Application of mutagenic treatment of active silt for oxidation of cellulose nitrate

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Abstract. The oxidation of native cellulose nitrate (13.38% N) and cellulose nitrate (CN) treated with UV radiation and ozone (UV+ozone) by a biocenosis of microorganisms of active silt (AS) from sewage disposal plants and by a combination of AS with sulfate-reducing bacteria Desulfovibrio desulfuricans BKM B-1388 and microscopic fungi Fusarium solani BKM F-819 was studied. The use of the preliminary treatment significantly increased the degree of decomposition of CN during its subsequent biodegradation by a symbiosis of AS microorganisms with D. desulfuricans and F. solani. The application of the mutagenic treatment of AS with nitrosomethylurea (NMU) allows the biocenosis of AS microorganisms with CN acting as a pollutant to retain high oxidation ability with good sedimentation properties for 65 days of incubation. The degree of decomposition was 24.36%. Substantial parameters were achieved during the incubation period of treated CN (CNₜₐₜ) within the first 5 days, which is promising from the point of view of practical use.

1. Introduction

The Materials characterized by high energy capacity, such as aromatic compounds, nitroethers, and nitroamines, are often serve as components of polymer composite materials. The scale of industrial use of cellulose di- and trinitrates with the nitrogen content higher than 10% (which are highly explosive) is being permanently increasing. Considerable amounts of sewage with wastes containing ecologically dangerous substances are formed during the industrial production of polymers of this kind. Waste waters are decontaminated at sewage disposal plants, the complete stage of operation in which is the biological treatment in aerotanks on active silt (AS). Active silt represents a complex biocenosis of many microorganisms, mainly bacteria, in which the cells exist in the medium of soluble or poorly soluble extracellular polymeric aggregates: polysaccharides, proteins, and ribonucleic and deoxyribonucleic acids.

The development of methods and technological approaches aimed at accelerating the decomposition of the compounds in the sewage of CN manufacturing would solve environmental problems related to CN accumulation and would diminish the negative load on the environment. Biological methods of utilization are most promising and environmentally friendly [1,2].
There are variants of application of chemical mutagenesis developed by Rapoport [3] increasing the oxidation ability of AS from sewage disposal plants toward poorly degradable or nearly non-degradable contaminating chemical substances [3-5]. After the mutagenesis, the enzymatic activity of AS microorganisms increases by more than two times [6]. For example, the use of the genetic method [7,8] made it possible to accelerate the destruction of benzene and synthetic grease substitutes when nitrosomethylurea (NMU) is applied as a mutagen.

We studied the biological decomposition of CN by sulfate-reducing bacteria Desulfovibrio desulfuricans [9] and mycelial fungus Fusarium solani [10,11] and also considered the influence on the process of the preliminary treatment of CN with UV radiation and ozone (CN\textsubscript{pre}).

The purpose of this work is the evaluation of the possibility of application of AS from sewage disposal plants subjected to the mutagenic treatment with NMU and in combination of AS with fungi F. solani and bacteria D. desulfuricans during the decontamination of sewage from industrial CN production and the choice of the most appropriate variant of microbiological destruction for the industrial use.

2. Experimental

2.1. Materials
The following reagents and solvents purchased from Sigma (USA), BioRad (USA), and Reakhim (Russia) were used for the preparation of cultural nutrient media and buffer systems and for carrying out physicochemical studies: KCl, NH\textsubscript{4}Cl, CaCl\textsubscript{2}x2H\textsubscript{2}O, K\textsubscript{2}HPO\textsubscript{4}, MgCl\textsubscript{2}x6H\textsubscript{2}O, NaNO\textsubscript{3}, sodium lactate, sodium salicylate, NaOH, glucose, tetrahydrofuran (THF), and acetone.

The CN samples kindly presented by the Kazan Gunpowder Plant (trade mark M/l 26 432-01, nitrogen content 13.38%, weight of the elementary unit of the macromolecule 284.4, degree of polymerization 1140, molecular weight 324 216, technical conditions GOST R 50461-92) were used.

2.2. Microorganisms and methods of study
Mycelial fungus Fusarium solani BKM F-819 and sulfate-reducing bacterium Desulfovibrio desulfuricans (strain BKM B-1388) obtained from the All-Russia Collection of Microorganisms (G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms of the Russian Academy of Sciences, Pushchino Research Center of Biological Investigation of the Russian Academy of Sciences) were used.

