Original Research

Examination of Biodegradation of Hydrocarbons in Emulsified Systems

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Received: 6 July, 2001
Accepted: 10 September, 2001

Abstract

This paper presents the biodegradation process carried out in water samples. Aliphatic hydrocarbons were degraded in the presence of the emulsifier. Non-ionic surfactant was used as the emulsifier. Two bacteria strains from the *Pseudononaceae* family were used in this process. We used Solid-Phase Extraction and gas chromatography for qualitative and quantitative determination of the components of the biodegradation process.

Keywords: solid-phase extraction, biodegradation, hydrocarbons, non-ionic surfactants.

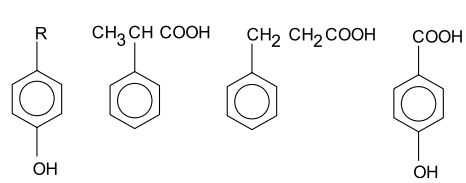
Introduction

Biotechnology is a discipline which has expanded rapidly in recent years. Biotechnological processes have found applications in the food industry, health protection (pharmaceutical industry), environmental protection (waste treatment), in agriculture (fodder, fertilisers) and in plant protection.

The degradation of hydrocarbons, especially petrochemical products, is an area where biotechnological techniques are commonly used.

There are many microorganisms which can biodegrade compounds from crude oil, especially n-alkanes having C_{12}-C_{16} chains, for example yeast from the *Candida* genus, *Deuteromycetes* fungus and bacteria genus: *Arthobacter, Brevibacterium, Corynebacterium,* and Pseudomonas. The main n-alkanes' biodegradation path is the oxidation of hydrocarbons to alcohols with oxygenase. Then, with the use of dehydrogenase, alcohols are oxidised to aldehydes and fatty acids, with following transmition to acetylo-CoA through the β-oxidation process [1].

Degradation processes can also be carried out in emulsified systems. Ionic or non-ionic surfactants are then used as emulsifiers. These surfactants are biodegraded, too. Among ionic surfactants very often linear alkylbenzene sulfonates (LAS) and branched-chain alkylbenzene sulfonates (BAS) are used. Some *Pseudomonas* strains degrade branched alkanes and linear alkanes. A *Pseudomonas aeruginosa* strain (W51D) is able to mineralise at least 70% of a BAS commercial mixture and completely degrade LAS. This strain is resistant to high concentrations of these surfactants. LAS are completely degraded in wastewater treatment plants. Different organisms participate in their mineralisation, each degrading a part of the molecule. The low rate of BAS biodegradation is due to the presence of highly branched alkyl groups. There are alcohols, alkylphenols and acids among the biodegradation products of BAS [2]:



R denotes linear or branched alkyl chain.

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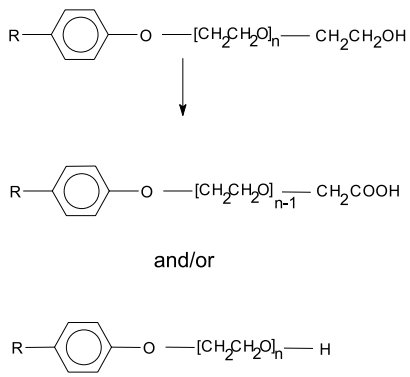
Nonylphenol polyethoxylates are one of the most often used surfactants. Two primary biodegradation processes may transform these compounds as follows:

a) shortening of the polyethoxy chain, resulting in the formation of alkylphenols and having shorter ethoxy chain alkylphenol polyethoxylates;
b) carboxylation of the terminal ethoxy unit, resulting in the formation of alkylphenol polyethoxycarboxylates [3].



The alkyl alcohol polyethoxylates can be transformed (biodegraded) in the same way. Mechanism of the biodegradation of chemicals is based on the conversion of available substrates into intermediates for the normal metabolic pathways. Degradation of the hydrophobic part of the surfactant molecule occurs via oxidation of the terminal carbon atoms. Oxidation leads to the aldehydes and carboxylic acids. There are two ways in which a polyglycol chain based on ethylene oxide can be attacked. The chain can be degraded from the end, one glycol unit at a time. Alternatively, the polyglycol chain can be broken at random into smaller glycol units [4, 5, 6].

