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APPROACHES FOR ACCELERATION OF WASTEWATER TREATMENT IN THE DAIRY INDUSTRY

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Abstract

The application of complex ecological approaches in biological wastewater treatment, assessment of its effect on the effectiveness of the process and comparison of the obtained data to the restrictions in the environmental legislation are key elements of the study. The processes in an anaerobic sequencing batch biofilm reactor, the first module from diphase anaerobic-aerobic biotechnology for dairy wastewater treatment, are investigated. The creation of highly specialized biological system by means of complex approaches based on adaptation, immobilization and bioaugmentation, as well as purposely designed process control are the eco-innovation in this study. A model wastewater with whey (a waste product from cheese manufacture when technologies for it utilization are missing) was used. The main components of whey are proteins and lactose. The dynamics of organic matter concentration (measured as chemical oxygen demand – COD), concentration of proteins and lactose were investigated during the process.
The obtained results showed that the anaerobic process with immobilized biomass was appropriate for initial decrease of organics. The most effective approach for creation of active biofilm was the combination of purposely accomplished adaptation with immobilization because it increased with 67% protein biodegradation and with 5% COD removal. In the same time, that approach accelerated COD removal process from 135 hours to 14 hours and protein hydrolysis from 135 hours to 72 hours.

Keywords: anaerobic-aerobic technology, biofilm, dairy wastewater

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1. Introduction

Dairy industry is one of the large sources of industrial wastewater in Europe (Tikariha and Sahu, 2014). One European enterprise generates approximately 500 m^3 wastewater per day (Demirel et al., 2005). High quantity clean water is used in all activities of dairy enterprises, including for washing of equipment; for disinfection; for warming and cooling of tanks (Tikariha and Sahu, 2014). Dairy wastewater is characterized with high organic matter concentration, measured as biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD). COD values vary between 500 and 9200 mgO_2/l (Demirel et al., 2005; Sirianuntapiboon et al., 2005). An exception of that is wastewater generated from production of white and yellow cheeses when the whey is discharged in wastewater (Demirel et al., 2005; Karadag et al., 2015; Omil et al., 2003; Tikariha and Sahu, 2014). The COD values in these cases are many higher. A considerable part from organic matter concentration and nutrients in dairy wastewater is a result of milk losses and release of specific wastes from milk processing. Organic pollutants in dairy wastewater are carbohydrates (lactose), milk proteins (caseins), whey proteins (α-lactalbumin and ß-lactoglobulin), and fats (predominant are triglycerides of butyric, capric, lauric oleic acids, etc.). Main by-product from cheese production is whey, which is lactose and protein rich (Carvalho et al., 2013). The lack of technologies for whey utilization in small and medium enterprises led to significant increase of COD

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in influent (above 68000 mgO_2/L), inhibition of activated sludge and biofilm during aerobic wastewater treatment processes, pollution of water receivers (Carvalho et al., 2013; Demirel et al., 2005; Karadag et al., 2015; Omil et al., 2003; Tikariha and Sahu, 2014). Application of anaerobic technologies for treatment of wastewater contained whey is one of the possibilities for problem-solving (Carvalho et al., 2013; Demirel et al., 2005; Karadag et al., 2015; Ribeiro da Silva et al., 2018). Other possibilities are introduction of technologies for whey utilization and production of dry whey, whey protein isolates, lactose, biodegradable plastics on basis of polylactic acid (Belen et al., 2018; Carvalho et al., 2013; Oymaci and Altinkaya, 2016; Panesar et al., 2007), etc.

Anaerobic biodegradation of carbohydrates, proteins and fats is presented on Fig. 1 with some of obtained metabolites. The first stage of biotransformation includes hydrolysis of carbohydrates, proteins and fats by extracellular enzymes (galactosidases, proteases and lipases). Some authors highlight the important role of this initial process because its duration is rate-limiting (Carvalho et al., 2013; Haridas et al., 2005; Vidal et al., 2000). The increase of the hydrolysis rate could be successful tool for acceleration of whole wastewater treatment process. The rate of hydrolysis of milk and whey proteins, and of fats is lower than rate of carbohydrate hydrolysis (Vidal et al., 2000). The main factor for that is lower bioavailability of their molecules (Fiume et al., 2017). Obtained monomers (glucose, amino acids, fatty acids, glycerol) are degraded to different organic acids (formic, acetic, propionic, lactic, etc.), alcohols (methanol, ethanol), ketones (acetone) in the second stage, acidogenesis. The main metabolites, obtained during acetogenesis, are acetate, hydrogen and carbon dioxide. In the last stage, they are transformed to biogas. The milk and whey proteins are general source of nitrogen in dairy wastewater. This nutrient is presented as organic (proteins, amino acids, urea, nucleic acids) and inorganic (ammonium, nitrite and nitrate) compounds. The major fluctuations in the concentration of total Kjeldahl nitrogen (TKN) and of total phosphorus reported Demirel et al. (2005) and Omil et al. (2003). The TKN varies between 50 and 150 mg/l and the total phosphorus between 40 and 80 mg/l. In the whole, the phosphorous is presented as organic (caseins, phospholipids) and inorganic compounds (orthophosphates). The nitrates and phosphates in the wastewater showed considerable seasonal variations because milk quantity is higher during summer months. Using acidic and alkaline cleansing products (especially those based on nitrous and phosphoric acid) for the hygienization of the equipment and the rooms additionally influences the wastewater characteristics and results in significant variations of the water pH (Demirel et al., 2005; Singh et al., 2007).

