DIFFERENT BIOLOGICALLY ACTIVATED SYSTEMS RESISTANCE TO INHIBITION

DAŽĀDU BIOLOĢISKI AKTIVĒTU SISTĒMU PRETESTĪBA INHIBĪCIJAI

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Abstract. *Biologically activated sorbent (BAS) are believed to be more efficient than separate conventional activated sludge and sorbents systems in removing phenols and others persistant organic pollutants from wastewater. These days, applications of biological activated systems treatment for various kinds of industrial wastewater are attracting greater attention as one of the efficient technologies. But the process is not very good understood, and there is not much parameter, which could describe stability and reliability of system and which could compare different BAS systems.*

The aim of this study was to investigate the resistance of different biologically activated sorbents to inhibition using respirometric measurements. To choose the most resistant to inhibition biologically activated system from five BAS: BAS-A, BAS-B, BAS-C, BAS-D and conventional activated sludge for comparing. Also to evaluate potential applicability of respirometric method for monitoring bioactivity in BAS systems.

The pesticide 3,5-dichlorfenol was chosen as inhibitor compound for experiment. The respiratory inhibition measurements were done with different biologically activated systems using different concentration of pesticide. The experiment was accomplished using respiratory inhibition method which described in Lithuanian normative document for environmental protection (Land 45-2001): activated sludge respiratory inhibition test. In parallel saprophyte bacterial counts were determined by spread plate technique and calculated as amount of saprophyte in one litter.

The results from both tests showed that the most resistance system to respiratory inhibition was BAS-A. Respirometric method is applicable for monitoring bioactivity in BAS systems.

Keywords: *biologically activated systems, respiratory inhibition, bacteria.*

Introduction

Environmental protection needs are facing a new phase that is why wastewater management requires a much higher purification level of industrial wastewater. These days, investigation and applications of biological activated carbon (BAC) treatment for various kinds of industrial wastewater is attracting greater attention as one of the efficient technologies [1, 2, 3, 6, 7, 8].

Wastewater usually contains various kinds of organic and/or inorganic pollutants and some of them may inhibit the microbial activity. Therefore, it is necessary for the design/operation of BAS systems to elucidate their resistance to inhibition. While the inhibition influence onto conventional biological treatment systems, e.g. the activated sludge have been studied for a long time, there are not so many studies carried in this area for the BAS systems [4, 5].

The aims of this study were: to investigate the resistance of different biologically activated systems to inhibition using respirometric method, to choose the most resistant to inhibition biologically activated system, to evaluate the potential applicability of respirometric method for monitoring bioactivity in BAS systems. In parallel saprophyte bacterial counts were performed by spread plate technique.

Materials and methods

Five experimental BAS systems were studied:

1. Microorganisms immobilized on sorbent 1(BAS-A).
2. Microorganisms immobilized on sorbent 2 (BAS-B).
3. Microorganisms immobilized on Zeolite (BAS-C).
4. Microorganisms immobilized on Anthracite (BAS-D).
5. Conventional activated sludge

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Experimental systems were prepared by placing certain amounts of the commercial media into the preparatory tank.
During few months" adaptation and cultivation of BAS systems took place. In the tanks continuous aeration was applied. Simulated composition wastewater prepared according [9] was fed to the tanks. Once every 24 hours tanks were half emptied and new portion of simulated wastewater was added.
After adaptation period respiratory inhibition experiment was performed using different concentrations of pesticide 3,5-dichlorfenol as inhibitor. The experiment was accomplished using respiratory inhibition test. In parallel BAS systems were evaluated performing saprophyte bacterial counts by spread plate technique.
Respiratory inhibition test method
This method assesses the impact of pollutant on microorganisms under determined conditions. Using different pollutant concentrations the intensity of respiration is measured. Usually this method is used for activated sludge to evaluate inhibition effect of certain pollutant. In our experiment resistance to inhibition of different biologically activated sorbent was evaluated.
As respiratory inhibitor pesticide 3,5-dichlorfenol was used. First 3,5-dichlorfenol concentration interval for the experiment has to be determined.
In the experiment two control samples without pesticide addition are included. One control test is performed at the beginning, another at the end of the experiment. Test results are reliable, if respiratory intensity values in both control samples differ less than 15%.
For the better representation of respiratory process kinetics, method was modified and oxygen consumption testing time was prolonged from 12 till 30 minutes. But in percentage value of respiratory inhibition calculations was used 12 minutes of oxygen consumption interval, because in that time oxygen consumption has linear profile.
The effect of respiratory inhibition of different kind of concentration and respiratory intensity was countable by 2 control values of respiratory intensity average percentage part:

