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Agnieszka GĄSZCZAK[1], Joanna KALETA[1], Elżbieta SZCZYRBA[1], Bożena JANUS[1] and Grażyna BARTELMUS[1]

KINETICS OF BUTYL ACRYLATE BIODEGRADATION BY SELECTED STRAIN OF MICROORGANISMS*

KINETYKA BIODEGRADACJI AKRYLANU BUTYLU PRZEZ WYSELEKCJONOWANY SZCZEP MIKROORGANIZMÓW

Abstract: The subject of research in the present study was biodegradation of butyl acrylate. Tests of microbiological degradation of this compound by selected environmental and laboratory strains were conducted. The *Pseudomonas fluorescens* PCM 2123 strain utilized the growth substrate most intensively and was selected for further research. Optimal growth conditions and toxic dose of the utilized substrate were determined for the selected strain. The research into kinetics of butyl acrylate biodegradation by *Pseudomonas fluorescens* PCM 2123 bacteria included a series of experiments conducted for different initial concentrations of acrylate in a batch reactor. The influence of initial substrate concentration on the specific growth rate was described by Haldan's equation. Constants of the equation were estimated on the basis of experimental data.

Keywords: biodegradation, batch culture, growth kinetic

The rapid development of civilization in the recent centuries caused a significant increase in the amount of the produced waste exceeding the self-purifying possibility of the environment. The development of methods of water treatment and waste-gases purification to eliminate pollution at its source, is the protection against the unfavourable for the environment phenomenon of waste accumulation. Methods based on the processes of biological decomposition of waste play a great role in such methods.

The processes of biodegradation are very common in the natural environment. Creating conditions different from natural ones aims at accelerating the process. A great advantage of biological methods is the fact that they do not shift pollution to another phase, which is the case in absorption and adsorption processes, but degrade it completely at relatively low investment and operating costs of the process.

The subject of analysis in the present study is the process of biological degradation of butyl acrylate. Acrylates - acrylate acids esters - are compounds widely used in industry. About 80% of the produced acrylate acid is converted to esters. 3.3 million Mg of acrylate acid esters [1] were produced in 2004, whereas in 2009 their production was 1.28 million Mg in China alone, out of which methyl and butyl acrylates made 320 000 Mg. A 4.3% increase in the consumption of acrylate acid esters is estimated in the years 2010-2015 [2].

Butyl acrylate is a colourless liquid having an unpleasant pungent odour. It dissolves in ethanol, diethyl ether and acetone. Its solubility in water at the temperature of 20ºC is 0.14 % vol. [3]. Butyl acrylate is used to obtain various types of polymers, to produce paints, lacquers, inks, glues as well as in leather and paper industries and in stomatology [1, 4]. The main ways in which butyl acrylate penetrates the body are breathing and digestive

[1] Institute of Chemical Engineering, Polish Academy of Sciences, ul. Bałtycka 5, 44-100 Gliwice, Poland, phone +48 32 231 08 11, email: gaszczak@iich.gliwice.pl
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systems and skin. Acrylate vapours can irritate respiratory tracts, eyes and mucosa of nose. When the concentration is high they can cause narcosis and edema of lungs. Butyl acrylate, due to its irritating pungent odour, can cause headaches, dysmnesia, hyperexcitability, arterial pulse drop, cholesterol growth and contact allergic skin inflammation among people exposed to work with this compound (among others dentists, printing-houses workers, chemical industry workers, workers in contact with glues [5, 6]. Accumulation of butyl acrylate has not been found in soil or living organisms according to literature reports. After penetrating surface water part of the substance is degraded by microorganisms and part is released into the air. According to US EPA classification butyl acrylate was classified in the group of easily biodegradable and moderately toxic substances [7].

Although acrylates are relatively easily biodegradable and do not cause great threat to human life and health, because of its characteristic unpleasant odour cause a problem to the people who live in the area where industrial plants producing acrylate acid esters or using such compounds during production are located. At the same time they are a problem to industrial plants workers employed to produce acrylates or process them and being subject to a long-lasting contact with these compounds. Increasing the comfort of work in the production processes using substances with pungent unpleasant odour involves applying proper ventilation systems, which carry away the air containing pollutants. The question is what to do with the air containing the undesirable substances whose penetration of the air makes the quality of living of the neighbouring people lower? Currently a bill regulating the emission of noxious odours is being elaborated. As a result, the regulations of quality assessment of air as regards the contents of odorous substances and standards will be introduced whose not obeying will result in a variety of sanctions [8].