The proteins and fats, basic components in the dairy wastewater, in high concentrations inhibit the biological system and decrease the effectiveness of the wastewater treatment processes (Vidal et al., 2000). They are adsorbed on biofilm and activated sludge; decrease the possibility for exchange of metabolites among the cells and the liquid phase, which results in decrease of the rate of biotransformation and biodegradation processes (Haridas et al., 2005; Vidal et al., 2000). These problems, as well as the slow start-up of the anaerobic processes require application of a unified, complex ecological approach for the management of the critical phases in the wastewater treatment using specialized biosystems.

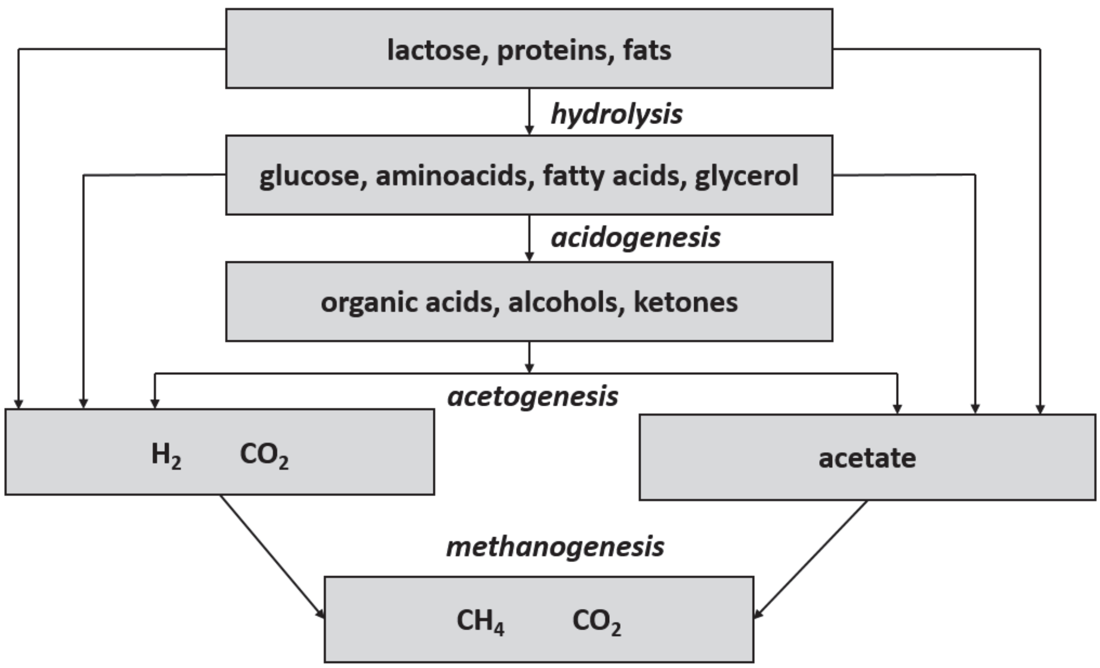

Fig. 1. Anaerobic biodegradation of main organic pollutants in dairy wastewater

In the scientific literature there are different possibilities regarding the creation of a highly specialized biological system (activated sludge or biofilm) such as: adaptation (Chen et al., 2008; Semrany et al., 2012; Sirianuntapiboon et al., 2005), immobilization (Semrany et al., 2012; Wang et al., 2005; Wijffels and Tramper, 1995; Zayed and Winter, 1998), addition of microorganisms (Chauchan and Dikshit, 2017; Foglar et al., 2005; Herrero and Stuckey, 2015; Loperena et al., 2006; Loperena et al., 2007; Loperena et al., 2009; Mongkolthanaruk and Dharmsthiti, 2002; Semrany et al., 2012), addition of enzymes as bioaugmentative factor (Dharmsthiti and Kuhasuntisuk, 1998; Leal et al., 2002; Leal et al., 2006; Mendes et al., 2006), but an overall strategy is missing. An example for eco-innovation in the creation of a highly specialized biofilm is the application of complex approaches, which include adaptation, immobilization and addition of specialized microorganisms. Missing biological methods for control of the anaerobic technologies is other critical problem and some new methods on basis of fluorescent analysis of co-factor F_{420} and methanogenic microorganisms are discussed in our investigations (Dinova et al., 2018a; Dinova et al., 2018b).

In Bulgaria, despite the intensive building of local wastewater treatment plants (WWTPs) during the period of joining the European Union and the introduction of the environmental management systems, there are still enterprises without WWTP that discharge wastewater in water receivers. The problems that have been observed in the building of local WWTPs to the dairy enterprises in Bulgaria were two. First, the big enterprises, which process over 200 tons milk daily don't have WWTPs and discharge in the municipal sewage. That was related to lesser financial expenses for fees and penalties in comparison to investment expenses for building of WWTP. Another more important reason is the location of some of these big enterprises. They are located in residential neighborhoods in the towns and the free area around the enterprises is limited. The second problem was that the small and medium enterprises from the sector introduce technologies, which are not in conformity with the content of the wastewater, namely aerobic reactors. It is necessary to offer new, more ecological solutions in conformity with the emerged situation. They must correspond to one of basic tendencies in the field of Environmental