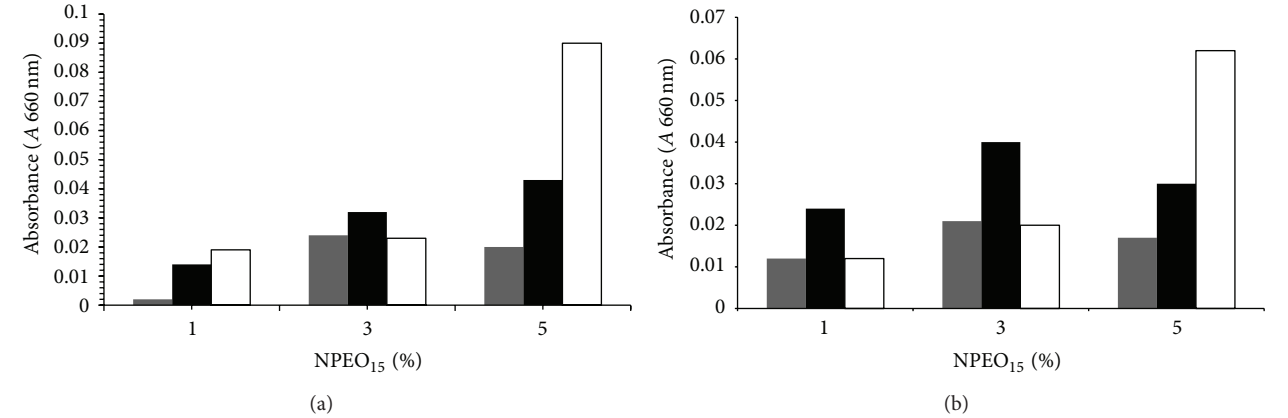

Figure 3: Bacterial growth at different $NPEO_{15}$ concentrations. Grey, black, and white bars indicate bacterial growth at 3, 6, and 9 h, respectively. (a) *Klebsiella pneumoniae* strain Yas1. (b) *Pseudomonas fluorescens* strain Yas2.

These facts indicated the cleavage of the $NPEO_{15}$ to lower molecular weight species. On basis of these data Yas1 and Yas2 degraded 0.0392 (0.0605–0.0213) and 0.0383 $NPEO_{15}$ moles L^{-1} (0.0605–0.0216) in 54.5 and 134 h, respectively. The extension of the degradation process has been reported as temperature dependent [6, 42]. In this study degradation was close to 65% at 27°C; similar values have been reported by other authors [6, 40, 41, 43].

It was also observed that foam appearing at the beginning of the incubations decreased at late incubation times. At 71 h (Yas1) and 163 h (Yas2) incubations, foam had totally disappeared and simultaneously, viscosity increases were evident

Table 2: Physical chemical parameters of the 0.0605 M $NPEO_{15}$ degradation by *Klebsiella pneumoniae* strain Yas1.

Incubation time (h)	Flow time (s)*	Remaining NPEO (moles L^{-1})	Density (g L^{-1})**	Viscosity (g cm^{-1} s^{-1})
0	283.9	0.0605	1.0200	1.1500
8.5	259.7 ± 1.689†	0.0348	0.9287 ± 0.0395†	0.9583
22.5	273.6 ± 1.376	0.0495	0.9964 ± 0.044	1.0831
32.5	250.0 ± 2.185	0.0245	0.9146 ± 0.0049	0.9084
49.5	256.0 ± 1.260	0.0308	0.9140 ± 0.0071	0.9296
54.5	247.0 ± 2.753	0.0213	0.9129 ± 0.0058	0.8959
71.0	285.5 ± 1.941	0.0622	1.0137 ± 0.039	1.1479

*Flow time of the FBF in the viscometer at 25°C, average of nine measurements. **Average of six measurements. †Standard deviation.

Table 3: Physical chemical parameters of the 0.0605 M $NPEO_{15}$ degradation by *Pseudomonas fluorescens* strain Yas2.

