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RAPID COMMUNICATION

Activated sludge-loaded polyvinyl alcohol microparticles for starch wastewater treatment in an airlift bioreactor

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Abstract−Emulsification followed by freezing and thawing cycles was applied to produce poly(vinyl alcohol) (PVA) microparticles and to simultaneously immobilize activated sludge. Activity of the obtained microparticles (~400 μm in diameter) was evaluated in glucose syrup solutions and heat-pretreated wastewater from a starch factory by measurements of permanganate index (COD_{Mn}) reduction. The reaction followed first-order kinetics, revealing slight mass transfer limitations in PVA microparticles as determined from the kinetic rate constant that was ~35% lower than that in the freely suspended activated sludge culture. Yet, efficiency of the microparticles increased almost five-fold in a laboratory airlift bioreactor operating either in batch or continuous regimes. Over 19 days of operation under high organic loadings in the industrial wastewater (~100 kg*COD* m^{-3} d^{-1}), PVA microparticles (12.4% volume fraction) remained active and induced a decrease of over 90% of biodegradable COD_{Mn} at the hydraulic retention time of 8 h.

Keywords: Poly(vinyl alcohol) Hydrogel, Freezing-thawing, Activated Sludge, Airlift Bioreactor, Starch Wastewater

INTRODUCTION

Poly(vinyl alcohol) (PVA) is a synthetic, hydrophilic polymer that has been widely investigated for a variety of applications including wastewater treatment [1]. PVA carriers in forms of beads, pellets, cubes, and membranes have been widely investigated for immobilization of variety of microorganisms for different wastewater treatment processes, such as nitrification (*e.g.* [2-5]), denitrification (*e.g.* [6-8]), phenol removal (*e.g.* [9,10]), etc. Activated sludge has also been successfully immobilized in PVA hydrogels, obtained either by dropwise extrusion of PVA-cell suspensions into saturated boric acid solution followed by gelation in sodium phosphate solution (*e.g.* [11,12]) or by microbial colonization of preformed, microporous PVA matrices. Such commercially available beads (Kuraray, Japan) with adsorbed anaerobic sludge have been used for treatment of high-strength corn steep liquor wastewater in an upflow anaerobic sludge-blanket reactor (UASB) [13] and an anaerobic fluidized-bed (AFB) reactor [14]. Airlift bioreactors are particularly convenient for light particles such as PVA carriers due to efficient mixing and high mass transfer rates at low shears [15], making this bioreactor type attractive for aerobic wastewater treatments at laboratory as well as industrial scales [16,17]. PVA beads produced by dripping methods generally have diameters of 2-4 mm, which were reported to induce non-uniform cell distribution (*e.g.* [18]) and a lower overall process rate in an airlift reactor as compared to a freely suspended sludge system [19]. Thus, production of microparticles (<1 mm in diameter) is desirable for achieving efficient internal mass transport and uniform cell distribution. PVA microparticles can be produced by the use of an emulsion method followed by freezing and thawing cycles as proposed first by Ficek and Peppas [20]. The particle size and size distribution depend on the aqueous to oil phase ratio, stirring rate and duration, and the nature and concentration of the emulsifier, so it is possible to obtain particle sizes ranging from a submicron scale to over 1 mm [20, 21]. This method has been investigated for controlled drug release but not yet for cell immobilization.

In this work, we have examined possibilities for application of the emulsion method followed by freezing-thawing cycles for immobilization of activated sludge in PVA microparticles. We studied the activity of the immobilized cells in glucose syrup in shaken flask cultures and then investigated possibilities for the use in treatment of high-strength wastewater from a starch factory in a laboratory airlift bioreactor.