Biotechnology, including pollution prevention by means of introduction of the best available and environmental friendly technologies (Gavrilescu, 2010). The aim of the present article is to compare the effect of the ecological approaches: i/ adaptation with immobilization and ii/ adaptation, immobilization with bioaugmentation on the biodegradation effectiveness and on the acceleration of processes in the created anaerobic module – type biofilter for dairy wastewater treatment. The questions are: "Is appropriate this module for small and medium dairy enterprises with local WWTPs on base of aerobic reactors which treat influent with high organics concentration (COD above 1000 mgO_2/L)? Is it possible to use as a pretreatment step in these local WWTPs for initial decrease of pollutants? Which ecological approach is more applicable for acceleration of biodegradation processes and for increase of its effectiveness?".

2. Materials and methods

2.1. Anaerobic sequencing batch biofilm reactor and model wastewater

The volume of anaerobic sequencing batch biofilm (ASBB) reactors was 0.5l and gravel carrier (with particle size between 8 and 16 mm) was used. Initially, the inert carrier was treated at 160 ^oC for sterilization. The anaerobic reactors operated for 282 days in sequencing batch regime because the whole volume was removed and replaced with fresh wastewater. The feeding cycle with fresh wastewater was 24±2 hours. The ASBB reactors were placed under dark conditions in thermostat at 28±2^{o}C.

A model wastewater was used for feeding of ASBB reactors. It contained 3.65 g/L dry whey and mineral medium, described from Loperena et al. (2006). The content of used model wastewater and it general characteristics were previously described (Schneider and Topalova, 2011).

2.2. Inocula for ASBB reactors

The specially treated and acclimated real activated sludge (AS) from the sludge thickener of Municipal Wastewater Treatment Plant of Sofia City (Bulgaria) was used as an inoculation material for biofilters. Detailed information about pre-treatment and adaptation procedure was presented in our previous published study (Schneider and Topalova, 2013). This AS was immobilized on carrier and used for investigation of the effect of the applied ecological approach of adaptation with immobilization on the biodegradation processes. The second approach (adaptation, immobilization with bioaugmentation) investigated biodegradation effectiveness of inocula, including the same AS, enriched with microbial preparations Laktazym and BiliKuk (Brave & Brave Ltd., Czech Republic). These commercial products contain high quantity of aerobic and anaerobic heterotrophic microorganisms with biodegradation activity to target pollutants in food processing wastewater (fats, proteins and carbohydrates).

The ASBB reactors were inoculated with three different biological systems. The inoculum for investigation of effect of adaptation with immobilization included as dry weight 10 g/L AS (Control). The inoculation materials for investigation of the effect of the combination adaptation, immobilization with bioaugmentation were two types. One of them included as dry weight 10 g/L AS, enriched with 3 g/L preparation Laktazym (L), and another included 10 g/L AS, enriched with 1.5 g/L Laktazym and 1.5 ml/l BiliKuk (LB).