The biodegradation products present in the postbiodegradation mixtures were analysed in different ways. The extraction techniques prior to chromatographic analysis were very often applied in environmental analysis [7, 8, 9, 10].

Solid Phase Extraction (SPE) was, among the extraction techniques, used for isolation and separation of the analytes. The sequenced SPE was used for the determination, preconcentration and separation of non-ionic polar compounds present in industrial matrices [11]. Using different sorbents (C18 phase in series with the styrene-divinylbenzene sorbent Lichrolut EN) and different eluents nonylphenol ethoxylates, alcohol ethoxylates, PEGs and phenols were separated. In the separation of anionic surfactants (LAS, BS, NPS) the styrene – divinylbenzene was used as the sorbent, compounds were eluted with 5 mL TEA and 5 mL acetic acid/methanol (1: 9, v/v). In the extraction procedure of non-ionic polar compounds two different sorbents were used: a C18 phase in the series with the styrene – divinylbenzene sorbent Lichrolut EN. Differential elution was applied to the octadecylsilica cartridge in order to obtain three fractionated extracts. n-Hexane was used to elute oxyethylated alcohols $A(EO)_n$ ($n > 15$), $A(EO)_n$ was eluted with dichloromethane/n-hexane (4:1, v/v), NPEO was eluted with methanol/dichloromethane (9:1, v/v). SDB sorbent was washed with methanol, to elute phenols and polyethylene glycols [11].

Graphitised carbon black was used as the SPE sorbent in the monitoring of aromatic surfactants and their biodegradation intermediates in the raw and treated sewage. The complex mixture was extracted from aqueous samples and it was fractionated by stepwise desorption [12].

These extraction techniques (SPE and LLE) were connected with such chromatographic techniques as: GC-FID, GC-MS, HPLC-DAD, HPLC-MS [11-14].

The aim of this work was to study the biodegradation process of hydrocarbons in the presence of emulsifier using SPE/GC-FID, and compare the effectiveness of two kinds of bacteria: *Pseudomonas putida* and *Pseudomonas aeruginosa*, in the biodegradation process.

Experimental Procedures

Chemicals

Aliphatic hydrocarbons ($C_{12}H_{26}$, $C_{16}H_{34}$) were obtained from Acros Organics (New Jersey, USA), oxyethylates $C_{12}(EO)_n$ ($n=3, 6, 7, 10$) from Sigma Chemical CO. (St. Louis, USA), solvents – n-hexane, dichloromethane, pure for analysis from Merck (Darmstadt, Germany), methanol, trichloromethane, ethyl acetate, and lauric acid pure for analysis from POCh (Gliwice, Poland). Derivatisation reagent – N,O-bis(trimethylsilyl)acetamid (BSA) and polyglycols with various number of oxyethylene groups (PEGn, $n=2, 3, 4, 9$) and lauryl acid were obtained from Fluka Chemika – BioChemika (Buchs, Switzerland). Inorganic salts: $(NH_4)_2HPO_4$ and NH_4NO_3 from POCh (Gliwice, Poland).

There were following initial concentrations of analytes in water samples:

– aliphatic hydrocarbons: 3 g/dm^3;
– oxyethylene ten lauryl ether (polydispersive $C_{12}(EO)_{10}$ will be signified with bold letters): 0.07 g/dm^3.

Biodegradation Process

The aliphatic hydrocarbons (C_{12} and C_{16}) and polyoxyethylene lauryl ether ($C_{12}(EO)_{10}$) were degraded with *Pseudomonas putida* and *Pseudomonas aeruginosa* bacteria strains isolated from soils contaminated with crude oil.

The examination of hydrocarbons' biodegradation was made in aseptic conditions.

Before addition to the biodegradation mixture microorganisms were incubated at 37°C in phosphoric solution ($(NH_4)_2HPO_4 + NH_4NO_3$) with 2-3 drops of hydrocarbons for 4-6 hours.

