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Efficiency of alcohols biodegradation in a membrane bioreactor

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ABSTRACT

Although alcohols are relatively easy biodegraded and many strains are capable of their degradation, the speed of these processes is not high. To achieve deep wastewater treatment required by standards, residence time in the classical flow reactors would have to be dozens or even hundreds of days. Therefore it is necessary to intensify the biodegradation by increasing biomass concentration which occurs in a membrane bioreactor. The efficiency of the process carried out in a membrane bioreactor is shown on the example of biodegradation of 1-butanol, 1-propanol, 2-propanol by the strain of *Pseudomonas fluorescens*. It was shown that the biomass concentration higher 5–6 times allows for shortening residence time up to 10–14 days. Further compaction is unnecessary and often poses unwanted problems.

Keywords: Biodegradation; Membrane bioreactor; Wastewater treatment; Alcohols; *Pseudomonas fluorescens*; Mathematical model verification; VOC

1. Introduction

Due to unfavorable effect of VOCs on the environmental, their emission and removal is a subject of legal grounds and research interest. To the classic methods for removal of VOCs from outlet gases belong first of all sorption methods [1–4] which are used to transfer a mass of an organic compound from the gas to solid or liquid phase. This procedure serves increasing VOCs concentration on/into sorbent. A problem is still its further processing, possibly in such a way that the undesirable compound does not go beyond the boundaries of the installation. When only one dominating component is released during desorption it is most reasonable to use it again. In an opposite case either catalytic burning or chemical or biological degradation can be applied [5–7].

Among the compounds belonging to the VOCs, alcohols are regarded as compounds of moderate toxicity [8] but there are very common in environment. Alcohols are emitted to the atmosphere from two sources: natural (fumes from some plant species, volcano eruptions, forest fires) [9] and anthropogenic (emissions from diesel engines, crude oil extraction and refining, wastewater from cosmetic, chemical, tobacco and other industries) [10–13]. In many countries, the production of alcohols exceeds the total production of all other groups of synthetic organic compounds. Within a year, in the US alone produces over 800 million lb of monohydric alcohols, of which the dominant group comprises 1-butanol [14].

Despite a significant emission of alcohols and a simple metabolic path of their decomposition by bacteria [15], the number of studies dedicated to alcohol biodegradation is not big. The studies concern biodegradation of 1-, 2-propanol [16–18] and 1-butanol [19–22] were carried out usually using one bacterial strain, rarely this was a group of microorganisms. The specific growth rates

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were very low and the substrate inhibition was observed starting from the concentration 5–6 g l^{-1} or less. Relative, high values of the specific growth rate (0.2–0.27 h^{-1}) were obtained for the solvent-tolerant *Enterobacter* sp. [21].

Because of small rate of the biodegradation process, it became necessary to intensify these processes for example by applying a membrane which allow concentration of microbial cells in the bioreactor zone which is known as membrane bioreactor. This allows great shortening of the residence time in the reactor preserving a satisfactory level of the substrate conversion [23,24].

Determination of fundamental kinetic constants of microbial transformation and biomass yield coefficient allows (based on the previously presented model of a membrane bioreactor [25]) for the selection of a membrane bioreactor operating parameters without unnecessary overstatement their value.

These procedures and membrane bioreactor efficiency were analyzed based on the process of alcohols biodegradation by the strain of *Pseudomonas fluorescens*.

2. Materials and methods

The strain of *Pseudomonas fluorescens* PCM 2123 from the collection of IITD Polish Academy of Sciences in Wroclaw (Poland) was used in the experiments. A culture medium was a broth consisting of 1 KNO_3, 1 KH_2PO_4, 1 K_2HPO_4, 1 NaCl, 0.02 CaCl, 0.2 $MgSO_4$ and 0.001 $FeCl_3$ (g l^{-1}) at pH 6.5. The strain was adapted to biodegradation by increasing the concentration of carbon sources (1-butanol, 1-propanol, 2-propanol). All reagents used in experiments and analysis were of high purity and were purchased from POCh (Poland) or Sigma (USA).

During batch processes cultures were grown at 30°C in shaken flasks (200 rpm). Due to the volatility of compounds tested, a typical aeration of culture was not possible. The flasks were tightly closed with ground-glass stoppers and H_2O_2 constituting the oxygen source was added to the system. Its optimal concentration was chosen on the basis of experiments carried out in a batch process in the range of H_2O_2 0.01–0.05% v/v.