2.3. Analytical methods and biodegradation effectiveness

COD, ammonium and phosphate concentrations were determined by means of standardized methods (APHA, 2012). The protein concentration was analyzed according to Kochetov (1974) and the lactose concentration was measured according to method, described from Miller (1959).

The effectiveness of organics biodegradation (Eff) was calculated by (Eq. 1):

$$Eff = \frac{Ct_1 - Ct_2}{Ct_1} \cdot 100 \quad [\%] \qquad (1)$$

where: Ct_1 is the organics concentration at the moment t_1 and Ct_2 is the organics concentration 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.

3. Results and discussion

3.1. Ecological approaches for acceleration of dairy wastewater treatment

The studied anaerobic module, which can be offered for small and medium dairy enterprises as a part of a diphase anaerobic-aerobic technology, requires a specialized biological system, adapted to the relevant parameters of the process. Three biological systems were studied, which are used for inoculation of the anaerobic biofilter: 1) activated sludge without adaptation; 2) activated sludge, acclimated to the model wastewater and immobilized on an inert carrier; 3) acclimated activated sludge, immobilized on the inert carrier, to which the microbiological preparations Laktazym and BiliKuk were added.

The used activated sludge without preliminary adaptation degraded 10% from the whey proteins (key pollutant from the milk processing with the lack of technologies for whey utilization) and 94% from the lactose for 135 hours (Fig. 2b, 2c). Respectively the decrease of COD for 135 hours was 20% (Fig. 2a). The same activated sludge (used as inoculation material) after adaptation to the model wastewater and immobilization on an inert carrier, degraded 77% of the proteins for 72 hours and 87% of the lactose for 14 hours. Respectively the decrease of COD was 25% for 14 hours. The initial addition of microbiological preparations to inoculum resulted in an increase of the effectiveness of protein biodegradation by 9%, i.e. 86% from the proteins were hydrolyzed for 72 hours. Respectively the lactose biodegradation was increased by 3% and the COD removal was increased by 10%, which for 14 hours was 35%.

The obtained results showed that the applied ecological approach, including a simple adaptation procedure of AS (as inoculation material), its immobilization on an inert carrier and its enrichment with microbiological preparations were an effective working mechanism for accelerating the wastewater treatment process. The preliminary adaptation is proved as a successful procedure regarding the acceleration of the protein hydrolysis (Fig.2b). It is found that the biodegradation time of the proteins was reduced from 135 hours to 72 hours. As it is seen from the data for the variant containing only acclimated inoculum, this was related above all with the adaptation procedure and not with the addition of the preparations. The reason was probably the shorter period of the lactose utilization (Fig. 2c) and the quicker activation of the proteases. Our results supported the claim of Vidal et al. (2000) and Semrany et al. (2012) that the acclimated biological system has a higher metabolic activity in comparison to the non-acclimated because of the shorter lag phase. Another positive factor for the acceleration of the process, besides the applied adaptation, was the immobilization.

It increased the stability of the system and created a greater variety of conditions and metabolic pathways for the organic matter biodegradation. As Zayed and Winter (1998) reported, the immobilized cells are more active and are preserved alive for a much longer period of time. Besides, biofilm is characterized with higher metabolic activity because of the concentration of substrates, biomass and enzymes on the inert carrier; higher stability towards unfavorable environmental conditions and quicker recovery after periods of stress.

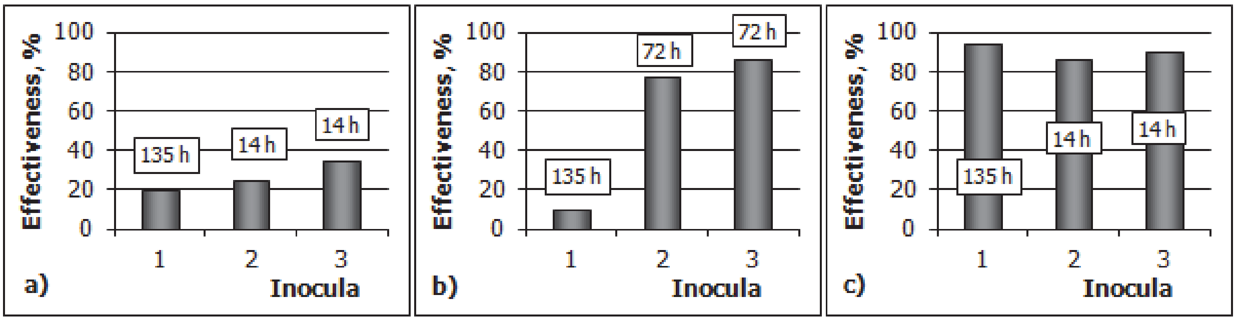

Fig. 2. Effectiveness of: a) COD removal; b) protein biodegradation and c) lactose biodegradation for different inocula (1- AS without adaptation; 2- acclimated AS with addition of inert material; 3- acclimated AS with addition of inert material and microbiological preparations BiliKuk and Laktazym)