$$S = \left(1 - \frac{2R_s}{R_{K1} + R_{K2}}\right) \times 100 \qquad (1)$$

Where:
S = percentage value of respiratory inhibition;
R_S = intensity of oxygen consumption, testing particular pollution concentration;
R_{K1} = intensity of oxygen consumption in 1 control sample;
R_{K2} = intensity of oxygen consumption 2 control sample.
Respiratory inhibition tests were performed in 1 litter glass beakers. Certain amount of BAS system was transferred to the beaker. After adding nutrition media in form of synthetic wastewater or nutrition media with 3,5-dichlorfenol and diluting to 500ml tested systems were continuously aerated for 3 hours (Fig 1-2). Aeration was then stopped, pH value was fixed and oxygen concentration change was measured every 6 min during 30 min period.

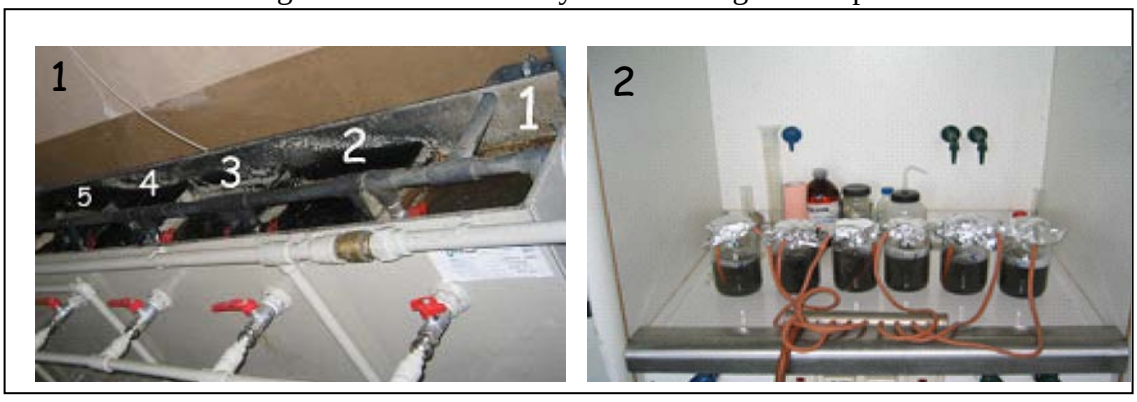

Fig. 1. **Respiratory inhibition experiment: 1 - preparatory tank for biological activated sorbets, (1. Conventional activated sludge, 2. BAS-A, 3. BAS-B, 4. BAS-C, 5. BAS-D); 2 - respiratory inhibition experiment (contact time 3 hours).**

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In addition to usual lab equipment for the experiment there were also used others instruments as aeration equipment, pH-electrode and measuring equipment (HI 9025 microcomputer), air pump (SLL-50), O_2 measuring instrument (Oxi 315i/SET).According method [9] recommended biomass concentration in the sample mixture – 0,8-1,6 g/l. Biomass concentration was measured using standard method [10]. Biomass concentration in the different biologically activated systems was measured using method [11].