The concentration of bacterial cells in the culture medium was monitored with a spectrophotometer PharmaSpec UV-1700 (Shimadzu, Japan) at the wavelength 550 nm. The equation derived on the basis of dry mass method was used in calculations:

$$\text{Abs}\,(550) = 2.13 \cdot X$$

where X is biomass concentration, g l^{-1}.

Alcohol concentration was determined by a GC-2014 gas chromatograph (Shimadzu, Japan) with a ZB-WAXplus capillary column (Zebron) 30 m long and 0.25 mm in diameter, covered with a 0.25 μm layer of polyethylene glycol. Samples were analyzed after centrifugation of cells (3000 rpm, 15 min) in isothermal conditions on the column (temperature of the column 55°C, feeder 180°C and detector 200°C).

A continuous process was carried out in the reactor (New Brunswick BioFlo, USA) — Fig. 1. The same bioreactor was used to build the system with a membrane module. The module (IBIB, Warsaw, Poland) containing polysulphone capillary membrane (d_{pore} = 0.05 mm, A = 0.0126 m^2) was used in the experiments.

The scheme of the membrane bioreactor is shown in Fig. 2. The bioreactor (1) (thermostatted stirred tank) is supplied with a stream of substrate (Q_1) by means of a dosing pump (P_1). The stream of the reaction mixture is directed to the external membrane module (2) by means of a circulating pump (P_2). To obtain proper transmembrane pressure, a pressure-reducing valve (3) is mounted. The retentate stream is returned to the reactor. Before the membrane module, branching is mounted and the required stream of the reactor mixture (Q_3) is removed from the bioreactor by a peristaltic pump (P_3). Under steady-state conditions the stream Q_1 has to be equal the sum of Q_3 and Q_6.

During the processes running in the membrane bioreactor, stability of the permeation flux (Q_6) and the concentrations in the bioreactor of cell and substrate were monitored. Measurements were carried out until steady state was obtained (usually it corresponded to the three exchanges of the reactor volume).

3. Results

3.1. Growth of Pseudomonas fluorescens cells in the presence of H_2O_2 as the only source of oxygen

In the case of biodegradation of volatile organic compounds, the classical aeration i.e. the flow of air through a bioreactor, causes significant losses in the mass of degraded substance because of its release to the atmosphere. One of the solutions to this problem is to supply bound oxygen, e.g. in the form of H_2O_2 to the culture. Because H_2O_2 decomposes to oxygen and water and is well soluble

Fig. 1. Bioreactor BioFlo (New Brunswick) used in the experiments.

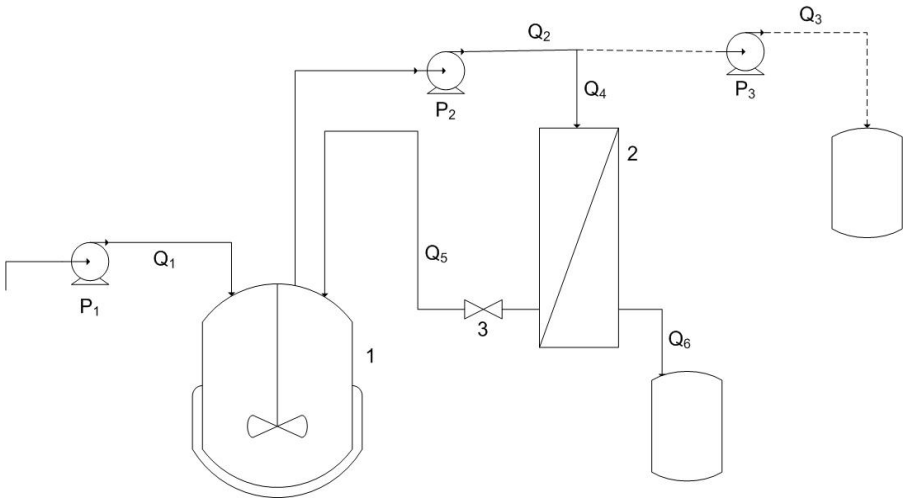

Fig. 2. Scheme of the membrane bioreactor — (1) bioreactor, (2) membrane module, (3) pressure-reducing valve, P_1, P_2, P_3 — pumps.

in water, ostensibly unlimited number of molecules of oxygen is available for biodegradation. A limitation is the use of H_2O_2 only for the culture of microorganisms equipped with appropriate enzymatic apparatus [26]. Among the microorganisms capable of using this alternative oxygen source is the strain of *Pseudomonas fluorescens* [27].