After growing on phosphoric nutrient 42 ml of the bacteria mixture was added to the water samples including hydrocarbons and emulsifiers (100 ml).

Such a biodegradation mixture was incubated with water bath at 37°C. The biodegradation mixture was analysed after the 3rd, 7th and 14th days of the biodegradation process. 10 ml of HCl was added to the sample and the analytes were extracted by SPE procedure.

Solid Phase Extraction

Polypropylene cartridges containing C8 and SDB-1 sorbents were produced by J.T. Baker (Deventer, Holland and supplied by Witko, Łódź, Poland).

C8 sorbent – silica bonded with octyl
Cartridge volume: 6 cm^3;
Sorbent mass: 500 mg.

SDB-1 sorbent – copolymer styrene-divinylbenzene
Cartridge volume: 6 cm^3;
Sorbent mass: 200 mg.

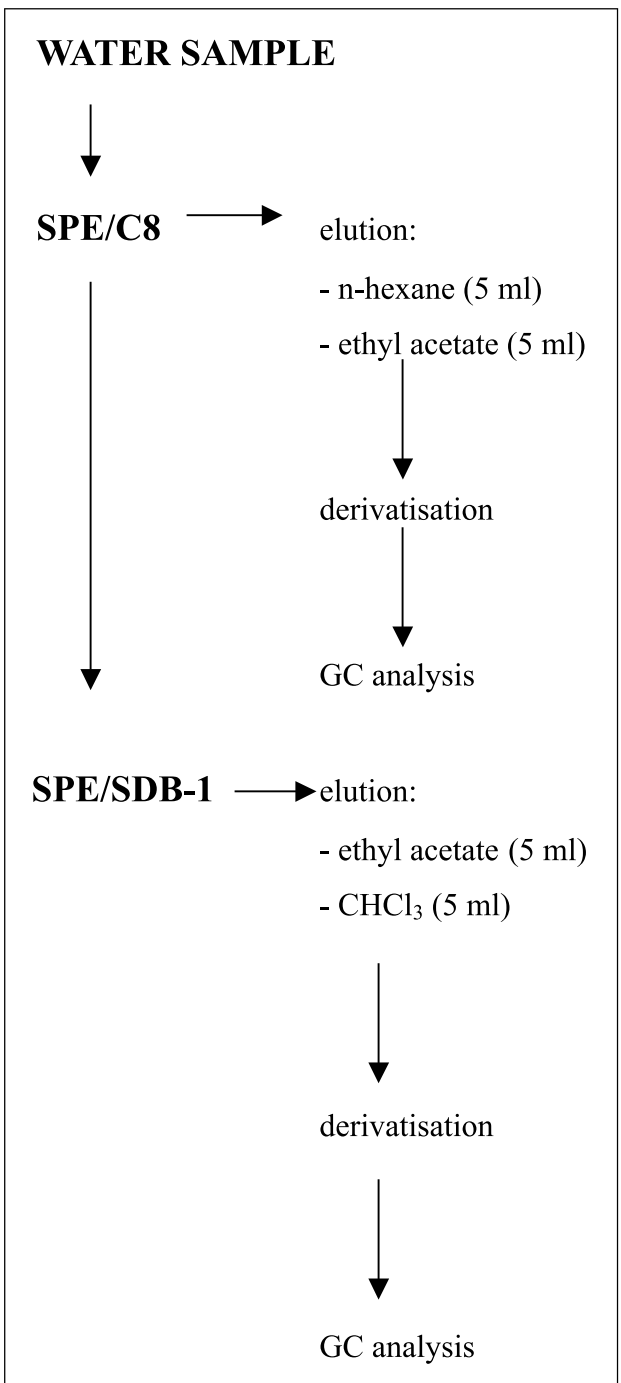


Fig. 1. The SPE procedure [15].

Extraction Procedures

We were looking for non-degraded initial compounds and biodegradation products present in the post-biodegradation mixture: different oxyethylates ($C_{12}(EO)_n$, n=(1-12)), lauryl alcohol and lauric acid, polyglycols (PEGn, n=(2-14)).