MATERIALS AND METHODS

1. Preparation of Activated Sludge

Activated sludge was kindly supplied by the petrochemical company Petrohemija (Pancevo, Serbia) and activated in three passages. The stock growth medium contained 0.4 g/*l* peptone, 4.4 g/*l* meat extract, 0.16 g/*l* $CaCl_2$ x $2H_2O$, 0.28 g/*l* NaClO, 0.12 g/*l* urea, 0.08 g/*l* $MgSO_4$ x $7H_2O$, 1.12 g/*l* KH_2PO_4, and 0.8 g/*l* glucose, while the growth medium was prepared before each passage by diluting 25 ml of the stock medium in 250 ml of distilled water. All chemicals were of chemical grade.

In the first passage, activated sludge was added to 275 ml of growth medium at the concentration of 1 mg/ml and mixed in a shaken bath at room temperature for 24 h. Second and third pas-

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sages were obtained by dilution of a sample (2.5 ml) of the previous passage in the growth medium at the dilution factor of 100 and incubation of the obtained suspensions in a shaken bath at room temperature for 24 h, so that the number of bacterial cells was approximately 10^8 CFU/ml.

2. Industrial Wastewater

Wastewater was obtained from the starch factory Jabuka (Pancevo, Serbia) with COD of 34,290 mg/*l*, BOD of 31,547 mg/*l*, Total Kjeldahl-N of 16,892 mg/*l*, total P of 944 mg/*l* and pH of 3.41. For the purpose of this research, wastewater was prepared by adjusting the pH at the value of 1.8 using 0.1 M HCl, and heating at 50-55 °C for 4 h. Finally, pH was adjusted to 3.4 by addition of Na_2CO_3. The resulting wastewater had a permanganate index (COD_{Mn}) of 50,733±2,245 mg/*l* (average of 15 samples) and was used in all experiments in this work.

3. Preparation of PVA Microparticles

Poly(vinyl alcohol) (PVA) powder (hot water soluble, P163-250G, Sigma, St Louis, MO) was dissolved in distilled water at the concentration of 10% w/v and heated at 90 °C for at least 6 h. Suspension of activated sludge from the third passage (2.5 ml, 1×10^8 CFU/ml) was mixed with 10 ml of the obtained PVA solution and added dropwise into the chilled and continuously stirred (1,500 rpm) oil phase, which consisted of 500 ml of soybean oil (Uvita, Pancevo, Serbia) with 4 g of lecithin (Sojaprotein, Becej, Serbia). After stirring for 2 h, the mixture was cooled to −20 °C for 72 h, thawed at +4 °C for 4 h and then frozen again at −20 °C for 18 h. The freezing - thawing cycles were then repeated six times in total. The obtained PVA microparticles with immobilized activated sludge (2×10^7 CFU/ml) were separated from the oil phase by filtration followed by washing in water. Size of the microparticles was determined by measuring at least 15 microparticles using an optical microscope.

4. Activity of PVA Microparticles

Activity of PVA microparticles with immobilized activated sludge was investigated in solutions of glucose syrup (hydrolysis degree of 65%, A.D Starch industry, Jabuka, Pancevo, Serbia) and in the industrial wastewater prepared as described above. The glucose syrup solution (2% v/v) exhibited similar initial COD_{Mn} (54,504±847 mg/*l*) as the wastewater used in this work. In shaken flask experiments, 10 ml of freshly prepared PVA microparticles was added to 250 ml of the glucose solution or wastewater in Erlenmeyer flasks (500 ml) and placed in a reciprocal shaking bath (100 strokes/min) at room temperature during 24 h. As a control, 2.5 ml of activated sludge from the third passage was added to 250 ml of wastewater yielding the sludge concentration of approximately 9.9×10^5 CFU/ml. All solutions were sampled (2.5 ml) at timed intervals and COD_{Mn} values were determined by the standard procedure [22]. All experiments were carried out in five replicates.