The retention of slow growing microorganisms as methanogens in biofilm structure was more effective in comparison to the processes with suspended biomass (Simeonov et al., 2010). The obtained results showed that the applied approach of two-stage microbial stimulation (adaptation with immobilization) was suitable for the acceleration of the anaerobic biofilm formation and its activation. The applied three-stage microbial stimulation (adaptation with immobilization and bioaugmentation) also was suitable for the acceleration of the anaerobic biofilm formation but it required more financial resources. The applied approaches could help to overcome the initial critical phases while starting the wastewater treatment processes in the anaerobic biofilter.

3.2. Dynamics of key chemical indicators during the simulated semi-continuous wastewater treatment process

The dynamics of the key parameters has been followed during an anaerobic semi-continuous wastewater treatment process for study of the duration of the effect from the added preparations. Three biological systems were used - control, L and LB. The changes in the concentrations of the key pollutants were presented with the change in their removal effectiveness (Fig.3, Fig.4 and Fig.5). The obtained results confirmed several leading tendencies. The semi-continuous process was accomplished in two phases. The early phase was ascertained during the first feedings (until the 85th day) of the ASBB reactor and it was critical for the recovery of the biodegradation activity in the transition from batch to semi-continuous process. The late phase was differentiated between the 85th and the 282nd day and in the course of it the biofilter operated steadily with high effectiveness. The dynamics of the key parameters during the anaerobic process was similar for all the three studied biological systems.

COD in the influent varied between 3514-4843 mgO_2/L. The effectiveness of the organic matter biodegradation in the transition to semi-continuous process was decreased with 28% for LB, with 10% for the control and with 2% for L. The combination of AS (as inoculum) and the preparations Laktazym and BiliKuk was the most effective for the dairy wastewater treatment at the early phase of the process, as the effectiveness of this biological system reached 70% on COD. A more long-term study showed however, that this variant was gradually replaced by the variant with Laktazym (Fig.3b). COD removal effectiveness reached to 90% at the end of the process. Our results were similar to these of Patil et al. (2012), which treated whey in anaerobic up-flow packed bed reactor.

The protein concentration in the influent varied between 0.83 and 1.7 g/L. The effectiveness of the protein biodegradation was low at the beginning of the process. The obtained values were negative and varied between 0 and - 40% (Fig.4b). With the advance of the process, after the 85th day, the protein biodegradation effectiveness was increased and the variations were between 40% and 80%. The variant with the preparation Laktazym there was the highest biodegradation activity**.**

The lactose concentration in influent varied between 2.5 and 3.2 g/l (Fig. 5). Its concentration in the effluent decreased below 0.1 g/L as a result of the microbial utilization. The three studied variants showed high effectiveness in the lactose removal, with a stable value above 95% (Fig.5b). The only exception was at the beginning of the semi-continuous process, when there was a decrease to 60-80%.

The decreased effectiveness of COD removal in the early phase of the semi-continuous process was related to the proteins accumulation (Fig.4a), as well as to a decreased effectiveness of lactose biodegradation (Fig.5a). On one hand, the accumulation of proteins was related to the restructuring of the microbial communities in the biofilter in this early phase, with the death of part of the organisms and the lysis of the cell structures, during which additional proteins were released in the water phase. On other hand, as it is seen from Fig. 5b, the lactose assimilation was incomplete (between 64% and 80%), which inhibits the synthesis of proteolytic enzymes.