In batch and continuous processes an optimum (non-limiting and non-inhibiting) concentration of H_2O_2 for the *P. fluorescens* culture was determined. Fig. 3 shows the results of the experiments carried out in the batch culture at initial H_2O_2 concentration ranging from 0.01 to 0.05% v/v, and for initial 1-butanol concentration equal to 2.2 g l^{-1}. The best growth was observed at the concentration of H_2O_2 equal to 0.02% v/v. A similar result was

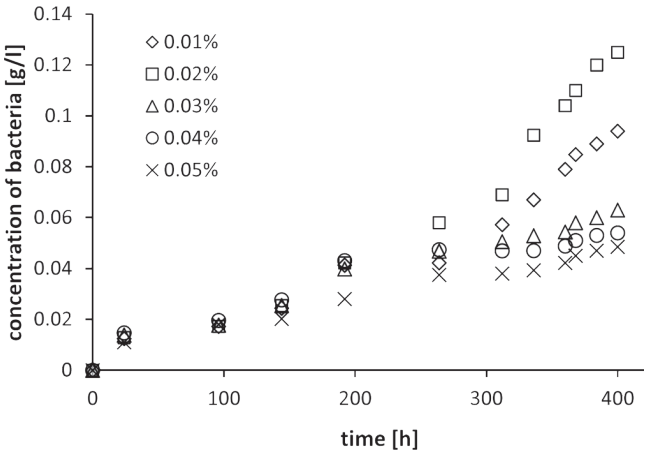

Fig. 3. Influence of concentration of H_2O_2 on *P. fluorescens* growth ($c_{0\ (1\text{-butanol})}$ = 2.2 g l^{-1}).

obtained for other initial concentrations of 1-butanol ranging from 0.13 to 3.2 g l^{-1}. In the case of a continuous process the relation was determined in respect to H_2O_2 concentration ranging from 0.015 to 0.05% v/v in the dosing tank. The most intensive growth of *P. fluorescens* strain was observed while dosing 0.03–0.04% v/v H_2O_2. In further studies, the H_2O_2 concentration equal to 0.02% and 0.04% v/v was applied, respectively, in the batch and continuous system (in a dosing tank).

3.2. *Kinetics of* Pseudomonas fluorescens *growth on monohydric alcohols*

Preliminary experiments were performed in a batch system, using single substrate (1-butanol, 1-propanol or 2-propanol). The initial substrate concentration was 0.12–4.6 g l^{-1}. During the process the concentrations of biomass and substrate were monitored. Fig. 4 illustrates a sample of the substrate and cell concentration changes in the experiment carried out at the initial 1-butanol concentration of 0.84 g l^{-1}.

Substrate inhibition was observed for all substrates tested which in the case of 2-propanol occurred already at 2.8 g l^{-1}, while for 1-butanol and 1-propanol it appeared at the concentration above 3.2 g l^{-1}.

Detailed kinetic studies were carried out in a continuous BioFlo reactor. The substrate concentration in the dosing stream was from 4 to 10 g l^{-1}. The residence time in the reactor in subsequent experiments was from 38.5 to 480 h.

According to the theory stating that for the kinetics with inhibition a steady state occurs on ascending arm only [28,29], points on the curve which could be described by the Monod equation [Eq. (1)] (without inhibition) were obtained. However, this equation applies only to a limited

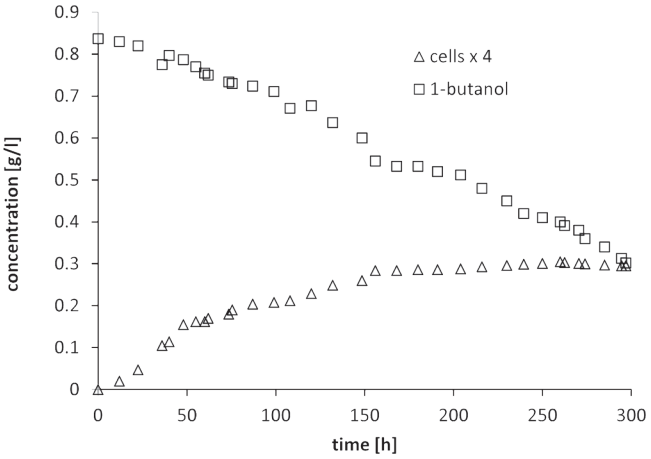

Fig. 4. Run of the batch process at $c_{0(1\text{-butanol})}$ = 0.84 g l^{-1}.

