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INITIATING BIODEGRADATION OF POLYVINYLPYRROLIDONE IN AN AQUEOUS AEROBIC ENVIRONMENT: TECHNICAL NOTE

ZAINICJOWANIE BIODEGRADACJI POLIWINYLOPIROLIDONU W ŚRODOWISKU WODNO-TLENOWYM: NOTATKI TECHNICZNE

Abstract: A synthetic polymer, polyvinylpyrrolidone (PVP - E 1201) primarily finds applications in the pharmaceutical and food industries due to its resistance and zero toxicity to organisms. After ingestion, the substance passes through the organism unchanged. Consequently, it enters the systems of municipal wastewater treatment plants (WWTP) without decomposing biologically during the waste treatment process, nor does it attach (through sorption) to particles of activated sludge to any significant extent, therefore, it passes through the system of a WWTP, which may cause the substance to accumulate in the natural environment. For this reason the paper investigates the potential to initiate aerobic biodegradation of PVP in the presence of activated sludge from a municipal wastewater treatment plant. The following agents were selected as the initiators of the biodegradation process - co-substrates: acrylamide, *N*-acethylphenylalanine and 1-methyl-2-pyrrolidone, a substance with a similar structure to PVP monomer. The biodegradability of PVP in the presence of co-substrates was evaluated on the basis of biological oxygen demand (BOD) as determined via a MicroOxymax $O_2/CO_2/CH_4$ respirometer. The total substrate concentration in the suspension equaled 400 mg·dm^{-3}, with the ratio between PVP and the co-substrate being 1:1, while the concentration of the dry activated sludge was 500 mg·dm^{-3}. Even though there was no occurrence of a significant increase in the biodegradation of PVP alone in the presence of a co-substrate, acrylamide appeared to be the most effective type of co-substrate. Nevertheless, a recorded decrease in the slope of biodegradation curves over time may indicate that a process of primary decomposition was underway, which involves the production of metabolites that inhibit activated sludge microorganisms. The resulting products are not identified at this stage of experimentation.

Keywords: polyvinylpyrrolidone, biodegradation, activated sludge, aqueous environment

Introduction

Polyvinylpyrrolidone (PVP) is a synthetic polymer that dissolves very well in water. From a chemical perspective, it is a polymer lactam with an internal amide bond and ranks

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amongst poly-*N*-vinylamides (Fig. 1). Soluble polyvinylpyrrolidone is known under different the names and abbreviations, for example [1]: Povidone - Pharmacopoeias (*eg* USP, Ph.Eur.); Polyvidon(e) - Pharmacopoeias (*eg* DAB); Povidonum - Pharmacopoeias (*eg* Ph.Eur.); Polyvidonum solubile - Pharmacopoeias (DAC 1986); Poly(1-vinyl-2-pyrrolidon) - Deutsches Arzneimittelgesetz 1984 §10(6); PVP general abbreviation, commercial name for cosmetics/technical grade. The diverse forms produced commercially are differentiated through the K-number denoted (*eg* PVP K12, K17, K25 and K30), indicating average molecular weight. With regard to monomer unit structure, PVP exhibits an amphoteric characteristic, due to it containing an amide group of high polarity and non-polar methylene groups in its main chain and ring, which indicates its hydrophilic and polar properties and hydrophobic properties, respectively.

An extensive body of toxicological data in animals supports the biological inertness of PVP. The acute, subchronic, and chronic toxicity of orally administered PVP is extremely low, with the only effect observed being diarrhea at high doses due to the osmotic action of PVP acting as a bulk purgative. Occasional observations of minimal absorption with storage in mesenteric lymph nodes seem to be of no toxicological importance. PVP is neither a sensitizer nor an irritant. There are no reported adverse effects following oral administration in humans. The currently permitted FAO/WHO ADI of 0-50 $mg \cdot kg^{-1}$ body weight for food uses provides an adequate margin of safety. There would appear to be no reason to restrict its oral or topical pharmaceutical use or topical cosmetic use in any way. There have been no reports of adverse effects following its use intravenously as a plasma expander, even after the administration of very large amounts. The only toxicological problems have involved the repeated injection of large amounts of the higher molecular weight material into poorly perfused sites such as subcutaneously and into the breast. If the use of PVP in injectables for repeated use is restricted to PVP with a molecular weight less than K-18 in limited amounts (*eg* 50 mg/i.m. dose) and the injection sites are varied, and intramuscular or intravenous routes are used, then these problems should not occur. The repeated use of PVP in depot preparations, which could lead to excessive storage, is not to be recommended [1].