In the next experimental series, the activity of one batch of PVA microparticles with immobilized activated sludge (2×10^7 CFU/ml) was investigated in a laboratory external-loop air-lift bioreactor (274 ml) described previously [23,24], operated in batch and continuous regimes (Fig. 1S, Supplementary material). In batch experiments (12 in total) 240 ml of wastewater was added to PVA microparticles (34 ml, 12.4% volume fraction), and water samples (2.5 ml) were taken at timed intervals over 24 hours. In the continuous mode experiment, the air-lift bioreactor was supplied with the wastewater (30 ml/h) by a peristaltic pump over seven days, and water samples at the bioreactor outlet were taken once a day. In all experiments, airflow of 0.1 *l*/min was introduced at the bottom of the reactor rising section providing continuous recirculation of the liquid and solid phases in the reactor loop.

RESULTS AND DISCUSSION

PVA microparticles with immobilized activated sludge at the approximate concentration of 2×10^7 CFU/ml were successfully produced by the emulsion method followed by freezing and thawing cycles. We applied the oil to the aqueous phase ratio of 50 : 1 under high stirring rate of 1,500 rpm to obtain small and uniform microparticles as suggested in the literature [20]. After six freezing-thawing cycles, white opaque and approximately spherical microparticles with the average diameter of 380±100 μm were obtained (Fig. 2S, Supplementary material) with the yield of about 70%. Rough surface of microparticles can be beneficial for starch removal by activated sludge since it proceeds by adsorption on flocs/particle surfaces followed by a slower process of enzymatic hydrolysis [25].

Activity of the obtained PVA microparticles was explored first in shaken flask cultures using glucose syrup solution and industrial wastewater from a starch factory. Suspended activated sludge in wastewater served as a control. In all cultures, the permanganate index, COD_{Mn}, decreased over time (Fig. 1), and the reaction rate was modeled by the first-order kinetics. However, it was shown that a certain quantity of COD_{Mn} in the wastewater amounting to 24,013±1,599 mg/*l* could not be further reduced by activated sludge (shown later in this manuscript, see Fig. 2(a)). Therefore, for deter-

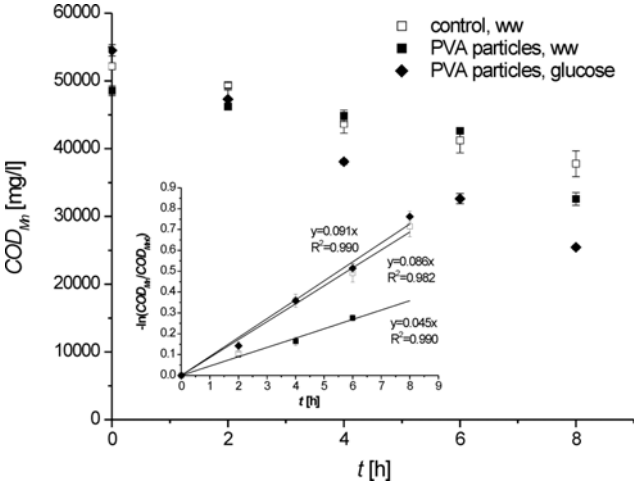

Fig. 1. Degradation of organic compounds in shaken flasks: COD_{Mn} as a function of time in the suspended activated sludge culture in wastewater (control) and immobilized activated sludge in PVA microparticles (i) in wastewater (PVA particles, ww), and (ii) in the glucose syrup solution (PVA particles, glucose). Inset: determination of the first-order kinetic rate constants of reducible COD_{Mn} decrease as slopes of the linear fits of the plots ($-\ln(COD_{Mn}/COD_{Mn0})$) *vs.* t applied to COD_{Mn} data decreased for the non-reducible COD_{Mn} value of 24,013 mg/*l*. All data are average of n=5.

