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Bioaugmentative Approaches for Dairy Wastewater Treatment

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Abstract: Problem statement: The achievement of a good ecological status of water receivers after discharge of waste or partially treated water from dairy industry requires harmonic interaction between water treatment technology and self-purification processes. **Approach:** The present research included two modules. First: an anaerobic treatment process for dairy wastewater in broadly spread sequencing batch bioreactor with fixed biomass was studied. As a source of active biological system specially treated and acclimated activated sludge from Sofia Wastewater Treatment Plant was used. The acclimation and immobilization of initially inoculated biomass, the addition of microbiological preparations and its modification for increase of the biodegradation activity to target pollutants were studied as opportunities for the stimulation of water treatment process in bioreactors and water receiver. Second: self-purification processes in a water receiver for partially treated dairy wastewater were investigated. The functional role and restructuring of the microbial communities in the water, sediment water and sediments were studied. **Results:** The results showed that the most important approaches for achieving high effectiveness of wastewater treatment process were both the acclimation and immobilization of biomass. In that aspect the data for the water receiver confirmed this conclusion. These two processes increased biodegradation effectiveness of the target pollutant (protein) with 67%. **Conclusion:** The effect of the added preparations was smaller (protein biodegradation was increased to 9% for the different biological systems). It was thoroughly related to low improvement of the rate of metabolism and functioning of the biological system mainly on an enzyme level.

Key words: Acclimation, immobilization, bioaugmentation, microbiological preparations, biofilm

INTRODUCTION

In general the problems of the dairy wastewater treatment in Bulgaria are related to: (i) The increase of the working capacity of the plants but not the one of the purification facilities; (ii) The choice of inappropriate technologies for treating the wastewaters which in real practice most often is carried out in aerobic conditions; (iii) Lack of technologies for utilizing the waste products including the whey; (iv) High concentration of organic matter and biogens in the wastewater. A topical issue is also the one of the big dairy processing plants, most of which still don't have purification installations and discharge the wastewater in the city sewerage. The outlined problems mainly in a technological aspect lead to severe ecological problems related to pollution of the water receivers. A combined management of the water resources is necessary (European Parliament and Council, 2000). On the one hand it is achieved by increasing the control at the source of pollution by introducing the best technologies available (when combining the best production and wastewater treatment practices in the processing plants). On the other hand it is achieved also by introducing limiting values for the concentration of the pollutants of the specific water body conformed to its self-purification potential.

In the aspects outlined above it is necessary to model an anaerobic treatment process with its maximum approximation to the real parameters of the wastewater and to the real requirements of the practice-creation of an anaerobic module with a specialized biological system. By means of applying the theory of Total Quality Management (via system process control and research of the control points) it is important to differentiate the risky moments in the modeling process (Horan, 1996). Examples of these are the continuing process of creating and activating the highly specialized biological systems, activated sludge and biofilm (Cresson *et al*., 2006; Demirel and

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Yenigun, 2006; Liu *et al*., 2002; Punal *et al*., 2000); the decreased biodegradation activity to the target pollutant (Dharmsthiti and Kuhasuntisuk, 1998; Demirel *et al*., 2005; Leal *et al*., 2002; Loperena *et al*., 2009; Lotrakul and Dharmsthiti, 1997); the destabilization of the biosystem (Cresson *et al*., 2006; Punal *et al*., 2000; Omil *et al*., 2003; Rajeshwari *et al*., 2000; Viraraghavan and Varadarajan, 1996). Development of active biofilm is a rate-limiting process and many scientific reports are related to factors that accelerate this process (Cresson *et al*., 2006; Demirel and Yenigun, 2006; Liu *et al*., 2002; Punal *et al*., 2000; Meier-Schneiders *et al*., 1993). The starting span is defined by the time needed for formation of the biofilms' compound trophic structure (including the complexes of microflora, microfauna and metafauna), for establishing of interrelations among the components and for their "synchronizing". The time-consuming growth of the anaerobic microorganisms and its greater sensitivity to the environmental factors also slow down the start-up of the process. Destabilization and inhibition of biofilm is risky moment in wastewater treatment with fixed biomass. This critical situation is result of sharp fluctuations in pollutant concentrations (alternation of high with low concentrations). This fact is more valid for small enterprises (around 40% from dairy industry in Bulgaria) which have inconstant business activity per day and haven't equalizer tank. The problem is that anaerobic wastewater treatment requires high concentration of organic matter (COD more than 1000 mg L^{-1}) while the less organic matter concentrations lead to starvation, endogenous respiration, accumulation of toxic metabolites and biofilm destabilization. For example, under aerobic conditions substrate concentration below which there is no biofilm growth (S_{min}) varies between 0.1 and 1 mg L^{-1}. The S_{min} under anaerobic conditions is much higher and ranges from 3.3-67 mg L^{-1} (Bitton, 2005).