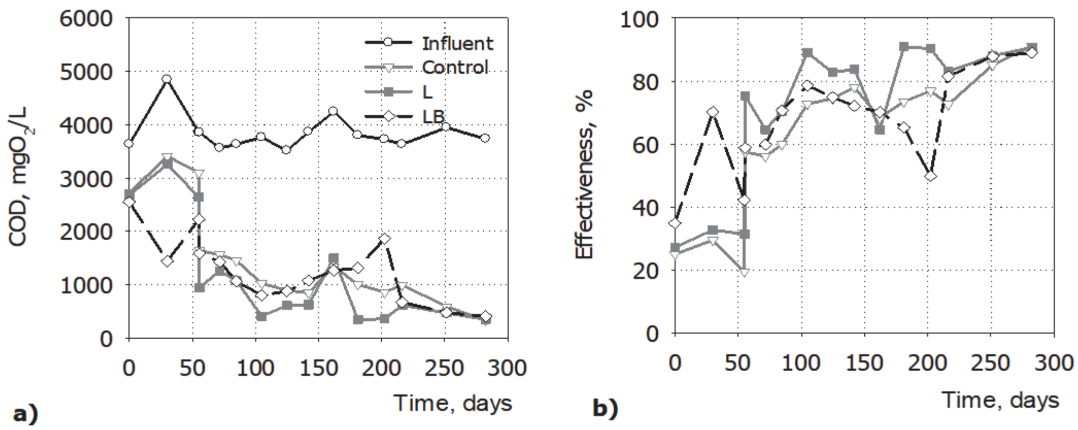

Fig. 3. Dynamics of a) the organic matter concentration (measured as COD) and b) the effectiveness of COD removal from the control, L and LB during the process (mean ± SD, n=3)

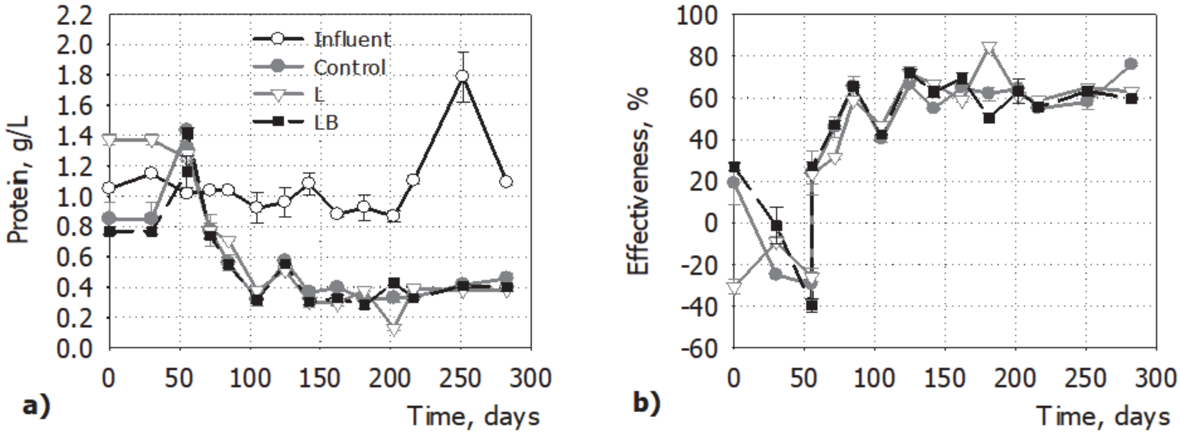

Fig. 4. Dynamics of a) the protein concentration and b) effectiveness of its removal from the control variant, L and LB during the process (mean ± SD, n=3)

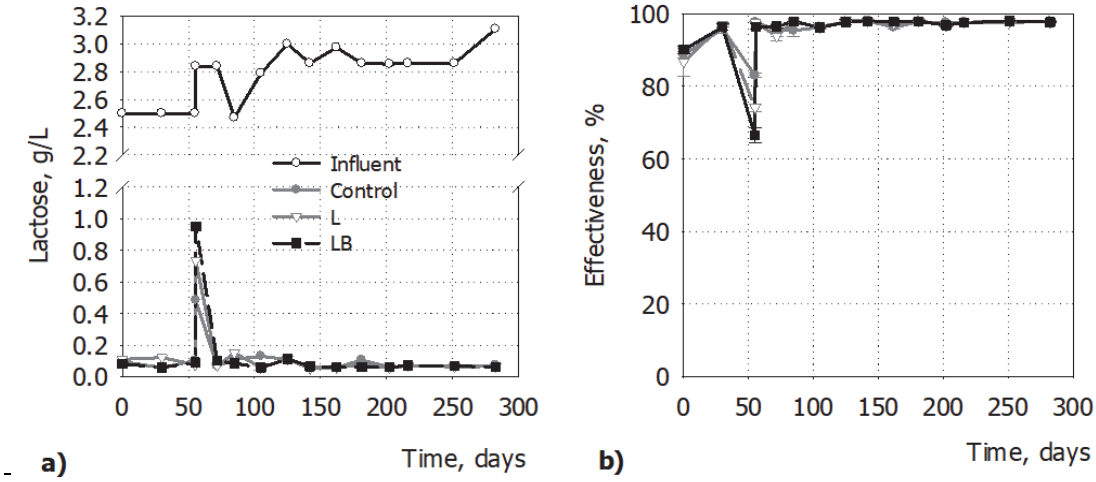

Fig. 5. Dynamics of a) the lactose concentration and b) effectiveness of its biodegradation for the control, L and LB during the process (mean ± SD, n=3)