Extraction of individual compounds was examined in different extraction systems. The optimal conditions were chosen. The conditions of chromatographic analysis were also optimised. It was impossible to separate and analyse all compounds by using only one kind of sorbent (SPE) and in one chromatographic step. So we have developed a procedure with a minimal number of steps [13, 15].

Taking into account the results of our preliminary investigations the scheme presented in Fig. 1 has been used in separation, cleaning concentration and analysis of the biodegradation mixture.

In this procedure two sorbents were used: C8 phase in series with the styrene-divinylbenzene sorbent. The same conditioning method was used for both cartridges. A water sample was loaded on C8, then the residual water was transported on SDB column. Different elution procedures were used for both cartridges. From C8 column hydrocarbons (accompanied by a small amount of polar compounds) were eluted with n-hexane while oxyethylene lauryl ether was eluted with ethyl acetate. SDB sorbent was used for separation of the part of oxyethylene lauryl ether (elution with ethyl acetate), and polyglycols (elution with trichloromethane). Small amounts of hydrocarbons were also eluted from SDB cartridge. After elution solvents were evaporated, and the sample was derivatised before GC analysis.

GC Analysis

GC analyses were made on HP 5890II gas chromatograph, (Hewlett Packard) on capillary column dimethyl-diphenyl polysiloxane with a film thickness of 0.25 μm, 30 m x 0.28 mm I.D. MXT-5 (Restek). Helium was used as the carrier gas at flow-rate of 2.5 ml/min and a head pressure of 140 kPa. The column temperature was held at 115°C for 1 min, then ramped at 10°C/min to 125°C, then at 25°C/min to 175°C where it was held for 2 min, then ramped at 30°C/min to 350°C, where it was held for 7 min.

Results

The SPE procedure (Fig. 1) allows the fast separation of the complex post-biodegradation mixture with the use of smallest volume of solvents and with smallest amount of analytical steps [15, 16].

The mixture may be separated in the sequential use of sorbent/eluent steps by the utilisation of the selectivity of the SPE sorbents. The subsequent application of gas chromatography (appropriate conditions of chromatographic analysis) allowed the quantitative and qualitative determination of the components in post-biodegradation mixtures.

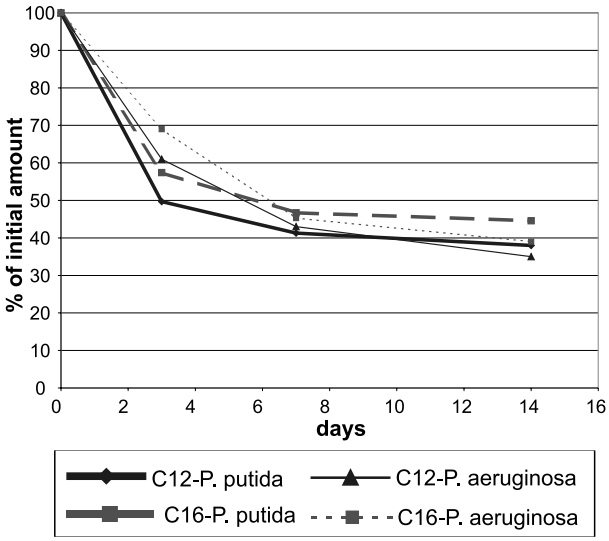


Fig. 2. The biodegradation of hydrocarbons (n-dodecane (C_{12}), n-hexadekane (C_{16})) with different bacteria strains (*P. putida, P. aeruginosa*).

