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BIODEGRADATION OF NON-SULPHATED AND SULPHATED NONYLPHENOL ETHOXYLATE SURFACTANTS

Biodegradation of the homologous series of branched nonylphenol-ethoxylate adducts used as nonionic in synthetic detergents, was evaluated. The course and rate of the decomposition were investigated by various analytical methods. Well adapted activated sludge was used as inoculum. Besides the length of the hydrophylic chain, the effect of sulphation or sulphonation upon the biological decomposition of these substances was also examined.

1. BIODEGRADATION TEST

Biodegradation was determined by means of a standard batch-test elaborated in the Department of Water Technology and Environmental Engineering [3]. Tested substances in concentrations corresponding to COD chemical oxygen demand of 100 mg/dm^3 were dissolved in 2000 cm^3 of synthetic biological medium containing a sufficient amount of inorganic nutrients. These compounds were the only source of organic carbon for the microbes of inoculum. The biological medium was inoculated with 100 mg/dm^3 of dry matter of adapted activated sludge. The samples of the mixture taken at suitable intervals, were centrifuged, and then analysed. Each experiment was conducted twice, and carried out until the content of the substances tested stopped to decrease.

The acclimation of activated sludge in the following way: the activated sludge placed in the 1000 cm^3 cylinder was fed with glucose and peptone, adding simulaneously the tested substances in concentrations increasing up to the value corresponding to the COD of 100 mg/cm^3. The total solids (dry matter) of the sludge in the cylinder amounted to 2.5 g/dm^3, the sludge age being 5 days.

2. ANALYTICAL METHODS

The course and degree of the biodegradation was examined indirectly by determining COD with 0,05 N $K_2Cr_2O_7$, and organic carbon on the Beckman analyser, as well as turbidimetrically with tetraiodobismuthate without any previous separation of surfac-

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tants [4], which were later separated by extraction with chloroform [1]. Their separation made by the Wickbold method was followed by potentiometric titration [6], and spectrophotometrical analysis made in UV region of 275 nm.

3. COMPOUNDS TESTED

The tests were performed with: branched nonylphenol ethoxylates made in CSSR under the trade name "Slovafoly", the number of ethoxyl groups in a molecule ranging from 3 to 35. In the sequel they will be reffered to as SF 03–SF 35. Their alkyl structure was determined, and the average number groups verified by means of NMR spectroscopy. The above compounds can be expressed by the following formula:

$$CH_3-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2-\underset{\displaystyle CH_3}{\underset{|}{CH}}-C_6H_4-O-(CH_2-CH_2-0)_nH$$

Adducts of ethylene oxide are not separate species but have always been regarded as a mixture of polymetric homologues. Their relative quantities in the substances investigated are seen on thin-layer chromatogram (fig. 1.). The chromatograms were made (under the following conditions using:) a layer of 0.5 nm silica gel G, initial concentration of 5–10 μg; elution being done with ethylmethylketone saturated with distilled water,

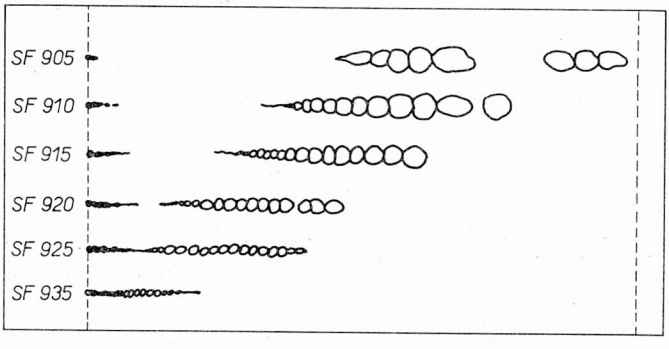

Fig. 1. Thin-layer chromatogram of branched-chain nonylphenol ethoxylates

Rys. 1. Chromatogram cienkowarstwowy rozgałęzionego łańcucha etoksylatów nonyfenolowych

and development by means of the Dragendorff reagent [1]. Free polyethylene glycols remain in the starting position, but the adducts having a small number of ethoxyl continue moving as fast as possible. The parameters of substances investigated are given in table 1.

Another series of samples which taken for tests consisted of non-sulphated and sulphated branched-chain nonylphenol ethoxylates average with the numbers of ethoxyl groups $n = 3$ to $n = 9$ (abbreviated to SF 03–SF 09). The relative quantities of polymeric homologues in non-sulphated adducts is seen on from the chromatogram given in fig. 2.

