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DYNAMICS OF CULTIVABLE HETEROTROPHIC BACTERIA ABUNDANCE AT THE WASTEWATER TREATMENT PLANT OF CERNAVODA, ROMANIA

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Abstract

The aim of this paper was to investigate and understand the relationship between several physical and chemical parameters such as temperature, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) on the one hand and dynamics of bacterial populations involved in the breakdown (hydrolysis) of macromolecular organic matter, on the other. The study was carried out during July-November 2009 at Cernavoda Wastewater Treatment Plant and included quantitative estimation of proteolytic, amylolytic, and lipolytic bacteria as major exoenzymes-producing bacteria. Significant populations belonging to these groups were detected in influent, effluent and in the aeration tank. Composition of exoenzyme-producing populations was different and variable in influent, aeration tank and effluent. No significant connections were found between the different bacterial group's abundance and COD whereas BOD was positively correlated mainly with the dynamics of lipolytic bacteria.

Key words: aeration, bacteria, dynamics, wastewater treatment

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1. Introduction

Wastewaters comprise a wide variety of organic substrates including low- and high molecular weight compounds (Cadoret et al., 2002; Covaliu et al., 2016). Organic matter existing in wastewater includes polysaccharides (25–50%), lipids (10-30%) and proteins (40-60%) (Nielsen et al., 1992).

Organic matter degradation is a complex process carried out by microbial communities in the activated sludge stage (Robescu et al., 2001). These microorganisms are able to produce and release hydrolytic enzymes and thus have the capacity to breakdown macromolecules to simpler compounds that can enter the cells for further degradation (Chrost, 1991; Sheng and Yu, 2006; Watson et al., 2004). Microbial enzymatic activity is not completely studied and understood, but it is known that microorganisms produce numerous enzymes among which very significant are proteases, lipases, and amylases (Nybroe et al., 1992).

Recently, efforts are made to extract the enzymes found in activated sludge for the potential use in biotechnology (Nabarlatz et al., 2012; Szilveszter et al., 2013; Yang et al., 2016). The paper aims to analyze and evaluate aspects related to the dynamics of amylolytic, lipolytic and proteolytic bacteria during July-November 2009 at the Cernavoda Wastewater Treatment Plant. These groups were quantitatively assessed and their time evolution was evaluated taking into account the temperature, chemical oxygen demand (COD) and biochemical oxygen demand (BOD). Enzymes-producing- bacteria may be a useful indicator of the organic matter degradation processes evolution.

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2. Material and methods

Samples were aseptically collected from three points: influent wastewaters, biological treatment stage activated sludge and effluent leaving the clarifier from July to November. During each month three samples were analyzed and the recorded results represent the average values. COD and BOD were determined according to the Romanian standards SR ISO 6060/1996, and SR EN 1899-1/2003. After collection, samples were shaken on a mechanical stirrer (5 min at 25 Hertz on a Vortex Zx^3), diluted in sterile saline water (decimal dilutions in sterile physiological water with 0.85% NaCl) and inoculated on solid culture media with specific composition depending on the bacterial group (Rodina, 1980; Benson, 1990). Dilutions were spread onto the solid media and incubated at 28ºC for 1-3 days.

The number of lipolytic bacteria was assessed on (Merck) Tributyrin Agar (peptone from meat 2.5 g; peptone from casein 2.5 g; yeast extract 3.0 g; agar-agar 12.0 g; distilled water 1000 mL; pH=7.6-7.8). Colonies surrounded by clear zone of tributyrin hydrolysis were counted. The amylolytic bacteria number was determined on Nutrient agar supplemented with soluble starch (Merck) (Bactopeptone Difco 5.0 g; beef extract Difco 3.0 g; soluble starch Merck 15.0 g; agar-agar Merck 15.0 g, distilled water 1000 mL; pH=7.6-7.8). After 48-72 h of incubation, the plates were flooded with iodine Lugol's solution and the colonies were surrounded by zones of starch hydrolysis were counted.