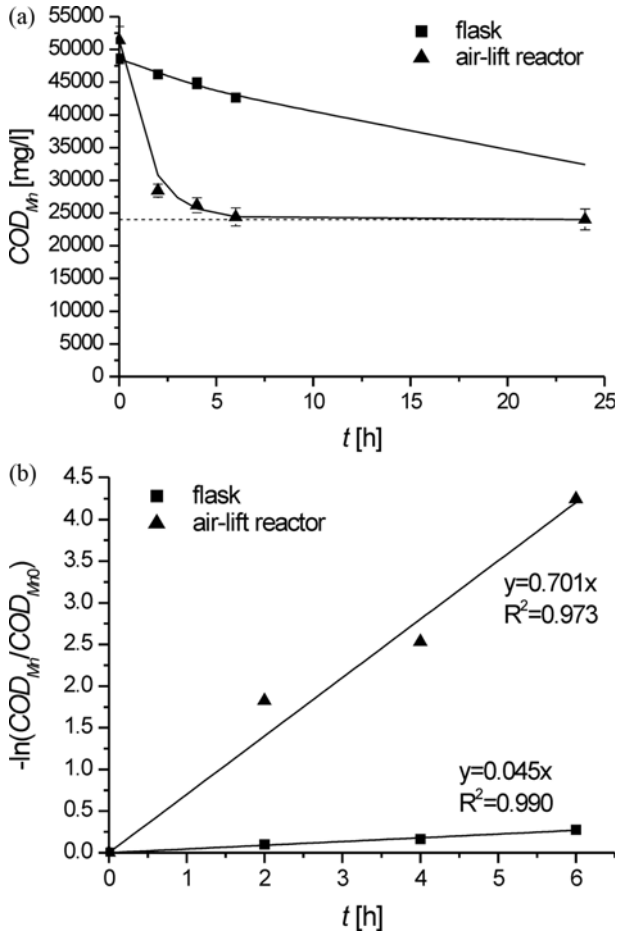

Fig. 2. Activity of PVA microparticles with immobilized activated sludge in wastewater in batch cultures. (a) COD_{Mn} as a function of time in shaken flasks and in the air-lift bioreactor: experimental data (symbols; average of at least n=5) and model predictions (solid lines); dashed line presents the non-reducible COD_{Mn} value (average of n=12); (b) determination of first-order kinetic rate constants *k* as slopes of the linear fits of the plots ($-\ln(COD_{Mn}/COD_{Mn0})$) *vs.* t applied to average COD_{Mn} decreased for the non-reducible COD_{Mn} value of 24,013 mg/*l*.

mination of kinetic rate constants for permanganate index reduction in the wastewater, from the experimental COD_{Mn} data, the value of 24,013 mg/*l* was subtracted and kinetics modeling was then applied to the reducible COD_{Mn}. Thus the kinetic constants, *k*, were then obtained from the slopes of linear fits of the plots ($-\ln(COD_{Mn}/COD_{Mn0})$) *vs.* t (Fig. 1 inset).

It can be seen that the degradation rate was the highest in the glucose syrup solution utilizing PVA microparticles ($k=0.091\ h^{-1}$), followed by the suspended activated sludge ($k=0.086\ h^{-1}$) and PVA microparticles ($k=0.045\ h^{-1}$) in the wastewater. The same trend was obtained when the kinetic rate constants were normalized per activated sludge content (*i.e.*, (9.9 *vs.* 7.7)×10^5 CFU/ml in the suspended and the PVA immobilized culture, respectively, section Materials and methods). Normalized kinetic rate constants were thus calculated as (4.55, 3.44, and 2.25)×$10^{-10}\ h^{-1}\ CFU^{-1}$ for PVA microparticles in the glucose syrup, the suspended control sludge in wastewater and PVA microparticles in wastewater, respectively. The kinetic constant in the suspended culture was still for about 35% higher than the kinetic constant determined in the PVA culture in wastewater, which can be attributed to diffusion limitations in the microparticles.

