

JANUSZ PLUCIŃSKI\*, MARIA PAWLACZYK-SZPILOWA\*\*, ELŻBIETA DZIENDZIEL\*\*, HENRYKA PRYSTASZ\*, OLGA STAROOJCIEC\*

## BIODEGRADATION OF THE TETRONIC-TYPE NONIONIC SURFACTANTS UNDER STATIONARY CONDITIONS

### PART II. DEGRADATION OF TECHNICAL GRADE AND PURE SURFACTANT AND SEPARATED BY-PRODUCTS

A technical-grade Tetronic-type block copolymer containing polyoxyalkylene glycols as by-products as well as pure fractions of the adduct and polyglycols have been subjected to biodegradation under stationary conditions in the presence of the bacterium strain *Bacillus* sp. The polyether was the sole source of carbon and energy for the bacteria. It has been found that under conditions used the polyglycols are the most easily degraded component of these surfactants.

#### 1. INTRODUCTION

In our previous paper [2] we have shown that polyoxyalkylene glycols present in the technical grade preparations of the Tetronic-type block copolymers interfere with the determination of their biodegradability. It has been also suggested that polyglycols, including polyoxyethylene glycols (PEG) and a copolymer of propylene oxide (PO) with ethylene oxide (EO) which are formed in the subsequent stages of synthesis, are the easiest degradable products, thus that bacteria adapt themselves mainly to this source of carbon.

In order to confirm the above-mentioned hypothesis, we have carried out degradation tests using a sample containing a high content of polyglycols as well as isolated fractions containing these polyglycols and the main copolymer being an adduct of alkylene oxides to ethylenediamine which was used as a polymerization initiator. Biodegradation was car-

---

\* Institute of Organic and Plastics Technology, Technical University of Wrocław, ul. Łukasiewicza 2, 50-370 Wrocław, Poland.

\*\* Institute of Environment Protection Engineering, Technical University of Wrocław, pl. Grunwaldzki 9, 50-370 Wrocław, Poland.

ried out under stationary conditions in the presence of bacterial strain *Bacillus* sp. The latter was isolated previously from wastewaters from the Chemical Works „Organika-Rokita” in Brzeg Dolny which, among other pollutants, contained similar nonionic surfactants. It has been shown that this strain is able to degrade many kinds of surfactants of the alkylene oxide copolymer type. In these experiments we have used a lower concentration of polyethers (0.3 wt. %) than in the previous ones [2] in order to lower their toxicity to bacterial cells. The changes in the bacterial growth, the amount of the product remaining after the predetermined time of biodegradation, and the changes both in the concentration of various types of carbonyl groups and ethylene oxide content in the polyether have been assumed as criteria of the progress of biodegradation.

## 2. EXPERIMENTAL

The Tetric-type copolymer 2300/20 having  $\bar{M}_n = 2700$  was obtained according to [2] and separated into the main alkaline adduct, and the neutral polyglycol fraction via ionic adsorption on a macroporous cation exchanger Amberlyst 15, as reported earlier [3]. The alkaline fraction constituted 81.9% of the product, the remaining 18.1% being the neutral fraction. The determined content of ethylene oxide in both these fractions was 20.9 and 36.7 wt. %, respectively.

Biodegradation tests were carried out according to the method given in [2] at 301 K. A flatbottomed bulb filled with broth was inoculated with such an amount of bacteria that at  $\lambda = 550$  nm the extinction measured with a Spekol (Zeiss-Jena, spectrophotometer) was 0.1. The growth of bacteria was assessed by determining optical density of the culture at the wavelength given above. The results obtained were then recalculated into the dry weight of bacteria by using a calibration curve.

The strain of *Bacillus* sp. isolated from industrial wastewaters was initially adapted to the starting copolymer 2300/20. This strain is an aerobic moving Gram+ bacillus, forming endospores; it decomposes glucose, saccharose, and lactose without gas evolution but with the formation of acid, it hydrolyses protein and starch and acidifies milk.

The IR spectra were recorded as reported in [2]. The absorption value for the individual bands was calculated from peak heights at  $\bar{\nu} = 1675$ , 1730, and 1760  $\text{cm}^{-1}$  using the base line drawn parallel to the abscissa and tangent to the absorption curve at  $\bar{\nu} = 1820$   $\text{cm}^{-1}$ .