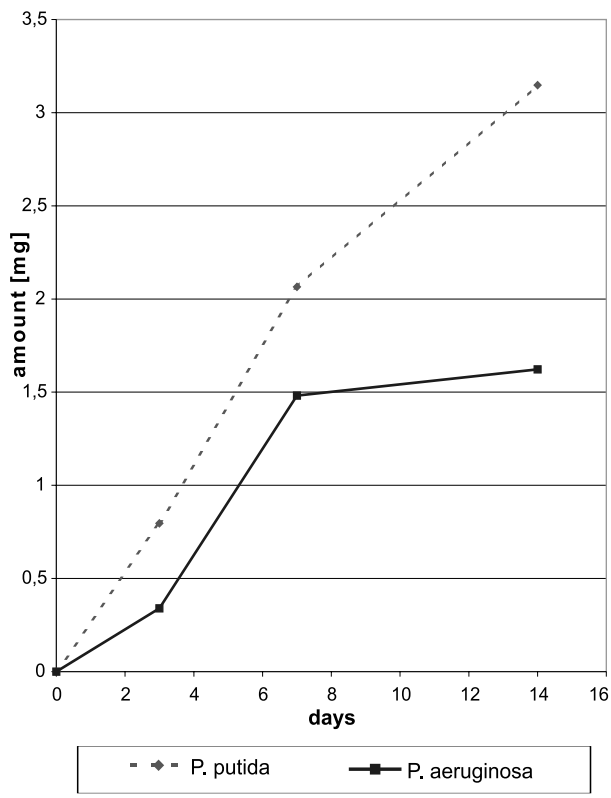


Fig. 3. The biodegradation of emulsifier ($C_{12}(EO)_{10}$) with different bacteria strains (*P. putida, P. aeruginosa*).

The effects of our research show that bacteria from the *Pseudomonaceae* family are suitable for the degradation of aliphatic hydrocarbons. Oxyethylene lauryl alcohol, present in biodegradation mixture as an emulsifier, facilitates the biodegradation of hydrocarbons, and as we can see, the emulsifier is also degraded (Figs. 2 and 3). It is possible that the decreasing content of emulsifier is one

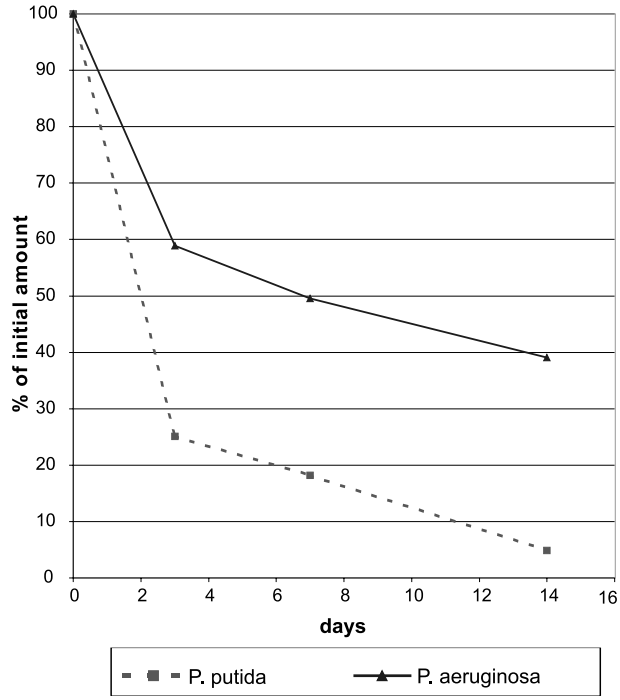


Fig. 4. The change of lauryl alcohol amounts during the biodegradation process with different bacteria strains (*P. putida, P. aeruginosa*).

of the reasons causing the slower biodegradation of hydrocarbons between 7 and 14 days of the process.

Comparison of the biodegradation process carried out with *P. putida* and *P. aeruginosa* shows that all compounds are degraded with different speed and the final amount of non-degraded compounds is most often lower for *P. putida*. With the use of these bacteria hydrocarbons are degraded better during the first 7 days, while the degradation process is slower between the 7^{th} and 14^{th} days of biodegradation. Polyoxyethylene lauryl ether is rapidly degraded by both bacteria strains during the first three days of the process while after the 7^{th} day biodegradation is slower (Fig. 2 and 3).

Among the biodegradation products lauryl alcohol was found. This is the product of the biodegradation of the emulsifier. As could be observed in Fig. 4, lauryl alcohol is produced faster in the biodegradation process carried out with *Pseudomonas aeruginosa*. This is connected with the fact that the biodegradation of the emulsifier is faster with *Pseudomonas aeruginosa* than with *Pseudomonas putida* (Fig. 3).