Next, activity of PVA microparticles was studied in a laboratory air-lift bioreactor in batch regime first and compared to that in shaken flasks (Fig. 2(a)). The non-reducible COD_{Mn} (24,013±1,599 mg/*l*) in the wastewater by activated sludge is clearly indicated (Fig. 2(a)). Thus, the first order kinetic rate model was applied also to the reducible COD_{Mn}, only, yielding the kinetic rate constant for the air-lift bioreactor of $0.701\ h^{-1}$ (Fig. 2(b)), which amounts to $10.3\times10^{-10}\ h^{-1}\ CFU^{-1}$ when normalized per the activated sludge content (24.8×10^5 CFU/ml). The obtained kinetic rate constant is almost five-fold higher than the value in the flask culture (*i.e.*, $2.25\times10^{-10}\ h^{-1}\ CFU^{-1}$) and three-fold higher than the value determined in the control suspended sludge culture (*i.e.*, $3.44\times10^{-10}\ h^{-1}\ CFU^{-1}$). Better results obtained in the air-lift bioreactor could be probably attributed to more suitable overall hydrodynamic conditions in this system.

Continuous air-lift bioreactor operation was performed at the wastewater flowrate of 30 ml/h, providing the hydraulic residence time (HRT) of 8 h sufficient to complete reduction of the reducible COD_{Mn}. Over seven days of operation, the outlet COD_{Mn} values slightly decreased over time oscillating around the average value of 25,807±1,707 mg/*l* (Fig. 3S, Supplementary material), thus achieving the maximal possible COD_{Mn} reduction in the wastewater used. Note that heat-pretreated, high-strength industrial wastewater from a starch factory was used in the present work. This type of wastewater is highly organic with chemical oxygen demand (COD) up to 30,000 mg/*l* with starch granules that are largely biodegradable [26,27]. Consistently, in this work, at the continuous operation of the airlift bioreactor at HRT of 8 h the organic loading rate was very high amounting to ~100 kg COD/(m^3·d). Under these conditions, the bioreactor provided a decrease of reducible COD_{Mn} for about 93.6%. In a comparable study, an inverse fluidized bed bioreactor using low density polypropylene particles loaded with activated sludge provided COD reduction in starch wastewater for 95.6% at a significantly lower organic loading rate of 1.35 kg COD/(m^3·d) and only 51.8% at a somewhat higher organic loading rate of 26.73 kg COD/(m^3·d) [28]. Remarkable performance of the system used in the present study could be attributed probably to better mixing and aeration in the airlift bioreactor as compared to the fluidized bed bioreactor. Still, utility of the immobilized system developed in this work should be investigated in more depth by analyses of additional water parameters (*e.g.*, BOD, N and P contents), optimization of wastewater pretreatment to decrease the non-degradable COD as well as to study operation of the bioreactor system over longer periods of time. However, in all present airlift bioreactor experiments the same batch of PVA microparticles was used, showing stable and even slightly increasing activity over 19 days in total, although some occasional agglomeration of microparticles was observed. Thus, the combination of PVA biocatalysts and the airlift bioreactor in these first studies showed promising performance under conditions resembling the situation in reality.

CONCLUSIONS

We have successfully applied the emulsion method followed by freezing-thawing cycles for immobilization of activated sludge into PVA microparticles approximately 400 μm in diameter. Cell viability was preserved during the production process so that the microparticles exhibited high activity in starch wastewater, especially under good mixing and aeration conditions in a laboratory airlift bioreactor. Over 19 days of operation under high organic loadings, the PVA microparticles remained active and induced a decrease of over 90% of biodegradable COD_{Mn}. Results of this study indicate that the immobilization method used could be attractive also for other cell types, while microsized PVA carriers in conjunction with airlift bioreactors could provide high mass transfer rates and thus high process efficiencies.