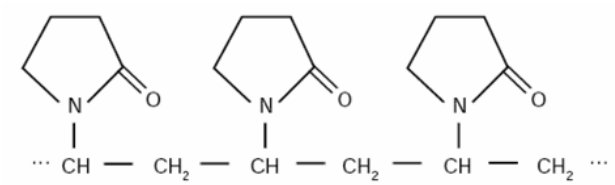

Fig. 1. The chemical structure of polyvinylpyrrolidone

Due to its physical, chemical and biological properties (bio- and hemocompatible, physiologically inactive), PVP finds applications in a range of technological processes. Due to its resistance and zero toxicity to organisms, the compound is mainly used in the pharmaceutical and food industries (in the European Union soluble PVP is labelled E 1201, within the system of E-numbers, when utilized in food supplements, pills and sweeteners). After ingestion, the substance passes through the organism unchanged [2]; consequently, it enters the systems of municipal wastewater treatment plants (WWTP) without decomposing

biologically during the waste treatment process, according to most recent studies [3-5]; nor does it attach (through sorption) to particles of activated sludge to any significant extent [6]. Therefore, it passes through the system of a WWTP, which may cause the substance to accumulate in the natural environment.

However, some studies do not completely reject its biodegradability, pointing out the importance of experimental conditions and the microbial cultures used. Abd El-Mohdy and Ghan [7], describe biodegradation of PVP in a soil environment (soil burial test). Their findings of a decrease in mass does not evidence the complete resilience of pure PVP, as regards decomposition, with such mass dropping to about 10% after 6 months. Marusincova [8] also notes some positive findings on eliminating PVP (Mr ~ 10 kDa) from a municipal WWTP by using anaerobic sludge; increased production of CH_4 was recorded for samples containing PVP after 24 days of testing compared with the endogenous production of CH_4.

As mentioned in the introduction, PVP contains a lactam ring in its structure, this being γ-lactam, a substance that may be subject to attack by γ-lactamase (an enzyme). Therefore, from both a theoretical and practical perspective, it would be of interest to use microorganisms producing the aforementioned γ-lactamase as a cornerstone for biologically decomposing PVP. Indeed, a number of microorganisms have been identified very recently, such as *Pseudomonas fluorescens* [9], *Microbacterium hydrocarbonoxydans* [10], *Sulfolobus solfataricus MT4*, *Rhodococcus sp. ENZA1*, *Aureobacterium sp. ENZA25*, *Pseudomonas solanacearum ENZA20*, *P. cepaecia* and *P. fluorescens ENZA22*, and *Comomonas acidovorans* [11]. Among other things, the work of Line et al [12] also found that some soil bacteria producing γ-lactamase are able to utilize N-acyl as a sole source of carbon and energy [12], while the study of Hickey et al [13] revealed increased activity by γ-lactamase in the presence of acrylamide substrate. This theoretically suggests that N-acethylphenylalanine or acrylamide might initiate the production of γ-lactamase in some types of microorganisms.

The literature study shows that the ultimate fate of PVP within a WWTP and subsequently in the environment has not been widely explored, so reiterating presumptions that PVP does not pose an environmental problem is not really an option. This paper investigates the potential to initiate aerobic biodegradation of PVP in the presence of mixed microbial cultures, in the form of activated sludge from a municipal wastewater treatment plant, with the following agents selected as the initiators of the biodegradation process - co-substrates: 1-methyl-2-pyrrolidone, a substance with a similar structure to PVP monomer, as well as acrylamide [13] with *N*-acethylphenylalanine [12], this based on literature research.