Table 1

List of investigated species (branched-chain nonylphenol ethoxylates)

Substance	DOC g C·g^{-1}	COD g O_2·g^{-1}	n	M
SF 05	0.72	2.32	5	440
SF 10	0.65	2.20	10	660
SF 15	0.64	2.13	15	880
SF 20	0.60	2.05	20	1100
SF 25	0.60	2.00	25	1320
SF 35	0.57	1.94	35	1760

DOC — disolved organic carbon,
COD — chemical oxygen demand,
n — average number of ethoxyl groups,
M — average molecular weight.

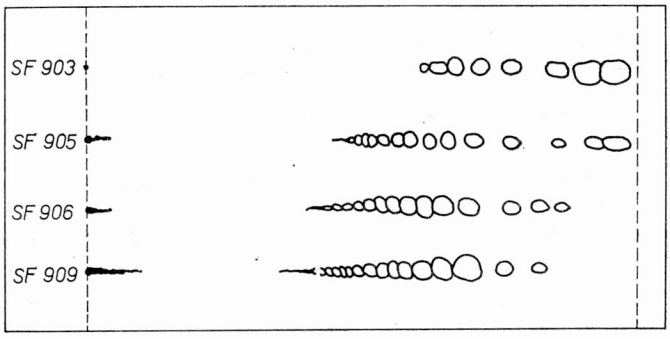

Fig. 2. Thin-layer chromatogram of branched-chain nonylphenol exthoxylates

Rys. 2. Chromatogram cienkowarstwowy rozgałęzionego łańcucha etoksylatów nonyfenolowych

Sulphation was carried out to two degrees and concerned, above all the terminal hydroxyl group of the hydrophylic chain, if however the reaction proceeds even the aromatic nucleus can be sulphonated. The samples with a lower degree of sulphation are by S_1, those with a higher degree by S_2. These substances are presented in table 2. Non-sulphated branched--chain nonylphenol ethoxylates shown in table 2 were of another batch than the substances given in table 1.

4. DECOMPOSITION OF NON-SULPHATED BRANCHED-CHAIN NONYLPHENOL ETHOXYLATES

After a rapid initial decrease in the content of branched-chain nonylphenol adducts, no further decomposition of these substances occurred after their incubation for 5–7 days. The maximum percentage of biological decomposition attained and determined by analytical methods, for individual adducts is summed up in table 3. The results of the two sets of experiments may be easily compared.

Table 2

Non-sulphated and sulphated branched-chain nonylphenol ethoxylates investigated

Substance	DOC g $C \cdot g^{-1}$	COD g $O_2 \cdot g^{-1}$	n	mg $S \cdot g^{-1}$ DOC
SF 03-S_1	0.410	1.37	3	80
SF 03-S_2	0.406	1.48	3	95
SF 05	0.576	1.92	5	0
SF 05-S_1	0.167	0.50	5	106
SF 06	0.548	1.81	6	0
SF 06-S_1	0.423	1.41	6	70
SF 06-S_2	0.397	1.36	6	73
SF 09	0.648	2.07	9	0
SF 09-S_1	0.486	1.44	9	48
SF 09-S_2	0.428	1.38	9	68

Table 3

The maximum degree of biodegradation of branched-chain nonylphenol ethoxylates

Sets of experiments	Substance	% COD	% DOC	% UV	% *A*	% *B*	% *C*
	SF 05	78.2	84.0	88.0	91.5	92.8	93.9
	SF 10	77.0	82.5	78.0	95.0	96.2	98.0
1st	SF 15	75.6	80.0	84.0	95.1	96.9	97.9
	SF 20	60.5	71.1	84.0	71.0	77.4	81.0
	SF 25	56.5	64.3	84.5	66.5	73.8	77.0
	SF 35	46.0	35.0	70.1	30.1	41.6	83.0
	SF 05	78.0	86.0	88.6	91.0	93.2	95.5
2nd	SF 10	78.2	84.0	83.5	95.0	96.3	100.0
	SF 15	80.0	77.4	88.8	95.0	96.3	97.4
	SF 20	58.6	74.0	86.6	73.0	73.7	77.0
	SF 25	50.0	70.7	82.5	67.1	70.0	71.0
	SF 30	48.0	40.5	72.0	32.2	43.8	84.5

UV — spectrophotometry in UV region,
A — direct turbidimetric method with tetraiodobismuthate,
B — turbidimetric method after extraction with chloroform,
C — Wickbold method.