Experimental procedure for each BAS system was performed as follows:

1. *First control sample mixture (K1).* At the time „0“ 16 ml of simulated wastewater was diluted with 300 ml of distillate water, then placed 25 ml of BAS sample. Finally mixture was diluted till 500 ml and aeration was started.
2. *Sample mixture with 5 mg/l of 3,5 – dichlorfenol (C1).* At the time „1 minute“ was everything repeated as in the first control sample, only before diluting sample till 500 ml, 5 ml of 3,5-dichlorfenol solution was added.
3. Analogically were prepared samples mixtures with 10, 20 and 40 mg/l of 3,5 – dichlorfenol (C2, C3, C4).
4. *Second control sample mixture (K2)* was made last and the same as sample K1.

In parallel after 30 min of oxygen measurements from the tested systems samples for the plate count test of saprophyte bacterial were taken.

Spread techniques for saprophyte bacterial counts

Standard plate count by Koch method was used during the experiment, in order to count saprotrophic bacteria [12]. The quantity of saprotrophic bacteria can approximately show the activity of biologically activated sorbent and active sludge. The major part of the procedure deals with a series of successive dilutions of the original culture in sterile test-tubes with sterile water. The diluted culture is poured into Petri dishes along with the nutrient agar. The number of colonies is counted after incubation.

Experimental procedure: dilutions of the original culture in sterile test-tubes with sterile water; diluted sample is spread on the surface of the nutrient agar in the Petri dishes; the plates are incubated in the inverted position at 37ºC for 24 hours; the number of colonies is counted after incubation; only plates between 15 and 300 colonies are counted.

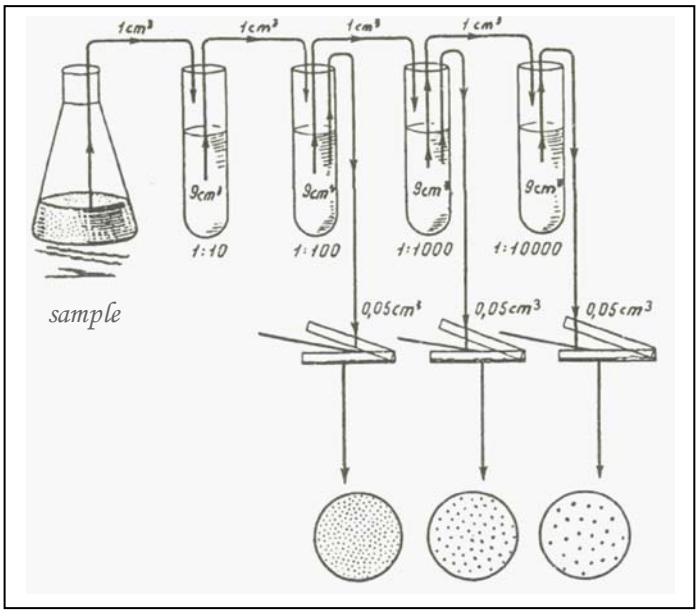

Fig. 2. **Dilutions scheme of sample by inoculatation microorganism”s suspension surface method**

The results obtained using a Plate Count Test are not an accurate assessment of total heterotrophic microorganisms concentrations.

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Possible explanations: the presence of some bacteria in a viable but non-cultivable state; media do not provide the complex nutritional requirements necessary for the growth of all heterotrophs.

Results and discussion

First 3,5-dichlorfenol concentration interval that is to be used in the main experiment was determined . Results showed that under the contact time of 3 hours 100% respiratory inhibition is reached then 3,5–dichlorfenol concentration is higher than 70 mg/l. It was decided for the further experiment to use lower pesticide concentrations - 5, 10, 20 and 40 mg/l. pH value of sample mixture before respiratory intensity measurements was 7-7,5. Under such conditions pH don"t give any negative impact for the intensity of biochemical process.

Figure 3 shows the oxygen consumption profiles of different BAS systems under several 3,5-dichlorfenol concentrations.