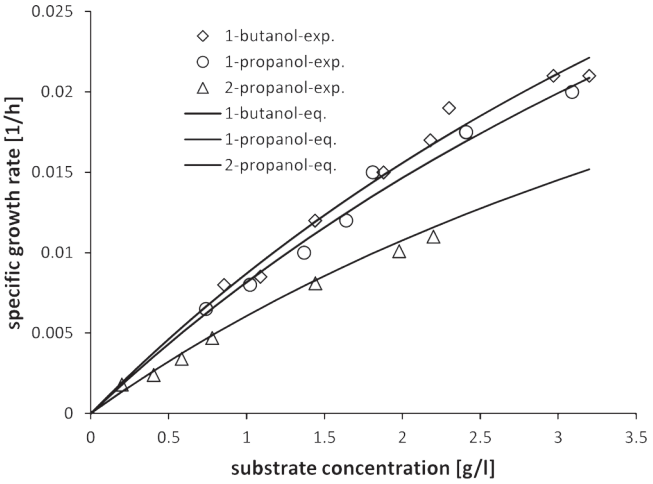

Fig. 5. Kinetics of 1-butanol, 1-propanol and 2-propanol biodegradation (points — experimental data from the continuous processes, line — the curve drawn on the basis of the kinetic constants).

range of concentrations in the bioreactor. At concentration above the limit steady state is not obtained.

$$\mu = \frac{\mu_{max} \cdot c_S}{K_S + c_S} \quad (1)$$

where μ — specific growth rate [h^{-1}], μ_{max} — maximum growth rate [h^{-1}], c_S — substrate concentration [g l^{-1}], K_S — Monod constant [g l^{-1}].

Biodegradation of particular compounds did not differ significantly; for 1-butanol and 1-propanol it was almost identical. In the case of all tested alcohols at the residence time shorter than 46 h, cells were eluted from the reactor. Table 1 gives constants in the Monod equation determined on the basis of stable points from continuous processes. These constants were used to draw kinetic curves, onto which experimental points were plotted (Fig. 5). The average relative error between experimental values and those resulting from the kinetic equation was 4.04% for 1-butanol, 4.97% for 1-propanol and 3.07% for 2-propanol.

In the processes with alcohol mixtures, when each alcohol was dosed at the concentration of 5 g l^{-1} and the residence time ranging from 46 to 192 h, values close to the kinetic curve for the particular substrate were obtained (Fig. 6). It looks that the process of biodegradation of each tested alcohol (1-butanol, 1-propanol, 2-propanol) is independent. The effect of substrate inhibition is not enhanced either.

Table 1
Constants in the Monod equation determined on the basis of points from continuous processes

	μ_{max} (h^{-1})	K_S (g l^{-1})
1-butanol	0.074	7.50
1-propanol	0.072	7.84
2-propanol	0.048	6.92

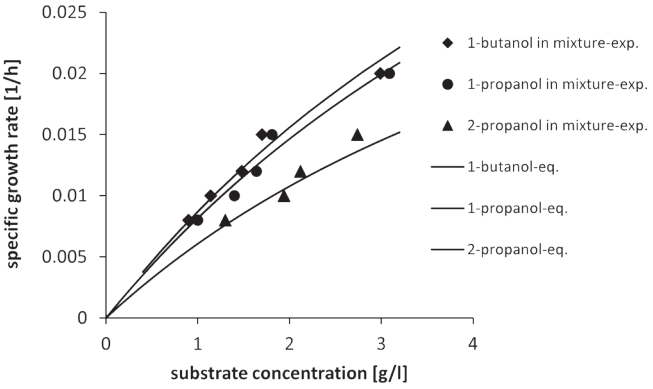

Fig. 6. Kinetics of the mixture alcohols biodegradation (points — experimental data from the continuous processes on alcohol mixtures, line — the curve drawn on the basis of the kinetic constants).