There was a necessity of a short period for adaptation of the microbial communities to the changes of the conditions in the bioreactor. The increase of the organics removal effectiveness during the process showed a gradual stabilization in the functioning of the biofilter and the activity of the biofilm. After the 85th day, a long-lasting increase of the effectiveness was established and at the end of the semi-continuous process was registered a 90% decrease of COD.

The obtained data were similar to those of Loperena et al. (2007), where such as inoculation material only the microbiological preparation Sybron Bi-Chem 1003FG has been used. The effectiveness of the organic matter removal during the process reached up to 89%, while COD from 1000 mgO_2/L was decreased to 283 mgO_2/L on the average. The minimal measured value of COD in the effluent from the biofilter in our study reached to 350 mgO_2/l in the L variant. Other two variants had lower effectiveness and a decrease of COD to 590 mgO_2/L for the control and to 480 mgO_2/L for LB was ascertained. The application of anaerobic wastewater treatment process and higher organics concentration in influent in our study (between 3514 and 4843 mgO_2/L) led to higher COD values in effluent. In comparison to us, Loperena et al. (2007) used wastewater with lower COD (1000 mgO_2/L) and investigated dairy wastewater treatment under aerobic conditions which led to more complete organics biodegradation. As a whole the reported values in our study favor the further treatment of the wastewater under aerobic conditions and further decrease of COD.

The obtained data for the semi-continuous process as a whole were close to those obtained from Yu and Fang (2001) for anaerobic activated sludge and model dairy wastewater. COD of the model wastewater was 4000 mgO_2/L. The reported effectiveness of COD decrease was above 92%, of the proteins was 80% and of the lactose was 98%. In our study such values were reached in stable functioning of the biofilm (the late phase of the process).

Loperena et al. (2009) investigated a mixed culture from *Bacillus pumilis*, *Pseudomonas sp.* and *Acinetobacter lwofii*. The three bacteria were isolated from bioreactor for dairy wastewater treatment. The biodegradation effectiveness of this mixed culture was compared to effectiveness of commercial microbiological preparation (Sybron Bi-Chem 1003FG). The effectiveness of COD decrease varied in close range, for mixed culture was 57% and for preparation was 63%. The mixed culture hydrolyzed 93% from proteins and 72% from fats while the microbiological preparation hydrolyzed 54% from proteins and 38% from fats. Also, differences between both biological systems were ascertained for lactose hydrolysis. The commercial preparation removed 99% from lactose for 48 hours and the mixed culture hydrolyzed 7% from milk sugar. In our study COD removal effectiveness reached to 90% (Fig. 3b), protein removal reached to 80% (Fig. 4b) and 95% from lactose was hydrolyzed (Fig. 5b) at the late phase of the process. The concentration of fats was not investigated because we used model wastewater with whey, which contained whey proteins and lactose. Results reported by Loperena et al. (2009) showed that usage of autochthonous mixed culture had higher stimulating effect for biodegradation of target pollutants (proteins and fats) than allochthonous commercial preparation. Semrany et al. (2012) explained this result with the necessity of acclimation of exogenous microorganisms to operational conditions (pH, temperature, redox potential, organic load, specific pollutants) and biotic relationships (competitions between indigenous and exogenous microorganisms for organics and nutrients, predatory press from protozoan community, partially inclusion of exogenous microorganisms in structure of activated sludge and biofilm).

The application of three-stage microbial stimulation, adaptation with immobilization and bioaugmentation with microbiological preparation Laktazym, had a stable and long-lasting effect on the wastewater treatment process. The presence of an inert carrier and the immobilization of the specialized inoculum helped to overcome the washing effect, reported by Loperena et al. (2007), and also helped to achieve a higher biodegradation activity in the studied ASBB reactor.

The obtained results for the three experimental variants showed that despite the similar character of the processes they differed by their intensity and effectiveness. This was a result from the differences in the structure of the biological system, which included a constant component (the acclimated activated sludge as inoculation material) and a variable component (different combinations from the microbiological preparations).