Active silt (AS) taken from the aerotank at the sewage installations of the Chernogolovka Research Center of the Russian Academy of Sciences (Chernogolovka, Moscow Region) was used as a consortium of microorganisms. The AS had a brown-grayish color with slightly boggy smell without predominating smell of chemical impurities.

Mutagenic treatment of AS. N-Methyl-N-nitrosoureia (NMU, reagent grade, Sigma (USA)) in a concentration of 0.07% was used as a mutagenic agent. The treatment of AS was made in 4 days of contact of the AS with the studied CN. The treatment was conducted for 18 h [3]. On the 29\textsuperscript{th} day from the experiment onset, the AS was repeatedly treated with NMU also for 18 h. The studies were carried out in parallel for both the initial and treated AS.

Growing of biomass was carried out according to the earlier described method [10].

Medium for experiments on biodegradation of CN. According to the described method [10], AS was diluted with the medium and CN, closed with a cap (in the case without oxygen purging), and left to stay in a warm place for 1 h.

Biological destruction of CN by the cultures of bacteria and fungi was carried out according to the published procedure [11]. The variants of the study of the biological degradation of CN using the microorganisms are presented in Table 1.

Destruction of CN by the mixed culture of fungi and bacteria was carried out according to the published procedure [11].
Oxidation of CN by AS. Experiments on the study of the biological oxidation of CN by the microbial consortium of AS (Table 1) were conducted in 3-L laboratory glass reactors under natural solar irradiation with air purging. The reactors contained the nutrient medium (1000 mL), AS (200 mL), and CN (10 g/L). In the first series of experiments, the reactors (reactors 1, 3, and 5) were loaded with the initial CN, whereas CN$_{\text{treat}}$ was loaded in the second series (reactors 2, 4, and 6).

In the variants providing the study of CN oxidation by AS, bacteria, and fungi, the cultures of bacterium $D.\ desulfuricans$ and fungus $F.\ solani$ were added to a solution of AS in the nutrient medium as described above (reactors 3, 4, and 6). Samples for analysis were taken on the 5th, 16th, 36th, and 65th day from the experiment onset in a volume of 50 mL after mechanical stirring of the solution.

Treatment of CN with ultraviolet and ozone was carried out according to the described procedure [10]. Isolation of CN from solution was conducted according to the described method [10]. Determination of nitrogen and other elements in CN was carried out according to the described procedure [10].

Determination of nitrates and nitrites was carried out according to the described procedure [10]. Determination of the heat release rate and heat in the thermal decomposition of the isolated CN samples was carried out according to the described procedure [12].

Table 1. Variants of the study of the biological degradation of CN

| Reactor no. | Conditions       | Volume of medium, mL | Volume of seeded bacteria, mL | Number of seeded fungi, shoals | Volume of AS, mL | Concentration of CN, g/L |
|------------|------------------|----------------------|-------------------------------|-----------------------------|----------------|-------------------------|
| Control    | AS               | 1000                 | -                             | -                           | 200             | -                       |
| 1          | AS+CN            | 500                  | -                             | -                           | 100             | 5.0                     |
| 2          | AS+CN$_{\text{treat}}$ | 1000                  | -                             | -                           | 200             | 10.0                    |
| 3          | AS+CN+bacteria+fungi | 1000                  | 50                            | 3x2                         | 200             | 10.0                    |
| 4          | AS+CN$_{\text{treat}}$+bacteria+fungi | 1000                  | 50                            | 3x2                         | 200             | 10.0                    |
| 5          | AS+NMU+CN       | 1000                 | -                             | -                           | 200             | 10.0                    |
| 6          | AS+NMU+CN$_{\text{treat}}$+bacteria+fungi | 1000                  | 50                            | 3x2                         | 200             | 10.0                    |

3. Results and discussion
It is seen from the data in Table 1 that the studies were carried out in parallel: (1) on CN and CN$_{\text{treat}}$ and (2) on AS and AS treated with NMU. In addition, the possibility to create a symbiosis of AS with the mixed culture of bacteria $D.\ desulfuricans$ and mycelial fungi $F.\ solani$ was considered.