Materials and methods

The materials used for tests were commercial products that are currently available: polyvinylpyrrolidone K15 (Mr ~ 10 kDa), the Aldrich Chemical Company; 1-methyl-2-pyrrolidone (1M-2P), acrylamide (AC) and *N*-acethylphenylalanine (APhA), at a purity of 98%, the Aldrich Chemical Company. Others chemicals employed were of analytical purity, produced or delivered by the Lachema Co., Brno, the Czech Republic.

A mixture of inorganic salts (biomedium) was used as an aqueous environment, which was prepared according to standards valid CSN EN ISO 9408 for evaluating biological aerobic degradability of organic substances in an aqueous environment [14]. Quantities

dosed into a 1 dm^3 volumetric flask were 800 cm^3 distilled water saturated with atmospheric oxygen, and always 1 cm^3 of stock solutions of $CaCl_2$ (27.5 $g \cdot dm^{-3}$), $FeCl_3 \cdot 6H_2O$ (0.25 $g \cdot dm^{-3}$), $MgSO_4 \cdot 7H_2O$ (22.5 $g \cdot dm^{-3}$), and of solutions of trace elements (0.75 $g \cdot dm^{-3}$ H_3BO_3, 0.05 $g \cdot dm^{-3}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.18 $g \cdot dm^{-3}$ $CoSO_4 \cdot 7H_2O$, 0.5 $g \cdot dm^{-3}$ $CuSO_4 \cdot 5H_2O$, 0.1 $g \cdot dm^{-3}$ $ZnSO_4 \cdot 7H_2O$, 3 $g \cdot dm^{-3}$ $FeSO_4 \cdot 7H_2O$). Quantities further added were 20 cm^3 solution of phosphate buffer (8.2 $g \cdot dm^{-3}$ KH_2PO_4, 21.75 $g \cdot dm^{-3}$ $K_2HPO_4 \cdot 12H_2O$, 44.7 $g \cdot dm^{-3}$ $Na_2HPO_4 \cdot 12H_2O$) and 5 cm^3 $(NH_4)_2SO_4$ (10 $g \cdot dm^{-3}$) solution. All were mixed and filled to the mark with distilled water saturated with atmospheric oxygen.

A mixed microbial culture in the form of activated sludge from a municipal wastewater treatment plant (WWTP Zlin-Malenovice, the Czech Republic) was applied as the inoculum (biological material). Prior to such testing, the aerobic sludge was first centrifuged at 4,500 rpm^{-1} for 10 minutes at 20ºC (Centrifuge MR23i, Jouan, France). After removing the supernatant, the biomass was suspended in the biomedium [14] and then aerated for 24 hours.

Biodegradability was determined using a respirometer, a MicroOxymax $O_2/CO_2/CH_4$ (Columbus Instruments corp., USA). The sample tested (PVP or PVP + co-substrate) was the sole source of organic carbon and energy in the environment. The conditions of the test were as follows: suspension volume 50 cm^3, dried sludge 500 $mg \cdot dm^{-3}$, concentration of PVP 200 $mg \cdot dm^{-3}$, concentration of the co-substrate 200 $mg \cdot dm^{-3}$, temperature 25±1°C and pH = 7. At the beginning and end of the experiment, sludge solids, pH and reference DOC (*dissolved organic carbon*; automatic analyzer Shimadzu TOC 5000A, Shimadzu corp. Japan) were determined for all samples. Allylthiourea as an inhibitor of nitrifying processes was not dosed when studying the biodegradation of PVP alone, even though the substance studied contains organically bound nitrogen. This procedure was chosen with regard to the work of Marusincova [8], where aerobic biodegradation of PVP via ordinary waste water treatment plant culture was not underway and, furthermore, the addition of allylthiourea was causing the process to become inhibited. All measurements were taken three times in parallel.