3.2. *The coefficient of biomass efficiency during biodegradation of monohydric alcohols by* Pseudomonas fluorescens

The values of coefficient of biomass efficiency ($Y_{X/S}$) were determined in continuous processes once the steady states were reached. It was determined from Eq. (2) by balancing the whole mass of cells and carbon substrate (in this case one of the tested alcohols):

$$Y_{X/S} = \frac{X_{reactor}}{c_{S,dosage} - c_{S,reactor}} \quad (2)$$

where $Y_{X/S}$ — coefficient of biomass efficiency; $c_{S,dosage}$ — concentration of *C*-substrate in the dosing stream [g l^{-1}]; $c_{S,reactor}$ — concentration of *C*-substrate in the reactor in steady state [g l^{-1}]; $X_{reactor}$ — concentration of biomass in the reactor in steady state [g l^{-1}].

It was observed that the values of coefficient $Y_{X/S}$ for all tested alcohols were similar, ranging from 0.14 to 0.16. It depended neither on substrate concentration in the reactor (in the range 0.2–3.2 g l^{-1} obtained in steady state) nor biomass concentration (0.18–1.4 g l^{-1}).

3.3. The membrane module characteristics

While selecting a membrane module, the following properties were considered:

- Size of membrane pores which has an immediate influence on the retention coefficient of biomass that should be close to unity ($R_X \approx 1$);
- Membrane surface area which has a linear influence on the obtained permeate streams that would allow us to obtain appropriate cell concentration in the system (Ψ = 3–6) [25];
- Fouling of cells on the membrane surface which would cause a decrease of the permeate stream during the process and unsteady state of the process.

After preselection, a module with polysulfone capillaries (d_{pore} = 0.05 mm, A = 0.0126 m^2), on which complete cell retention was obtained irrespective of filtration conditions, was used in the experiments. The permeate stream for distilled water was 2.2×10^{-3}–13.2×10^{-3} l h^{-1} depending on the pressure applied (0.007–0.055 MPa) – Fig. 7.

During circulation of bacteria ($V_{reactor}$ = 2.7 l, X = 0.87–1.14 g l^{-1}), the permeate stream decreased by 12.4–19.7% in relation to the stream obtained for pure water, but it was stabilized after 7–8 h already and until the end of the process (even up to several days) it remained practically unchanged (Fig. 8). The surface cell concentration 56.7–88.7 g m^{-2} estimated from mass balance in subsequent experiments corresponded to 5.7–6.8% mass of cells present in the system. It is important that similar to the permeate stream, this concentration did not change significantly (> 5%) after about 8 h. Such a result allowed us to obtain steady-state conditions without the necessity of implementing a procedure of membrane surface regeneration during the process.

3.4. Biodegradation in the membrane bioreactor

According to the directive [30], an admissible concentration of 1-butanol directed to water or soil is 14.2–96.5 mg l^{-1} (depending on an industrial plant) while for 2-propanol it is 15.3–104 mg l^{-1}.

When analyzing kinetic constants (Table 1) and kinetic curves (Fig. 5) it was estimated that in order to meet the imposed standards the residence time in the classical flow

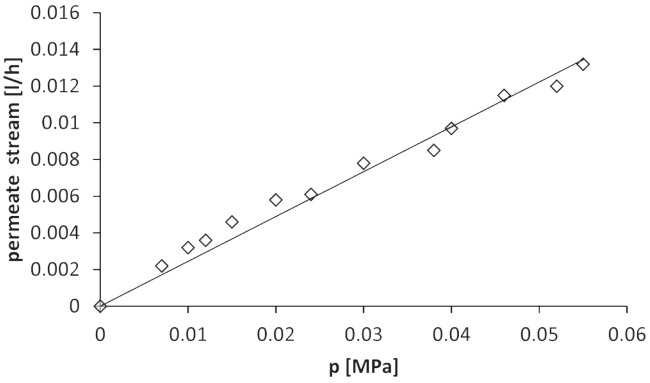

Fig. 7. Characteristic of the membrane module with polysulfone capillaries (A = 0.0126 m^2).