3.1. Degree of biological oxidation of CN
Biodestruction was evaluated by a change in the characteristic parameters: pH, viscosity, MWD, contents of C, H, and N in CN, the formation of nitrates and nitrites in the solution, and changes in the value of heat release of the samples.
Table 2. Contents of carbon and hydrogen in CN and nitrate and nitrite ions in the microorganism growth medium and in the AS after biodegradation

| Reactor no. | Incubation time, day | pH  | C, wt.% | H, wt.% | NO$_3^-$, µg/mL | NO$_2^-$, µg/mL |
|-------------|----------------------|-----|---------|---------|-----------------|-----------------|
| Control     | 0                    | 6.5 |         |         |                 |                 |
|             | 5                    | 7.04|         |         |                 |                 |
|             | 16                   | 7.15|         |         | 2.13            | 0.15            |
|             | 38                   | 7.45|         |         | 1.62            | 0.44            |
|             | 65                   | 7.68|         |         | 2.63            | 0.35            |
|             | 0                    | 6.5 |         |         |                 |                 |
|             | 5                    |     | 31.23   | 3.570   | 4.30            | not found       |
|             | 16                   | 8.39| 27.07   | 3.264   | 3.64            | not found       |
|             | 38                   | 5.98| 26.46   | 3.135   | 2.02            | 12.48           |
|             | 65                   | 5.73| 25.70   | 2.794   | 16.03           | not found       |
| 1           | 0                    | 5.0 |         |         |                 |                 |
|             | 5                    |     | 29.44   | 3.744   | 12.90           | not found       |
|             | 16                   | 8.15| 24.92   | 2.606   | 7.58            | not found       |
|             | 38                   | 6.99| 25.80   | 3.038   | 2.02            | 0.18            |
|             | 65                   | 6.40| 25.16   | 2.721   | 17.50           | 44.6            |
| 2           | 0                    | 6.0 |         |         |                 |                 |
|             | 5                    |     | 29.92   | 3.810   | 3.80            | not found       |
|             | 16                   | 8.60| 26.01   | 2.993   | 8.08            | not found       |
|             | 38                   | 6.84| 25.91   | 2.759   | 3.03            | 0.15            |
|             | 65                   | 6.34| 25.00   | 2.865   | 6.62            | 23.4            |
| 3           | 0                    | 4.50|         |         |                 |                 |
|             | 5                    |     | 32.52   | 4.333   | 10.86           | not found       |
|             | 16                   | 8.21| 27.59   | 2.970   | 16.90           | 0.57            |
|             | 38                   | 7.00| 27.55   | 3.396   | 7.37            | 0.99            |
|             | 65                   | 5.41| 26.58   | 3.213   | 4.04            | 2.52            |
| 4           | 0                    |     |         |         |                 |                 |
|             | 5                    |     | 28.54   | 3.550   |                 |                 |
|             | 16                   |     | 25.01   | 2.621   |                 |                 |
|             | 38                   |     | 25.55   | 2.976   |                 |                 |
|             | 65                   |     | 25.96   | 2.845   |                 |                 |
| 5           | 0                    |     |         |         |                 |                 |
|             | 5                    |     | 29.65   | 3.683   |                 |                 |
|             | 16                   |     | 26.49   | 2.902   |                 |                 |
|             | 38                   |     | 25.91   | 3.477   |                 |                 |
|             | 65                   |     | 26.09   | 2.895   |                 |                 |

As can be seen from the data in Table 2, sulfur was observed in none of the studied CN samples taken from reactors 1–6.

The initial CN in water is characterized by pH 6.0. The content of NO$_3^-$ found in it ranged from 5.94 to 6.24 µg/mL, and no NO$_2^-$ was observed.

The pH value increases from 6.5 to 7.68 within 65 days of incubation in the control reactor containing AS only. The concentrations of nitrates and nitrites in this reactor are insignificant: [NO$_3^-$]$_{\text{max}}$ = 2.63 µg/mL and [NO$_2^-$]$_{\text{max}}$ = 0.44 µg/mL.
In reactors 1–4, pH increases from neutral and weakly acidic to 8.15–8.60 from the experiment onset to the 16th day, whereas on the 65th day pH decreases to 5.41–6.40. During biodegradation, the amount of nitrate ions in the AS growth medium in reactor 1 on the 38th day insignificantly differs from that in the control reactor and only on the 65th day increases considerably: to 16.03 µg/mL. The situation is principally different in reactor 2 containing CN_treat on AS: after 5 days of storage, the content of NO_3^- is 12.9 µg/mL, then smoothly decreases almost to the values for the control reactor, and increases again to 17.5 µg/mL only on the 65th day (Table 2).

