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PROPERTIES OF THE ACTIVATED SLUDGE AFTER LIPASE BIOAUGMENTATION

Bioaugmentation of the activated sludge with microflora, which is capable of biochemical oxidation of lipids, influences the physicochemical parameters of the wastewater treatment process. The results present not only the changes related to the quality and quantity of the sludge, but also loads of the aeration chambers and the sludge with the load of contaminants.

It seems possible to dose the substrate, which produces bacterial lipase in aquatic environment, in such a way as to maximize the activity of this enzyme or to maximize the time it is present inside the reactor. Simulation of the reactor operation, based on measurements, enables the kinetics of the biological wastewater treatment process to be determined. The modelling of the processes facilitates the decision making in order to maintain high efficiency of plant operation through bioaugmentation; for example, by using the relative biomass growth rate with respect to substrate concentration, together with approximation for the Haldane's model, information about strong substrate inhibitory action has been gained.

1. INTRODUCTION

In the era of more and more efficient water and water sewage disposal, an increased contamination of wastewater fed into treatment plants is being observed. The presence of lipids (ether extract) in the sewage discharged into the municipal sewage systems in the concentration exceeding 300g/ dm^3 is particularly troublesome [7].

The presence of lipids in wastewater makes its biological treatment difficult. In aerobic processes, lipids have detrimental effects on oxygen transfer. They reduce the rates of oxygen transfer to the biological floc by formation of lipid coat around

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the floc [2]. Moreover, in activated sludge systems the amount of lipids has been shown to be related to the occurrence of the filamentous microorganisms, i.e., *Microthrix parvicella*, which are involved in the formation of scum and stable foams. In addition, long-chain fatty acids, the primary products of hydrolysis of fats and oils, severely inhibit the activity of the biomass in biological treatment processes. Moreover, lipid depositions in pipes and other plant components frequently cause operational disturbances. Therefore, the presence of lipids in wastewater is still a major problem in wastewater treatment plants.

The presence of bacterial flora suitable in terms of the quality, quantity and adaptation to the conditions of the decomposition characteristic of a given substance is a condition required for a proper course of the reaction. The oxidation process can be substantially impeded, when a non-adopted microflora is used [3].

One of the methods for obtaining the maximum effects of waste decomposition at minimum costs is to increase the biomass activity by influencing its metabolism or by bioaugmentation with microflora capable of processing certain type of waste. According to many authors, it is possible to dose the substrate that activates bacterial lipase in aquatic environment in such a way as to maximize the activity of this enzyme or to maximize the time it is present inside the aeration reactor. Information about the occurrence of substrate inhibition enables optimal control of the bioreactor operation [1].

The purpose of this research was to determine the way in which the lipase activity can be modelled in the water environment and to define the influence of the bioaugmentation with the specimen of high lipase activity on efficiently nitrifying activated sludge in the model Fill-and-Drow reactor with 24-h aeration.

Relative rate of the biomass growth may be used to construct the kinetics model of the biomass growth rate, and indirectly to estimate the properties of the substrate inhibitory action [4].

2. MATERIALS AND METHODS

2.1. LIPASE SOURCES AND LIPASE ACTIVITY MEASUREMENT

The lipase used in this study was the biospecimen known under the trade name PBA 702.052, produced by APB Environnement Fontenay le Vicomte (France).

The lipase activity was determined using titrimetry methods. Activated sludge from each reactor was filtered and then 5 cm^3 samples of liquid with 10 cm^3 olive emulsion were thermostabilized (37 °C). 0.1 M NaOH was used as a titrant (1 cm^3 of 0.02 N NaOH corresponded to 100 lipase units) [6].

Table

Lipase-producing microorganisms in PBA 702.052 biospecimen

Bacillus subtilis	*Lactococcus lactis*
Bacillus licheniformis	*Acinetobacbacter* sp.
Baccilus megaterium	*Comamonas* sp.
Bacillus sp.	*Rhizobium* sp.
Pseudomonas putida	*Deinococcus rediodurans*
Pseudomonas fluorescens	
Pseudomonas sp.	*Botrytis cinerea*
Alcaligenes sp.	*Saccharomyces cerevisiae*
Acetobacter sp.	*Sporotrichum dimorphosporium*
Nitrosomonas sp.	*Trichoderma roseus*
Nitrobacter sp.	
Lactobacillus helveticus	

