Effect of bioaugmentation on anaerobic wastewater treatment in the dairy industry

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ABSTRACT

The creation of a biofilm as a specialized biosystem, its development, and activity were studied at 3 critical control points of biofilter operation: start-up, transition from batch to sequencing batch regimen, and set-up of stable sequencing batch process. Five variants of biosystems were investigated with an inoculum from specially adapted real activated sludge, enriched with various combinations of 3 microbial preparations. A stable and working biofilm was developed in the phase of stabilized sequencing batch process. The differences among biodegradation effectiveness of the 5 variants were insignificant during that phase and the effect of the preparations was low. The effectiveness of organic removal for the 5 bioaugmentation approaches reached 60% for protein, 70% for chemical oxygen demand, and 97% for lactose. Commercial inocula did not improve final reactor performance over an inoculum from a municipal wastewater treatment plant alone.

Key words: bioaugmentation, dehydrogenase activity, dairy wastewater, anaerobic sequencing batch biofilm reactor

INTRODUCTION

Diphase technologies comprising anaerobic and aerobic bioreactors are widely applicable in dairy wastewater treatment because of the high amount of OM (including carbohydrates, proteins, and lipids). The purpose of the anaerobic bioreactor is to initially reduce the organic content from chemical oxygen demand (COD) values from 4,000 to 5,000 mg/L to <1,000 mg/L. The function of the aerobic bioreactor is to further decrease the concentration of the pollutants to the allowance for outfall into the receiving water body or sewage system (Chan et al., 2009). The object used in this study was

an anaerobic bioreactor module with fixed-film biomass in near-to-real conditions.

Biochemically, the anaerobic biodegradation of OM takes place through reduction of final acceptors of electrons; that is, nitrate (in denitrification), sulfates (in sulfate reduction), carbon dioxide (in methanogenesis), organic molecules (in fermentations), and so on (Bitton, 2005). The heterogenic biofilm structure and the presence in it of microhabitats of diverse conditions (including a redox gradient and various microzones of different enzyme activities, organics, and nutrient concentrations) enable simultaneous development of these processes. Their realization is also determined by the occurrence in the biofilm and the activity of assorted physiological groups of microorganisms whose metabolic pathways complement each other. The complex microbial structure of the biofilm allows, depending on the conditions (redox gradient, and concentrations of oxygen, carbon dioxide, nitrate, sulfates, and so on) in the bioreactor, one microbial group to become active while another physiological group is resting. For example, under aerobic conditions, anaerobic bacteria are present but resting. These microorganisms become active and dominate functionally in the structure of biofilm when oxygen is depleted.

A critical moment in wastewater purification is the start-up of the process, because the biofilms develop for an extended period from 2 to 9 mo (Lauwers et al., 1990). The starting span is defined by the time needed for formation of the biofilm's compound trophic structure (including the complexes of microflora, microfauna, and metafauna), for establishing interrelations among the components, and for their synchronization. The time-consuming growth of the anaerobic microorganisms and greater sensitivity to the environmental factors also slow down the start-up of the process. One possibility for decreasing reactor start-up time is bioaugmentation by addition of specialized bacteria to native inocula (activated sludge). For example, Ma et al. (2009) ascertained that the addition of specialized bacteria to activated sludge (AS) lasted only 20 d when the effectiveness of COD removal was 84% compared

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with 30 d for an AS system with effectiveness of COD removal of 74%.

