Conference Paper

Development of a Wastewater Treatment Biotechnology Based on Immobilised Microorganisms

A.A. Goncharova and A. Yu. Ignatova

Kuzbass State Technical University, Kemerovo, Russia

Abstract

The wastewater treatment processes involving immobilised microorganisms of the activated sludge have been studied. An experimental laboratory unit, which is a flow channel-like reactor operating in a recirculation mode with biomass attached to the nozzle, was developed and assembled. Plant residues of straw and sawdust with the immobilised microflora of the activated sludge from the treatment plants of PAO Koks PJSC, Kemerovo were used as a nozzle. The unit consists of a cascade of tanks placed on a special stand. The results showed an increase in the number of microorganisms during the experiment and a decrease in the concentration of phenol, total ammonia and COD in the treated water, which is indicative of the effectiveness of water purification based on the technology.

Keywords: sewage, phenol, activated sludge, microorganisms, immobilisation.

Corresponding Author:
A.A. Goncharova
anasta.novoselova@yandex.ru

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1. INTRODUCTION

The annual growth of industrial volumes negatively affects the environment.

Wastewater is one of the main and hazardous wastes of the chemical industry. For example, phenols, petroleum products, nitrogen, zinc, iron, copper, manganese compounds as well as organic compounds in terms of COD and BOD and suspended substances are the typical pollutants of rivers in industrial regions the concentrations of which regularly exceed the permissible concentrations by several times [1].

Biological treatment is one of the most common and effective wastewater treatment methods. This method is widely used for the sewage treatment at companies representing the pulp-and-paper, hydrolysis, coke chemical, chemical and food industries as well as domestic sewage [2]. However, its use is associated with a number of complications, e.g. the constrained biological treatment system, the need to control pollutant concentrations in the effluent entering the biological treatment and the large volumes of treatment facilities.

1.1. Purpose of the research

Development of an effective, economically beneficial and environmentally safe biological wastewater treatment method for using natural associations of microorganisms immobilised on plant residues.

1.1.1. Research objectives

R&D related

- Study of the survival of microorganisms in contact with pollutants.
- Selection and approbation of the most promising immobilisers.
- Determination of the pollutant content dynamics in water with immobilised microorganisms;
- Development and assembly of a laboratory unit for experimental research;

Practice related:

- Enhancement of the efficiency of sewage treatment from organic pollutants at chemical enterprises;
- Improvement of the environmental conditions in regions.

The studies were carried out with the sewage water of PAO Koks PJSC, and after the sewage regulator, it goes to the biochemical treatment facilities. The sewage received at the treatment facilities contains phenol, resinous substances, pyridine, thiocyanates, ammonia, cyanides, etc.

1.2. Scientific relevance

lies in the idea of stimulating the natural associations of destructor microorganisms by creating optimal conditions for them. An effective method of stimulating the vital activity of microorganisms is their immobilisation. In this study, plant residues, which are simultaneously a source of readily available nutrients for microorganisms thus making it easier for them to adapt to high concentrations of toxic substances in industrial effluent, are used as an immobiliser.

2. METHODS OF RESEARCH

The active sludge of the treatment facilities of PAO Koks PJSC was taken as a target of research. The active sludge is represented by microorganisms belonging to various systematic groups (bacteria, fungi, protozoa, rotifers, etc.) between which complex ecological and physiological connections are established. The organisms that predominate in such a community are bacteria and protozoans but the main role in the biochemical oxidation of contaminants belongs to the heterotrophic bacteria [3].

The active sludge samples were analysed by microbiological methods. For isolation and identification of microorganisms, conventional tests were used [4, 5]. Microorganisms were grown on liquid and agar media.

The ability of microorganisms to split phenolic compounds was determined on elective media, where 0.1% phenol was added as the sole source of carbon and energy. Fractional application of phenol (0.1 g/l) was carried out as long as it was consumed by microorganisms. To cultivate destructor bacteria of phenol, a mineral medium of the following composition (g/l) was used, Na_2HPO_3 – 0.73; KH_2PO_4 – 0.35; $NaHCO_3$ – 0.25; NH_4NO_3 – 0.75; $MnSO_4$ – 0.002. The number of viable cells in 1 ml of the culture medium with phenol was determined during the exposure time at 4°C, 10°C, 20°C, 32°C once a day. For this purpose, a series of ten-fold dilutions of an aliquot of the suspension was prepared up to 10,000 cells/ml. 0.1 ml of the culture liquid was plated on the surface of the agar medium. The number of the grown colonies was counted.