The ethylene oxide content in the polyether was determined by the  $^1\text{H-NMR}$  spectroscopy according to [1] using a Tesla BS 497 100 MHz spectrometer.

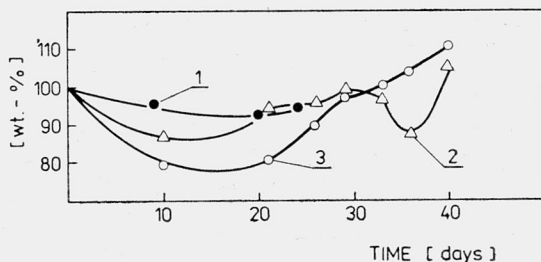
## 3. RESULTS AND DISCUSSION

Preliminary biodegradation tests of Tetric-type copolymer 2300/20 and the isolated alkaline and neutral fractions with the strain *Bacillus* sp. have shown that the loss of polyether weight determined by the extraction method is relatively low (fig. 1). For the initial

preparation this loss was ca. 20%, being similar to the results already reported [2]; for the polyglycol fraction, however, it did not exceed 10%. Like in the case of mixed populations of bacteria [2], after a certain maximum value of the weight loss was exceeded, the amount of extracted polyether increased again. The second maximum of the weight loss,

Fig. 1. Amount of extracted biodegradation products versus the time of the process pure polyglycol fraction (1), surfactant freed of polyoxyalkylene glycols (2), technical grade Tetric-type surfactant 2300/20 (3)

Rys. 1. Zależność ilości wyekstrahowanych produktów biodegradacji od czasu procesu czysta frakcja poliglikolu (1), surfaktant uwolniony z glikoli polioksyalkilenowych (2), techniczny surfaktant typu Tetricu 2300/20 (3)



so characteristic of the mixed bacterial populations, was observed only in the presence of the main adduct (the alkaline fraction). At the present stage of our studies it is difficult to decide definitely whether this phenomenon depends on the strain of bacteria or on the lower concentration of polyether in the medium (0.3 vs. 1.0 wt. %). The amount of extracted degradation products exceeding 100% has not been explained either.

In view of our previous studies [2] it might be expected that the polyglycol fraction should exhibit a markedly higher material weight loss than the starting Tetric. If, however, the degree of decomposition (fig. 2) is measured by the increase in the bacterial mass, then it can be seen that the degradation of polyglycols proceeds many times faster than that of the remaining two polyethers, for which an increase in the dry weight of bac-

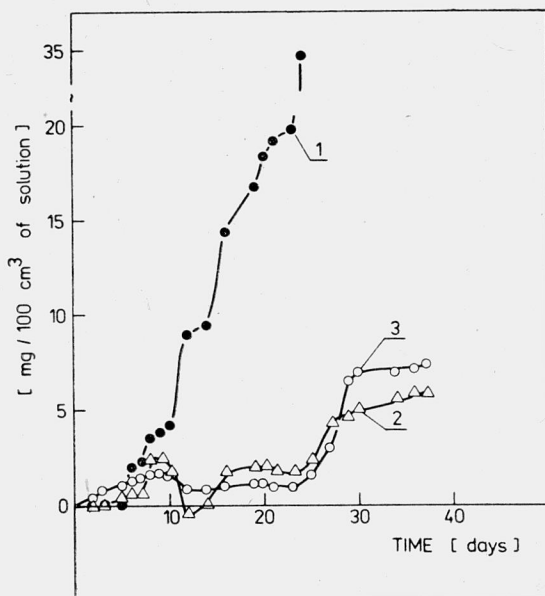


Fig. 2. Dry weight of bacteria versus biodegradation time

For explanations see fig. 1

Rys. 2. Sucha masa bakterii w zależności od czasu biodegradacji

Objaśnienia jak na rys. 1

teria differs only slightly. In the case of these two last preparations some changes in bacterial growth seem to indicate that readaptation of this strain takes place.

It should be noticed that in the presence of the starting preparation, i.e. the one used for the adaptation of the strain, bacterial mass begins to increase markedly after 1–2 days of biodegradation, and that in the case of adduct and polyglycols the same growth rate is observed as late as after 5–6 days of incubation. As far as the adduct is concerned this phenomenon is quite understandable but, according to our earlier data [2], it is difficult to explain the induction period in the case of polyglycols.