In the BAS-A sample mixture without inhibitor after 24 min, oxygen concentration decreased by 95%. Increasing of inhibitor concentration to 5 and 10 mg/l the oxygen consumption was very similar and reached 90%. When the concentration of inhibitor was 20 mg/l the oxygen consumption was 75%. Under the 40 mg/l of pesticide concentration inhibition effect was high and oxygen consumption was just 39%. That means 2,5 times less comparing with mixture without inhibitor.

In BAS-B system control sample - without added inhibitor - during 24 minutes after stopped aeration, oxygen concentration decreased by 90% comparing to the initial value. Then the 3,5-dichlorfenol concentration was 5 and 10 mg/l inhibiting influence was not very obvious - oxygen consumption was a bit slower and after the same period of time as in previous case it composed 71 and 75% of initial oxygen saturation. Pesticide concentrations – 20 and 40 mg/l make bigger influence to the system. It"s resistance decreases and oxygen consumption by microorganisms after 24 minutes was 45 and 22% respectively. This is 2-4 times less than in the system without inhibition.

Control sample in the BAS-C system oxygen concentration within 24 minutes decreased by 88% from the initial level. Under the inhibitor concentrations 5 and 10 mg/l, oxygen consumption was 78 and 60% respectively. After adding higher 3,5-dichlorfenol amount reaching - 20 mg/l, oxygen consumption was slower and after 24 minutes composed 31%. Then inhibitor concentration rose to 40mg/l almost no changes in oxygen consumption were observed. It means that BAS-D system lost its resistance to inhibition at this concentration.

The BAS-D control sample during 24 minutes consumed period oxygen consumption reached 98%. Under the 5 and 10 mg/l of pesticide concentration oxygen consumption was slower and it composed 88% and 70% respectively. Pesticide concentration - 20 mg/l make bigger influence to the system. Oxygen consumption by microorganisms after 24 minutes was 42%. Reaching 40 mg/l of 3,5-dichlorfenol concentration inhibition of system was significant and oxygen was consumed nearly 10% of the initial value.

As we can see from figure 3 the in activated sludge during 24 minutes period the oxygen concentration decreased 90% in the control sample. Quite low negative influence on oxygen consumption can be seen under 5 mg/l and 10 mg/l concentration of pesticide. Oxygen consumption reached 80% and 85% respectively. But existing 20 mg/l and 40 mg/l of pesticide concentration evidently effected activated sludge system and oxygen consumption during 24 minutes was only 27% and 8% respectively.