The phenol concentration in the medium was determined spectrophotometrically (SF-40) at λ = 272 nm. A calibration curve was constructed for an aqueous phenol solution.

To determine COD, the bichromate oxidation method was used, according to HDPE F 14.1, 2.100-97. The total ammonia was determined according to the federal environmental regulatory document PND F 14.1:2.100-97. The COD of the initial water was 2667 mg O^2/l.

3. RESULTS AND DISCUSSION

The research was carried out in several stages.

At the first stage, we studied microorganisms capable of recovering organic compounds, in particular phenol. The active sludge of the treatment facilities of PAO Koks PJSC in Kemerovo provided the basis for the microbiological community. Previously,

we shed strains of microorganisms most adapted to growth on the media containing phenol, Pseudomonas pictorum, Dfcillus pseudococcus, Pseudomonas fluorestens, Rhodococcus opacus, Aureobacterium saperdae [6]. The revealed cultures were identified according to the aggregate of cultural-morphological and physiological-biochemical features [7].

At the second stage, substrates for fixing microorganisms were chosen. Wood sawdust, straw, activated carbon filter cartridge to household filter and polyurethane (foam rubber) were used as carriers in the research.

The dynamics of the number of immobilised microorganisms in water with phenol was studied. The adsorbent with the immobilised microflora was placed in a capsule made of nylon tissue, then in 250 ml flasks. Ammonium nitrogen was added as mineral source of nutrition in the calculation of 40 mg/l and phosphates in the calculation of 16.5 mg/l. The initial concentration of phenol was 500 mg/l. The results of studying the dynamics of the number of microorganisms are presented in Table 1.

The effect of various adsorbents on the viability of microorganisms was studied. The results are shown in Table 1.

Table 1: Number of the immobilised microorganisms in tap water samples with phenol (the number of cells in 1 ml).

Adsorbent	Cell count in 1 ml					
	1 day	3 days	5 days	7 days	10 days	12 days
Sawdust	3.1×10^{7}	2.7×10^{7}	4.2×10^{8}	1.6×10^{8}	1.4×10^{7}	2.5×10^{7}
Straw	1.5×10^{7}	5.4×10^{8}	7.2×10^{9}	2.3×10^{11}	4.1×10^{11}	1.6×10^{10}
Activated carbon	7.8×10^{6}	7.3×10^{6}	5.0×10^{6}	2.3×10^{5}	6.3×10^{5}	1.3×10^{5}
Polyurethane	6.3×10^{6}	6.6×10^{6}	7.1×10^{5}	4.4×10^{5}	3.8×10^{5}	3.2×10^{4}

The number of microorganisms immobilised on plant adsorbents increases over time whereas the use of activated carbon and polyurethane does not. Microorganisms use plant residues as a nutrient substrate.

Dynamics of the phenol concentration in experiments with various types of immobilisers was determined. The initial concentration of phenol was 500 mg/l (Table 2).

In all cases, the concentration of phenol decreases. However, in the experiment with plant immobilisers, the reduction occurs faster.

Further research was carried out directly with the active sludge of the treatment facilities of PAO Koks PJSC.

Table 2: Dynamics of phenol concentration in the samples with tap water.

	Content of phenol in the sample, mg/l		
	1 days	3 days	5 days
Control (tap water) + mixture of cultures	351±2.2	144±1.6	51±0.3
Sawdust + immobilised microflora	248±2.5	48±0.7	5.1±0.04
Straw + immobilised microflora	263±1.9	83±0.4	12±0.08
Activated carbon+ immobilised microflora	269±2.4	143±1.4	53±0.6
Polyurethane+ immobilised microflora	293±1.09	158±1.3	64±0.4

At the third stage of the research, the task was to set up a series of experiments on the purification of waste water from phenol and other organic impurities using a vegetable immobiliser as well as a chemical and microbiological analysis of the water under study.