The number of proteolytic bacteria was estimated on Calcium caseinat agar (Merck) (Peptone from meat 0.4 g; meat extract 0.2 g; peptone from casein 0.2 g; calcium casein at 3.5 g; calcium chloride 0.2 g; tri-potassium citrate monohydrate 0.35 g; di-potassium hydrogen phosphate anhydrous 0.105 g; potassium dihydrogen phosphate 0.035 g; sodium chloride 5.0 g; agar-agar 13.0 g; pH= 7.4-7.6) (Benson, 1990; Rodina, 1980). Colonies of bacteria with proteolytic activity with clear zones of hydrolysis were counted. Statistical analysis was carried out with Statistica software v. 8. and included Student *t* test (number of datasheet n=15) and Pearson product moment correlations coefficient, *r*.

3. Results and discussion

Water temperature in the aeration tank decreased from July to November from 26.2ºC to 17.5ºC (Fig. 1). This temperature interval might affect the microorganism's activity in the activated sludge. With the exception of July, COD varied insignificantly in influent waters and had an average value of 241.2 mg/L (Fig. 2). In the effluent water, COD fluctuated from a minimal value of 13.8 mg/L (November) to maximal value of 22.1 mg/L (August). COD values according to the national legislation limits were obtained in the effluent. The COD concentration evolution can be seen in Fig. 2, both for the influent and effluent. BOD values were relatively stable in the influent during the evaluated period and they decreased strongly in effluent, being about 20-40 times lower than in influent ($p<0.05$) (Fig. 3).

Generally, bacterial groups able to decompose macromolecules (polysccharides, lipids, and proteins) presented large fluctuations both in influent as well as in effluent. The evolution may be justified by the intermittent discharge of wastewaters. These periodic discharges are able to induce specific enzymatic systems (proteases, lipases and amylases) simultaneously with the increase of bacterial populations involved in such activities.

Proteolytic bacteria. The mean density of this group in the influent was around $6.1x10^4$ CFU/mL (Fig. 4a), but over time the proteolytics abundance of varied between $2x10^4$ CFU/mL and $9x10^4$ CFU mL (Fig. 4a). Proteolytic bacteria density increased to approximately $9x10^5$ in the aeration tank, sometimes reaching a maximum value of $1.3x10^6$ CFU/mL (Fig. 4b).