2.2. REACTORS AND BACTERIAL CULTURE

Reactor R1 of 1.5 dm^3 volume was prepared by dissolving 1.88 g of PBA 702 in distilled water and adding to this solution 7.5 cm^3 of substrate. Substrate consisted of 25 g of ACT 606 and 20 cm^3 of ACT 706 completed with demineralized water to the volume of 1 dm^3. The second reactor (R2) was operated with bioaugmentation. The water containing activated endogenous efficiently nitrifying sludge (TSS, 166 mg/dm^3) was inoculated with the same preparation as the previous reactor. Inoculated sludge was prepared approximately 30 days before using the same preparation. After 4 days of cultivation at a temperature of 30 °C and feeding with the substrate as mentioned before, the temperature was decreased to 20 °C and feeding with the substrate of the loading of 200 mg/dm^3 COD (peptone) and ammonia nitrogen concentration of 10 mg/dm^3 $N–NH_3$ begun. Before commencing the experiment, nitrification was maintained stable. All ammonia was consumed and practically fully converted to nitrate (nitrite concentration lower than 0.1 mg/dm^3). Reactors were operated under aeration at oxygen concentration of at least 2.5 mg O_2/dm^3, and at stabilized temperature (30 °C). After 24 hours of aeration, each day 250 cm^3 of sludge was pump out and refilled to previous volume with dematerialized water and 27 cm^3 of substrate. During this operation HRT of reactor was 6 days.

2.3. DISCUSSION OF RESULTS

The result of the contaminant removal depends on the activity of the biomass. The rate of the contaminant removal in the activated sludge reactor increases along with the increase in the amount of suspended solids. Usually, in the aeration chamber the concentration of contaminants was 3–3.5 g /dm^3 [3].

In reactor R1, a gradual increase in the content of suspended solids of activated sludge takes place. After 10 days, the amount of suspension is about 5 g/dm^3 at 70% of organic sludge. Large amount of inorganic part of sludge makes stirring and aeration difficult as the sludge is liable to sedimentation (figure 1).

An increase in the volume index in reactor R2 can be justified by a substantial loss of dry matter of sediments with simultaneous disintegration of flocs. In reactor R1 a crucial increase in the biomass, especially in its inorganic part, can be observed (figure 2).

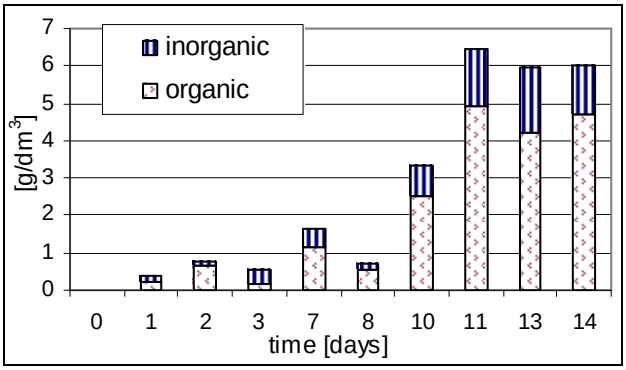

Fig. 1. The content of suspended solids in reactor R1 [g/dm^3]

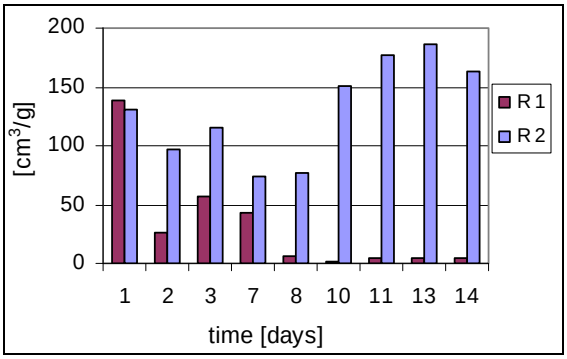

Fig. 2. Stirred sludge volume index (SV) [cm^3/g]

Along with the sludge loading rate the activity of bacterial biocenosis changes. When the volumetric loading rate of the systems is relatively constant (figure 3) and the content organic suspension in the sediment changes, it is possible to observe considerable fluctuations in the substrate load, especially in reactor R1 (figure 4).

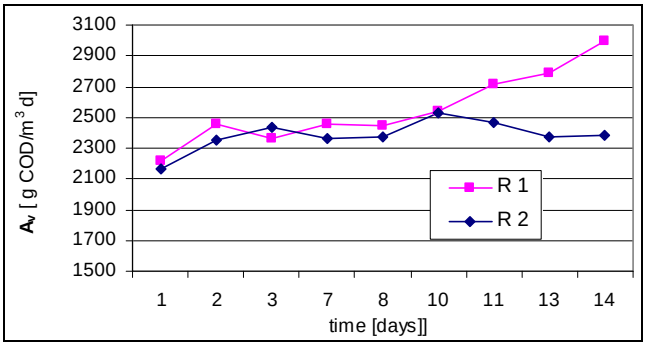

Fig. 3. Volumetric loading rate (A_v) in reactors R1 and R2 [g COD/ m^3d]