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SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

REFERENCES

1. C. M. Hassan and N. A. Peppas, *Adv. Polym. Sci.*, **153**, 37 (2000).
2. J.-K. Seo, I.-H. Jung, M.-R. Kim, B. J. Kim, S.-W. Nam and S.-K. Kim, *Aquacult. Eng.*, **24**, 181 (2001).
3. W. M. Rostron, D. C. Stuckey and A. A. Young, *Water. Res.*, **35**, 1169 (2001).
4. Y. Chen, Y. Wang, M. Fan and H. Jin, *Procedia Environ. Sci.*, **11**, 1171 (2011).
5. H. Bae, H. Yang, Y.-C. Chung, Y. J. Yoo and S. Lee, *Bioprocess Biosyst. Eng.*, **37**, 1115 (2014).
6. Z. Zhang, Z. Lei, X. He, Z. Zhang, Y. Yang and N. Sugiura, *J. Hazard. Mater.*, **163**, 1090 (2009).
7. H. Uemoto and H. Saiki, *Appl. Environ. Microbiol.*, **66**, 816 (2000).
8. J. Trogl, A. Bouskova, J. Mrakota, V. Pilarova, J. Krudencova, J. Měchurova, S. Krízenecka and R. Stloukal, *Desalination*, **275**, 82 (2011).
9. M. H. El-Naas, S. A. Al-Muhtaseb and S. Makhlouf, *J. Hazard. Mater.*, **164**, 720 (2009).
10. Y. J. Liu, A. N. Zhang and X. C. Wang, *Biochem. Eng. J.*, **44**, 187 (2009).
11. K.-C. Chen, S.-C. Lee, S.-C. Chin and J.-Y. Houng, *Enzyme Microb. Technol.*, **23**, 311 (1998).
12. L. P. Ramteke and P. R. Gogate, *J. Chem. Technol. Biotechnol.*, **91**, 456 (2014).
13. Z. Wenjie, W. Dunqiu, K. Yasunori, Y. Taichi, Z. Li and F. Kenji, *Bioresour. Technol.*, **99**, 8400 (2008).
14. W. Zhang, Q. Xie, J. D. Rouse, S. Qiao and K. Furukawa, *J. Biosci. Bioeng.*, **107**, 49 (2009).
15. J. Merchuk, *Trends Biotechnol.*, **8**, 66 (1990).
16. C. Nicolella, M. C. M. van Loosdrecht and S. J. Heijnen, *Trends Biotechnol.*, **18**, 312 (2000).
17. C. Nicolella, M. C. M. van Loosdrecht and S. J. Heijnen, *J. Biotechnol.*, **80**, 1 (2000).
18. S. Guobin, X. Jianmin, G. Chen, L. Huizhou and C. Jiayong, *Let. Appl. Microbiol.*, **40**, 30 (2005).
19. S.-Y. He, Y.-H. Lin, K.-Y. Hou and S.-C. J. Hwang, *Bioresour. Technol.*, **102**, 5609 (2011).
20. B. J. Ficek and N. A. Peppas, *J. Control. Release*, **27**, 259 (1993).
21. J. K. Li, N. Wang and X. S. Wu, *J. Control. Release*, **56**, 117 (1998).
22. International Organization for Standards (ISO) "*Water quality - Determination of permanganate index*" ISO 8467:1993(E), Geneve, Switzerland (1993).
23. M. Milivojevic, A. Dudukovic, B. Obradovic, A. Spasic and B. Bugarski, *Hem. Ind.*, **58**, 10 (2004).
24. M. Milivojevic, S. Pavlou, I. Pajic-Lijakovic and B. Bugarski, *Chem. Eng. J.*, **123**, 117 (2007).
25. O. Karahan, A. Martins, D. Orhon and M. C. M. van Loosdrecht, *Biotechnol. Bioeng.*, **93**, 964 (2006).
26. L. P. Huang, B. Jin, P. Lant and J. Zhou, *J. Chem. Technol. Biotechnol.*, **78**, 899 (2003).
27. B. K. Rajbhandari and A. P. Annachhatre, *Bioresour. Technol.*, **95**, 135 (2004).
28. M. Rajasimman and C. Karthikeyan, *J. Appl. Sci. Environ. Manage.*, **11**, 97 (2007).