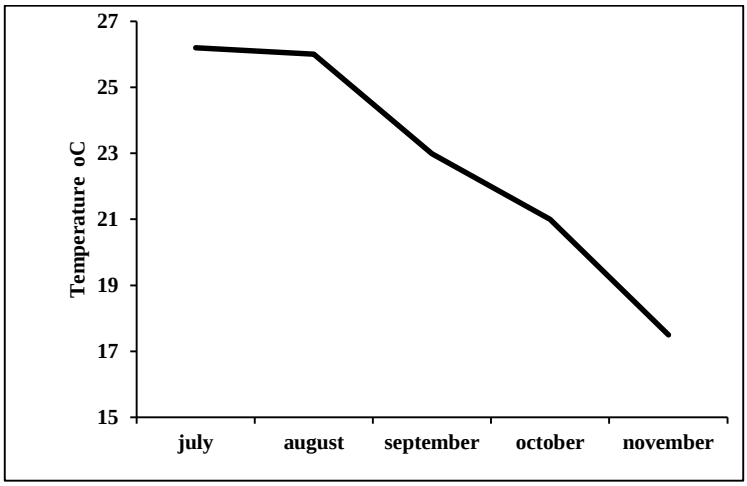


Fig. 1. Temperature dynamics

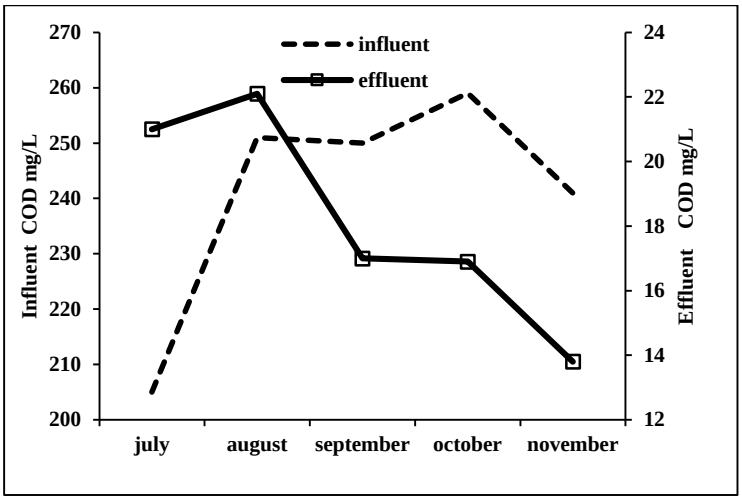


Fig. 2. COD value dynamics

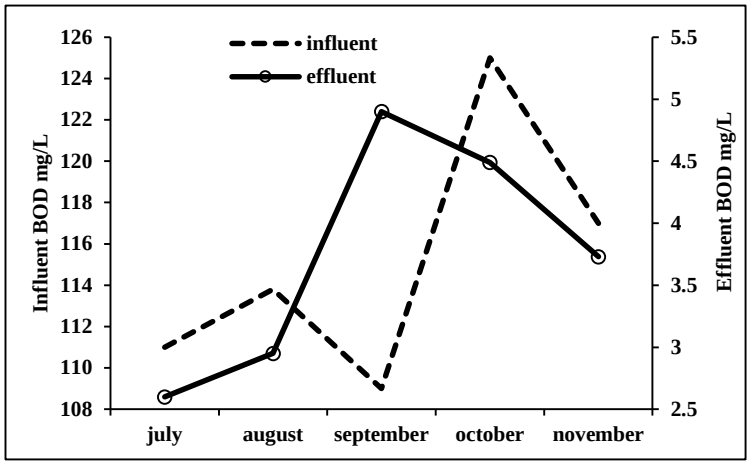


Fig. 3. BOD value dynamics

In some cases, the effluent leaving the clarifier carried on between $2x10^4$ CFU/mL and $6x10^4$ CFU/mL proteolytic bacteria (Fig. 4c). Protease-producing microorganisms are important members of the global activated sludge bacterial community (Kim et al., 2002; Yu et al., 2009). While the primary role of the protease produced by proteolytic microorganisms is to process proteinaceous substrate from wastewaters, its activity may be extended in further steps of activated sludge treatment, such as reduction of excess sludge by heat treatment (Yan et al., 2008).

Lipolytic bacteria. The density of this group was characterized by significant fluctuations in the plant's influent between $3x10^4$ CFU/mL and $1.5x10^5$ CFU/mL (Fig. 5a-5c). Their abundance in aeration tank was important, ranging between $1.2x10^6$ CFU/mL and $8x10^6$ CFU/mL (Fig. 5b). In general, lipids are degraded more slowly in comparison to proteins and this fact allows their accumulation, enhancing numerically the lipase-producing microbiota. Lipase-producing microorganisms and lipase itself was extensively studied (Li and Chrost, 2006; Yu et al., 2007). Lipases are not only important in treating the wastes, but also in other practical applications (Gessesse et al., 2003). Therefore, activated sludge biomass might provide a valuable source of lipase extraction with a variety of applications.

Amylolytic bacteria. This group was characterized by relatively high densities in influent (Fig. 6a). The abundance was almost double in the aeration tank where their number had an average of $3.7x10^4$ CFU/mL (Fig. 6b). The effluent also comprised significant numbers of amylolytic bacteria, between $1x10^4$ CFU/mL and $6x10^4$ CFU/mL (Fig. 6c). Starch-degrading bacteria are important components of activated sludge populations (Verstraete et al., 1975; Yu et al., 2008). Amylolytic bacteria was *in situ* detected by molecular techniques and it was observed that it might represent up to 11% of the total bacterial biomass (Xia et al., 2008), stressing their important role in overall metabolism of activated sludge.

Degradation of macromolecules (starch, proteins, lipids) plays an essential role not only in wastes degradation, but also in maintaining optimal conditions for excess N and P removal in wastewater treatment systems (Morgenroth et al., 2002). It was not observed an obvious trend in abundance over time, from summer to end of autumn.