Different opportunities for acceleration and improvement of effectiveness of wastewater treatment process as acclimation (Chen *et al*., 2008; Sirianuntapiboon *et al*., 2005), immobilization (Muyima and Cloete, 1995; Wang *et al*., 2005; Zayed and Winter, 1998), addition of alochtonic microorganisms (Loperena *et al*., 2006; 2007; 2009), addition of enzymes (Dharmsthiti and Kuhasuntisuk, 1998; Leal *et al*., 2002; 2006; Lotrakul and Dharmsthiti, 1997; Mendes *et al*., 2006) were reported in the scientific literature. However, an entire integral strategy for decision of that problem is missing. In these aspects, it is necessary to develop a complete strategy for overcoming of critical situations in dairy wastewater treatment. The purpose of the present article was by application of specific bioaugmentatative approaches, based on acclimation, immobilization and addition of microorganisms, to develop working solutions for overcoming the critical situations. Their application in practice would provide for a higher degree of prevention of pollution in the water sources-receivers of wastewaters from dairy industry. The response of the microbial communities in the water receiver to the chronic pollution with partially treated wastewaters from dairy industry was studied in parallel. The purpose was to determine if the self-purification potential is a sufficient condition for restoring the ecological state of the river.

MATERIALS AND METHODS

Experimental design:

Bioreactors and processes: Two critical situations during anaerobic treatment of dairy wastewater in a reactor with fixed biomass were studied-initially biofilm activation and its destabilization as a result from one monthly period of starvation. A sequencing batch reactor (diameter 75 mm and height 110 mm) with fixed biomass and gravel bed carrier (diameter 8-16 mm) was used. The reactors were sealed airtight and placed in a thermostat at 28-30°C. Thirty reactors for first critical situation were used in every variant of biological system-six repetitions of each treatment. The experiments for the second critical situation were accomplished in triplicate. The feeding mode during two experiments was batch. The obtained data for first situation was discussed at 72nd h and for second situation at 14th h. The model wastewater contained dried whey-3.65 g L^{-1} (P.I.C. Co Ltd., Bulgaria) as a key pollutant. The mineral composition of model wastewater was prepared according to Loperena *et al.* (2006). It contained (in g L^{-1}): NH_4Cl 0.57, KH_2PO_4 0.43, K_2HPO_4 1.09 and Na_2HPO_4 1.33, $MgSO_4.7H_2O$ 0.023, $CaCl_2$ 0.028 and $FeCl_3.6H_2O$ 0.025. The concentration of whey corresponded to COD values of about 4,000 mgO L^{-1}, because COD of dairy waste effluents from full-scale operations varies between 500, 9,200 and or 3,811 mg L^{-1} on average (Demirel *et al*., 2005).

An activated sludge from a sludge thickener of Sofia Wastewater Treatment Plant (Schneider and Topalova, 2009a; Topalova *et al*., 2007), microbiological preparations HydroPacks, Laktazym and BiliKuk (Schneider and Topalova, 2008; 2009a; Topalova *et al.*, 2007) and two pure microbial cultures (Schneider and Topalova, 2008) were used for inoculation. The inoculum amount was calculated to reach 10% (v/v), which is peculiar for anaerobic processes because of the lower growth rate of those microorganisms. The commercial preparations designed for fat, protein and carbohydrate biodegradation were supplied by Brave and Brave Ltd. (Czech Republic).

Leva River as a water receiver: A chronically polluted receiver (Leva River, NW Bulgaria) with dairy wastewater during the critical period of summer low water was studied (Schneider and Topalova, 2009b; 2009c). In the river was discharged wastewater from the dairy processing plant “Milk paradise 99” Ltd. (Bulgaria), which produces yoghurt and milk, cheese, curds, yellow cheese and butter. The production capacity of the plant is up to 100 t day^{-1}. The preliminary processing of the wastewater includes a grease trap. The functional role and restructuring of the microbial communities in water, sediment water and sediments after dairy wastewater discharge were investigated. The five river sampling stations are shown on Fig. 1. Station 1 is located before dairy wastewater discharge from “Milk paradise 99” Ltd. Station 1a is the zone of mixture between river and dairy wastewater. Station 2, 3 and 4 are located 5, 50 and 100 m after the mixture between the river water and dairy wastewater, respectively.