One of the factors of great importance for the start-up of the wastewater treatment process is the type of inoculation material for the biofilter. The scientific literature contains many papers on bioaugmentation at the start-up of the process; that is, introduction of exogenous microorganisms proven to display specific and controllable activity toward the target pollutants. The studies are based mainly on the application of AS from wastewater treatment plants (Chen and Shyu, 1996; Ince, 1998; Haridas et al., 2005; Sirianuntapiboon et al., 2005; Demirel and Yenigun, 2006; Loperena et al., 2006) or of commercial microbial preparations (Carta et al., 2002; Mongkolthanaruk and Dharmsthiti, 2002; Rajeshkumar and Jayachandran, 2004; Loperena et al., 2006; Kosseva et al., 2007). Information is lacking, however, on the possible combination of these 2 approaches for dairy wastewater treatment. The AS as a source of inoculum has the advantage of broad biodiversity, on the genetic level as well as on metabolic, enzyme, and organism levels. The selection of AS is therefore of great importance. For example, the use of methanogenous sludge is not recommended because of its poor diversity of physiological groups of microorganisms due to the presence of strongly synergetic acetotrophs and methanogens. At the same time, the application of AS from a municipal wastewater treatment plant, which contains a rich variety of microorganisms, enzymes, and co-factors, would provide for co-metabolic and syntrophic supplementation of the metabolism of certain microbial groups and for the synergetic accomplishment of, for example, hydrolysis, fermentation, acetogenesis, methanogenesis, denitrification, and sulfate reduction. The commercial microbial supplements, for their part, contain a set of pure cultures and they have the advantage of high biodegradation activity toward the target pollutant. An increase of functional biodiversity and metabolic activity toward target pollutants, because of the addition of microbiological preparations to the AS, is expected. The use of AS as a source of inoculum is economically more beneficial, whereas the application of commercial preparations is more expensive. The combination of approaches in the management of biological processes is beneficial and is often used in practice. In this regard, it is important to define the effect of the preparations on the formation and degradation activity of the biofilm.

The present work is a comparative study on the degradation activity of 5 biological systems initially inoculated with purposely adapted AS and then enriched with the microbial preparations HydroPacks, Laktazym, and BiliKuk (EKO-PF, České Budějovice, Czech Republic). An anaerobic treatment process of dairy

wastewater was studied at 3 critical control points: (1) reactor inoculation and active biofilm formation in the start-up period; (2) transition from batch to sequencing batch process and instability of the biofilm; (3) set-up of stable sequencing batch process and stable biofilm development. The aim of the study was to evaluate the effect of initial bioaugmentation on the effectiveness of biodegradation of OM during these 3 phases.

MATERIALS AND METHODS

Bioprocesses and Biological Systems

An anaerobic process for dairy wastewater treatment was carried out in 0.5-L glass reactors (75 mm diameter and 110 mm height) of the biofilter type placed in a temperature-controlled chamber at 28 to 30°C (Figure 1). The reactors were sealed airtight and metallic clamps on hoses (outlet for biogas escape) were used to prevent air from entering the system through the biogas outlet. The carrier in the anaerobic sequencing batch biofilm (ASBB) reactor was gravel, with particle size of 8 to 16 mm, and it was presterilized at 160°C. The reactors were fed with synthetic wastewater, the whole volume being removed and replaced with fresh wastewater. The reactors were fed with synthetic wastewater by removing the entire reactor volume and replacing with fresh wastewater every 19 ± 5 d. Thirty reactors were used in every variant of biological system, with 6 repetitions of each treatment. During the batch process, each sampling was done in a separate reactor. During the sequencing batch process, a blended sample from the 6 independent repetitions was analyzed because the results obtained for each separate treatment were close to the data obtained for the average sample. The variability among reactors receiving the same treatment is much smaller than the observed differences among treatments. The estimate of variability was accomplished with results from a single set of measurements made on the effluent from all 30 reactors during one sampling period. The conditions in the reactors were aerobic at the beginning, but only for a short time. The oxygen available in the bioreactor was soon exhausted and the conditions shifted to anaerobic. Such an approach was adopted to achieve a simulation model that corresponded to conditions in actual wastewater treatment practice.

The ASBB reactor was in operation for 282 d. The complete operational period was divided into 3 stages corresponding to 3 critical control points in the biological wastewater treatment. The first critical control point (CCP1) was start-up (from d 0 to 30) with active biofilm development. The second critical control point (CCP2) was transition from batch to sequencing batch

Figure 1. Anaerobic sequencing batch biofilm reactor.

mode (from d 30 to 105) with instability of biofilm. The third critical control point (**CCP3**) was stabilization of sequencing batch process (from d 105 to 282) with stable biofilm development. The reactors operated in sequencing batch mode because the whole volume was removed and replaced with fresh wastewater.