For immobilization in a 250 ml flask, 10 g of the carrier (straw or sawdust) and 100 ml of the activated sludge with a baseline size of 107 cells/ml were added. The suspension was incubated with the adsorbent for 6 hours without stirring at room temperature 18-20°C. The carriers with the immobilised microflora were placed in capsules made of nylon tissue, then wastewater was added and kept at room temperature for 7 days.

Tap water with the activated sludge added was used for control.

Figure 1: A series of experiments with the immobilised microorganisms.

The initial concentration of microorganisms in 1 ml was 10^6 cells. Fig. 1 shows experimental flasks with wastewater.

In the initial wastewater, a fairly low number of microorganisms was found, 1.8 10^3 cells/ml, which indicates that the sewage water of PAO Koks PJSC cannot be sufficiently purified only due to the vital activities of the microflora of treatment facilities.

Tables 3, 4 show the dynamics of the number of microorganisms in waste water when using an immobiliser.

Table 3: Number of microorganisms in the sewage water of PAO Koks PJSC with the introduction of a consortium of microorganisms immobilised on straw.

Initial number of microorganisms in waste water	1 day	2 days	3 days	4 days	5 days	6days	7 days
$1.8 \cdot 10^3$	$7.3 \cdot 10^7$	$1.4 \cdot 10^9$	$9.2 \cdot 10^{11}$	$4.2 \cdot 10^{12}$	$2.4 \cdot 10^{10}$	$6.9 \cdot 10^{10}$	$3.4 \cdot 10^9$

Table 4: Number of microorganisms in the sewage water of PAO Koks PJSC with the introduction of a consortium of microorganisms immobilised on sawdust.

Initial number of microorganisms in waste water	1 day	2 days	3 days	4 days	5 days	6 days	7 days
$1.8 \cdot 10^3$	$6.5 \cdot 10^7$	$6.9 \cdot 10^8$	$3.1 \cdot 10^{10}$	$4.3 \cdot 10^{10}$	$9.5 \cdot 10^9$	$1.4 \cdot 10^9$	$6.6 \cdot 10^8$

The maximum number of microorganisms was recorded on the 3rd and 4th days of the experiment using straw as an immobiliser and amounted to 9.2 10^{11} and $4.2 \cdot 10^{12}$ 1012 cells/ml.

In experiments using sawdust as a carrier, the number of microorganisms reached 10^{10} cells/ml.

In parallel with microbiological studies, the concentration of phenol and the COD of wastewater were determined. The data are presented in Tables 5, 6.

Table 5: Indicators of wastewater treatment by microorganisms immobilised on straw.

Sampling time, days	COD, mg O_2/l	Concentration of phenol, mg/l
initial	2,668	291±1.35
3 days	827	71±0.5
7 days	352	0

Table 6: Indicators of wastewater treatment by microorganisms immobilised on sawdust.

Sampling time, days	COD, mg O_2/l	Concentration of phenol, mg/l
initial	2,668	290±1.35
3 days	1,697	49±0.3
7 days	843	10±0.02

The intensive development of immobilised destructor microorganisms in the wastewater treatment process made it possible for a relatively short period of time to achieve a high degree of phenol removal and a decrease in COD.

It was found that under the influence of high concentrations of phenol on the association of microorganisms immobilised on plant substrates, the reduction in the number of microbial cells does not occur. In extreme conditions, microorganisms endure and multiply. The combination of a mixture of cultures on an immobiliser, which is also a nutrient substrate, is most favourable for the survival of microorganisms.

At the following stage, a laboratory unit was developed and assembled.

In developing a model unit, the type of reactor with a fixed biofilm is taken as a basis. In this case, the biomass of microorganisms grows on the surface of the nozzle. The nozzle should have a high specific surface area to increase the area suitable for the growth of microorganisms, and a large porosity conducive to the passage of air and liquid. The inlet flow of the wastewater, which has passed the preliminary sedimentation, is introduced by a switchgear.

To improve the efficiency of the cleaning system, we have chosen the recirculation mode. Recirculation involves dilution of inlet effluents with outlet effluents. The recirculation ratio was constant at 1:1.

To assemble the unit, plastic containers with a capacity of 10 litres were chosen. Each container is closed with a lid to reduce the evaporation of water and eliminate odour. The unit is placed on a special stand in the form of a cascade of containers at various levels which allows the fluid to flow from one container to another through pipelines with control devices (cranes).

