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KINETICS STUDIES OF THE BIODEGRADATION OF VOLATILE ORGANIC COMPOUNDS IN A BATCH REACTOR

BADANIA KINETYKI REAKCJI BIODEGRADACJI LOTNYCH ZWIĄZKÓW ORGANICZNYCH W REAKTORZE OKRESOWYM

Abstract: The aim of this work is to present Volatile Organic Compounds degradation by microorganisms. Vinyl acetate was utilized by laboratory strain *Pseudomonas fluorescens* PCM 2123 or environmental strain EC3_2001, identified as *Pseudomonas putida*. Styrene was decomposed by bacteria from genus *Pseudomonas*, described as E-93486. The experiments were conducted for different initial concentration of vital substrate in a batch reactor. During research influence of organic compound concentration on microorganisms' growth was studied.

Keywords: biodegradation, batch culture, growth kinetic

The consequences of civilization progress are changes in our environment, for example the composition of air. Our atmosphere contains gases, solid particles and liquids which can be harmful both to people and living organisms. Natural air pollutants come from forest fires and eruption of volcanoes. Pollutants whose source is human activity are more dangerous. A large group of air pollutants are volatile organic compounds (VOCs). They are any organic chemical compounds having vapor pressures (under normal conditions) more than 0.07 kPa and an initial boiling point less than or equal to 260°C. Harmfulness of emission of these compounds to the atmosphere derives not only from their toxicity for living organisms but also from the fact, that they participate in photochemical reactions. Products of these reactions (ozone, hydrogen peroxide, peroxyacetylnitrate (PAN)) cause photochemical smog to form and they have influence on human health, plants and climate.

There are several different ways to reduce the emission of harmful organic compounds. In recent years many common materials and products used indoors (paints, coatings, cleaning solvents, wood preservatives) have been improved and they have low VOCs content. Also some new methods of removal pollutants from exhaust gases have been developed. Some of these base on the possibility of using microorganisms to degrade VOCs. Biological methods are effective, efficient and they do not generate secondary pollutants. Biodegradation is especially positive when gas stream is large, unstable in time and the concentration of volatile organic compounds is not high.

Vinyl acetate and styrene belong to the group of VOCs and they are on the list of hazardous air pollutants. Vinyl acetate is commonly used in industry to produce polyvinyl acetate and other polymers or resins. It is intermediately used in paints, coatings, textiles and acrylic fibers. Breathing its vapor causes symptoms of intoxication.

Styrene is an important monomer which is used for the production of polystyrene or rubber (SBR, SAN). Irritation of the upper respiratory system, reduced time response, weakness, headache, nervousness are observed in persons exposed to styrene.

Both styrene and vinyl acetate can be the source of energy and carbon for the microorganisms and they can be utilized for carbon dioxide and water [1, 2].

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Experiments

A bioreactor Biostat B was used for the batch processes. The reactor was filled with a mineral salts medium, which contained (per 1 dm^3) EDTA-Na (Titriplex) 0.2 g; $MgSO_4 \cdot 7H_2O$ 0.58 g; $CaCl_2 \cdot 2H_2O$ 0.067 g; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0002 g; $FeSO_4 \cdot 7H_2O$ 0.002 g; $(NH_4)_2SO4$ 1 g; KH_2PO_4 3.4 g; $Na_2HPO_4 \cdot 12H_2O$ 4.5 g. Then, the suspension of biomass was added in the amount ensuring a constants value of cell mass concentration. After inoculation a certain volume of vinyl acetate or styrene was added to the medium directly. The equipment of the bioreactor enables to control and to keep fixed process parameters (pH, oxygen saturation, stirring, temperature). Experiments were conducted at the temperature 30°C, agitation 300 rpm, oxidation 5 $mg \cdot dm^{-3}$ and pH = 7. The pH was maintained by adding either KOH or KH_2PO_4. All experiments were carried out without aeration so, to overcome the oxygen limitation problem, H_2O_2 was used as an additional oxygen source in water.

Liquid samples were periodically withdrawn from the reactor. The experiments were conducted for different initial concentrations of vital substrate in the batch reactor.

Analytical methods

Cell concentrations in samples were estimated by measuring the absorbance A at λ = 550 nm. The A values were converted to dry cell mass using a calibration curve.