The course of biodegradation was evaluated on the basis of determining biochemical oxygen demand [14], expressed as the ratio of the biological oxygen demand measured vs. theoretical oxygen demand - BOD/TOD [%], according to equation (1):

$$\text{Biodegradation of PVP} = \frac{\left(\frac{BOD_{PVP/CO-SUBSTRATE} - BOD_{COSUBSTRATE}}{c_{PVP}}\right)}{TOD_{PVP}} \times 100 \quad (1)$$

where $BOD_{PVP/CO\text{-}SUBSTRATE}$ and $BOD_{CO\text{-}SUBSTRATE}$ are experimentally found values pertaining to biological oxygen demand of PVP biodegradation in the presence of the co-substrate, respectively merely the co-substrate (all in [$mg \cdot dm^{-3}$]),TOD is theoretical oxygen demand (for PVP = 2020 $mg \cdot g^{-1}$), and c_{PVP} is the concentration of PVP in the suspension tested in [$g \cdot dm^{-3}$].

Results and discussion

The first part of the experimental work focused on observing the biodegradability of PVP and each of the co-substrates. The results of these measurements were then used to

evaluate the impact of the co-substrates on the biodegradation of PVP. Due to the fact that the retention time of wastewater in WWTP aeration tanks is in the order of several hours (depending on the type of WWTP facility), testing was carried out only for 30 days.

The BOD_{max}/TOD values for each co-substrate (data not presented) revealed that the substances are highly biologically degradable. Acrylamide decomposed from 100% within as little as 170 hours of testing, the lag phase being 60 hours. Similarly, 1M-2P was fully decomposed after 300 hours, with the lag phase equaling 30 hours. As regards APhA, 83% biodegradation was achieved after approximately 200 hours, with the lag phase being 30 hours. BOD attributable to the decomposition of PVP alone, without any additional co-substrate, progressed almost at the level of endogenous respiration (Fig. 2). Despite the above, about 3.19 ± 2.91% of biodegradation was achieved. Nonetheless, this value cannot be considered significant due to its high standard deviation, and it may be assumed that under the given conditions decomposition occurs only through the production of unreacted *N*-vinylpyrrolidone, a monomer contained in commercial PVP.

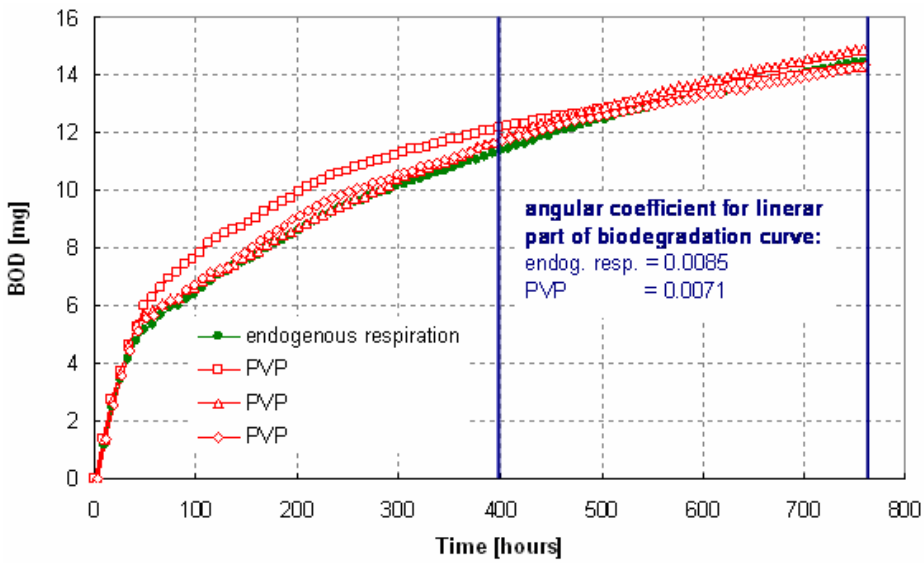

Fig. 2. Respiration of activated sludge (endogenous respiration) and respiration of activated sludge in the present of PVP (suspension volume 50 cm^3, sludge dry 500 $mg \cdot dm^{-3}$, concentration of PVP 200 $mg \cdot dm^{-3}$)

Figures 3-5 show the impact of the co-substrate on the biodegradation of PVP alone. It should be emphasized that each curve (Fig. 6) and column (Fig. 7), as shown in the diagrams, are based on the BOD value for merely PVP, *ie* after deducting oxygen consumption attributable to the decomposition of the co-substrate itself and endogenous respiration of activated sludge.