Incubation time (h)	Flow time (s)*	Remaining NPEO (moles L^{-1})	Density (g L^{-1})**	Viscosity (g cm^{-1} s^{-1})
0	283.9	0.0605	1.0200	1.1500
6.0	278.9 ± 1.969†	0.0552	1.0064 ± 0.001†	1.1152
12.0	280.9 ± 2.320	0.0573	1.0067 ± 0.002	1.1235
36.0	270.6 ± 1.056	0.0464	1.0141 ± 0.0047	1.0903
60.5	264.9 ± 1.357	0.0403	0.9805 ± 0.0132	1.0320
71.5	261.6 ± 1.069	0.0368	0.9098 ± 0.0029	0.9456
112.5	255.4 ± 0.853	0.0302	0.9190 ± 0.0053	0.9325
117.5	251.3 ± 0.506	0.0259	0.8714 ± 0.0365	0.8700
134.0	247.3 ± 2.573	0.0216	0.8641 ± 0.0216	0.8490
163.0	285.9 ± 1.354	0.0626	0.9855 ± 0.0488	1.1194

*Flow time of the FBF in the viscometer at 25°C, average of six measurements. **Average of four measurements. †Standard deviation.

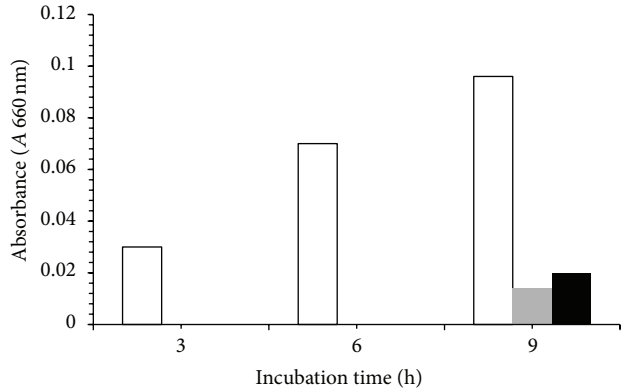

Figure 4: Growth of a mixture culture of Yas1/Yas2 on 1% $NPEO_{15}$. Grey and Black colors correspond to the individual cultures of Yas1 and Yas2 respectively.

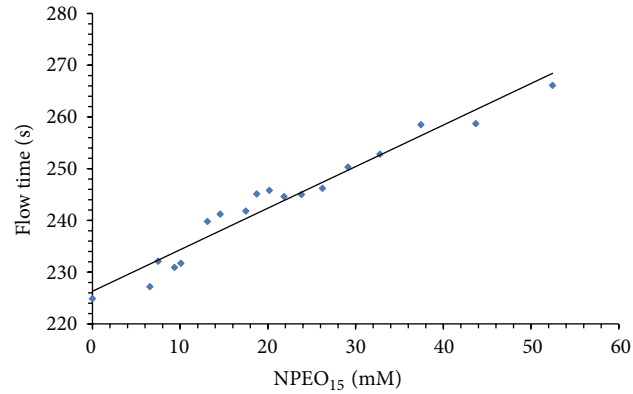

Figure 5: Calibration curve relating flow time to $NPEO_{15}$ concentration. Each point represents the average of three determinations.

in both cultures. It is well known that several bacterial genera are able to produce viscous polymers (mucopolysaccharides, dextrans, proteins, poly-β-hydroxybutyrate, polyphosphates, and xanthans) as strategies to retain nutrients and water, as energy reserves and for defense purposes [44–48]. Another aspect for consideration is the observed viscosity changes with the incubation times; Yas1 decreased the viscosity from 1.15 g cm^{-1} s^{-1} to 0.8959 in 54.5 h, a relative short time; at 8.5 h of incubation the viscosity fell, but it suddenly rose (22.5 h) to fall again at 32.5 h incubation. This apparent data dispersion could be due to the well-known mucogenic properties of the *Klebsiella* genus [44, 46, 47], which was also observed in this study (see Table 1). On the other hand, Yas2 changed the viscosity from 1.15 to 0.849 g cm^{-1} s^{-1}, in 134 h, a relative longer time, without abrupt changes. Thus, Yas1 produced viscous materials at early and late incubation times, whereas Yas2 did it only at late times. Synthesis of dextrans and alginates by *Klebsiella* and *Pseudomonas*, respectively, has been reported [49–51]; therefore, viscometry studies to evaluate degradation of viscous substances seem to be inappropriate when the bacteria are capable of synthesizing viscous polymers during the whole incubation time, such as *Klebsiella* did. However, if the bacteria yield viscous molecules at relatively late times during the incubation, then the viscosity