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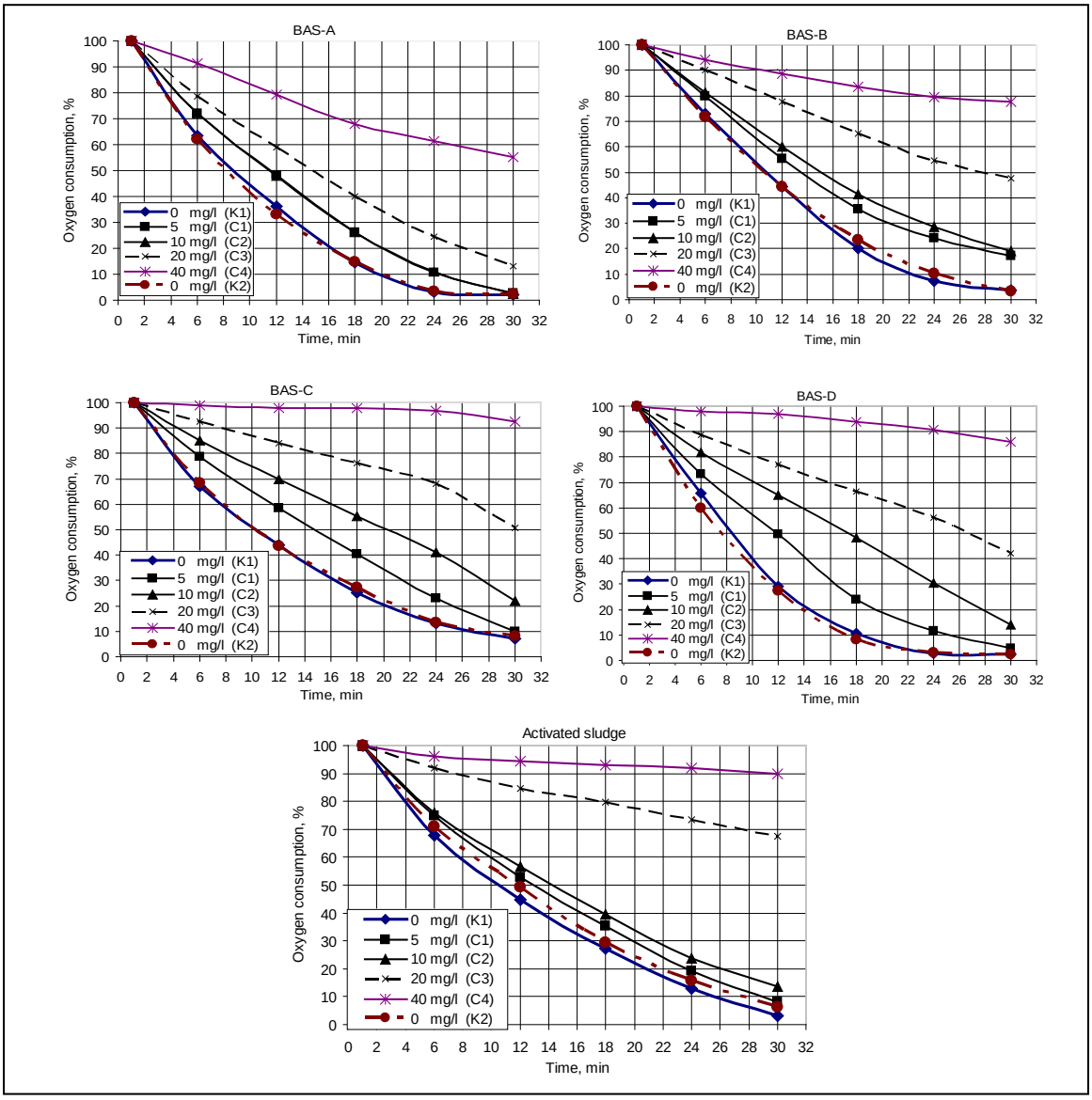

Fig. 3. **Oxygen consumption during 30 min by different BAS and with different amount of 3.5-dichlorfenol concentration**

Comparing between each other different systems it is obvious that systems with microorganisms immobilized on (BAS-A and BAS-B) in general demonstrated higher stability than the other systems, especially in the range of higher inhibitor concentrations. In comparison to the BAS-B system BAS-A is more resistant to inhibition.

Table 1 gives values of oxygen consumption during 12 minutes in the different BAS systems, under different 3,5-dichlorfenol concentrations. It was evaluated reliability of experiment results. Results of experiment are reliable, if values of respiratory intensity in both control experiments differ less than 15%. Values obtained satisfy such requirement.

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Table 1.

Values of oxygen consumption during 12 minutes in the different BAS systems

3,5-dichlorfenol concentration., mg/l	Oxygen consumption, mg/l during 12 min				
	BAS-A	BAS-B	BAS-C	BAC-D	Activated sludge
0-K1 (Control sample)	3,9	5,0	4,4	4,8	3,8
5	3,7	4,4	3,4	4,1	3,7
10	3,4	4,4	2,7	3,0	3,2
20	2,0	3,6	1,5	2,0	1,3
40	1,2	1,9	0,2	0,3	0,5
0 – K2 (Control sample)	3,9	5,4	4,2	4,7	3,6
Percentage difference of respiratory intensity values in both control mixtures	0	8	5	2	2