Utility diagram of the experimental laboratory unit is shown in Fig. 2.

The unit is operated as follows. Sewage is poured into receiving tank 1, from receiving tank 1 the liquid flows through untreated water pipe 2 to distributor 7 and into biological purification tank 3. In biological purification tank 3 there are upper and lower meshes 4 and 5, between which packing material 6 with biofilm from the activated sludge. The raw sewage from distributor 7 flows through packing material 6, where it is treated by the activity of the microorganisms of the biofilm, then flows through conduit 9 into the water receiving tank after biological treatment 10. From tank 10, water after biological treatment 11 through water circulation pipe 13 is supplied by pump 14 under pressure to distributor 7.

Overflow line 8 is designed to avoid liquid overflowing through the edge of biological treatment tank 3. For water sampling, water tank after biological treatment 10 is provided with sampler 12. For discharge of the treated water, the unit has valve 18. The unit is equipped with power supply unit 16 that converts 220 to 12 V. There is also rechargeable battery 15 for backup power. The selection of the power source and the

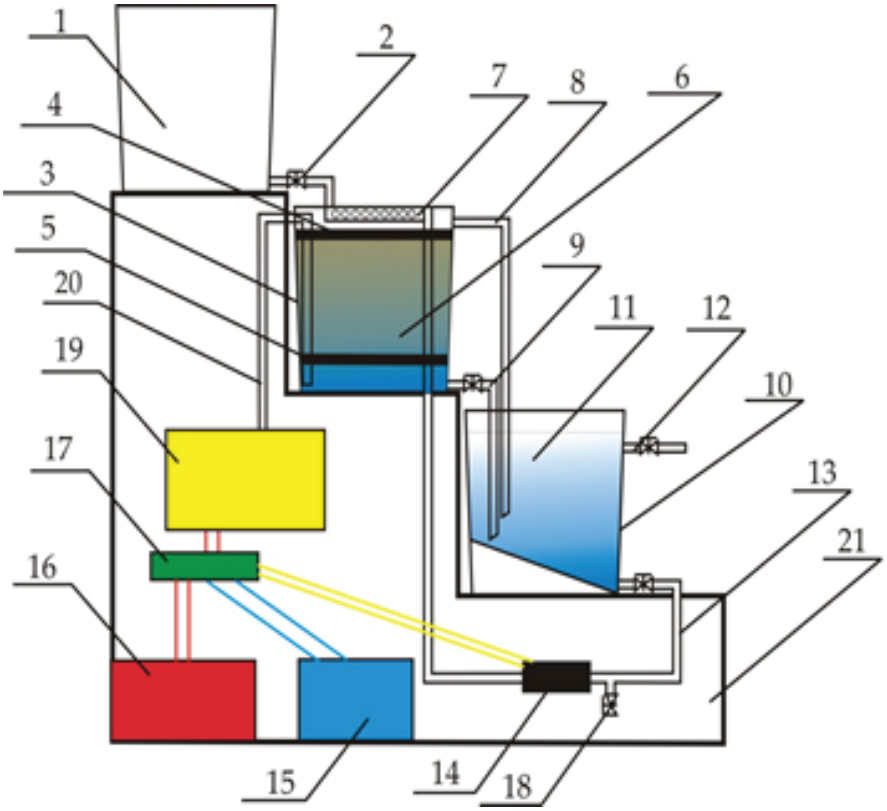

Figure 2: Utility diagram of an experimental laboratory unit for water purification: 1 - receiving tank, 2 - untreated water pipe, 3 - biological purification tank, 4 - upper mesh, 5 - lower mesh, 6 - packing material with biofilm, 7 - wastewater distributor, 8 - overflow pipeline, 9 - pipeline with water after biological treatment, 10 - tank for receiving water after biological purification, 11 - water after biological purification, 12 - sampler 13 - water circulation pipe, 14 - pump, 15 - battery, 16 – power supply unit 220 – 12 V, 17 - pump control unit, 18 - valve for the treated water discharge, 19 – compressor, 20 - hose with air, 21 - stand.

water delivery rate of the pump is controlled by pump control unit 17. For aeration of the liquid into biological treatment tank 3, hose with air 20 supplied from compressor 19 is inserted. The laboratory unit is located on stand 21. In the course of the experiment, in addition to microbiological studies, the phenol concentration in wastewater was measured, the COD index, which indicates the total content of organic impurities in water, and the total ammonia content. The indicators of wastewater treatment by microorganisms immobilised on plant residues in dynamics are given in Tables 7, 8, 9 for the first cleaning cycle and in Tables 10, 11, 12 for the second treatment cycle.