The most significant influence on the degradation of PVP was that of acrylamide (Fig. 3). The biodegradation curve showing the combination of PVP and acrylamide was located above the curve of merely the co-substrate. The difference between both curves pertains to the biodegradation of PVP alone (about 20% of biodegradation).

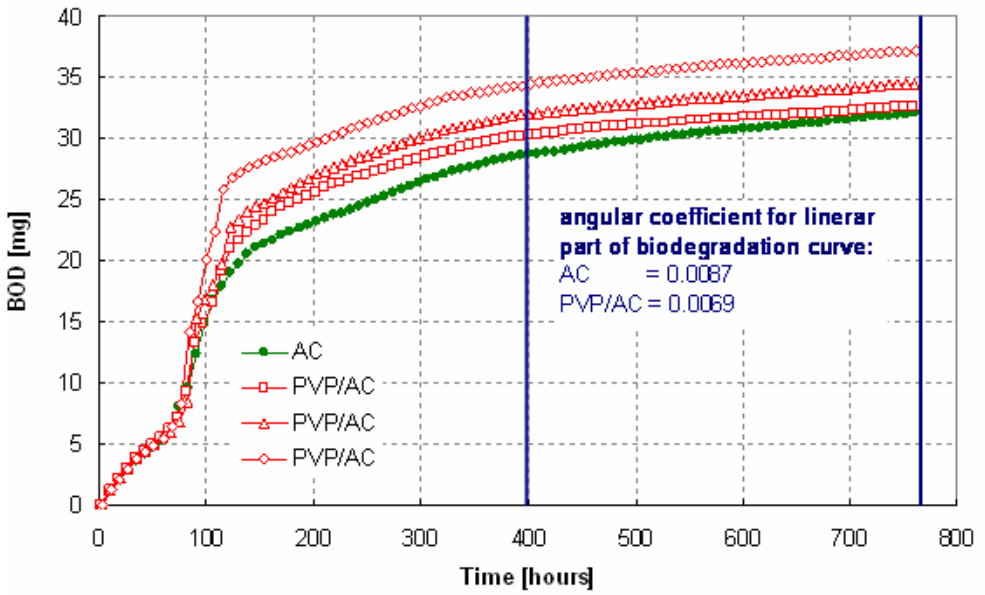

Fig. 3. The course of AC biodegradation and PVP biodegradation in the presence of AC (PVP/AC); suspension volume 50 cm^3, sludge dry 500 $mg \cdot dm^{-3}$, concentration of PVP 200 $mg \cdot dm^{-3}$ and AC 200 $mg \cdot dm^{-3}$