Analytical methods: The Chemical Oxygen Demand (COD) (Eaton and Franson, 2005), concentration of lactose (Miller, 1959) and protein (Kochetov, 1980) were determined. The number of Aerobic (AH) and Anaerobic Heterotrophs (AnH), Denitrifiers (DN) and anaerobic spore-forming bacteria (AnS) in commercial preparations and the quantity of AH in the water receiver were counted by plate techniques (Kuznetzov and Dubinina, 1989).

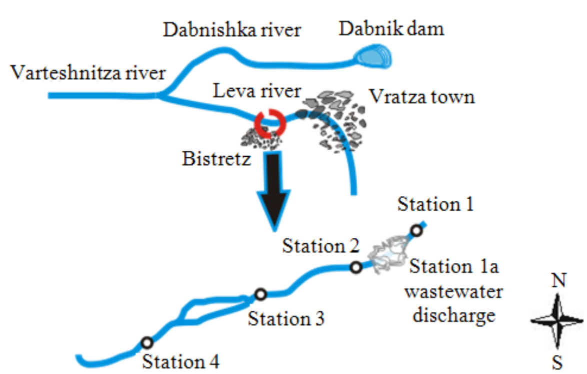


Fig. 1: Location of sampling stations in the water receiver-Leva river

Anaerobic microbial groups were incubated in anaerobic jars (Merck and Co., Inc.,) with Anaerocult A (Merck and Co., Inc.) for creation of anaerobic conditions. The immobilized biomass for microbiological analyses was detached from preparation bran and sediment particles by ultra sonic disintegration with UD-20 automatic (3×5 sec with frequency 22 kHz and vibration amplitude 8 µm) in saline solution. An indicator for the total rate of the cell metabolism as dehydrogenase activity was used (Gabbita and Huang, 1984). Nitrate-Reductase Activity (NRA) as an indicator for denitrification potential was measured according to Kochetov (1980) method. The quantity of biomass for determination of specific enzyme activity was measured as concentration of cell protein (Kochetov, 1980). The preliminary processing of the samples for Scanning Electronic Microscope (SEM) was carried out after the Herbert and Chui (1993) method and the microscope preparations were treated with golden dust.

RESULTS

Microbiological and enzymological analysis of commercial preparations: The quantity of microorganisms in preparation Laktazym was higher by two to three orders in comparison to these in HydroPacks and BiliKuk (Fig. 2a). The higher number of anaerobic heterotrophs in preparations BiliKuk and Laktazym assumes that its combination will increase the treatment effectiveness. The quantity of denitrifiers on the other hand was more in HydroPacks and Laktazym than in BiliKuk. The combination of lyophilized preparations with liquid preparation could ensure higher denitrification rate for nitrogen removal from wastewater.

The three preparations showed high dehydrogenase activity-DHA (Fig. 2b). The activities of BiliKuk and Laktazym were with similar values and were three times higher in comparison to HydroPacks. Nitrate Reductase Activity (NRA) of HydroPacks was two times higher than the activity of Laktazym. The intentional combination of the three microbiological preparations could ensure higher treatment effectiveness and process acceleration.

The preparations contain anaerobic spore-forming bacteria (Fig. 2a). SEM photographs of microbial preparations HydroPacks and Laktazym are shown on Fig. 3. A particle from the preparation and attached spores were observed.