Shortly, every plant (also those whose production deals with acrylates) will be forced to limit the emission of noxious substances/odours by modernizing the production process or introducing innovative ways of removal of compounds which are noxious from ventilated air or waste gases. Taking the above into consideration, it seems purposeful to work out efficient ways of biopurification of big streams of industrial gases polluted with substances noxious for the environment. Butyl acrylate is undoubtedly one of them.

The choice of microorganisms for which butyl acrylate will be the growth substrate and the determination of the kinetics of this growth must be the first stage of research.

Materials and methods

Microorganism

No study was found in literature which would show that microorganisms degrade butyl acrylate effectively. The aim of this stage of the study was the selection of a microorganism for which the degraded compound would be the growth substrate. The tests were conducted on two strains of microorganisms isolated from the soil samples collected on the site of a Chemical Company Dwory S.A. in Oświęcim, Poland and two laboratory strains. The environmental strains, as a result of biochemical tests, were identified as belonging to *Pseudomonas putida* species. The following laboratory strains underwent tests: *Pseudomonas fluorescens* PCM 2123 from the collection of Institute of Immunology and Experimental Therapy in Wroclaw, Poland and a strain from the *Pseudomonas* genus

(showed 97% homology with *Pseudomonas putida* and 97% with *Pseudomonas stutzeri* from 16S r DNA analysis), coming from VTT Culture Collection (Finland). The research procedure whose aim was to appoint the strain which uses the degraded substrate most effectively was presented in the earlier study [9]. Among the tested microorganisms the *Pseudomonas fluorescens* PCM 2123 strain used butyl acrylate as the only source of carbon and energy most effectively and was appointed for further tests. The activity of the chosen strain was tested at changing in a vast range of pH values, temperature and the concentration of butyl acrylate in the solution.

They made it possible to determine the most favourable for the growth of microorganisms conditions (pH = 7, T = 303 K) and the toxic dose of the utilized pollution. Testing toxicity confirmed that the utilised compound may be degraded by *Pseudomonas fluorescens* PCM 2123 strain even in very big doses (up to 10 000 ppm).

Preparation of inoculum

Pure strain of *Pseudomonas fluorescens* PCM 2123 bacteria chosen to biodegrade butyl acrylate, stored in a lyophilized form, was activated during a 24-hour culture on the nutrient agar foundation (King B) - on Petri dishes. Next the bacteria which grew on the dishes are carried to test tubes (Falcon type) containing 5 cm^3 of LB medium. After 24 hours the suspension was carried to hermetic flasks filled with 10 cm^3 of mineral salts solution. Butyl acrylate (3 mm^3), an organic compound introduced into the solution, was the source of carbon and energy for the culture. The composition of the mineral medium used for cultivation was (g/dm^3 distilled water): 0.067 $CaCl_2x2H_2O$, 0.2 EDTA-Na, 0.58 $MgSO_4x7H_2O$, 1 $(NH_4)_2SO_4$, 3.4 KH_2PO_4, 4.5 $Na_2HPO_4x12H_2O$ and 1 ml trace elements and vitamin solution. After another 24 h, the suspension was carried to a hermetic bottle replenished with mineral medium to 100 cm^3, and 30 mm^3 of butyl acrylate was added. In subsequent days, the absorbance was measured, and 52 mm^3 of substrate was added every day. Cultures were renovated every week. The adaptation of PCM 2123 to butyl acrylate lasted ~5 weeks.

Batch experiments

Experiments were conducted in Biostat B fermenter (Sartorius, USA) with working volume of 2 dm^3. The tests were conducted at 303.2 K, pH = 7, and stirred speed 300 rpm. A constant value of pH was maintained by feeding a 10% solution of KOH or KH_2PO_4. The following procedure was performed before every experiment. The sterile biostat was filled with mineral medium and centrifuged and washed out with deionized water cells. Butyl acrylate grown bacteria were inoculated into the biostat in the amount which enables the start of each culture at a similar concentration of cells in the solution (0.2 absorbance at λ = 550 nm). After the process parameters had been stabilized, a proper doze of liquid butyl acrylate (S_{L0}), as the only source of carbon and energy for bacteria, was introduced into the solution. During the experiments, at regular intervals (15 min), biomass, growth substrate and intermediate products (acrylic acid and butanol) concentrations were determined.

The experiments were performed for the initial concentration of butyl acrylate changed within the range of 5-50 mg/dm^3 by 5 mg/dm^3.