In reactor 4 (AS+CN_treat+bacteria (b)+fungi (f)), the content of nitrate ions observed on the 5th day of incubation is significant (10.86 µg/mL), increases more to the 16th day (up to 16.9 µg/mL), and begins to decreases on the 38th day.

Figure 1 demonstrates that the residence of CN on AS results in a decrease in the nitrogen content in time. The preliminary UV+ozone treatment of CN increases the rate of CN decomposition on AS. For example, after 38 days of incubation in reactor 2, the nitrogen content is considerably lower (10.38%) than that in reactor 1 (10.97%). Similarly, reactor 4 contains a lower amount of nitrogen than reactor 3, whereas the nitrogen content in reactor 6 is lower than that in reactor 5.

The treatment of AS with the mutagen (NMU) exerts an additional positive effect. To the 38th day of incubation, the nitrogen contents in reactor 5 (10.12%) and the more so in reactor 6 (9.97%) are significantly lower than those in reactor 1 (10.97%) and reactor 2 (10.38%).

The introduction of bacteria and fungi into AS, i.e., an attempt to create a symbiosis of microorganisms, does not significantly improve the decomposition of CN. The nitrogen content in reactor 3 was stable and higher within the whole observation period (from 5 to 38 days) than that in reactor 1 containing AS only.

It follows from the results presented in Fig. 1 that the low nitrogen content in the samples is achieved already to the 16th day in reactor 4 in which bacteria and fungi were added to AS and CN_treat was used. The nitrogen content in this reactor decreases to 10.68% on the 38th day of exposure. The lowest nitrogen content is achieved within 38 days in a similar variant of treatment (CN_treat+b+f) but on the AS treated with NMU, i.e., in reactor 6, where the content of N was 10.12%, the maximum nitrogen loss was 3.26%, and, correspondingly, the degree of decomposition with respect to nitrogen was 24.36%.

The sum of all effects is observed in reactor 6: AS+NMU+b+f on CN_treat. However, the decrease in the nitrogen content is this reactor differs insignificantly from that in reactor 4. Therefore, it seems
doubtful and unreasonable to introduce an additional technological procedure into the real technological process.

3.2. Heat release rates and heats for the thermal decomposition of CN

The kinetics of thermal decomposition of CN subjected to different variants of microbiological treatment in order to decrease the degree of nitration was studied. We found in our previous study [12] that the total heat of the heat release of the initial CN was about 4080 J/gCN on the average. The heat of the heat release of CN_treat is somewhat lower being about 3560 J/gCN. This can indirectly indicate that nitro groups are eliminated upon the UV-irradiation of CN.

The results of studying the kinetics of thermal decomposition of CN and CN_treat upon the storage on AS are presented in Figs. 2–5 along with the curves for the initial CN and CN_treat samples shown for comparison. The decomposition of both the initial CN and all CN subjected to the microbiological treatment and then isolated from an acetone solution proceeds via the autocatalytic reaction law as described earlier [12].

![Figure 2](image-url)

**Figure 2.** Time dependences of the heat release rate during the thermal decomposition of the CN samples at 139.8°C isolated after 38 days of the microbiological treatment of CN: 0, CN_init; 1, reactor 1 (AS+CN); 2, reactor 2 (AS+CN_treat); 3, reactor 3 (AS+CN+b+f); 4, reactor 4 (AS+CN_treat+b+f); 5, reactor 5 (AS+NMU+CN); 6, reactor 6 (AS+NMU+CN_treat+b+f).

It is seen from Fig. 2 that after 38 days of different variants of biological treatment the heat release rate in all reactors is lower than that of the initial CN (curve 0). The highest heat release rate is retained in reactor 5 (AS+NMU+CN) and is close to that in reactor 1 (AS+CN).