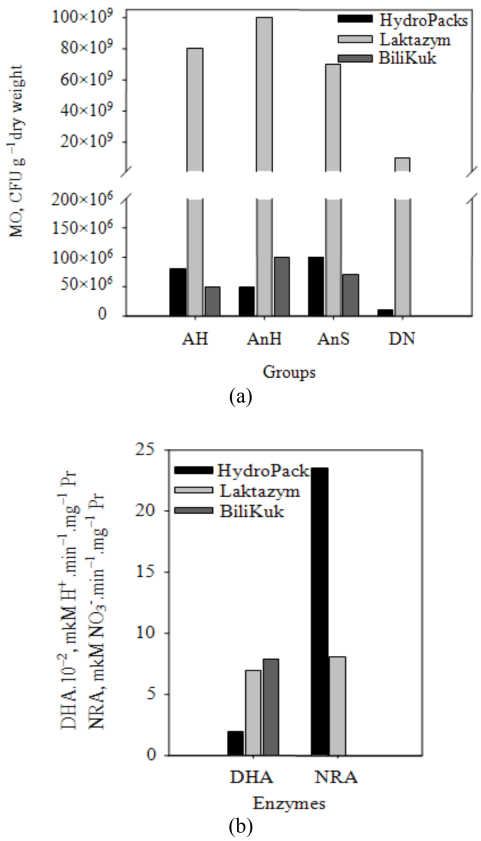


Fig. 2: Characterization of commercial preparations HydroPacks, Laktazym and BiliKuk by: (a) microbiological (aerobic heterotrophs-AH, anaerobic heterotrophs-AnH, anaerobic spore-forming bacteria-AnS, denitrifiers-DN) and (b) enzymological (dehydrogenase activity-DHA, nitrate-reductase activity-NRA) parameters

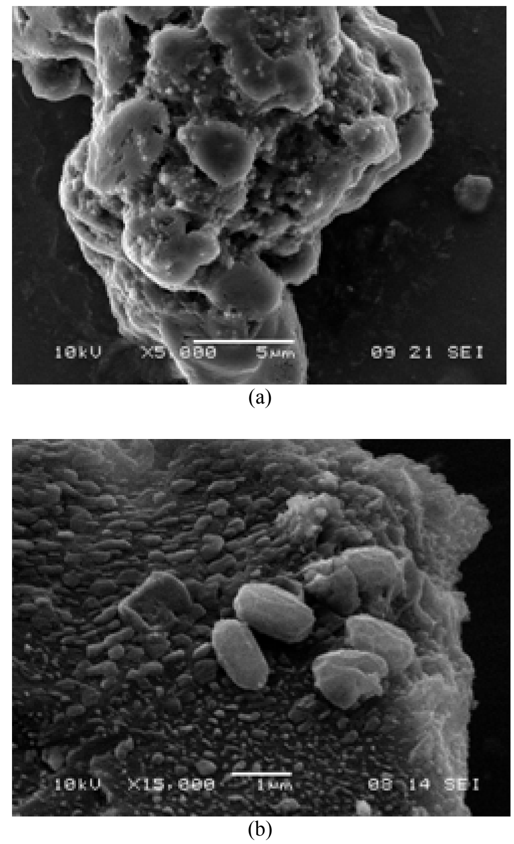
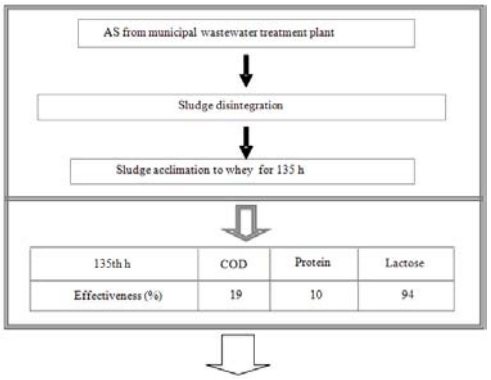


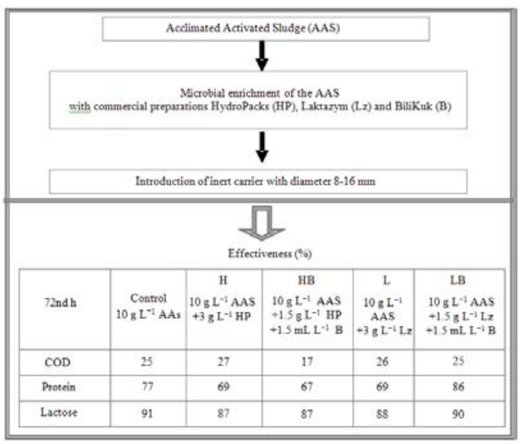
Fig. 3: Structure of microbial preparation HydroPacks (a) SEM-5 000X and microbial preparation Laktazym; (b) SEM-15 000X

Two approaches are used in the real wastewater treatment practice for the initial reactor inoculation. The first includes the introduction of microbiological preparations, commercial products for wastewater treatment. The second approach includes the introduction of activated sludge from well working wastewater treatment plants. The combination between these two approaches was a new decision, which has more financial benefits than the first approach. On the basis of the obtained results for the microbiological and the enzymological parameters of the three preparations the following combinations were studied: Acclimated Activated Sludge (AAS) as a control one; AAS and preparation HydroPacks (H); AAS and preparation Laktazym (L); AAS and preparations HydroPacks + BiliKuk (HB); AAS and preparation Laktazym + BiliKuk (LB).