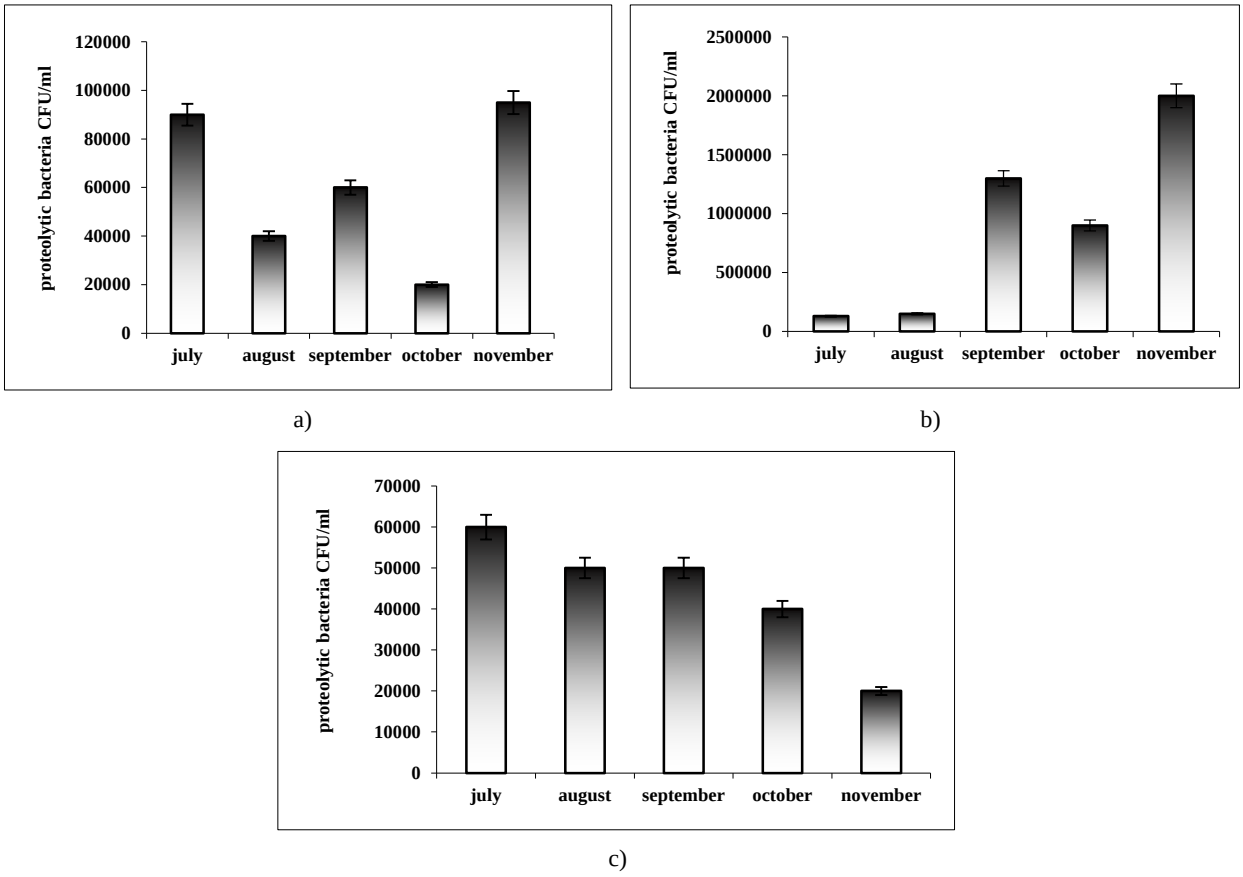
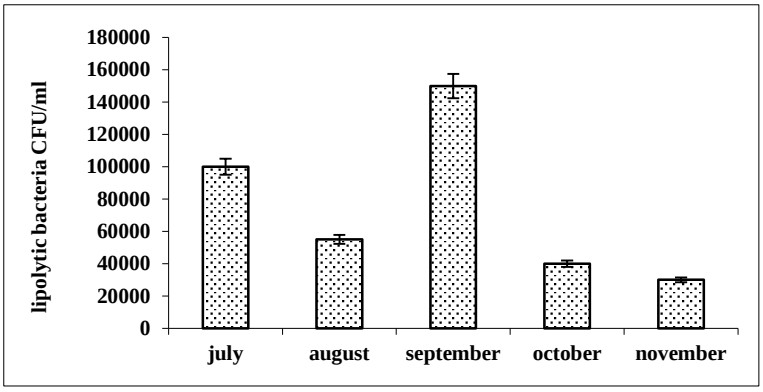