3.3. Comparison of the obtained results for the effluent from the ASBB reactor with the European laws

The obtained values for COD in the effluent allow discharge in the municipal sewage without a WWTP according to GR (2000b) but do not allow discharge in a water receiver according to GR (2000a). The concentration of the ammonia nitrogen was from three to five times higher than emission standards for discharge in a municipal sewage, while the concentration of the phosphate phosphorus was about seven times lower.

The comparison of the obtained results for the effluent with the restrictions in the ecological legislation (Table 1) confirmed that to create a completed technology (without risk for the waters and the sediments in the water receivers) it is necessary to combine the anaerobic module with an aerobic one to further lowering the pollutants concentration to the norms for discharge in a water receiver. Dairy wastewater is rich in proteins (caseins, α-lactoalbumin, β-lactoglobulin), during the degradation of which high concentrations of nitrogen are released. The pollution control in this case is based on systems for prevention of their release in the environment and for their removal from the wastewater. The concentration of the ammonia ions in the used wastewater exceeds 200 mg/L. The ammonia nitrogen decreases from 190 mg/L to 111-163 mg/L for different variants during anaerobic wastewater treatment process. It was related to anabolic processes of synthesis of new biomass. The ammonia nitrogen concentration was found that after the ASBB reactor exceeds from three to five times emission standards for discharge in the sewage (Table 1), which imposes the necessity of an aerobic bioreactor for the complete removal of the ammonia nitrogen.

Table 1. Values of key parameters in influent and effluent at the end of the process and emission standards for dairy wastewater discharged in a water receiver or a municipal sewage

Parameters	*Influent*	*Effluent*	*Emission standards for industrial effluent discharged in:*		
			water receiver GR, (2000a)	*sewage without municipal WWTP GR, (2000b)*	*sewage with municipal WWTP GR, (2000b)*
COD, mgO_2/L	3801	587 for C 472 for L 477 for LB	250	700	*[a]
NH_3-N, mg/L	190	135 for C 111 for L 163 for LB	-[b]	35	35
phosphate (as phosphorus), mg/L	1.06	2.44 for C 2.34 for L 1.9 for LB	-[b]	15	15

[a] *The emission standards are determined for each case depending on the capacity and loading of municipal WWTP*
[b] *no data*

The biological removal of the phosphorus from the wastewater is based on the potential of some bacteria (*Acinetobacter sp., Pseudomonas sp.* etc.) to accumulate the excessive phosphorus, regardless of their metabolic needs (Brdjanovic et al., 1998). Under anaerobic conditions in the reactor, these bacteria accumulate (as polyhydroxyalkanoates) the organic acids, released as a result of the activity of the fermentative microorganisms and release phosphates in the water (Wagner and Loy, 2002). Under aerobic conditions, the bacteria accumulate phosphates as organic polyphosphates in their cells. Higher rate of the release of phosphates under anaerobic conditions determines the higher rate of the accumulation of polyphosphates under aerobic conditions (Brdjanovic et al., 1998). In our case, the obtained results regarding the anaerobic phase confirmed the release of phosphates in the water, since the concentration of the phosphate phosphorus increased to approximately 2 times (Table 1). Despite that the concentration of the phosphate phosphorus was about 7 times lower in comparison to the emission standards for discharge in the sewage system.

4. Conclusions

The obtained data leads to the following conclusions:

1. The combination of adaptation and immobilization significantly accelerated the wastewater treatment process and increased the biodegradation activity of the biological system. The application of this combination was more effective and financially more affordable than the application of bioaugmentation, which requires the purchase and addition of microbiological preparations.
2. In all the three studied biological systems of ASBB reactor the organic matter /measured as COD/ was decreased to values below 1000 mgO_2/L, which are favorable for the following aerobic treatment and discharge in a water receiver.

In Bulgaria during the last years local WWTPs were built to the small and medium dairy processing enterprises. They were based on aerobic sequencing batch reactors, which are not suitable for wastewater with high organic matter concentration. The effectiveness of the WWTP could be increased by offering a partial innovation or the so-called innovation by adding a new component /for minimizing the negative effect on the environment without changing the whole system/. This innovation includes transformation of aerobic equipment in two bioreactors first anaerobic biofilter and second aerobic biobasin. The introduction of an inert material with the possibility of biofilm development in the first anaerobic reactor has to be accomplished.

Our results undoubtedly confirmed: i/ the ASBB reactor as a first module of diphase anaerobic-aerobic technology, is appropriate and possible for dairy industry; ii/ the improvement of this technology by the proved ecological approach /adaptation with immobilization/ for the regulation of the anaerobic biofilm functioning is an applicable mechanism for the management of the critical phases in the wastewater treatment.

Acknowledgments

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