Steps for governing of two critical situations during biological treatment for dairy wastewater: Two critical situations during the operation of a sequencing batch reactor with fixed biomass were investigated. Some relevant solutions for each of them were developed on the basis of the applied ecological approaches. The first critical situation was related to the start-up period of local wastewater treatment plants to dairy enterprises, as well as to acceleration of prolonged period for active biofilm formation. The governing of this critical situation included four steps (Fig. 4). First: The preliminary flocs disintegration of real activated sludge from well operated Wastewater Treatment Plant (WWTP) aimed obtaining of homogenic microbial suspension with high diversity of bacteria. The selection of the activated sludge 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.



(A)



(B)

Fig. 4: Steps for initial formation and activation of biofilm-approaches of (A) Acclimation; (B) immobilization and addition of microbiological preparations

The application of activated sludge from a municipal WWTP or from a local WWTP to some dairy firm, 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 different anoxic and anaerobic processes (hydrolyses, fermentations, acetogenesis, methanogenesis, denitrification, sulfate-reduction). The activated sludge from a sludge thickener of Sofia Wastewater Treatment Plant was used in our study. This plant treated municipal and industrial wastewater including wastewater from big dairy enterprises as “Danon-Serdika”, “LB Bulgaricum” and other. Second: acclimation of the treated activated sludge, as it was described above, to whey for 135 h; Third: bioaugmentation with added microbiological preparations HydroPacks, Laktazym and BiliKuk; Fourth: immobilization of the acclimated biomass on inert material and initial biofilm formation.

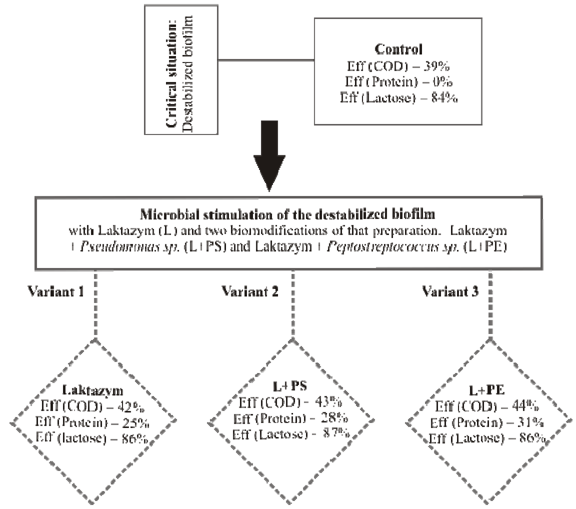


Fig. 5: Steps for activity increasing of destabilized biofilm-an approach by addition of Laktazym and microbial cultures/Eff is effectiveness of biodegradation for each pollutant at 14th h

The activated sludge at the end of acclimation (135th h) had low effectiveness for decreasing total organic matter (measured as COD). This was related to protein utilization and its lower rate of biodegradation-10%. The availability of lactose in model wastewater leads to suppression of proteases (Yu and Fang, 2001). This statement explains the lower speed of biodegradation of the proteins than the one of the carbohydrates.

Another critical situation during biofilter operation is biofilm destabilization and its detachment from the inert material as a result of the change of organic matter concentrations (Cresson *et al*., 2006; Punal *et al*., 2000; Omil *et al*., 2003; Rajeshwari *et al*., 2000; Viraraghavan and Varadarajan, 1996). The governing of this critical situation included the addition of preparation Laktazym or its biomodifications with microbial cultures. This microbiological preparation was chosen as an object for purposeful biomodification because of the more lasting positive effect on the water treatment process (Schneider and Topalova, 2008). The added cultures were purposefully selected for biodegradation of milk and whey proteins (Schneider and Topalova, 2008). The selected bacterial dominants were determined as *Pseudomonas sp.* (isolated as denitrifier) and *Peptostreptococcus sp.* (isolated from the group of anaerobic heterotrophs). The control variant was without preparation and cultures. The protein accumulation in the control variant was ascertained (Fig. 5). The reason was the decreased activity of destabilized biofilm.