The inocula were prepared from specially treated and adapted real AS enriched with microbial preparations – HydroPacks, Laktazym, and BiliKuk (EKO-PF). An anoxic AS was taken from the sludge thickener of Sofia Wastewater Treatment Plant (Sofia, Bulgaria; processing municipal and industrial wastewater, including wastewater from several large dairy companies). It was acclimated to the model wastewater for 135 h. In this case, the adaptation was based on the natural mechanisms for community reconstruction to the wastewater. The acclimation was related to replacement of dominated groups of organisms in the biocenose, increase of bacterial numbers, and expression of enzymes with high activity against target pollutants. The main aspects that determine the type of adaptation are the nature of pollutants, the physicochemical conditions, and metabolic potential of the microbiota. The dairy wastewater composition included compounds of natural origin (proteins, lipids, and carbohydrates) that do not require a specialized method for acclimation. The commercial preparations designed for fat, protein, and carbohydrate biodegradation were supplied by Brave & Brave Ltd. (Tabor, Czech Republic). The preparations contained aerobic and anaerobic heterotrophic bacteria, some of which are spore forming. The solid microbial preparations HydroPacks and Laktazym contained between 1.10^9 and 7.10^{10} cfu/g aerobic and anaerobic heterotrophic bacteria, and the microbial count in the liquid preparation BiliKuk was 1.10^9 cfu/

mL. The ASBB reactors were inoculated with the following 5 biological systems: (1) control: 10 g/L AS as dry weight (**dw**); (2) 10 g/L AS as dw + 3 g/L preparation HydroPacks (**H**); (3) 10 g/L AS as dw + 3 g/L preparation Laktazym (**L**); (4) 10 g/L AS as dw + 1.5 g/L HydroPacks + 1.5 mL/L BiliKuk (**HB**); (5) 10 g/L AS as dw + 1.5 g/L Laktazym + 1.5 mL/L BiliKuk (**LB**). The inoculum amount was calculated to reach 10% (vol/vol), which is unusual for anaerobic processes because of the lower growth rate of those microorganisms. The ASBB reactors in the sequencing batch process were not reinoculated.

Wastewater

Whey is a byproduct of the dairy industry, and it is one of components of wastewater in Bulgaria. Consequently, a critical problem of dairy wastewater treatment plants in this country is the effectiveness of whey biodegradation. Synthetic wastewater was used for influent fed in the ASBB reactor and it contained NH_4Cl 0.57 g/L, KH_2PO_4 0.43 g/L, K_2HPO_4 1.09 g/L, and Na_2HPO_4 1.33 g/L (served also as buffer agents), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.023 g/L, CaCl_2 0.028 g/L, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.025 g/L, and whey 3.65 g/L. The mineral composition of model wastewater was prepared according to Loperena et al. (2006). Dried whey (P.I.C. Co Ltd., Sofia, Bulgaria) was used as a sole source of carbon and energy for microbial growth. The concentration of whey corresponded to COD values of approximately 4,000 mg of O/L, because the COD of dairy waste effluents from full-scale operations varies between 500 and 9,200 mg/L, or 3,800 mg/L on average (Demirel et al., 2005). The general characteristics of synthetic wastewater were pH 7.62; COD 3,863.67 mgO/L; protein 0.95 g/L; lactose 2.67 g/L; N-NH_4 186.74 mg/L; N-NO_2 0.009 mg/L; N-NO_3 0.37 mg/L; P-PO_4^{3-} 0.48 mg/L.

Analytical, Microbiological, and Enzymological Methods

The COD and the concentrations of ammonium, nitrite, and nitrate were analyzed according to APHA (1989). The determination of ammonium and nitrite concentration was based on a colorimetric method with reagent of Nessler and Cleve's acid, respectively. The concentration of nitrate was determined by a spectrophotometric method using sulfosalicylic acid. The protein content was measured by the micro-biuret method (Kochetov, 1974). The lactose concentration was determined by a colorimetric method with 3,5-dinitrosalicylic acid according to Miller (1959). The biomass for determining the enzyme activities and quantity of anaerobic heterotrophs (**AnH**) was harvested on d 3,

56, and 216, corresponding to the 3 critical processes. The AnH and dehydrogenase activity (**DHA**) were investigated in the 2 components of the biological system: (1) immobilized microorganisms on the carrier and (2) suspended biomass in the liquid. Immobilized biomass for microbiological analyzes was detached from gravel by ultrasonic disintegration with a UD-20 automatic (3×5 s with frequency 22 kHz and vibration amplitude 8 μ m; Techpan, Warsaw, Poland) in saline solution. The microbial numbers were counted by plate techniques according to routine practices (Kuznetsov and Dubinina, 1989). Anaerobic heterotrophs were cultivated on nutrient agar (Brit. Pharm.; Scharlau Chemie, Barcelona, Spain) and incubated in anaerobic jars with Anaerocult A (Merck & Co., Inc., Darmstadt, Germany) for creation of anaerobic conditions. The microbial biomass for enzyme analyses was harvested according to Feist and Hegeman (1969). Dehydrogenase activity was determined according to the method of Gabbita and Huang (1984). The quantity of biomass for determination of specific enzyme activity was measured as concentration of cell protein according to Kochetov (1974).