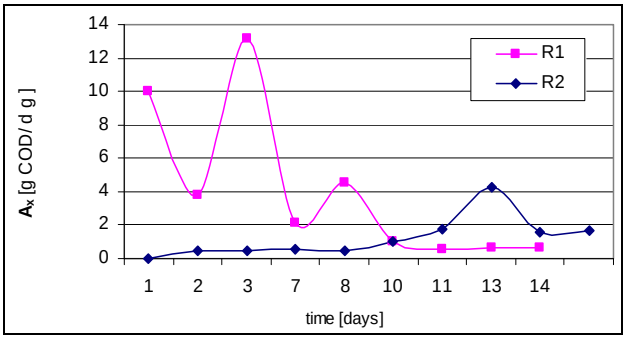

Fig. 4. Sludge loading rate (A_x) in reactors R1 and R2 [g COD/ g d]

In reactor R2, a decrease in the lipase activity till the moment of bioaugmentation was observed. On the 7th day of the process, an additional amount of the biospecimen was added, which caused a temporary increase in activity in the systems followed by its almost complete decline on the 14th day of the experiments (figure 5).

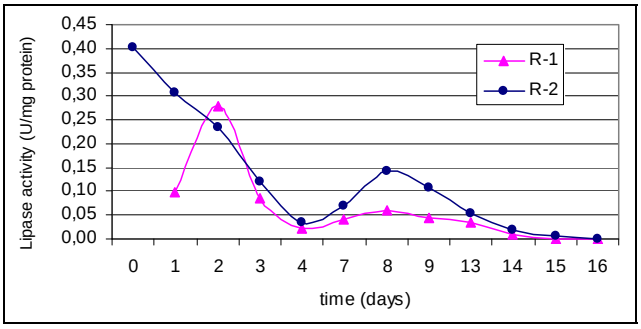

Fig. 5. Lipase activity as lipase units per mg of protein in reactors R1and R2 (1 cm^3 of 0.02 N NaOH corresponded to 100 lipase units) [6]

2.4. MICROSCOPIC EXAMINATION OF ACTIVATED SLUDGE

A microscopic examination of activated sludge was performed using the microscope with phase contrast. Activated sludge in reactor R2 during the whole experiment consisted of primary particles with amorphous structure and not numerous flocs whose number successively increased. Initially the structure of sludge in reactor R2 was flocculent. At the end of test we dealt with amorphous structure and a large number of protozoans such as: *Vitreoscilla* sp., *Arcella* sp., *Lionotus* sp., *Aspidisca* sp., *Colpidium* sp., *Paramecium sp*.

2.5. MODELLING

The concentration of biomass during its cultivation is described by the following equation:

$$\frac{dC_x}{dt} = \mu C_x - DC_x,$$

where:

μ – specific growth rate,

C_x – biomass concentration,

D – dilution rate.

In order to calculate a specific growth rate versus time, the following function is used:

$$\mu(t) = \frac{\ln\left(\frac{C_{Xk+1}}{C_{Xk}}\right)}{t_{k+1} - t_k}.$$

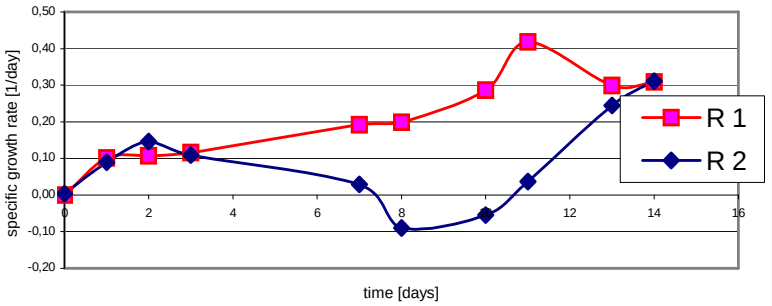

Fig. 6. Estimated specific growth rates in reactors R1 and R2

However, for a small number of samples and increasing time intervals, it is very difficult to calculate the differential function. Therefore, μ was estimated based on an extended Kalman filter [5]. The Kalman fiter is a recursive algorithm which estimates the state of a dynamic system from a series of noisy measurements using minimal-variational estimator of linear dynamic system discretised in time domain modelled by linear operators perturbed by Gaussian noise. The algorithm updates both measurements and time domain. Figure 6 shows estimated specific growth rate in particular reactors.

The estimated specific growth rates were used for determining the models of biomass growth kinetics. For determining the substrate inhibition the following Haldane's-type model was initially investigated:

$$\mu = \frac{\mu_{\max} S}{K_S + S + \frac{S^2}{K_I}}.$$

Two parameters defining substrate influence are present in this model: K_S – affinity, K_I – inhibition. Very high values of K_I testify to a weak substrate inhibitory action, and the equation approaches Monod's kinetics description. Because the lipase durability is short and because this enzyme can exclusively be derived from bacterial source, it was assumed that its activity is proportionally dependent on the substrate concentration that is absorbed for lipase release. Data approximation was performed by nonlinear regression using MATLAB *nlinfit* function. The results of measurements were treated as a set of experiments for individual days. The approximating function, based

on Haldane's model, has the following form:

haldanef = inline('$p(1).*x./(p(2)+x+(x.*x./p(3)))$','$p$','$x$'),

where:

$p(1)$ – μ_{max} [1/day],
$p(2)$ – K_S [mg/dm^3],
$p(3)$ – K_I [mg/dm^3].