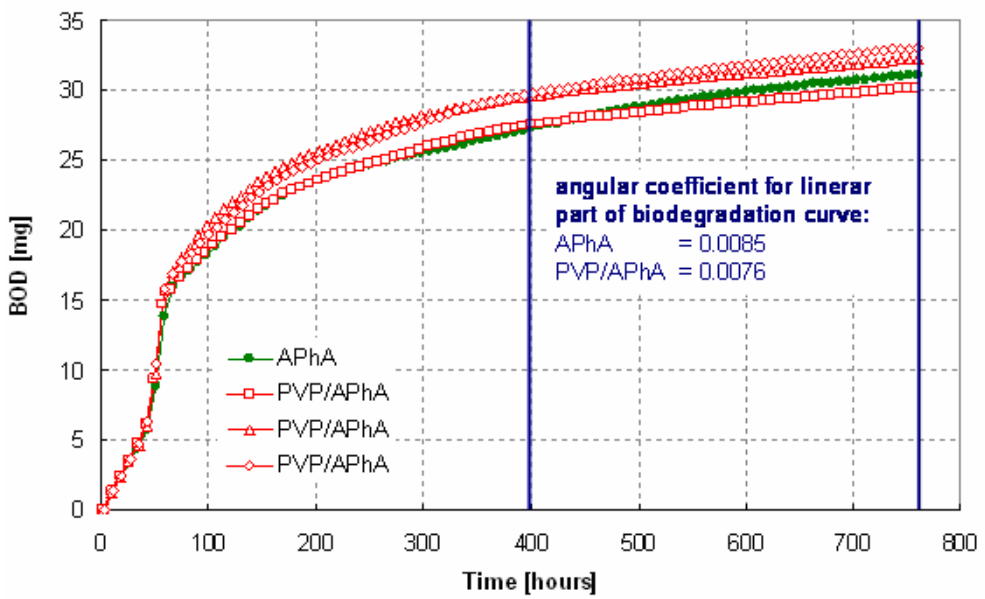

Fig. 4. The course of APhA biodegradation and PVP biodegradation in the presence of APhA (PVP/APhA); suspension volume 50 cm^3, sludge dry 500 $mg \cdot dm^{-3}$, concentration of PVP 200 $mg \cdot dm^{-3}$ and APhA 200 $mg \cdot dm^{-3}$

Adding APhA to the PVP sample did not induce the presumed support of decomposition of the latter (Fig. 4). The values of biological oxygen demand attributable to the decomposition of PVP in the presence of APhA varied only slightly to those of BOD attributable to the decomposition of the co-substrate alone. As with acrylamide, there was also a reduction in the biodegradation curves over time in this case.

Although 1M-2P is a substance with a structure greatly resembling that of PVP monomer, there was clearly no formation of γ-lactamase or other enzymes capable of decomposing PVP throughout the biodegradation process. Concurrently, adding 1M-2P suppressed the decomposition of merely PVP (Fig. 5).

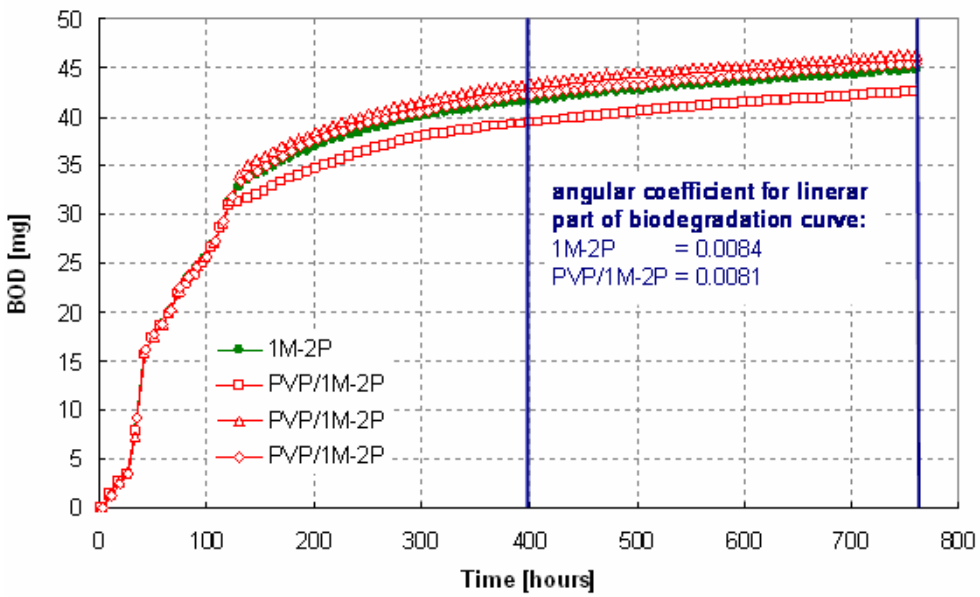

Fig. 5. The course of 1M-2P biodegradation and PVP biodegradation in the presence of 1M-2P (PVP/1M-2P); suspension volume 50 cm^3, sludge dry 500 $mg \cdot dm^{-3}$, concentration of PVP 200 $mg \cdot dm^{-3}$ and 1M-2P 200 $mg \cdot dm^{-3}$

Nevertheless, the sample containing PVP + the co-substrate showed a stagnation of BOD values over time compared with endogenous respiration, which resulted in a drop in the biodegradation curves (Fig. 6). The results displayed in Figure 2 thus indicate that there might be primary decomposition of PVP accompanied by the production of metabolites inhibiting microorganisms in the activated sludge.