It is seen that the time of achieving the maximum heat release rate somewhat decreases when using various types of biological treatment as compared to reactor 1 (AS+CN). All variants are rather close. The lowest heat release rate was established in reactor 4 (AS+CN_treat+b+f). In this case, the time of achieving maximum is also minimal (12 h). For other variants of the treatment, the time of achieving maximum ranges from 16 to 18 h. A comparison of reactors 5 and 6 in which AS was subjected to the mutagenic treatment shows that within the indicated time (38 days of incubation of CN on AS) the performed mutagenic treatment of AS does not accelerate the decomposition of CN. It is seen that the preliminary treatment of CN induces the decrease in the heat release rate to a higher extent: pairs 1 and 2; 3 and 4; and 5 and 6. A comparison of reactor 4 (AS+b+f) and reactor 6 with the preliminary mutagen treatment (AS+NMU+b+f) also does not show a decrease in the rate on the treated AS.
Figure 3. Time dependences of the heat released to the given moment upon the thermal decomposition at 139.8°C of the CN samples isolated after 38 days of the microbiological treatment of CN (numbers at the curves are variants of the microbiological treatment of CN): 1, reactor 1 (AS+CN); 2, reactor 2 (AS+CN_treat); 3, reactor 3 (AS+CN+b+f); 4, reactor 4 (AS+CN_treat+b+f); 5, reactor 5 (AS+NMU+CN); 6, reactor 6 (AS+NMU+CN_treat+b+f).

The time dependences of the heat released to the given moment in the thermal decomposition reaction are presented in Fig. 3. Reactor 4 (AS+CN_treat+b+f) can be distinguished by a minimum amount of the isolated heat and a minimum time of achieving the maximum heat release rate, which corresponds to the data in Fig. 2.

The initial \((dQ/dt)_{t=0}\) and maximum \((dQ/dt)_{max}\) heat release rates, times of achieving maximum rates \(t_{max}\), and total heats of decomposition \(Q_{tot}\) for the studied CN samples are presented in Table 3. The weak dependences of the initial heat release rate, maximum heat release rate, time of achieving it, and total heat of the process on the microbiological treatment conditions show that the nitrogen content in the CN fraction dissolved in acetone differs slightly from the nitrogen amount in the initial CN. Under the conditions of microbiological processes with nitrogen consumption during the living activity of microorganisms that are present in the initial AS and seeded bacteria and fungi, the balance with respect to nitrogen should be calculated taking into account the amount of nitrogen consumed in the growth of population of all types of microorganisms, nitrogen transited to the mother liquor in the form of nitrite and nitrate anions and sugar nitrates, and nitrogen in the CN fraction that was not dissolved in acetone after 38 days of incubation.

Of the whole array of microbiological conditions of the CN treatment, the conditions in reactor 4 (AS+CN_treat+b+f) result in a lower heat of thermal decomposition and, hence, in a lower content of nitrogen in the CN dissolved in acetone after 38 days of incubation.

Table 3. Kinetic parameters of the thermal decomposition of CN after 38 and 65 days of biological degradation.

| Reactor no. | after 38 days of biological degradation | after 65 days of biological degradation |
|-------------|----------------------------------------|----------------------------------------|
|             | \((dQ/dt)_{t=0}\), \(mW/g\) | \((dQ/dt)_{max}\), \(mW/g\) | \(t_{max}\), \(h\) | \(Q_{tot}\), \(kJ/g\) | \((dQ/dt)_{t=0}\), \(mW/g\) | \((dQ/dt)_{max}\), \(mW/g\) | \(t_{max}\), \(h\) | \(Q_{tot}\), \(kJ/g\) |
| initial     | 9.6 | 77.2 | 20.8 | 4.1 |
| initial     | 9.4 | 72.2 | 18.3 | 4.1 |
The time dependences of the heat release rate and heat released to the given moment in the thermal decomposition of CN after 65 days of incubation in the reactors with different variants of microbiological treatment are presented in Figs. 4 and 5. As can be seen from Fig. 4, the maximum rate of heat release still remains in reactor 1 (AS+CN). The high rate of heat release is also observed in reactor 3 (AS+CN+b+f). However, the amount of the released heat in reactor 3 is substantially lower and almost the same as in reactors 2, 5, and 6. Reactor 4 (AS+CN\textsubscript{treat}+b+f) can be distinguished in which the heat release rate and the amount of released heat are minimum.