The highest percentage of decomposition was determined by structural-analytical methods, above all, by the Wickbold method; this method does not allow to determine free polyethylene oxides formed as intermediate decomposition products [2]. In low-ethoxylated adducts, the decomposition exceeds 95%. In the biological medium, however, more than 5% of the initial content of surfactants is not decomposed, as follows from the data of COD and DOC dissolved organic carbon. In case of COD, the decomposition ranges from 75.6 to 80.0%, in case of DOC, from 77.4 to 84.0%. The greatest difference was observed in SF 35, when the decomposition carried out by the Wickbold method was almost twice as high as that in the case of COD or DOC.

From table 3 it is apparent that structural-analytical methods are not suitable for evaluation of the biodegradation of nonionic surfactants. These methods give essentially higher results, which differ from the real amount of these substances when removed biologically. Thus it is recommended to evaluate from the decrease of DOC or COD.

Dependence of the biodegradation expressed in COD or DOC percentage upon the average number of ethoxyl groups is given in fig. 3. Spectrophotometry used in the UV region has proved the fission of the aromatic nucleus of nonylphenol. With the exception of SF 35 adduct, the decomposition was relatively constant within 78.0 to 88.8% UV (tab. 3).

The rates of decomposition before and after 5 days incubation (when the major part of biological decomposition had been finished) have been compared. The values of the average specific rate of decomposition given in mg of COD and DOC removed by one gram of the initial total solids of inoculum after one hour, are shown in fig. 4 and 5. The dependence of the specific rate of decomposition upon the length of the hydrophylic chain is found to be the most conspicuous (fig. 3). With the increasing number of ethoxyl

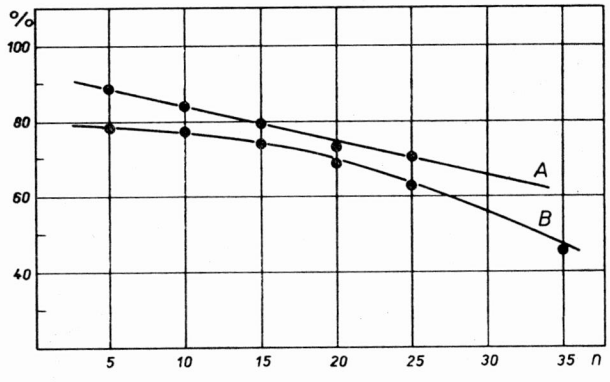

Fig. 3. Biodegradation in DOC (*A*) or COD (*B*) percentage, vs. the average number of ethoxyl groups (*n*)

Rys. 3. Biodegradacja w procentach DOC (*A*) lub CHTZ (*B*) w zależności od przeciętnej liczby grup etoksylowych (*n*)

grups, the rate of decomposition decreases considerably. But even the relatively readily decomposable low-ethoxylated branched-chain nonylphenol ethoxylates SF 05 and SF 10 are very slowly decomposed if compared with many aliphatic and aromatic compounds. For example, under the same experimental conditions, various phenols show specific rates of decomposition ranging from 15 to 120 mg of $COD \cdot g^{-1} \cdot h^{-1}$, for the basic rates being high as 200 mg $COD \cdot g^{-1} \cdot h^{-1}$ [3].

As to the way of fission of the hydrophylic part of the molecule of ethylene oxide adducts, attention has been paid to this point in experiments with the biological degradation of polyethylene oxides [5].

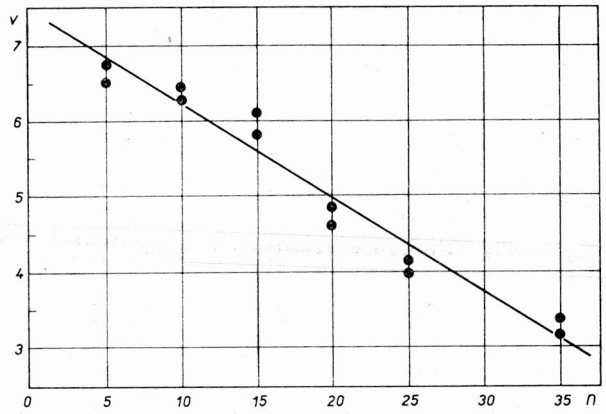

Fig. 4. Specific rate decomposition (v) vs. the average number of ethoxyl groups (n): v—mg COD·g^{-1}·h^{-1}