A markedly higher biodegradability of polyglycols was further confirmed by determining the changes in the absorption bands for carbonyl groups of the carboxyl ( $1760\text{ cm}^{-1}$ ), ester ( $1730\text{ cm}^{-1}$ ), and amide ( $1675\text{ cm}^{-1}$ ) types (fig. 3). Some content of the latter groups in the starting materials resulted from the oxidation with atmospheric oxygen during their purification and extraction as well as from evaporation of solvent. The presence of amides in the polyglycol fraction is related to their neutral characteristics.

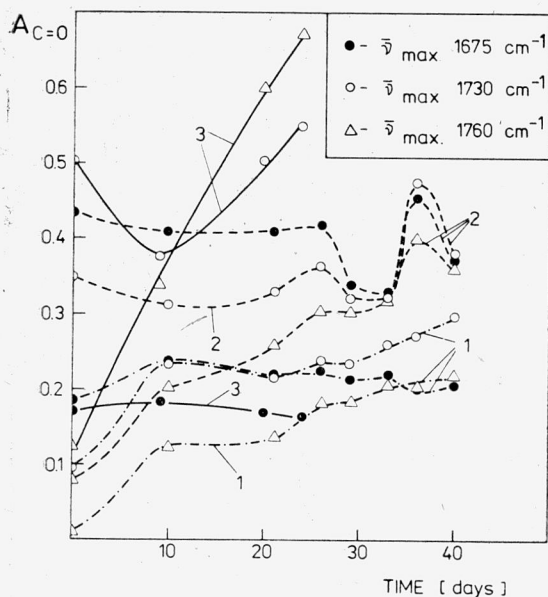


Fig. 3. Changes in absorbance (IR) of carbonyl groups during biodegradation of surfactants

technical grade Tetronic-type surfactant 2300/20 (1), surfactant freed of polyoxyalkylene glycols (2), separated polyglycols (3)

Rys. 3. Zmiany absorbancji (IR) grup karbonylowych podczas biodegradacji surfaktantów techniczny surfaktant typu Tetronic 2300/20 (1), surfaktant uwolniony z glikoli polioksyalkilenowych (2), wydzielone poliglikole (3)

In general, the changes in intensity of bands at  $\nu_{\max} = 1675$  and  $1730\text{ cm}^{-1}$  are considerably smaller than at  $1760\text{ cm}^{-1}$ . The intensity change of the  $1760\text{ cm}^{-1}$  band is particularly large in the case of polyglycol fraction. This may indicate that biodegradation of polyethers leads to the increasing content of carboxylic groups. This observation is in agreement with the known mechanism of biodegradation of nonionic surfactants belonging to the group including polyoxyethylates and polyglycols [4]. A temporal decrease in the intensity of all the bands mentioned above, observed for the adduct after 30 days of biodegradation, was manifested in the loss of its dry weight. Similar correlations have been observed when mixed populations of bacteria were used [2].

The ethylene oxide content in the polyglycol fraction decreases in the course of the process, but in the adduct it increased temporarily after the first 10 days of biodegradation (fig. 4). The decrease of ethylene oxide fraction may indicate that the decomposition rate of the polyoxyethylene groups present in both the fractions studied at the ends of macro-

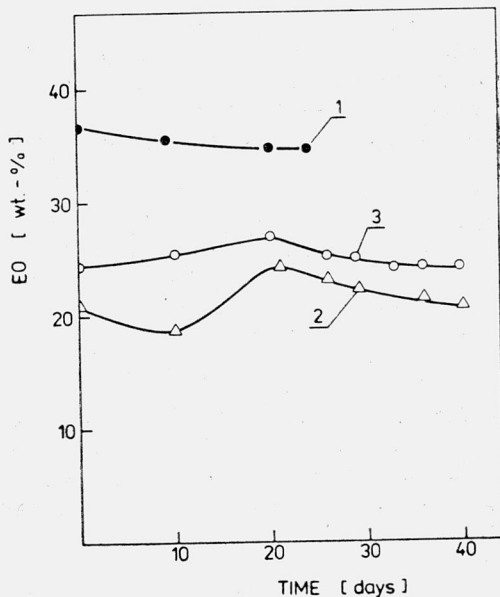


Fig. 4. Changes in ethylene oxide content during biodegradation

For explanations see fig. 1

Rys. 4. Zmiany w zawartości tlenku etylenu podczas biodegradacji  
Objaśnienia jak na rys. 1

molecules is higher. The mentioned temporal increase of the ethylene oxide fraction in the adduct seems to be closely related to the bacterial mass loss (fig. 2) as well as to the increasing amount of the extracted polyether (fig. 1). In such a case, however, the partly decomposed polyether, which initially should be accumulated in these bacteria and subsequently released because of their destruction, should contain a different ratio of both alkylene oxide units, namely a marked predominance of ethylene oxide.