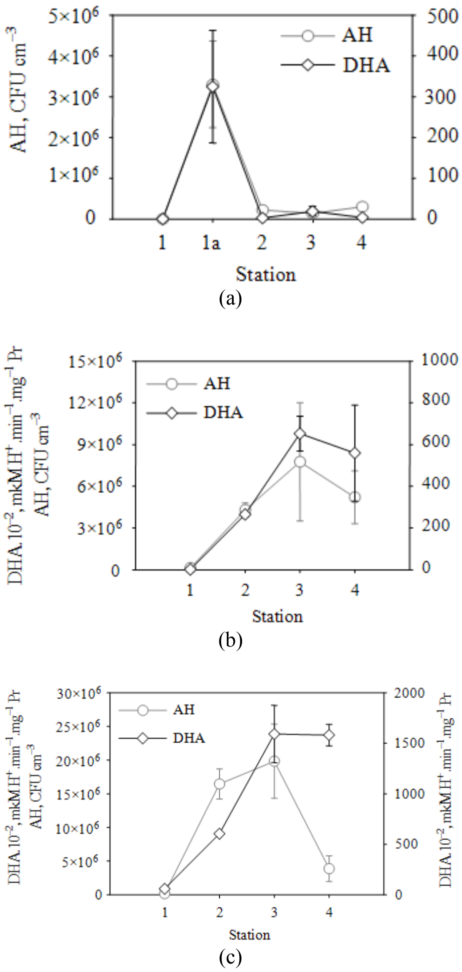


Fig. 6: Dynamics of Aerobic Heterotrophs (AH) and Dehydrogenase Activity (DHA) in: (a): water; (b) sediment water and (c) sediments in the water receiver

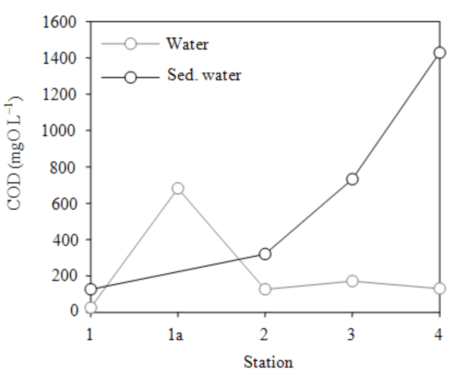


Fig. 7: Dynamics of total organic matter concentrations (measured as COD) in stream water and sediment water

The recovery of biofilm activity was accomplished by means of three different microbial stimulations-addition of: (1) Preparation Laktazym; (2) Laktazym + *Pseudomonas sp.* (PS) and (3) Laktazym + *Peptostreptococcus sp.* (PE). The effectiveness of protein biodegradation was increased by 25-31% via the three variants of preparation Laktazym (Fig. 5). Besides, the protein utilization began at the 14th h in comparison to the control variant-at the 72nd h (data not shown) (Schneider and Topalova, 2008).

Self-purification potential of a water receiver for dairy wastewater: The self-purification potential of the real water receiver-Leva River has been studied as a key ecological approach. The microbial communities, a key factor for accomplishment of self-purification processes in water receiver, were studied during the critical period of summer low water. The restructuring and functioning of microbial communities in water, sediment water and sediments were investigated.

The quantity of aerobic heterotrophs after dairy wastewater discharge was considerably increased in comparison to the control station-Station 1 (Fig. 6). The bacterial amount in water (Fig. 6a) was the highest in Station 1a (the zone of mixture of river water with dairy wastewater), while the bacterial amount in the sediment zone was the highest in Station 3 (Fig. 6b and c). The stations with the highest values of microorganisms corresponded to the stations with higher concentration of biodegradable organic matter (Fig. 7). In vertical aspect the microbial quantity was higher in the sediment zone (5×10^6-2×10^7 CFU cm^{-3} for sediment and 3-9×10^6 CFU cm^{-3} for sediment water) in comparison to the stream water (3×10^5 and 4×10^6 CFU cm^{-3} in Station 1a).

DISSCUSION

About biological treatment of dairy wastewater: The obtained data about the simulated anaerobic process confirmed the hypothesis that the acceleration of lactose utilization will accelerate protein hydrolysis (Fig. 4B). The biochemical explanation is that use the lactose first in this bi-substrate media is due to the fact that it takes less enzymes to use lactose than it does to use proteins. The utilization of lactose provides more energy for a shorter period in which the synthesis of proteolytic enzymes is carries out.