Rys. 4. Specyficzna szybkość rozkładu (v) w zależności od przeciętnej liczby grup etoksylowych (n)

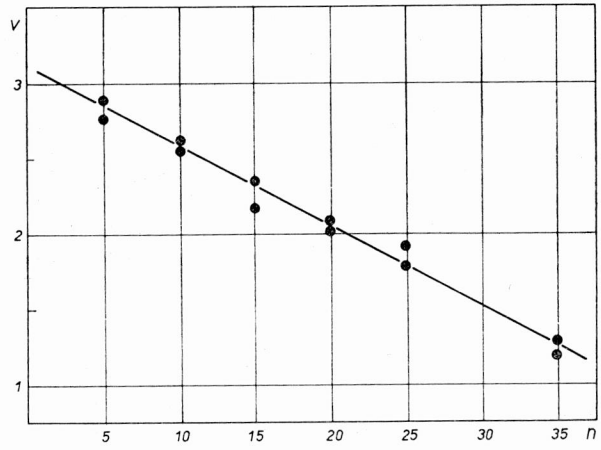

Fig. 5. Specific rate of decomposition (v) vs. the average number of ethoxyl groups (n): v—mg DOC·g^{-1}·h^{-1}

Rys. 5. Specyficzna szybkość rozkładu (v) w zależności od liczby grup etoksylowych (n)

5. EFFECT OF SULPHONATION UPON THE DECOMPOSITION OF BRANCHED-CHAIN NONYLPHENOL ETHOXYLATES

The maximum percentages of biological decomposition attained in sulphated and non-sulphated nonylphenol ethoxylates and determined by various analytical methods, are presented in table 4. Sulphated adducts of ethylene oxide have anion-active properties. The sensitivity of these adducts being rather low, they can not be determined by structural--analytical methods applicable to nonionic surfactants. Thus the decomposition was determined by means of COD, DOC and UV spectrophotometric methods.

Table 4

The maximum degree of biodegradation of sulphated and non-sulphated nonylphenol ethoxylates

Substance	1st set of experiments			2nd set of experiments		
	% COD	% DOC	% UV	% COD	% DOC	% UV
SF 03-S_1	43.5	55.0	—	44.5	53.2	—
SF 03-S_2	42.0	50.1	—	41.2	47.7	—
SF 05	71.5	80.0	—	70.5	85.0	—
SF 05-S_1	41.5	45.6	57.7	48.8	48.1	61.6
SF 06	62.5	83.2	84.0	62.0	84.4	85.0
SF 06-S_1	34.2	41.3	72.5	34.0	43.6	71.4
SF 06-S_2	32.9	40.2	71.8	35.1	44.1	73.7
SF 09	68.9	87.6	88.1	70.5	88.0	89.5
SF 09-S_1	34.5	37.6	64.2	33.8	36.8	66.0
SF 09-S_2	25.2	26.8	33.7	23.9	24.9	32.2

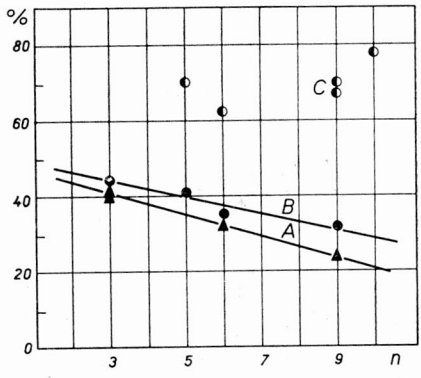

Fig. 6. Maximum degree of biodegradation expressed of COD vs. the average number of ethoxyl groups (*n*):

A — sulphated nonylophenol ethoxylates (samples having a higher percentage of sulphation (series S_2)), *B* — sulphated nonylphenol ethoxylates (samples having a lower percentage of sulphation (series S_1)), *C* — non-sulphated nonylphenol ethoxylates

Rys. 6. Maksymalny stopień biodegradacji wyznaczony jako zależność CHZT od przeciętnej liczby grup etoksylowych (*n*)