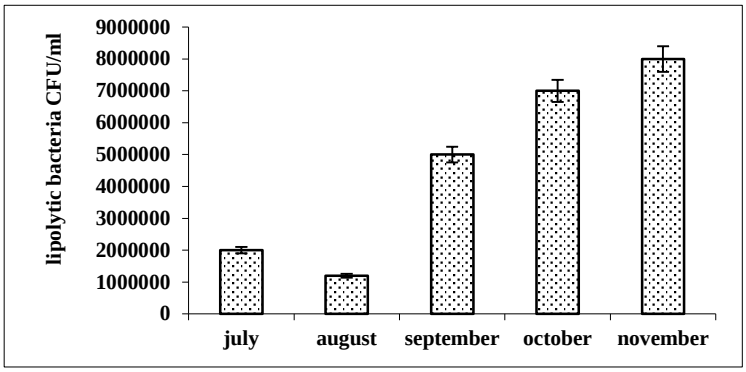
Fig. 4. (a) Temporal distribution of proteolytic bacteria in the influent, (b) temporal distribution of proteolytic bacteria in the aeration tank, (c) Temporal distribution of proteolytic bacteria in the effluent

Some groups presented maximum density values in the summer time, while others in autumn, without a direct link to the decreasing temperature of wastewaters. Although the temperature decreasing from 20°C to 17°C may affect the metabolism rate, this variability does not influence significantly the cells viability. Each group was characterized by particular fluctuations of abundance in the influent, aeration tank, and effluent, fluctuations which could not be associated to a single factor. It should be noted that the bacterial abundance recorded after the samples inoculation on laboratory media might reflect only in part the real bacterial abundance due to the following major factors: (i) only a percent of bacterial populations can grow on solid artificial media (Ellis et al., 2003; Kell et al., 1998); (ii) bacteria are entrapped on the aggregate within the structure of the flocs (Snaidr et al., 1997; Yu et al, 2009). The mean density values indicated some differences in bacterial composition as proportion among different groups (Fig. 7).

Thus, while a high percent of amylolytic bacteria was detected in the influent, the lipolytic bacteria as a group was dominant in the aeration basin. The temperature decrease affected negatively all the groups in the aeration tank, lipolytic bacteria ($r=-0.88$) ($p<0.05$), amylolytic bacteria ($r=-0.91$) ($p<0.05$), and proteolytic bacteria ($r=-0.91$) ($p<0.05$).



(a)



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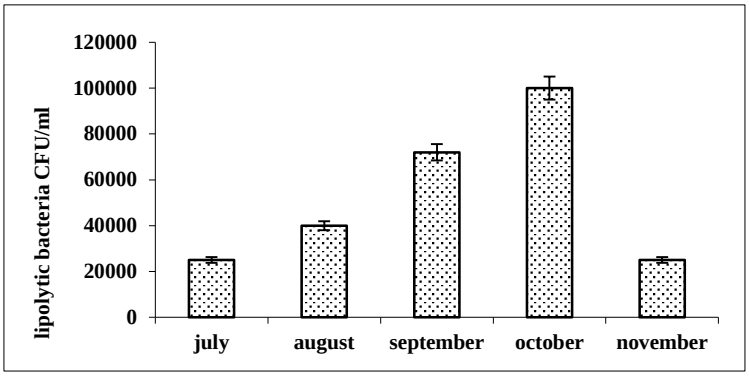


(c)

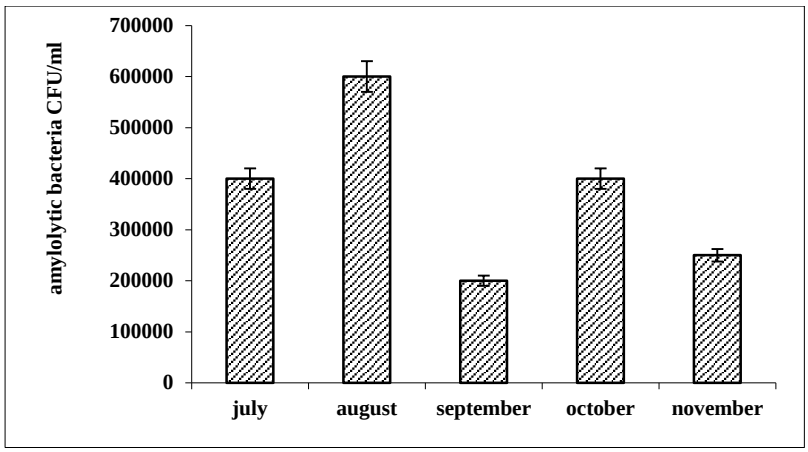
Fig. 5. (a) Temporal distribution of lipolytic bacteria in the influent, (b) Temporal distribution of lipolytic bacteria in the aeration tank, (c)Temporal distribution of lipolytic bacteria in the effluent