The changes in the substrate and by-products concentration were determined by gas chromatography. Samples were taken from the cultures and directly analyzed by injection of 0.15 mm^3 samples on a Varian 3800 gas chromatograph, equipped with a 30 m length, CP-wax 52 CB column and a flame ionization detector FID. Helium was used as the carrier gas.

Microorganisms

In the research on the vinyl acetate biodegradation, laboratory strain *Pseudomonas fluorescens* PCM 2123 coming from the Polish Collection of Microorganisms (IITD Wroclaw) or bacteria isolated from samples of soil, described as EC3_2001 and identified as *Pseudomonas putida* were used. To isolate bacterial strain able to degrade vinyl acetate the classical enrichment techniques were used [3]. Strains were kept on agar slopes at 4°C. Procedure of activation and adaptation to use organic compound present in medium, was the same for both strains. It is described in detail in [4].

For the microbiological decomposition of styrene bacteria from genus *Pseudomonas*, coming from VTT Culture Collection (Finland) and described as E-93486, were chosen.

Results and discussion

The first part of the present work was focused on vinyl acetate biodegradation. We have attempted to compare the efficiency of microbiological breakdown of vinyl acetate in aerobic conditions by laboratory strain and by environmental strain. Multiplication of adapted bacteria was carried out on 500 cm^3 Erlenmeyer's flasks in standard conditions (temp. 30ºC, shaking 130 rpm, vinyl acetate concentration 400 ppm).

During experiments the influence of organic compound concentration on the growth of microorganisms was studied. Cultures in the batch reactor included the same number of cells in medium but the initial substrate concentration was changed.

In the research on vinyl acetate biodegradation the initial concentration of this compound was changed from 30 to 185 ppm. The smallest dose of substrate was used on maintenance and no biomass growth was observed. Increasing doses of substrate allowed to observe differences in biomass growth. Regular growth was obtained by introducing vinyl acetate to *Pseudomonas fluorescens* culture.

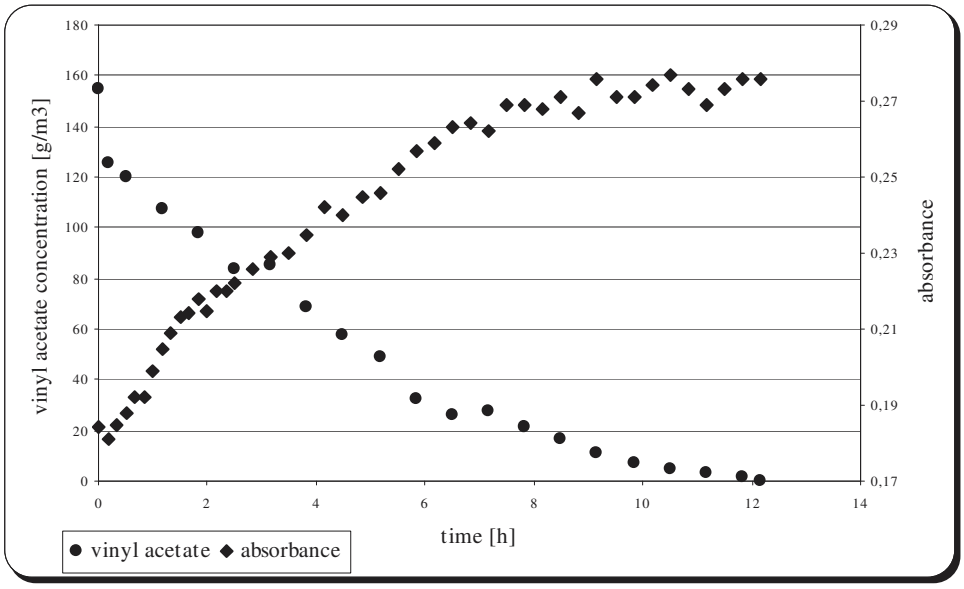

Fig. 1. Changes of the vinyl acetate concentration and absorbance during the aerobic biodegradation by *Pseudomonas fluorescens* in a batch bioreactor for initial substrate concentration 155 ppm

Rys. 1. Zmiany stężenia octanu winylu i absorbancji w czasie tlenowej biodegradacji przez *Pseudomonas fluorescens* w reaktorze okresowym dla początkowego stężenia substratu 155 ppm

In EC3_2001 cultures initial intensive growth of bacteria was stopped after a short time. It is an effect of metabolites appearing in cultures with initial substrate concentration higher than 50 ppm. Hydrolyses of ester bond caused vinyl acetate to decompose into acetic acid and vinyl alcohol. Vinyl alcohol is unstable and, under normal conditions, tautomerizes to acetaldehyde. This compound can be oxidized to acetic acid or reduced to ethanol. In samples from *Pseudomonas fluorescens* cultures acetaldehyde and ethanol were not present. Acetic acid appeared in cultures with initial substrate concentration equal or higher than 124 ppm. The formed acetaldehyde is immediately oxidized to acetic acid.