The initial rate of heat release is 14–15 cal/(h g) on the average, and its deviations toward overestimation are observed for the variants of microbiological treatment in reactor 4. The times of achieving the maximum rate of heat release during CN decomposition after the microbiological treatment for 65 days are shorter than the corresponding times during the treatment for 38 days. The shortest time of achieving the maximum rate of heat release during CN decomposition after the microbiological treatment for 65 days was observed for reactor 3.

| UV+ozone irradiation | 1     | 2     | 3     | 4     | 5     | 6     |
|----------------------|-------|-------|-------|-------|-------|-------|
|                      | 21.5  | 65.5  | 14    | 3.8   | 19.8  | 103.3 |
|                      | 18.1  | 72.6  | 16.7  | 4.3   | 7.1   | 74.6  |
|                      | 11.2  | 65.2  | 17.3  | 4.1   | 19.3  | 74.7  |
|                      | 4.3   | 64.7  | 17.3  | 4.1   | 21.6  | 59.8  |
|                      | 23.7  | 57.5  | 13.5  | 3.6   | 20.8  | 77.9  |
|                      | 10.6  | 73.7  | 17.5  | 4.3   | 6.6   | 72.6  |

Figure 4. Time dependences of the heat release rate during thermal decomposition at 139.8°C of the CN samples treated for 65 days in the microbiological reactor: 0, CN\textsubscript{init}; 1, reactor 1 (AS+CN); 2, reactor 2 (AS+CN\textsubscript{treat}); 3, reactor 3 (AS+CN+b+f); 4, reactor 4 (AS+CN\textsubscript{treat}+b+f); 5, reactor 5 (AS+NMU+CN); 6, reactor 6 (AS+NMU+CN\textsubscript{treat}+b+f).

The heat of CN decomposition for all variants of treatment and both treatment times is about 1000 cal/g on the average. It is seen from the data in Table 3 that the amount of the heat released during CN decomposition after 65 days of incubation under various conditions differs weakly from that released in the specific variant of biological treatment after 38 days of incubation, i.e., an additional time of the microbiological process does not result in a significant decomposition of CN. The data in Figs. 2 and 4 and Table 3 show that a comparison of reactors 5 and 6 in which AS was
subjected to the mutagenic treatment revealed weak dependences in both the initial rates of heat release, maximum heat release rates, times of achieving it, and total heat of the process on the time of the microbiological process.

The observed parameters of the heat release rates and total heats of the process allow us to conclude that the preliminary UV treatment affects substantially the decrease in the nitrogen content in the CN fraction subjected to the microcalorimetric study: the subsequent biological treatment of these samples makes it possible to decrease the nitrogen content compared to the initial CN. Of the whole array of microbiological conditions of the CN treatment for 38 days, the conditions in reactor 4 (CN\textsubscript{treat}+b+f) still result in a lower heat of thermal decomposition and, hence, a lower nitrogen content in the CN subjected to the microbiological treatment.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Time dependences of the heat released to the given moment upon thermal decomposition at 139.8°C for the CN samples treated for 65 days in the microbiological reactor (numbers at the curves indicate the variants of the microbiological treatment of CN): 0, CN\textsubscript{init}; 1, reactor 1 (AI+CN); 2, reactor 2 (AS+CN\textsubscript{treat}); 3, reactor 3 (AS+CN+b+f); 4, reactor 4 (AS+CN\textsubscript{treat}+b+f); 5, reactor 5 (AS+NMU+CN); 6, reactor 6 (AS+NMU+CN\textsubscript{treat}+b+f).}
\end{figure}

4. Conclusion
It is found in the present study that the biological destructors (microorganisms) decompose CN. The degree of biodegradation of CN by the studied microorganisms was 24.36% within a period of up to 38 days of residence under the action of various microorganisms, potential biological destructors. Among particular types of microorganisms, the most substantial results on the CN decomposition were achieved for the oxidation of CN by mycelial fungus \textit{Fusarium solani} BKM F-819. The use of the preliminary UV+ozone treatment significantly increases the degree of decomposition of CN.

A symbiosis of microorganisms \textit{Desulfovibrio desulfuricans} 1388 and \textit{Fusarium solani} BKM F-819 and a biocenosis of AS microorganisms are efficient for biodegradation. It is shown that the application of the mutagenic treatment of AS with NMU allows the biocenosis of AS microorganisms to retain the high oxidation ability with good sedimentation properties within up to 65 days of incubation with the highly toxic and poorly degradable contaminator CN.

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