Another biodegradation product found in the post-biodegradation mixtures were polyethylene glycols with different chain length (Fig. 6). These are biodegradation products of oxyethylene lauryl ether. The amount of individual polyglycols changes during the process (Fig. 6). This means that different polyglycols are formed during the first days of the process – the amount of all polyglycols increases, and they are degraded in the following days of the biodegradation process.

Oxyethylene lauryl ethers with different amounts of oxyethylene groups may be found among the biodegradation products (Fig. 7). The amount of individual oxyethylates changed during the biodegradation process.

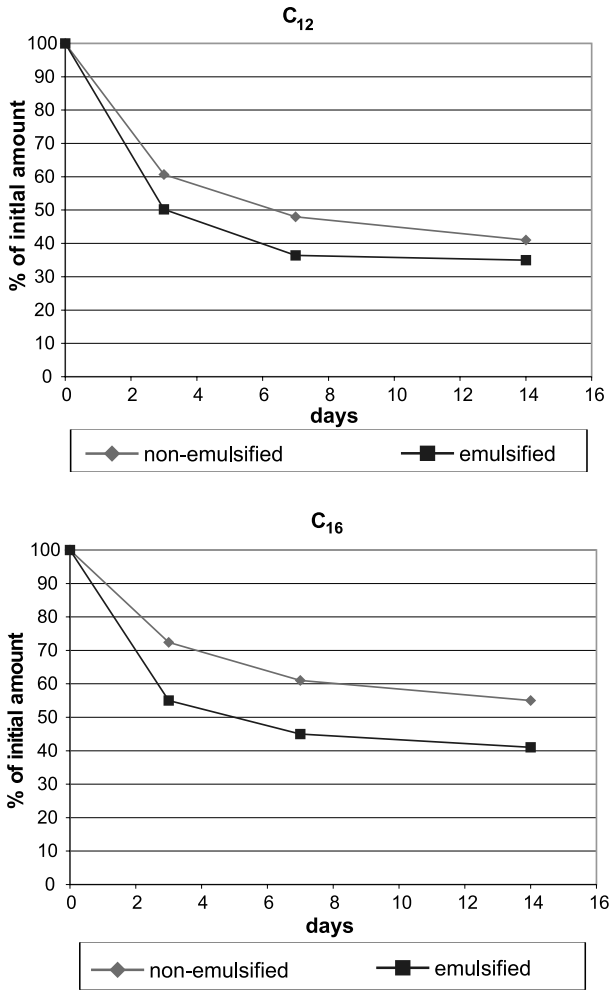


Fig. 5. The comparison of hydrocarbons' (C_{12}, C_{16}) biodegradation degree in the simple (non-emulsified) and in the emulsified systems (*P. putida*).

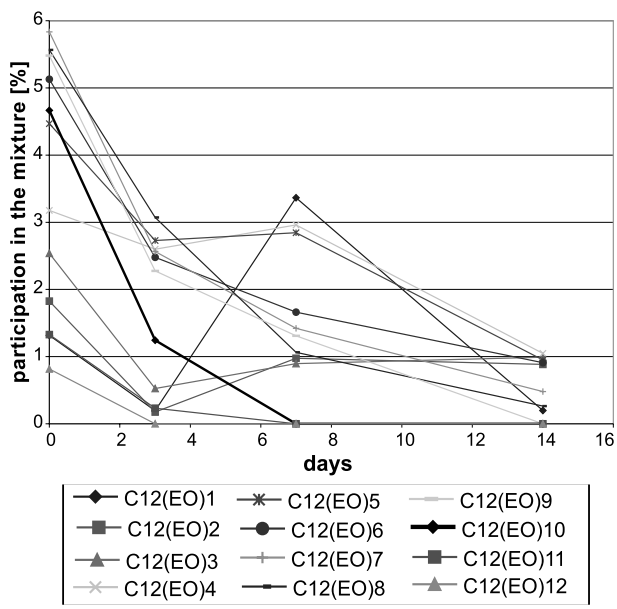


Fig. 7. The change of different oxyethylene lauryl ether amounts during the biodegradation process (*P. putida*).