* **Results of experiment are reliable, if values of respiratory intensity in both control experiments differ less than 15%**

Results presented in Table 1 were used to calculate percentage of respiratory inhibition for the different BAS systems according equation1. Obtained values are presented in Figure 4. As it can be seen from the Figure 4 percentage of respiratory inhibition calculated from the oxygen consumption during first 12 minutes data showed the same tendency as oxygen consumption curves in Figure 3 representing measurements during 30 minutes. Systems BAS-A and BAS-B demonstrated higher resistance to inhibition than the BAS-C, BAS-D and activated sludge. Higher percentage of respiratory inhibition in the latest systems show negative effect of 3,5-dichlorfenol on microbial activity in these systems. Under the highest 3,5-dichlorfenol concentration used in experiment percentage of respiratory inhibition for the system BAS-A reached only 56%, BAS-B - 73% and for BAS-C, BAS-D and activated sludge it was 95, 92 and 89% respectively. Therefore it can be concluded that the media causes lower level of inhibition. The fact that biologically activated systems with activated carbon media tend to reduce negative effects of inhibition was also observed studying influence of heavy metals [5].

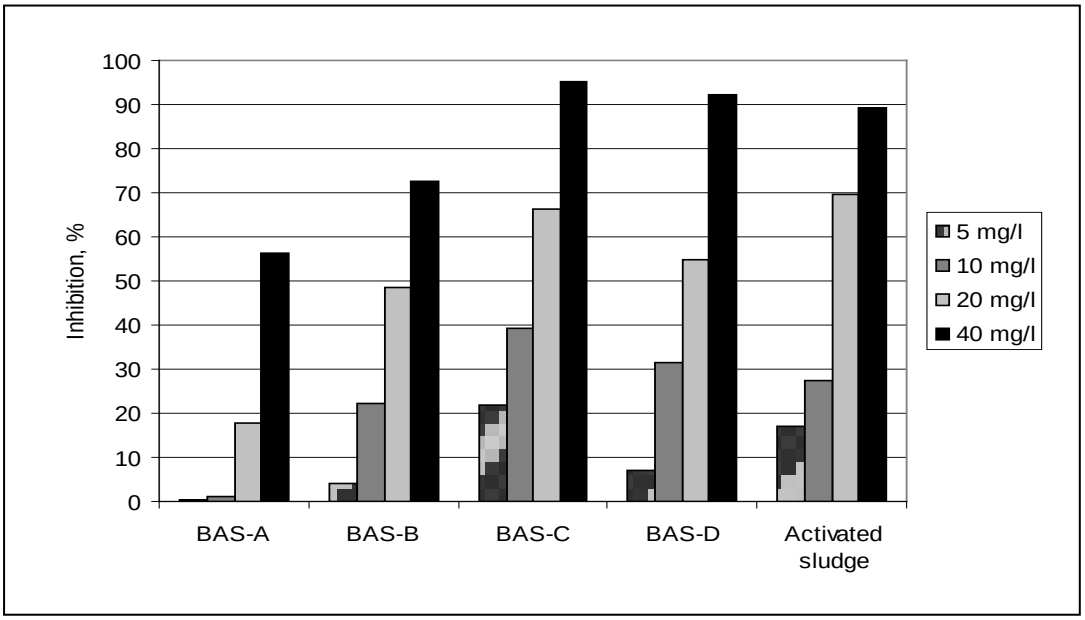

Fig. 4. **Percentage of respiratory inhibition for the different BAS systems**

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As it was mentioned earlier in parallel to the respirometric measurements saprophyte bacterial counts were performed in all inhibited systems by spread plate technique. It was noticed, that increase of 3,5-dichlorfenol concentration causes decrease in number of microorganisms in all tested systems (Figure 5). At the same time it is obvious that sensitivity to inhibition is not the same. In BAS-C and BAS-D systems microorganisms cultures demonstrated low resistance even to small concentrations of inhibitor. Under the higher concentrations - 20 and 40 mg/l microorganisms cultures in these systems were nearly gone. Activated sludge, BAS-A and BAS-B systems appeared to be more resistant to inhibition than BAS-C and BAS-D. The most stable and viable system is BAS-A, which is almost completely resistant to small doses of inhibitor. Only concentration of 40 mg/l more noticeably affected vital functions of microorganisms and their ability to multiply and to form colonies.