Analytical methods

The concentration of biomass was determined by measuring the optical density (OD) of the fluid culture (λ = 550 nm). Next the suspension absorbance was converted into grams of dry mass of microorganisms according to the calibration which was performed earlier.

Butyl acrylate and intermediate products concentrations were determined by gas chromatography using Varian 3800 (USA) chromatograph with a 30-m length CP-wax 52CB column and a flame ionization detector (FID). Helium was used as the carrier gas.

Every experimental point was repeated three times in identical conditions, and the values were averaged to get the true experimental value.

Results

Figure 1 shows, as an example, the course of changes in concentration of the analyzed components during one individual experiment.

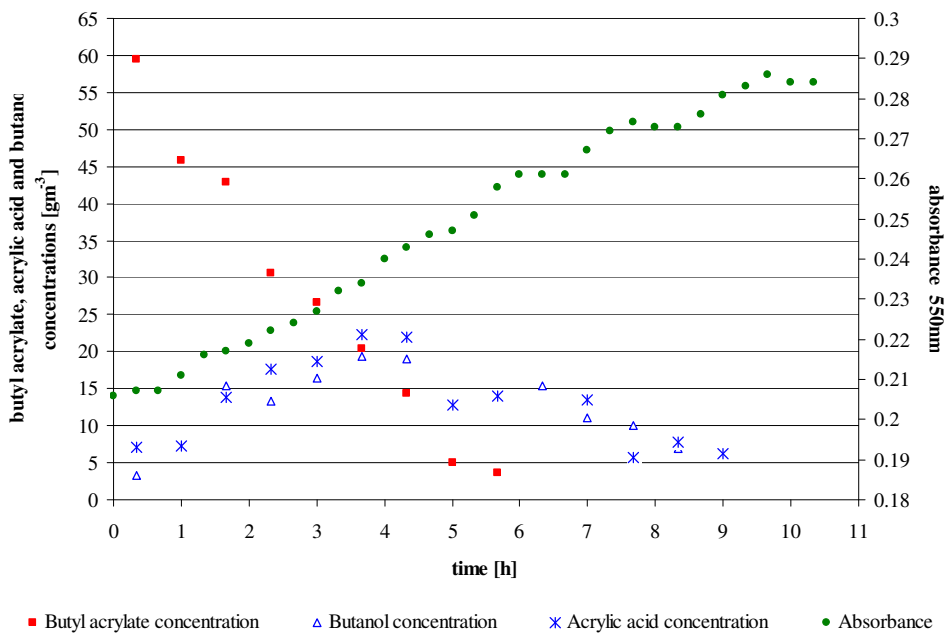

Fig. 1. Variation of butyl acrylate, acrylic acid and butanol concentrations as well as biomass for aerobic biodegradation of butyl acrylate in a batch laboratory reactor (for S_0 = 50 gm^{-3})

For the exponential growth phase, where the specific growth rate of biomass remains constant, the growth rate of biomass is first order and can be defined as:

$$\frac{dX}{dt} = \mu_{net} X \tag{1}$$

Integration of Equation (1) with boundary conditions $X = X_0$ at $t = 0$ yields:

$$\ln X = \mu_{net} \cdot t + \ln X_0 \tag{2}$$

For every experimental point, that is, for every initial dose of substrate, the graph representing the dependence $\ln X = f(t)$ is expected to take the form of a straight line whose slope is μ_{net}.

A data base making it possible to draw up a diagram of the $\mu_{net} = f(S_0)$ dependence and the choice of the form and estimation of kinetic equation constants can be obtained by changing, in the following experiments, the initial concentration of the growth substrate. The diagram of the $\mu_{net} = f(S_0)$ dependence shown in Figure 2 indicates the inhibition of biomass growth by increasing concentration of the substrate. Thus, the Haldane model was used to describe the growth kinetics of *Pseudomonas fluorescens* PCM 2123 strain on butyl acrylate and kinetic equation parameters were estimated using last-square error method with the help of NLREG - nonlinear regression analysis program - yielding the dependence:

$$\mu = \frac{0{,}1515 \cdot S}{8{,}5591 + S + \dfrac{S^2}{16{,}2144}} \tag{3}$$

Equation (3) with the mean percentage error not exceeding 5% approximates the experimental data.