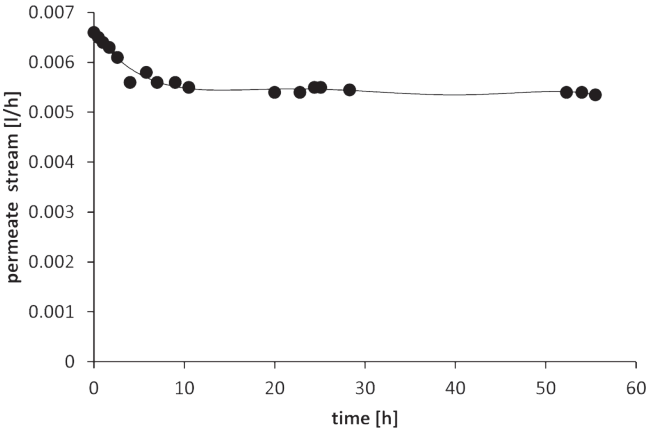

Fig. 8. Change of the permeate stream during circulation of bacteria (ΔP = 0.024 MPa, $V_{reactor}$ = 2.7 l, X = 1.14 g l^{-1}).

reactors would have to be from dozens to several hundred days. Such long biodegradation processes, despite environment-friendly, are not very attractive compared to decomposition by chemical methods. A solution to this problem is application of membrane bioreactor working at higher biomass concentration.

In the previous study [25], a mathematical model of the microbial membrane bioreactor was developed. The system consists of a membrane module integrated with a mixing tank bioreactor (Fig. 2). Stream partition coefficient (Ψ) was introduced to the model:

$$\Psi = \frac{Q_3 + Q_6}{Q_3} = \frac{Q_1}{Q_3} \tag{3}$$

A domain of this coefficient is the range [1, ∞], with the value of 1 reflecting the classical flow bioreactor. The coefficient Ψ is in close relationship with a sludge age q_X, the term used often in sewage technology:

$$\theta_X = \tau \cdot \frac{\Psi}{1+(\Psi-1)\cdot(1-R_X)} \quad (4)$$

In the case when $R_X = 1$ (which usually occurs) the above equation is simplified to the form:

$$\theta_X = \tau \cdot \Psi \quad (5)$$

where τ is residence time [h, d]; q_X — sludge age [h,d].

The higher is the value of Ψ the higher is biomass concentration in the reactor under given conditions:

$$X_2 = Y_{X/S} \cdot \Psi \cdot \left(c_{S,1} - c_{S,2}\right) \quad (6)$$

where $c_{S,1}$, $c_{S,2}$ — substrate concentration in stream Q_1 and Q_2 (Fig. 2), respectively [g l^{-1}]; X_2 — biomass concentration in stream Q_2 (and in bioreactor) [g l^{-1}].

When R_S = 0 substrate concentration in the reactor ($c_{S,2}$) equal to its concentration in the permeate stream ($c_{S,6}$) according to the model [25] is described by Eq. (7):

$$c_{S,2}(R_S = 0) = c_{S,6}(R_S = 0) = \frac{K_S}{\mu_{max} \cdot \tau \cdot \Psi - 1} \quad (7)$$

The concentration of the particular alcohol calculated from the above formula was compared with experimental data. Experiments were made for 1-butanol (Fig. 9) and 2-propanol (Fig. 10) at the concentration of alcohol in the dosing stream 5.6–9.14 g l^{-1}.

It was shown in both cases that due to multiple increase of biomass concentration (X_2 = 4.12–8.07 g l^{-1} at Ψ = 5–6) already at the residence time of 10–14 d, the values specified in the standards can be obtained.

Good agreement was obtained between the model and experimental values (the mean relative error was 8.81% for 1-butanol and 10.03% for 2-propanol), which confirmed correct determination of kinetic constants and positive verification of the model. This model can be used in designing any biodegradation process with known basic kinetic data, which is carried out with a single strain or with mixed culture, in one- or multi-component systems, when the interaction between substrates is negligible, as it was in the considered case.