The degree of purification from phenol in the first cycle on day 3 was 97.8; 98.7% respectively.

The degree of purification of water from phenol in the second cycle on day 3 was 96.3% and 97.45% respectively. These data indicate the possibility of using a packing material (immobiliser) with microorganisms in several cycles.

Table 7: Phenol concentration dynamics (treatment cycle 1).

	Content of phenol in the sample, mg/dm^3			
	initial	1st day	2nd day	3rd day
Active sludge immobilised on straw	315±3.12	158±1.43	52±0.45	6.9±0.063
Active sludge immobilised on sawdust	320±3.01	143±1.12	76±1.71	4.3±0.03

Table 8: COD dynamics (treatment cycle 1).

	COD, mgO_2/l		Degree of purification, %
	initial	3rd day	
Active sludge immobilised on straw	2675	1069	60.03
Active sludge immobilised on sawdust	2675	1213	54.65

Table 9: Total ammonia dynamics (treatment cycle 1).

	NH_3, mg/l		Degree of purification, %
	initial	3rd day	
Active sludge immobilised on straw	590	54	90.8
Active sludge immobilised on sawdust	590	75	87.3

Table 10: Phenol concentration dynamics (treatment cycle 2).

	Content of phenol in the sample, mg/dm^3			
	initial	1st day	2nd day	3rd day
Active sludge immobilised on straw	350±1.03	178±1.37	31±0.14	12.8±0.01
Active sludge immobilised on sawdust	350±1.04	162±1.5	70±1.23	8.9±0.02

The dynamics of the number of microorganisms in sewage water of PAO Koks PJSC during the purification process is presented in Tables 13, 14.

At the end of the process, the treated water was drained from the unit, and the unit was loaded with another portion of the initial wastewater and the following treatment

Table 11: COD dynamics (treatment cycle 2).

	COD, mgO_2/l		Degree of purification, %
	initial	3rd day	
Active sludge immobilised on straw	2,980	1,230	58.7
Active sludge immobilised on sawdust	2,980	1,480	50.3

Table 12: Total ammonia dynamics (treatment cycle 2).

	NH_3 mg/l		Degree of purification, %
	initial	3rd day	
Active sludge immobilised on straw	575	35	93.9
Active sludge immobilised on sawdust	575	61	89.4

Table 13: Dynamics of microorganisms in wastewater (treatment cycle 1).

Option	Number of microorganisms, cells/ml		
	Initial value	2nd day	3rd day
Active sludge immobilised on straw	1.5×10^6	5.2×10^8	6.1×10^8
Active sludge immobilised on sawdust	1.8×10^6	6.9×10^7	4.1×10^7

cycle was started without replacing the packing material with immobilised microorganisms.

Table 14: Dynamics of microorganisms in wastewater (treatment cycle 2).

Option	Number of microorganisms, cells/ml		
	Initial value	2nd day	3rd day
Active sludge immobilised on straw	6.1×10^5	6.3×10^7	3.2×10^9
Active sludge immobilised on sawdust	5.7×10^5	4.1×10^7	7.3×10^8

From the data (Table 7-14) it can be seen that the repeated use of immobilisers with microorganisms does not reduce the microorganism multiplication rate, and a

high degree of wastewater treatment from organic and inorganic compounds can be preserved.

4. CONCLUSION

The use of wood industry and agriculture waste as immobilisers made it possible to mitigate the aggressive environment and speed up sewage treatment.

Experiments with the laboratory unit close to production conditions showed he efficiency of water purification in a flow channel-like reactor using plant residues (straw and sawdust) as a nozzle.

It is possible to implement the developed method at the chemical industry enterprises. The use of biotechnology is much cheaper than traditional methods, and pollutants can be recovered without the accumulation of toxic substances as the final products of the life of destructor microorganisms are simple compounds, in particular carbon dioxide and water.

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