Biodegradation Effectiveness

The biodegradation effectiveness (Eff) in the 3 critical process periods was calculated for the total organic content (measured as COD), protein, and lactose. The following formula for determination was used:

$$Eff = \frac{C_{t_1} - C_{t_2}}{C_{t_1}} \cdot 100\%, \quad [1]$$

where C_{t_1} is the concentration of pollutant at the moment t_1 ; C_{t_2} is the concentration of pollutant at the moment t_2 . The moment t_1 is just after a feeding and the moment t_2 is just before the next feeding.

The biodegradation effectiveness was presented for the transition period as an average value from the first 4 feedings and for the stable ASBB reactor operation as an average value from the following 9 feedings.

RESULTS AND DISCUSSION

The focus of this research was not on the bioreactor operation but on the influence of microbial preparations on the effectiveness of biodegradation and on the changes in structural and functional characteristics of biological systems during the 3 periods.

Effectiveness of Biodegradation

The effectiveness of OM biodegradation was lowest at start-up and did not exceed 30% (Figure 2a), which

Figure 2. Biodegradation effectiveness (mean $\pm$ SD, $n = 3-6$) of (a) organic matter (measured as chemical oxygen demand, COD), (b) proteins, and (c) lactose in period of start-up [critical control point (CCP) 1], instability of biofilm (CCP2), and stable operation (CCP3) during anaerobic treatment process. The standard deviations are based on repeated composite measurements made over time. H = HydroPacks; HB = HydroPacks + Bilikuk; L = Laktazym; LB = Laktazym + Bilikuk (EKO-PF, České Budějovice, Czech Republic).

was due to the changes in the microbial community structure after biofilter inoculation. The COD values for all 5 variants studied were decreased for that period to about 2,700 to 3,000 mg/L. The COD removal measured up to 60% during the transition from batch to sequencing batch process, and it reached 80% during stable operation. A gradual development with time was observed for metabolic potential of the microbial community (the capability of the microbial community to destroy a given pollutant due to adaptation and syntrophic complementation of the metabolic pathways) because of regular feeding of the anaerobic bioreactors with fresh synthetic wastewater and stable biofilm formation. The most effective for removal of OM during CCP2 was LB, whereas the most effective in CCP3 was L. The COD values decreased to 1,000 to 1,500 mg/L during the second period, and they varied between 350 and 600 mg/L during the third period. In particular, COD in the effluent decreased to 350 mg/L for L, to 480 mg/L for LB, and to 590 mg/L for the control. The overall COD achieved during the second period was favorable for the further aerobic treatment of wastewater to reach the quality parameters complying with the national regulations for outfall into the receiving water body.

Low effectiveness of protein biodegradation (<20% during CCP1 and <10% during CCP2) and its accumulation in the system (Figure 2b) was observed during the first 2 periods. The degradation of whey proteins was difficult because of their lower bioavailability compared with that of carbohydrates, and because of the suppression effect of lactose on the synthesis of proteolytic enzymes (Yu and Fang, 2001; Demirel et al., 2005). An exception was for LB during the start-up period, when the effectiveness of protein biodegradation reached 50%. That high activity for LB probably resulted from the higher quantity of immobilized AnH. Our previous investigations revealed formation of active biofilm in the start-up period (Topalova et al., 2007). However, the presented results in the current study indicated that a period longer than 2 mo was required to establish a microbial community with stable metabolic activity. Protein removal during the stabilized sequencing batch process varied between 60 and 64%.

Lactose was biodegraded at a high rate, leading to its nearly complete removal from the synthetic wastewater (Figure 2c). The effectiveness of lactose removal during the start-up period exceeded 80%, whereas during stable biofilm development it was more than 97%.

The anaerobic degradation of OM takes place through reduction of various final acceptors of electrons, such as nitrate (in denitrification), organic molecules (in fermentations), or carbon dioxide (in methanogenesis).