A — siarczanowe etoksylaty nonyfenolowe (próbki o wyższym procencie zasiarczenia (serie S_2)) *B* — siarczanowane etoksylaty nonyfenolowe (próbki o niższym procencie zasiarczenia (serie S_1)) *C* — niesiarczanowane etoksylaty nonyflenolowe

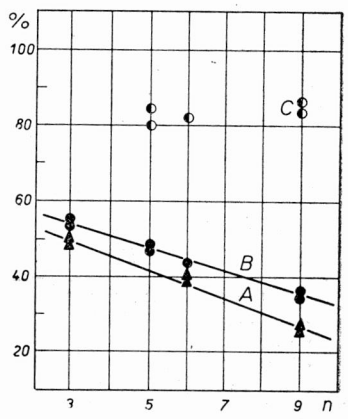

Fig. 7. Maximum degree of biodegradation expressed in DOC vs. the average number of ethoxyl groups (*n*):

A — sulphated nonylphenol ethoxylates (samples having a higher percentage of sulphation (series S_2)), *B* — sulphated nonylphenol ethoxylates (samples having a lower percentage of sulphation (series S_1))

Rys. 7. Maksymalny stopień biodegradacji wyrażonej jako zależność DOC od przeciętnej liczby grup etoksylowych (*n*)

A — siarczanowane etoksylaty nonyfenolowe (próbki o wyższym procencie zasiarczenia (serie S_2)) *B* — siarczanowane etoksylaty nonylfenolowe (próbki o niższym procencie zasiarczenia (serie S_1))

Experiments were made in two groups under the same conditions. The maximum degree of biological decomposition, expressed in the percentage of COD and DOC, against the number of ethoxyl groups is given in figs. 6 and 7. These figures show quite apparently that sulphation and its degree evaluated from the content of organic sulphur (tab. 1) affects the decomposition of these adducts in a remarkably negative way. Analogous relation was observed also in average specific rates of decomposition (figs. 8 and 9). Beca-

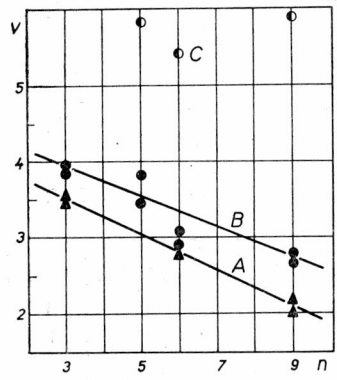

Fig. 8. Specific rate of decomposition (v) vs. average number of ethoxyl groups (n)

v — mg COD · g−1 · h−1
A — sulphated nonylphenol ethoxylates (series S_2), B — sulphated nonylphenol ethoxylates (series S_1), C — non-sulphated nonylphenol ethoxylates

Rys. 8. Specyficzna szybkość rozkładu (v) w zależności od przeciętnej liczby grup etoksylowych (n)

A — siarczanowane etoksylaty nonyfenolowe (próbki o wyższym procencie zasiarczenia (serie S_2)) B— siarczanowane etoksylaty nonyfenolowe (próbki o niższym procencie zasiarczenia (serie S_1)) C — niesiarczanowane etoksylaty nonylfenolowe

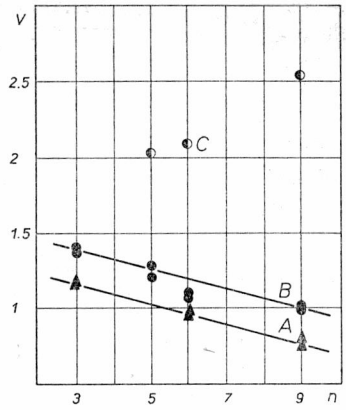

Fig. 9. Specific rate of decomposition (v) vs. the average number ethoxyl groups (n):

v — mg DOC · g−1 · h−1
A — sulphated nonylphenol ethoxylates (series S_2), B — sulphated nonylphenol ethoxylates (series S_1), C — non-sulphated nonylphenol ethoxylates

Rys. 9. Specyficzna szybkość rozkładu (v) w zależności od przeciętnej liczby grup etoksylowych (n)

A — siarczanowane etoksylaty nonyfenolowe (próbki o wyższym procencie zasiarczenia (serie S_2) B — siarczanowane etoksylaty nonyfenolowe (próbki o niższym procencie zasiarczenia (serie S_1)) C — nie siarczanowane etoksylaty nonylfenolowe

use of a rather low of decomposition degree after sulphation determined spectrophotometrically in UV region, it may be stated that even the fission of the hydrophobic part of the molecule of alkylphenol (tab. 4) is unfavourably affected by sulphation.