Supporting Information

Activated sludge-loaded polyvinyl alcohol microparticles for starch wastewater treatment in an airlift bioreactor

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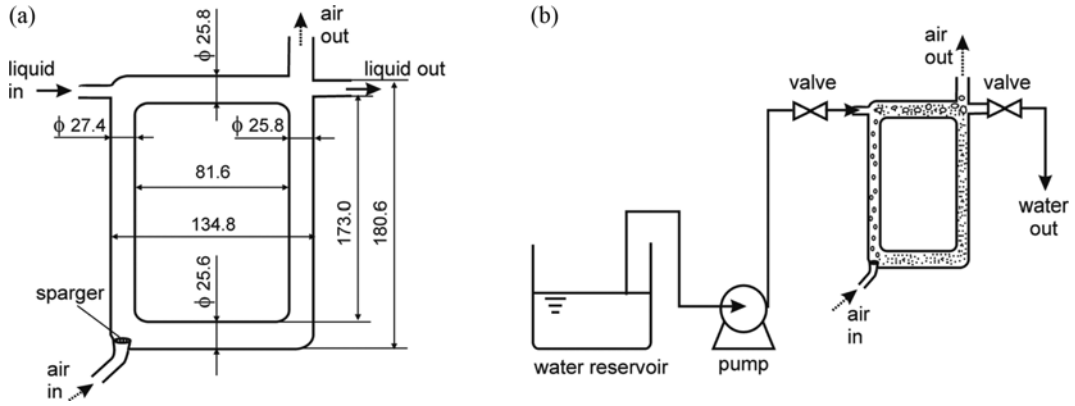

Fig. S1. Experimental air-lift bioreactor: (a) Bioreactor geometry [all values are in mm]; (b) continuous experimental system: wastewater was supplied by a peristaltic pump at the flowrate of 30 ml/h in the 3-phase air-lift bioreactor with the volume fraction of PVA microparticles of 12.4%.

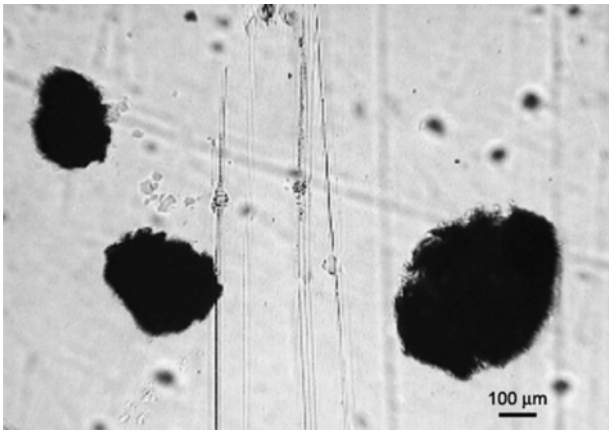

Fig. S2. PVA microparticles with immobilized activated sludge (scale bar=100 μm).

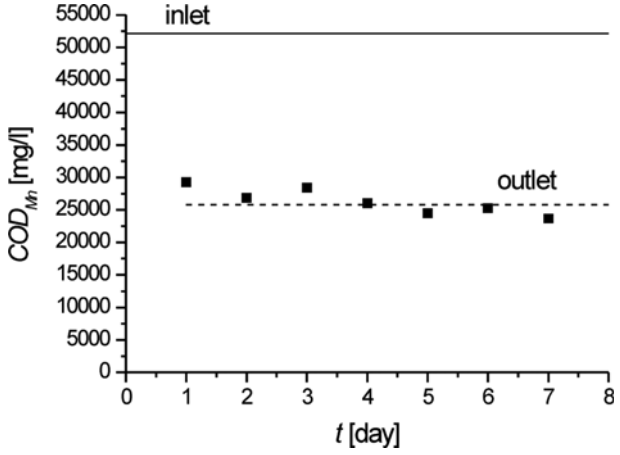

Fig. S3. Continuous operation of the air-lift bioreactor with PVA microparticles at the wastewater flowrate of 30 ml/h and inlet COD_{Mn} of 52,140 mg/*l* (solid line) and outlet COD_{Mn} (symbols) averaging at 25,807±1,707 mg/*l* (dashed line).