The results imply (Fig. 7) that the aerobic environment is probably one in which partial PVP lactam ring cleavage has the potential to occur through the activities of microorganisms and influence of the AC and APhA co-substrates, and which may not be accompanied by polymer chain decomposition; it was not studied whether this is caused by γ-lactamase production or not. The declining trend of the curves indicates the aforementioned production of metabolites inhibiting microorganisms in the activated sludge. Any resulting intermediates were not identified at this stage of experimentation.

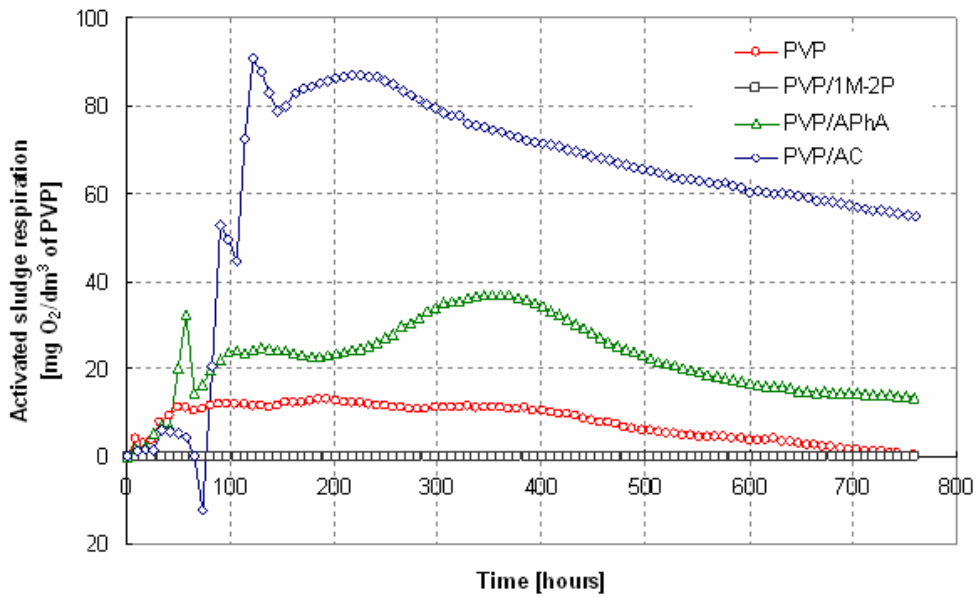

Fig. 6. The course of PVP biodegradation in the presence of co-substrates expressed as the respiration of activated sludge per instance of decomposition of PVP alone; that is after deduction of endogenous respiration and respiration attributable to the decomposition of merely the co-substrate