In some cases was observed a considerable deterioration of the biological biodegradation of branched-chain nonylphenol ethoxylates caused by sulphation. For example, in SF 09 adducts, the percentage of decomposition was reduced from about 70% to 25% of COD, from 88% of DOC, and from 88% to 33% UV.

When the average number of ethoxyl groups is the same, but these groups come from different batches, the determination of biological decomposition give often different results. This is due to the fact that the products made under different conditions have very different distribution curves of polymetric homologues; e.g., two adducts denoted by SF 05 (figs. 1 and 2). This fact should be taken into account when the decomposition of these surfactants is determined and evaluated versus the number of ethoxyl groups.

6. CONCLUSIONS

Biodegradation of nonionic surfactants of the branched-chain nonylphenol ethoxylate series examined by structural-analytical methods in which the initial unchanged molecule is determined seems to be over-valued. This fact concerns especially the Wickbold method.

Decomposition should be estimated by the methods allowing to determine organic substances as a whole, including various intermediate products of biodegradation. Hence the determination of DOC and COD may be taken into consideration.

Biodegradation of the branched-chain nonylphenol ethoxylates having the average numbers of 5 to 25 ethoxyl groups in molecule ranged from 80% to 46% of COD, or 86% to 35% of DOC.

During the decomposition, the fission of both the hydrophobic and hydrophylic parts of the molecule takes place. In highly ethoxylated adducts, it is the hydrophylic chain which makes the decomposition difficult.

Sulphation of nonylphenol ethoxylates deteriorates the biodegradation of these substances. This negative effect increases with the increasing percentage of sulphation.

Decomposition rate is very low with respect to the majority of simple aliphatic and aromatic compounds.

Biological decomposition of nonylphenol ethoxylates depends upon the distribution of polymetric homologues. The products having the same average number of ethoxyl groups may be characterized by different distribution curves.

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ROZKŁAD BIOLOGICZNY NIE SIARCZANOWANYCH I SIARCZANOWANYCH NONYLOFENOLOWYCH ETOKSYLOWANYCH ZWIĄZKÓW POWIERZCHNIOWO CZYNNYCH

Określono rozkład biologiczny szeregu homologicznego rozgałęzionych nonyfenolowych aduktów etoksylowanych, stosowanych w syntetycznych detergentach jako niejonowe surfaktanty. Przebieg i szybkość rozkładu badano różnymi metodami analitycznymi. Jako inoculum użyto osadu czynnego adaptowanego przez długi okres czasu. Ponadto zbadano długość łancucha hydrofilowego oraz wpływ sulfatacji na rozkład biologiczny tych substancji.

BIOLOGISCHER ABBAU VON SULFURIERTEN UND NICHT SULFURIERTEN, NONYLPHENOLIGEN, ETOXYLIERTEN, OBERFLÄCHENAKTIVEN SUBSTANZEN

Ermittelt wurde der biologische Abbau einer homologen Reihe verzweigter nonylphenoliger etoxylierter Addukte, die als nichtionogene, oberflächenaktive Mittel in synthetischen Waschmitteln verwendet werden.

Der Abbauverlauf und die Abbaurate wurde mittels verschiedener Analysenmethoden ermittelt. Als Impfmittel diente ein lange Zeit adaptierter Belebtschlamm. Ausserdem wurde die Länge der hydrophilen Kette sowie der Einfluß der Sulfonierung auf den biochemischen Abbau dieser Substanzen getestet.

БИОЛОГИЧЕСКОЕ РАЗЛОЖЕНИЕ НЕ СУЛЬФИТИРОВАННЫХ И СУЛЬФИТИРОВАННЫХ НОНИЛФЕНОЛОВЫХ ЭТОКСИЛИРОВАННЫХ ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ

Определено биологическое разложение гомологического ряда разветвленных этоксилированных нонилфеноловых аддуктов, применяемых в качестве неионных поверхностно-активных веществ для изготовления синтетических детергентов. Ход и скорость разложения исследовались различными аналитическими методами. Материалом для прививки служил активный осадок, приспособляемый в течение продолжительного времени. Исследована, сверх того, длина гидрологической цепи и влияния сульфирования на биологическое разложение этих веществ.