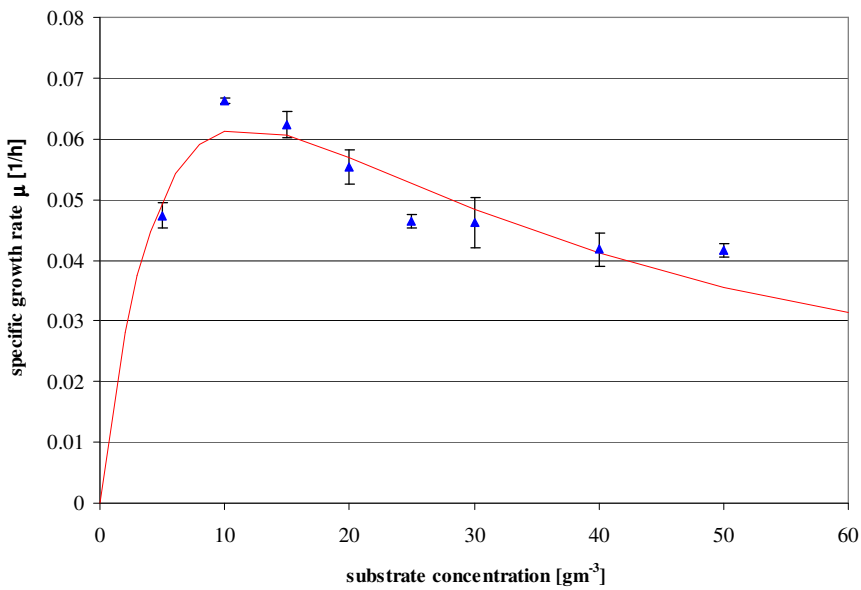

Fig. 2. Effect of initial butyl acrylate concentration on specific growth rate

Conclusions

A bacteria strain using effectively the utilized pollution as a growth substrate was selected. Optimal for the selected strain growth conditions and its kinetics were determined.

The Haldane model, in the form presented in equation (3), was used to describe the dependence of the specific growth rate of *Pseudomonas fluorescens* PCM 2123 strain on the initial concentrations of butyl acrylate. Research into the process of purifying air polluted with butyl acrylate in biotrickling filter using *Pseudomonas fluorescens* PCM2123 will be the next step. Kinetic data determined in the presented work will be used in the mathematical model of the reactor.

Symbols

μ_{net} - specific growth rate [h^{-1}]
S_0 - initial substrate concentration [g m^{-3}]
t - time [h]
X - biomass concentration [g m^{-3}]

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KINETYKA BIODEGRADACJI AKRYLANU BUTYLU PRZEZ WYSELEKCJONOWANY SZCZEP MIKROORGANIZMÓW

Instytut Inżynierii Chemicznej, Polska Akademia Nauk, Gliwice

Abstrakt: Celem prezentowanej pracy było wyselekcjonowanie szczepu bakterii, który efektywnie mineralizuje akrylan butylu, oraz określenie kinetyki tego procesu. Testy porównawcze przeprowadzono dla czterech gram-ujemnych szczepów bakterii. Szczep EC3_2001 był wyizolowany z próbek gleby, pobranych w Oświęcimiu z terenu firmy chemicznej Synthos S.A., szczep *Pseudomonas fluorescens* PCM 2123 pochodził z Polskiej Kolekcji Mikroorganizmów (Wrocław), natomiast szczepy *Pseudomonas putida* mt-2 i szczep E-93486 zakupiono w Finlandii z Kolekcji VTT. Wszystkie testowane szczepy posiadały zdolność do wykorzystania akrylanu butylu jako jedynego źródła węgla i energii w procesie aerobowym. Głównym kryterium selekcji mikroorganizmów była szybkość produkcji biomasy, a tym samym konwersji substratu. Badania prowadzono w kolbach, zawierających 300 cm^3 roztworu soli mineralnych i zaadaptowany do biodegradacji akrylanu butylu szczep bakterii. Do kolb co 24 godziny dodawano określone dawki substratu. Kolby były termostatowane (30°C) i mieszane (130 rpm). Szczep *Pseudomonas fluorescens* PCM 2123 najefektywniej degradował akrylan butylu, zatem dla tego szczepu wyznaczono kinetykę biodegradacji. Badania kinetyczne prowadzono w bioreaktorze Biostat B firmy Sartorius, w stałych i optymalnych dla wybranych bakterii warunkach (temperatura, pH, natlenienie, skład pożywki). Podczas pojedynczego eksperymentu wyznaczano krzywe wzrostu mikroorganizmów oraz konsumpcji substratu. Zgromadzona baza danych eksperymentalnych umożliwiła wybór postaci równania kinetycznego (model Haldana) oraz estymację parametrów tego równania.

Słowa kluczowe: akrylan butylu, biodegradacja, hodowla okresowa