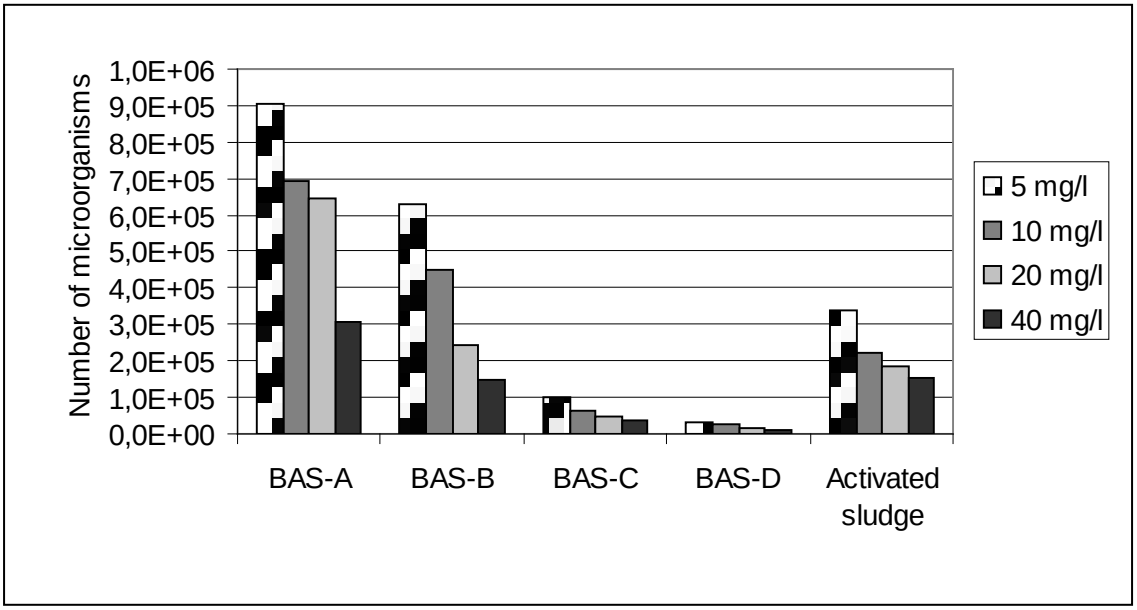

Fig. 5. **Saprophyte bacterial counts in all inhibited systems by spread plate technique**

Comparing all the systems by their viability, number of multiplying microorganisms and decrease in their number under the influence of rising inhibitor concentration shows, that the most resistant to inhibition system is BAS-A.

Experimental results obtained by respirometric measurements and bacterial counts by spread plate technique presented in Figure 4 and Figure 5 show the same tendency. It may be concluded that respirometric method can be used for monitoring bioactivity in BAS systems.

Conclusions

1. From tested five biologically activated systems the higher resistance to inhibition was determined in systems with BAS-A and BAS-B.
2. BAS-A showed the highest resistance to inhibition. Percentage of respiratory inhibition under 5 and 10 mg/l of 3,5-dichlorfenol was only 0 and 1% respectively. Concentrations of 20 and 40mg/l caused respiratory inhibition of 18 and 56%.
3. Results obtained by respirometric measurements and bacterial counts by spread plate technique show the same tendency. Respirometric method is applicable for monitoring bioactivity in BAS systems.

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