Figure 7 presents the relationship between a relative biomass growth rate and the substrate concentration approximated to Haldane's model for reactor R1. The parameters of approximations are: $p(1) = 2163.7$, $p(2) = 3807$ and $p(3) = 0.00036721$, respectively. Low value of $p(3)$ testifies to a strong substrate inhibitory action. Analogous analyses carried out for reactor R2 led to similar results, but these correspond to the first 7 days of the process. As is shown in figures presenting suspension concentration, on the 8th day a rapid decrease in organic suspension took place, and a microscopic examination testified to a decay of amorphous sludge. This may suggest that the sludge was utilized as a carbon source.

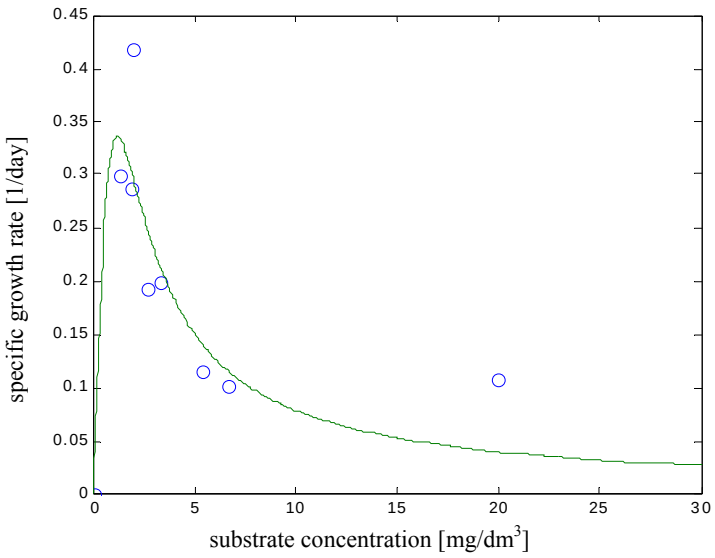

Fig. 7. Biomass growth rate in reactor R1 versus substrate concentration

3. CONCLUSIONS

On the basis of the results obtained, the following conclusions can be drawn:

- Bioaugmentation of efficiently nitrifying activated sludge in reactor R2 with the

lipolytic biospecimen causes a decrease in dry matter content and imbalance between the organic and mineral parts. Microscopic examination testifies to a successive disintegration of sludge flocks, which in connection with the changes in sludge loading and the changes in Mohlman index may suggest that the sludge is used as the source of organic carbon. Approximation of the Haldane's model supports this presumption.

- When the sediment is cultivated in order to remove fats with high lipase activity, it loses its properties (reactor R1, lipase activity diminishes reaching zero). Simultaneously, the sediment is produced whose morphology and qualitative composition are similar to these of the sludge in conventional wastewater treatment plants. The dependence of a relative growth rate on the substrate concentration approximated to Haldane's model reveals a strong inhibitory activity of the substrate.
- The suggestion of substrate inhibition constrains the set of optimal control strategies of bioreactor operation.
- Further research should be continued in the direction of substrate dosing and composition that would catalyze bacterial activity of lipase in aquatic environment (without immobilization), so that the highest enzyme activity is obtained or the time it is present inside the reactor is maximized.

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WŁAŚCIWOŚCI OSADU CZYNNEGO Z BIOAUGMENTACJĄ LIPAZY

Bioaugmentacja osadu czynnego mikroflorą zdolną do biochemicznego utleniania tłuszczów wpływa na parametry fizykochemiczne procesu oczyszczania ścieków.

Wydaje się możliwe takie dozowanie substratu wytwarzającego lipazę bakteryjną w środowisku

wodnym, które pozwala uzyskać najwyższą aktywność tego enzymu lub jak najdłuższą jego obecność w reaktorze. Symulowanie pracy reaktora na podstawie pomiarów umożliwia określenie kinetyki procesów biologicznego oczyszczania ścieków. Modelowanie ułatwia podjęcie decyzji, aby utrzymać wysoką efektywność pracy instalacji dzięki bioaugmentacji, np. wykorzystując względną szybkość wzrostu biomasy w zależności od stężenia substratu z aproksymacją dla modelu Haldane'a, uzyskano informację o silnym działaniu substratu jako inhibitora.