In the first stage EC3_2001 bacteria only hydrolyzes vinyl acetate and acetaldehyde concentration increases. Now, all energy is used to transform toxin to acetic acid and excess is transformed to ethanol. When concentration of acetaldehyde decreases, alcohol is oxidized to acetaldehyde and farther to acetic acid. These stages of vinyl acetate degradation reflect biomass growth. Initial, intensive increase of the number of cells is

inhibited by the appearance toxicological acetaldehyde. When toxin concentration decreases, growth is more intensive but not as fast as earlier.

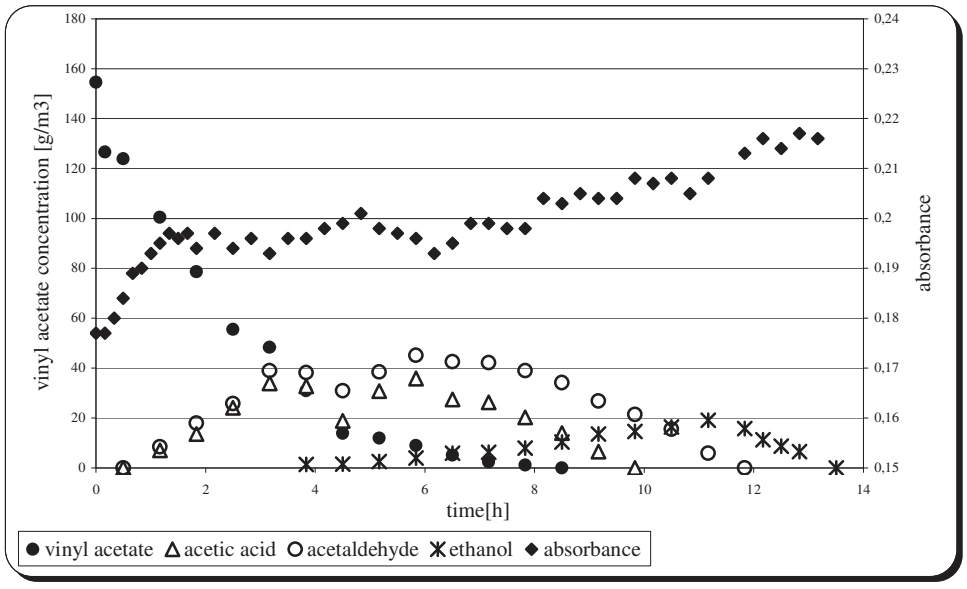

Fig. 2. Changes of the vinyl acetate concentration and absorbance during the aerobic biodegradation by EC3_2001 strain in a batch bioreactor for initial substrate concentration 155 ppm

Rys. 2. Zmiany stężenia octanu winylu i absorbancji w czasie tlenowej biodegradacji przez szczep EC3_2001 w reaktorze okresowym dla początkowego stężenia substratu 155 ppm

Taking into consideration the above *Pseudomonas fluorescens* strain was chosen to farther research on air purification in trickle bed reactor.

Next, styrene biodegradation was studied in the batch reactor. Trials of strain *Pseudomonas fluorescens* adaptation to use this pollutant as energy and carbon source resulted in failure. The adaptation of environmental strain EC3_2001 was not successful, either although this strain was isolated from soil samples collected in the area of chemical company.

In connection with above results, we decided to buy E-93486 strain, coming from VTT Culture Collection (Finland). Activation and adaptation of this strain was conducted according to the instructions from VTT Culture Collection.

Biodegradation of styrene by E-93486 strain was studied in the batch reactor. Initial substrate concentration was changed from 5 to 90 ppm. Introducing styrene to E-93486 strain culture obtained regular growth. A clear lag phase was observed in culture with initial substrate concentration higher than 15 ppm. The metabolism of microorganisms from genus *Pseudomonas,* which are capable of styrene biodegradation, proceeds through styrene oxide. Other metabolites are phenylacetaldehyde and phenylacetate. Only styrene appeared in liquid samples collected from reactor whereas metabolites were not present. Inhibitory influence wasn't noted in the given range of initial styrene concentration.