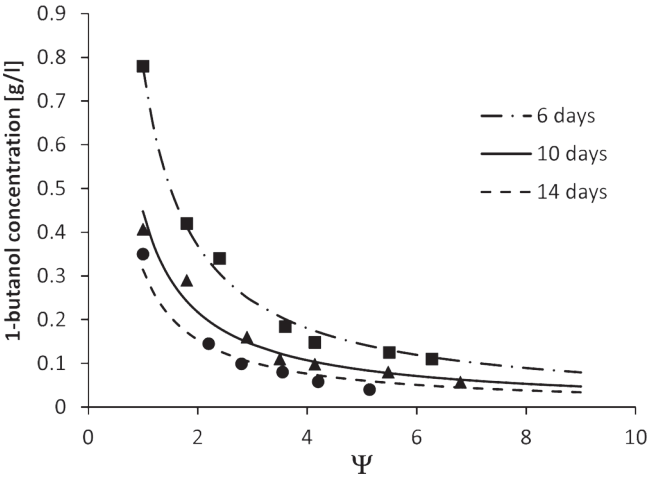

Fig. 9. Influence of Ψ on 1-butanol concentration in outlet stream. Line — the model calculations, points — experimental data.

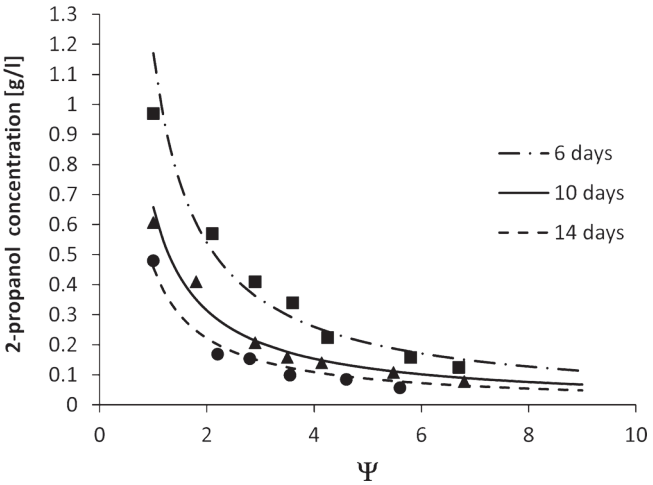

Fig. 10. Influence of Ψ on 2-propanol concentration in outlet stream. Line — the model calculations, points — experimental data.

4. Conclusion

Although alcohols do not belong to the most harmful organic pollutants, standards which refer to these compounds, like to the most substances belonged to VOCs, require deep treatment of their gaseous and liquid outlet streams. Sorption methods used frequently to gas purification are related directly to the process of sorbent regeneration. As a result a liquid stream which contains concentrated toxic substances arises.

This study describes the intensification of degrading alcohols process in a membrane bioreactor. Although alcohols are easily biodegradable, i.e. enzymes necessary for this process are not specific and there is a large group of bacteria which possess them, the rate of their decomposition is not high. Using the strain of *Pseudomonas fluorescens*, required the residence time in the reaction zone which lasted for several dozen or several hundred days. Hence, these processes must be intensified. A known solution is to use a membrane bioreactor with a microfiltration unit increasing the concentration of biomass in a reactor.

A model of such a system was presented in the literature [25] and now it has been positively verified. Knowing the basic kinetic constants, with the use of the mentioned model, one can choose such operation parameters of the

bioreactor (streams, degree of biomass concentration, residence time) which would allow us to obtain the necessary degree of wastewater treatment and to avoid accumulation of biomass over the required level. Obtaining a high value of coefficient Ψ is not a problem. However, the high concentration of biomass complicates conducting of the process. At a high value (>15 g l^{-1}) of bacteria concentration, physiology of microbial growth is changed [31,32]. In addition come the problems of disturbed flow, sedimentation of cells and a strong membrane fouling. As the study showed in the case of alcohols Ψ = 5–6 was found to be sufficient. It corresponds to a biomass concentration of about 4.12–8.07 g l^{-1} at alcohol concentration in the dosing stream of 5.6–9.14 g l^{-1}. Usually the effect of intensification is significant in the range of Ψ from 1 to 3; a further biomass concentration is required when it is necessary to meet the standards.

It is expected that if the application of a membrane bioreactor brought such tangible benefits in the biodegradation of alcohols, its use will be even more effective for compounds harder (slower) degraded. A particularly strong intensification in the membrane bioreactor could be observed for these processes which corresponded to low substrate conversion degrees in the classical bioreactor, when the area to intensification is broad.

Acknowledgments

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