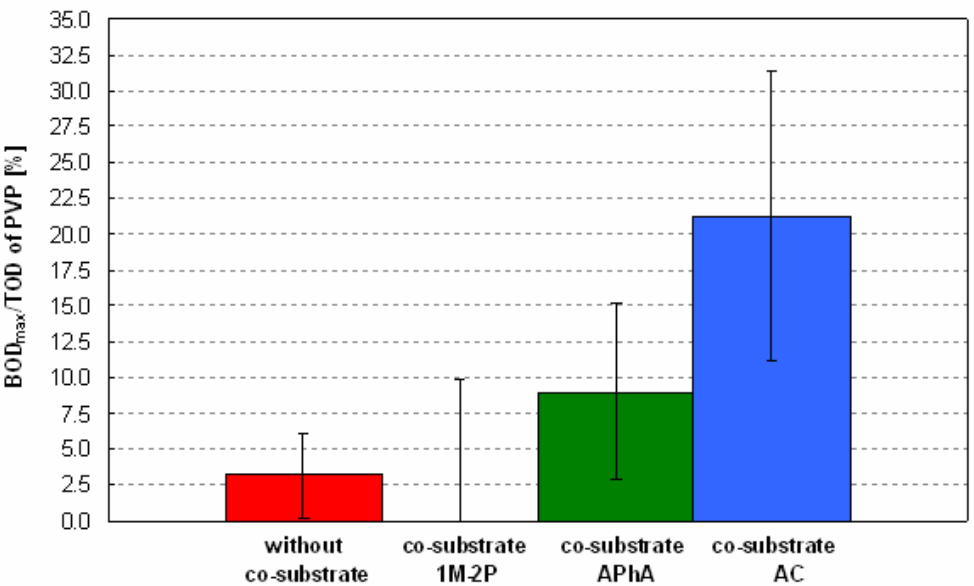

Fig. 7. The percentage of PVP biodegradation expressed as BOD_{max}/TOD; ±standard deviation

Conclusion

1. The results of this preliminary research need to be viewed with reservation (due to the high values of standard deviations). However, in light of the knowledge obtained, it can be concluded that the biodegradation of PVP is influenced by the presence of other substances. Toxic metabolites may form during the PVP decomposition process. This fact will require verification in future research using advanced instrumental techniques (GC or HPLC with MS detection). At the same time it would be appropriate to extend the testing period.
2. None of the selected co-substrates proved a significant accelerator of PVP biodegradation. The efficacy per co-substrate may be arranged as follows: AC > APhA > 1M-2P, with an astonishing 20% of biodegradation achieved in the case of acrylamide.

Acknowledgments

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ZAINICJOWANIE BIODEGRADACJI POLIWINYLOPIROLIDONU W ŚRODOWISKU WODNO-TLENOWYM: NOTATKI TECHNICZNE

Abstrakt: Syntetyczny polimer, poliwinylopirolidon (PVP - E 1201), znajduje zastosowanie przede wszystkim w przemyśle farmaceutycznym i spożywczym ze względu na jego odporność i brak toksyczności dla organizmów. Po spożyciu substancja ta przechodzi przez organizm niezmieniona. W związku z tym, że w procesie przetwarzania odpadów w komunalnym systemie oczyszczalni ścieków (OŚ) polimer ten nie ulega rozkładowi biologicznemu ani nie jest znacząco sorbowany w osadzie czynnym, może on gromadzić się w środowisku naturalnym. Ze względu na istnienie tych problemów w artykule przedstawiono możliwości aerobowej inicjacji biodegradacji PVP w obecności osadu czynnego miejskiej oczyszczalni ścieków. Jako inicjatory procesu biodegradacji zostały wybrane następujące środki: kosubstraty, akryloamid, N-acetylofenyloalanina i 1-metylo-2-pirolidon, substancje o strukturze podobnej do monomeru PVP. Biodegradację PVP w obecności kosubstratów oceniano na podstawie biologicznego zapotrzebowania na tlen (BOD), określonego za pomocą respirometru MicroOxymax $O_2/CO_2/CH_4$. Całkowite stężenie substratu w zawiesinie wynosiło 400 mg · dm^{-3} dla stosunku PVP i kosubstratu wynoszącego 1:1 oraz dla stężenia suchego osadu czynnego wynoszącego 500 mg · dm^{-3}. Chociaż nawet w tym układzie nie stwierdzono wzrostu biodegradacji samego PVP w obecności kosubstratu, to sam akryloamid okazał się najbardziej efektywnym rodzajem podłoża. Niemniej jednak, zauważony spadek nachylenia krzywych biodegradacji w czasie może wskazywać, że zachodzi proces rozkładu pierwotnego, który wiąże się z produkcją metabolitów hamujących aktywowane mikroorganizmy osadu. Otrzymane produkty nie zostały zidentyfikowane na tym etapie badań.

Słowa kluczowe: poliwinylopirolidon, biodegradacja, czynny osad ściekowy, środowisko wodne