The proposed acclimation was successful, protein hydrolysis was accelerated and the percent of protein removal was increased from 10% for 135 h to average 74% for 72 h. The short-lasting time for protein biodegradation is mainly related to acclimation procedure as it can see from the data for control variant which contained only AAS. The time for lactose biodegradation was shorter (14 h) and the protease activation was quicker (Topalova *et al*., 2007). The other positive factor for the process acceleration was immobilization. It increases biosystem stabilization, creates more diverse conditions and metabolic pathways for degradation of organic matter and the biofilm has higher activity for a longer period (Zayed and Winter, 1998; Ozaki *et al*., 1991). Besides, the positive role of the immobilization is related to concentration of: target substrates, active biomass and enzymes for biodegradation

The most important approaches for achieving high effectiveness of dairy wastewater treatment process were both the acclimation and immobilization of biomass. This was related to restructuring microbial communities by changes in qualitative and quantitative composition; increasing enzyme activities for biodegradation of target pollutants; creation of microhabitats with diverse conditions and increasing metabolic diversity of biological systems. The protein degradation for the first critical situation was increased by 67% by applying acclimation and immobilization while addition of microbiological preparations increased it by 9%. The protein utilization for the second critical situation was increased by 25-31% by the addition of microorganisms. The preparations and cultures accelerated the metabolism of biological systems. This was related to additional introduction of enzymes, activators and protectors, mutual interactions of metabolic pathways by syntrophy, synergism, co-metabolism.

The used ecological approaches such as acclimation, immobilization and addition of microbiological preparations are easily applicable in the real wastewater treatment practice. The biomodification of a microbiological preparation by adding specialized biodegradants requires highly skilled staff and specialized laboratory. The technological and ecological circumstances as well as the financial possibilities of the dairy enterprises determine the choice of method for overcoming critical situations.

The studied anaerobic reactor and developed solutions require a second step of water treatment-aerobic reactor. The role of the aerobic reactor will be decreasing chemical oxygen demand to least 250 mgO L^{-1} (for wastewater discharge in receivers); decreasing the high concentration of ammonium nitrogen (about 100 mg L^{-1}) in the anaerobically treated wastewater; decreasing the phosphate concentration.

About self-purification potential of a water receiver for dairy wastewater: The vertical distribution of microorganisms and it domination in sediment zone was related to higher concentrations of suspended solids for biomass immobilization; more stable environmental conditions and higher concentrations of organic matter. The quantity of aerobic heterotrophs in Leva River was higher with one to two orders in comparison with other researches but the tendencies in vertical distribution of microorganisms were the same (Todorova and Topalova, 2008; Wakelin *et al*., 2008).

As a result of the increased levels of biodegradable organic pollution along the river the microbial communities in water and sediments accelerated the rate of transformation processes (Fig. 6). The biodegradation of organic matter was accomplished with higher rate in the sediment zone than in the stream water. Although the increasing COD value in Station 4 in sediment water was ascertained that the rate of transformation processes decreased. At the same time, the protein concentration in this station decreased and lactose in the stream and sediment water wasn’t determined (Schneider and Topalova, 2009b). The results showed the availability of some kind of refractory or non-biodegradable organic matter that was included in the COD value. As a whole the obtained data for dehydrogenase activity and microbiological analysis showed that the essential part of self-purification processes was accomplished in sediment zone. The highest rate of self-purification was ascertained for biofilm of the river sediments. These results are a confirmation of data obtained from Todorova and Topalova (2008) and Wakelin *et al*. (2008).

CONCLUSION

Acclimation, immobilization and addition of microbiological preparations are appropriate for bioaugmentation of dairy wastewater treatment. The used ecological approaches can be applied to a broad range of dairy companies because of the similar composition of wastewater. The proposed methods are more reliable, more ecologically friendly and have important financial advantages. Their application in the real wastewater treatment practice will accelerate purification process; will increase effectiveness of biosystem functioning in the risky situations and will ensure a higher prevention against pollution of water receivers.

The results showed that the most important approaches for achieving high effectiveness of wastewater treatment process were both acclimation and immobilization of biomass. In this aspect the obtained data for water receiver confirmed this conclusion. The effect of the added preparations was not high. It was thoroughly related to improving the rate of metabolism and functioning of the biological system mainly on an enzyme level.

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