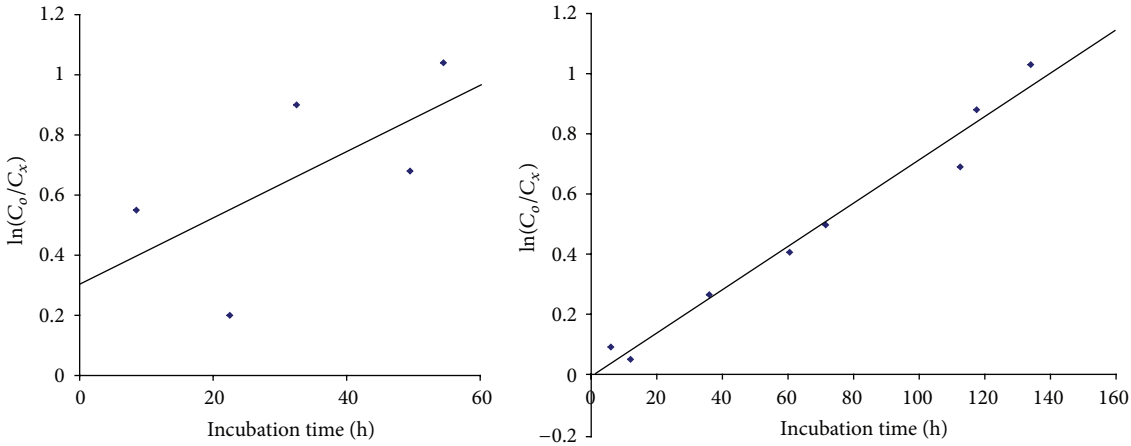

Figure 6: Kinetic order for the $NPEO_{15}$ biodegradation reaction. (a) *Klebsiella pneumoniae* strain Yas1 ($Y = 0.309 + 0.0109X$; $R^2 = 0.4028$); (b) *Pseudomonas fluorescens* strain Yas2 ($Y = 0.0072X - 0.0053$; $R^2 = 0.9728$).

Table 4: Chemical oxygen demand.

Incubation time (h)	COD values (mg O_2 L^{-1})	
	Strain Yas1	Strain Yas2
0	20,230 ± 4,303†	20,230 ± 4,303†
6	2,000 ± 1,058	3,333 ± 1,890
24	666.4 ± 462	3.066 ± 1.285

†Standard deviation.

Table 5: Biological oxygen demand.

Incubation time (h)	BOD values (mg O_2 L^{-1})	
	Strain Yas1	Strain Yas2
0	8.675	14,000 ± 5,515†
6	2,245 ± 1,874†	1,992 ± 1,295
24	60.11 ± 51.3	67.8 ± 46.9
48	5.26 ± 4.12	5.11 ± 3.11

†Standard deviation.

changes could be a useful, cheap, and rapid method to detect biodegradation of viscous xenobiotic polymers, as in the *Pseudomonas* system. Regardless of whether the bacteria degrade $NPEO_{15}$ or the bacterial polymers, differences in degradation times displayed by the bacterial strains would imply that Yas1 cleaved chemical bonds near the aromatic ring in the $NPEO_{15}$ molecule, producing low molecular weight species and causing a rapid change in the viscosity, whereas Yas2 seems to exert its action progressively on bonds near to the hydroxylated end of the surfactant ethoxylated chain, thus the decrease of the molecular mass was not as abrupt and the viscosity decrease should be slow. An alternative possibility is to assume Yas1 is a better enzyme producer. The reported data in Tables 2 and 3 allowed obtaining kinetic information about the $NPEO_{15}$ biodegradation. Taking the initial (C_o) and remaining (C_x) $NPEO_{15}$ concentrations at different incubation times it was possible to determine the kinetic order for the $NPEO_{15}$ biodegradation according to first- and second-order kinetic equations for a chemical reaction [39], also the kinetic rate constant k and the half life time τ for the NPEO biotransformation were calculated.