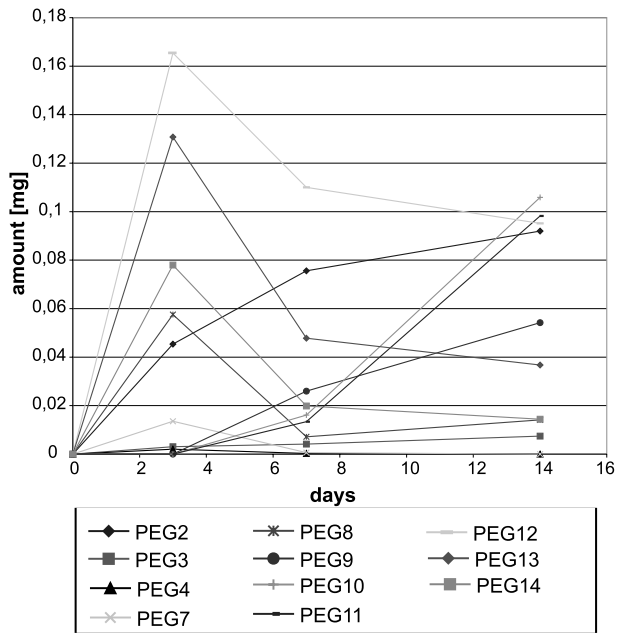


Fig. 6. The change of different polyethylene glycol amounts during the biodegradation process (*P. putida*).

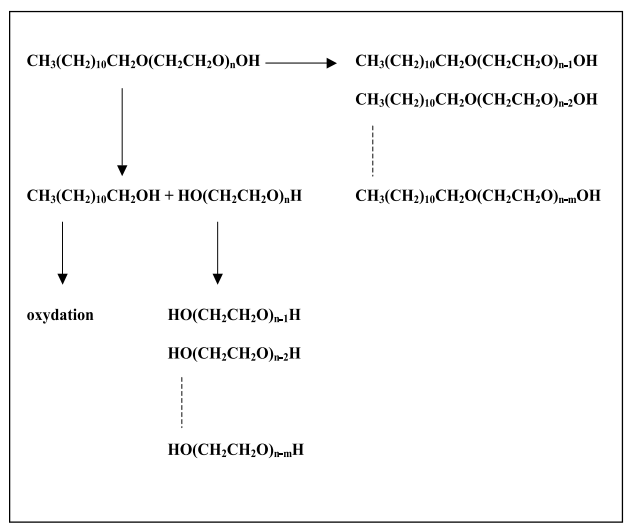


Fig. 8. Oxyethylene lauryl ether biodegradation pathways.

The amount of long oxyethylates decreased rapidly after the first 3 days, whereas the amount of shortest oxyethylates increased during the first step of the biodegradation process with a following decrease after 14 days.

Judging by the determined amounts of biodegradation products two biodegradation pathways of polyoxyethylene lauryl ether may be proposed (Fig. 8). The first one is central fission in which different polyethylene glycols and free fatty alcohol are formed. Another pathway is ω-oxidation of the oxyethylene chain leading to shorter oxyethylene chain in the polyoxyethylene lauryl ether molecule. This means that both biodegradation pathways [2-6] occur in the same biodegradation process.

Conclusion

1. The advantage of the SPE technique is its selectivity – one may separate individual compounds by using appropriate sorbents and solvents. Biodegradation products and non-degraded compounds may be easily separated.

2. Aliphatic hydrocarbons and emulsifier $C_{12}(EO)_{10}$ were well degraded with the bacteria from *Pseudomonaceae* family (Fig. 2 and 3).

3. Lauryl alcohol, polyethylene glycols and the series of oxyethylates with different lengths of oxyethylene chain were produced during the biodegradation process and were present in the biodegradation mixture (Fig. 4).

4. Determined biodegradation products allowed to describe the oxyethylene lauryl ether's biodegradation pathways (Fig. 8).

Acknowledgement

This work was supported by DPB 32/018/2001.

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