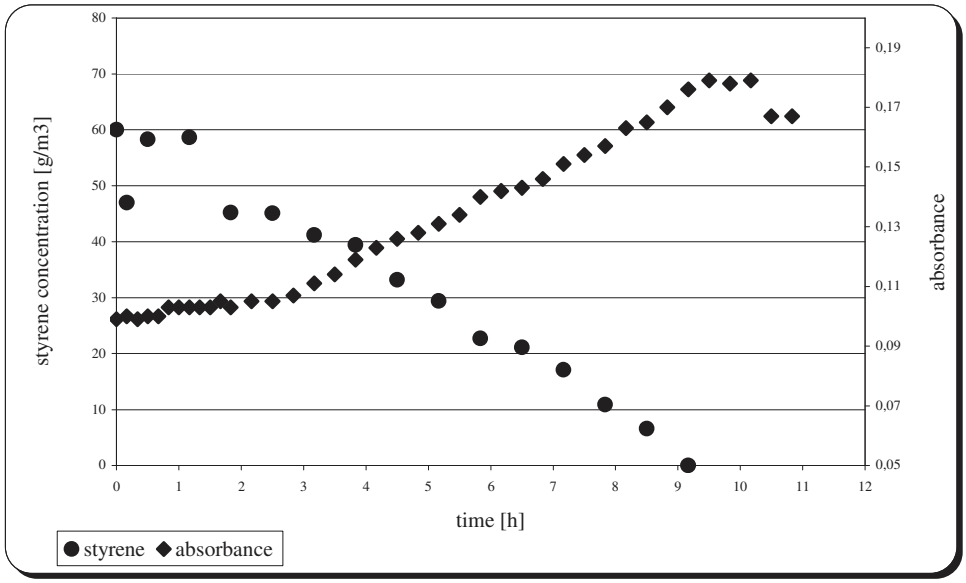

Fig. 3. Changes of the styrene concentration and absorbance during the aerobic biodegradation by E-93486 strain in a batch bioreactor for initial substrate concentration 60 ppm

Rys. 3. Zmiany stężenia styrenu i absorbancji w czasie tlenowej biodegradacji przez szczep E-93486 w reaktorze okresowym dla początkowego stężenia substratu 60 ppm

Conclusions

Vinyl acetate and styrene could be degrade by selected microorganisms.

Growth in the presence of vinyl acetate is not only limited by the initial concentration of substrate, but also by presence of intermediates. Especially acetaldehyde has toxic influence on living organisms.

The adapted bacteria, able to grow in the presence of vinyl acetate, were not able to grow in the presence of styrene as vital substrate. Strain E-93486 degrades styrene well in concentration below 100 ppm.

Acknowlegement

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BADANIA KINETYKI REAKCJI BIODEGRADACJI LOTNYCH ZWIĄZKÓW ORGANICZNYCH W REAKTORZE OKRESOWYM

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Abstrakt: Przedstawiono wyniki badań procesu degradacji lotnych związków organicznych przez mikroorganizmy. Badania dotyczyły rozkładu octanu winylu przez dwa różne szczepy: laboratoryjny *Pseudomonas fluorescens* oraz EC3_2001, zidentyfikowany jako *Pseudomonas putida*. Druga część badań dotyczyła degradacji styrenu przez szczep E-93486. Omówiono wyniki pomiarów prowadzonych w reaktorze okresowym, w którym utrzymywano stałą temperaturę 30°C, pH = 7, prędkość mieszania 300 rpm oraz natlenienie podłoża na poziomie 5 $mg \cdot dm^{-3}$. W czasie trwania hodowli okresowo pobierano próbki płynu hodowlanego w celu określenia aktualnego stężenia substratu i ewentualnie produktów pośrednich (za pomocą chromatografu gazowego Varian 3800) oraz stężenia biomasy (poprzez pomiar absorbancji). Badania przeprowadzono w reaktorze okresowym dla szerokiego zakresu zmian początkowego stężenia substratu życiowego. Zgromadzona baza danych eksperymentalnych umożliwi wyestymowanie stałych równania opisującego szybkość wzrostu mikroorganizmów oraz współczynników wydajności biomasy dla obu analizowanych substratów wzrostowych.

Słowa kluczowe: biodegradacja, VOCs, hodowla okresowa