#### 4. CONCLUSIONS

1. Under stationary conditions the biodegradation of polyoxyalkylene glycols (in the forms of homo- and copolymers) being the by-products in the technical grade Tetric-type surfactants is considerably faster than that of the main copolymer based on ethylenediamine as a polymerization initiator.

2. The evaluation of the biodegradation degree based on the polyether weight loss, determined via extraction, can lead to erroneous results when the process is carried out under stationary conditions.

## LITERATURE

- [1] JANIK R., PLUCIŃSKI J., *Zastosowanie spektroskopii  $^1\text{HNMR}$  w analizie kopolimerów blokowych tlenku propylenu i tlenku etylenu*, Przemysł Chem., Vol. 59 (1980), pp. 555-556.
- [2] PLUCIŃSKI J., PAWLACZYK-SZPIŁOWA M., SEBASTIAN M., STAROJCIEC O., KARPIŃSKA-SMULIKOWSKA J., *Biodegradation of the Tetronic-type nonionic surfactants under stationary conditions*. Part I, Environ. Prot. Eng., Vol. 9 (1983), No. 2, pp. 49-62.
- [3] PLUCIŃSKI J., LEFLER A., PRYSTASZ H. *Über die Reaktion von Propylenoxid mit Äthylendiamin*, Proceeding of the VII International Congress on Surface Active Substances, Vol. 1, pp. 299-312, Moscow 1977.
- [4] SWISHER R. D., *Surfactant biodegradation*, Marcel Dekker Inc., New York 1970.

BIODEGRADACJA NIEJONOWYCH SURFAKTANTÓW TYPU TETRONIKÓW  
W WARUNKACH STACJONARNYCH  
CZEŚĆ II. ROZKŁAD TECHNICZNEGO I CZYSTEGO SURFAKTANTU  
ORAZ WYDZIELONYCH PRODUKTÓW UBOCZNYCH

Techniczny kopolimer blokowy typu Tetroniku, zawierający glikole polioksyalkilenowe jako produkty uboczne oraz czyste frakcje poliglikoli, poddano biodegradacji w warunkach stacjonarnych w obecności szczepu *Bacillus* sp. Polietyery były jedynym źródłem węgla i energii dla tego szczepu. Wykazano, że w badanych warunkach poliglikole są najłatwiej rozkładanym składnikiem.

BIOLOGISCHER ABBAU NICHTIONOGENER TENSIDE  
VON TETRONIC-TYP UNTER STATIONÄREN VERSUCHSBEDINGUNGEN  
TEIL II. ABBAU EINES TECHNISCHEN UND REINEN TENSIDS  
SOWIE DER NEBENPRODUKTE

Es wurde versucht, ein technisches Blockpolymer von Tetronic-Typ, als auch das eigentliche Additionsprodukt der Alkylenoxide an Ethylendiamin und die als Nebenprodukte gebildeten Polyoxylenglykole, in Gegenwart des Stammes *Bacillus* sp. biochemisch abzubauen. Für den erwähnten Bakterienstamm waren die Polyether die einzige Kohlenstoff und Energiequelle. Unter den angeführten Bedingungen wurden Polyoxylenglykole bevorzugt abgebaut.

БИОДЕГРАДАЦИЯ НЕИОННЫХ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ ТИПА  
ТЕТРОНИКОВ В СТАЦИОНАРНЫХ УСЛОВИЯХ  
ЧАСТЬ II. РАЗЛОЖЕНИЕ ТЕХНИЧЕСКОГО И ЧИСТОГО ПАВА  
И ОБРАЗОВАННЫХ ПОБОЧНЫХ ПРОДУКТОВ

Технической блок-сополимер типа Тетроник, содержащий полиоксикаленгликоли как побочные продукты, а также и чистые фракции собственно аддукта и полигликоли, подвергались биодegradации в стационарных условиях и при наличии штамма *Bacillus* sp. Единственным источником углерода и энергии для этого штамма были полиэфиры. Доказано, что в исследуемых условиях полигликоли являются лучше всего разлагаемым компонентом.