The realization of these processes and the interrelations among them are determined by the local redox potential and the concentrations of protons and electron acceptors in the heterogeneous environment in the biofilters. Shen and Guiot (1996) noted that the possibility for a simultaneous course of strictly anaerobic (at redox potential between -0.520 and -0.600 V) and anoxic processes (at redox potential between $+0.110$ and -0.430 V) is due to the fact that the facultative anaerobic bacteria predominate in the outer layer, whereas oxygen-sensitive organisms inhabit the innermost zones of the biofilm or AS. The transformation of carbon and nitrogen compounds is in close relationship. The simultaneous removal of these 2 biogenes require C:N ratio to be 140:1 for anaerobic processes (Lettinga, 1995). The source of nitrogen in the model wastewater was dried whey, which introduces ammonium, nitrite, and nitrate in the system, as well as whey proteins. The initial 2 phases of protein biodegradation, hydrolysis and deamination of amino acids, increase ammonium concentration. However, decreasing concentration of ammonia was ascertained during the treatment process (Figure 3a). The most probable reasons are partial nitrification to nitrite and ammonium assimilation for new biomass formation. It is possible that part of the ammonium is removed by the biological process of anaerobic ammonium oxidation (ANAMMOX), where nitrite and ammonium are converted directly into dinitrogen gas (Fernández et al., 2008). The prerequisite for this hypothesis is heterogenic structure of biofilm and the possibility for formation of microzones with less organic concentration, which stimulated these processes. The trends in changes in nitrite and nitrate nitrogen contents during the 3 periods are presented in Figure 3. The decrease in nitrate indicates that a process of denitrification is taking place in the bioreactors. The nitrite concentration during the 3 periods was increased, which is logically related to accomplishment of the partial nitrification and denitrification where nitrite is a metabolic product. Nitrite and nitrate were still revealed in the medium, albeit in low concentrations: nitrite was, on average for the 5 variants, <0.04 mg/L, and nitrate was, on average, <0.16 mg/L. This finding indicated that nitrogen was undergoing further transformation in the experimental bioreactors, as affected by trace amounts of oxygen, and a certain level of nitrite and nitrate was still maintained sufficient to provide for the parallel development of diverse anaerobic processes. The ammonium concentration in effluent was high (around 80 mg/L). An aerobic reactor for additional decrease of ammonium to 35 mg/L is required for discharge into the sewage system to be in compliance with national regulations.

Figure 3. Trends in dynamics of ammonium nitrogen (a, b, c), nitrite nitrogen (d, e, f), and nitrate nitrogen (g, h, i) in period of start-up (a, d, g), instability of biofilm (b, e, h), and stable operation (c, f, i). The central line shows median, the box shows 25th and 75th percentiles, and the whiskers show 10th and 90th percentiles. LB = Laktazym + Bilikuk; L = Laktazym; HB = HydroPacks + Bilikuk; H = HydroPacks (EKO-PF, České Budějovice, Czech Republic); MWW = model wastewater.

Effect of the Initial Bioaugmentation on the Biodegradation Processes

The effect of microbial preparation on the effectiveness of the dairy wastewater treatment process was estimated, taking the values of the control variant as a reference (100%). The positive effect of microbial stimulation for COD removal was 4 to 8% during the initial period of biofilm formation, 25 to 49% during CCP2, and 3 to 10% during the stable sequencing batch process (Figure 4a). The applied microbial preparations increased protein biodegradation in the ASBB reactor by 8% for H and by 238% for LB at start-up, by 30 to 116% during CCP2, and by 4 to 5% during the stable ASBB reactor operation (Figure 4b). The variants with microbial preparations were less effective in lactose degradation compared with the control (Figure

4c). However, the 5 investigated biosystems as a whole were sufficiently effective for carbohydrate removal (Figure 2c). The obtained results confirmed the initial reactivity of the microbial preparations and their positive role for COD and protein removal. An important factor for increasing the effectiveness of biodegradation of the wastewater treatment process in conditions of biological instability was the enrichment of the ASBB reactor with selected and adapted active bacterial communities. The differences for the 5 variants during the stabilized sequencing batch process were insignificant, and the effect of the preparations was lower.