Figure 6(b) shows the $\ln(C_o/C_x)$ versus t plot corresponding to the Yas2 data (Table 3). The obtained straight line ($Y = 0.0072X - 0.0053$, $R^2 = 0.9728$) indicated the $NPEO_{15}$ biodegradation obeyed first-order reaction kinetics; data from other laboratories indicated that $NPEO_1$ and $NPEO_2$ also obeyed the same order kinetics [32]. A similar analysis for the Yas1 system (Figure 6(a)) did not allow assigning any reaction order because of the viscous material synthesized at early and late time incubations by the Yas1 strain. The estimated values of the rate constant k and time τ for Yas2 were 0.0072 h^{-1} and 96.3 h, respectively. For these calculations the corresponding data at 163 h were not considered because the observed viscosity increments were probably due to the synthesis of viscous bacterial polymers and not due to $NPEO_{15}$ present in the media.

Although the maximal bacterial growth was obtained at 5% $NPEO_{15}$, the following experiments were executed at 1% $NPEO_{15}$ because the principal aim of this study was to propose a satisfactory solution to the real environmental NPEO contamination which according to several reports should be less than 1 mg L^{-1} [23, 52–55].

The chemical and biological oxygen demands, determined in broths previously used by microorganisms, are indirect measurements of the carbonaceous substrate degradation by a microbial population. Tables 4 and 5 shows the chemical oxygen demand (COD) and the biological oxygen demand (BOD) of both bacterial cultures in MM/1% $NPEO_{15}$ medium, pH 8 at different incubation times.

After 24 h incubation, the COD values decreased from an initial value of 20,230 mgO_2 L^{-1} (zero time) to 666 and 3,066 mgO_2 L^{-1}, which represent 96 and 85% $NPEO_{15}$ degradation for Yas1 and Yas2 strains, respectively (see Table 4). These low COD values, representing 4 and 15% of the initial values, indicate that both bacterial strains can probably degrade $NPEO_{15}$ and its low ethoxylated derivatives, including nonylphenol, as it has been reported for other bacterial

strains [6, 26, 43, 56]. The BOD_5 data (Table 5) indicated that both bacterial strains consumed 99% (60.11/8,675 and 67.8/14,000) and 99.9% (5.26/8,675 and 5.11/14,000) of the available oxygen in 24 and 48 h, respectively, which implies that the $NPEO_{15}$ derivatives did not exert appreciable toxic effects on the bacterial strains and are biodegradable.

$NPEO_x$ and its derivative degrading bacterial strains have been isolated from several natural environments and wastewater treatment plants [1, 5, 21, 30, 57–59]. In this paper the reported data indicated that indigenous bacterial strains, isolated from soil, are able to use $NPEO_{15}$ as the sole carbon source. Although the viscosity descent is indicative of bond cleavages in the $NPEO_{15}$ molecule, it does not imply the use of the degradation products for sustaining the bacterial viability. However, the changes in the chemical and biological oxygen demands and the increments (viability) in the CFU mL^{-1} number in $NPEO_{15}$ complemented media indicate that Yas1 and Yas2 must use the $NPEO_{15}$ degradation products to satisfy their metabolic requirements and support cellular division. *P. fluorescens* (Yas1) and *K. pneumoniae* (Yas2) thus appear to be useful biotechnological tools to bioremediate NPEO contaminated waters and soils.

4. Conclusions

Five bacterial strains, isolated from soil, grew on a minimal mineral medium supplemented with $NPEO_{15}$ (0.0365 M and 0.365 M) as the sole carbon source, being *Pseudomonas fluorescens* and *Klebsiella pneumoniae* the two most efficient $NPEO_{15}$ degrading strains. The extent of $NPEO_{15}$ degradation after 24–48 h incubations, evaluated by COD and BOD_5 assays, was, 85–95% and 99.9% respectively. The kinetic rate constant (k) and the half life time (τ) for the $NPEO_{15}$ biotransformation by *P. fluorescence* were estimated to be 0.0072 h^{-1} and 96.3 h, respectively, and the process followed first-order kinetics.

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