Microbial and Enzymological Parameters

The microbial count of suspended cells in CCP2 was much higher than that in CCP1 and CCP3. The transi-

tion from batch to sequencing batch process led to a partial destruction of the biofilm and to an increase of suspended cell numbers in the liquid phase (Figure 5a). Other researchers have reported similar results (Demirel and Yenigun, 2006). The lower biodegradation activity and the protein accumulation during the first 2 critical control processes (Figure 2b) confirmed that observation. The stabilization of microbial communities during CCP3 was related to a decrease of AnH counts in the water phase because of detention of microorganisms on the inert carrier.

The preparations were added with the aim of increasing the biodiversity in the community on a structural and a functional level. Although additional numbers of microorganisms entered the systems, what portion would become active and survive in the new environment was important, because microbial numbers are not the same as microbial activity. For example, the number of anaerobes in the control was greater (despite the smaller inoculum) compared with the number of suspended microorganisms in HB (Figure 6a) and with the immobilized microorganisms in H (Figure 6b).

The microbiological and enzymological parameters outlined some tendencies:

- (1) The highest metabolic activity of the suspended cells during the start-up period was observed for the variant LB (Figure 6a). This fact determined a much higher effectiveness of protein biodegradation in this variant (Figure 2b).
- (2) The quantity of AnH was highest during CCP2, but the biodegradation activity was lowest. The increase in numbers of AnH during the transition period was probably a compensatory mechanism to the decreased biological activity. However, this period was the most critical for the dairy wastewater treatment because of the suppressed microbial community and the accumulation of whey proteins (less than 10% was degraded).
- (3) The highest metabolic activity was observed during the stable biofilm development, when the count of immobilized AnH decreased sharply. The lowest quantity of ANH in the biofilm was registered for variant L, which had the highest biodegradation activity. The observed paradox between activity and microbial quantity was due to the high activity of the thin biofilms (Lazarova et al., 1992). Nikolov et al. (2005) suggested presence of highly active cells on the biofilm surface because of more favorable liquid flow and because of stable formation of synergetic consortia. The quantity of microorganisms in this consortium is lower but it possesses higher biodegradation activity.

Figure 4. Effect of commercial inocula on biodegradation effectiveness for (a) chemical oxygen demand (COD), (b) protein, and (c) lactose during period of start-up [critical control point (CCP) 1], instability of biofilm (CCP2), and stable operation (CCP3). Value of control variant is 100%. H = HydroPacks; HB = HydroPacks + Bilikuk; L = Laktazym; LB = Laktazym + Bilikuk (EKO-PF, České Budějovice, Czech Republic).

Figure 5. Anaerobic heterotrophs (AnH) in (a) water phase and (b) biofilm during period of start-up [critical control point (CCP) 1], instability of biofilm (CCP2), and stable operation (CCP3) (mean $\pm$ SD, $n = 3-6$). H = HydroPacks; HB = HydroPacks + Bilikuk; L = Laktazym; LB = Laktazym + Bilikuk (EKO-PF, České Budějovice, Czech Republic).

- (4) The highest dehydrogenase activity of suspended biomass also was registered during CCP3. The reason is that detached particles from the biofilm continue to be active zones in the biofilter (Nikolov et al., 2005). Thus, these 2 closely linked biosystems (suspended and immobilized cells) ensure high effectiveness of pollutant removal.

CONCLUSIONS

The most critical periods for dairy wastewater treatment were the initial period, when an effective biosys-

Figure 6. Dehydrogenase activity (DHA) of (a) suspended biomass and (b) biofilm in period of start-up [critical control point (CCP) 1], instability of biofilm (CCP2), and stable operation (CCP3) during anaerobic treatment process. H = HydroPacks; HB = HydroPacks + Bilikuk; L = Laktazym; LB = Laktazym + Bilikuk (EKO-PF, České Budějovice, Czech Republic).

tem (biofilm) was forming, and the biofilm's consequent development and stabilization in the ASBB reactor. At the same time, the positive effect of the accomplished microbial stimulation was most significant in those periods. The obtained data about effectiveness of OM biodegradation, microbial biomass, and DHA proved the successful development of a stable biofilm during the stabilized sequencing batch process. The differences among biodegradation effectiveness of the 5 variants were insignificant during that phase and the effect of

the preparations was low (about 3–10% for decreasing COD). The effectiveness of organic removal for the 5 bioaugmentation approaches reached 60% for protein, 70% for COD, and 97% for lactose. The obtained results showed that commercial inocula did not improve final reactor performance and their effect was insignificant over an inoculum from a municipal wastewater treatment plant alone.

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