There was no significant link identified between COD and different bacterial groups in influent and effluent. However, a weak negative relationship seemed to exist in the influent between BOD and lipolytic bacteria (r=-0.77) indicating that competition could exist between different bacterial groups in the wastewater entering the plant. A relatively significant positive relationship was found in the effluent between BOD and lipolytic bacteria (r=0.84) on the one hand and BOD and amylolytic bacteria (r=0.82) on the other. There was no relationship identified between COD and BOD neither in influent nor in the effluent.

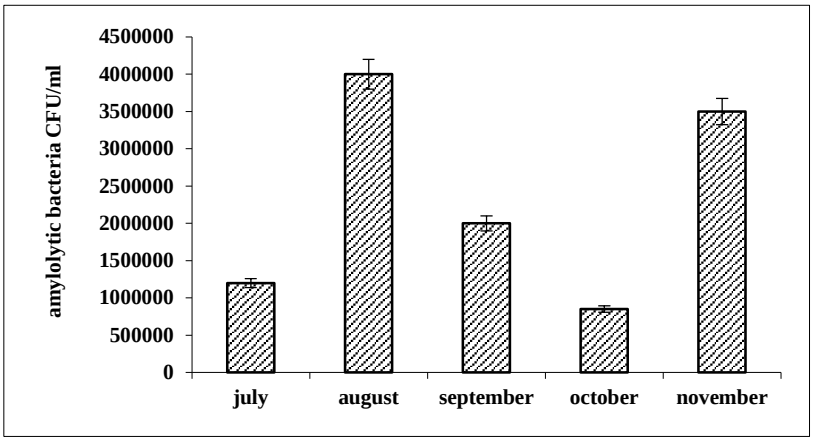
In fact, COD may account for the refractory organic matter which is degraded slowly by bacteria while BOD for labile substrate easily available to bacterial metabolism. Lipolytic bacteria was an important functional group at WWTP Cernavoda during the study period, indicating that lipids and lipase-driving activities accounted for a major fraction of bacterial metabolism.

4. Conclusions

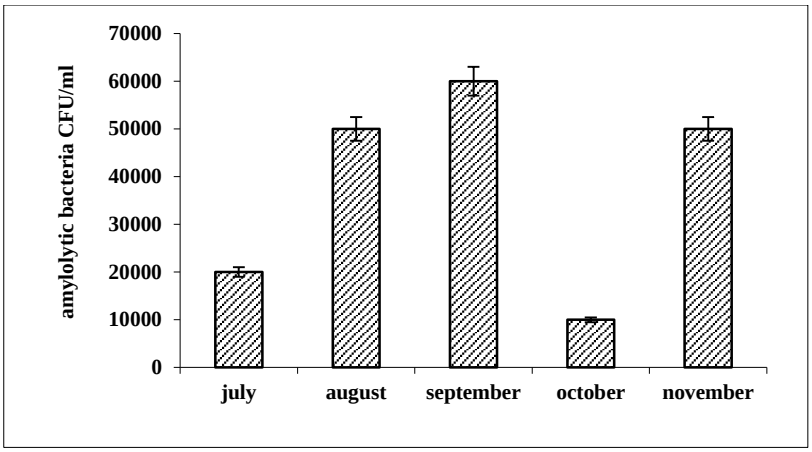
Density of exoenzyme-producing bacteria was variable and did not always follow the temperature pattern.



(a)



(b)



(c)

Fig. 6. (a) Temporal distribution of amylolytic bacteria in the influent, (b) Temporal distribution of amylolytic bacteria in the aeration tank, (c) Temporal distribution of amylolytic bacteria in the effluent

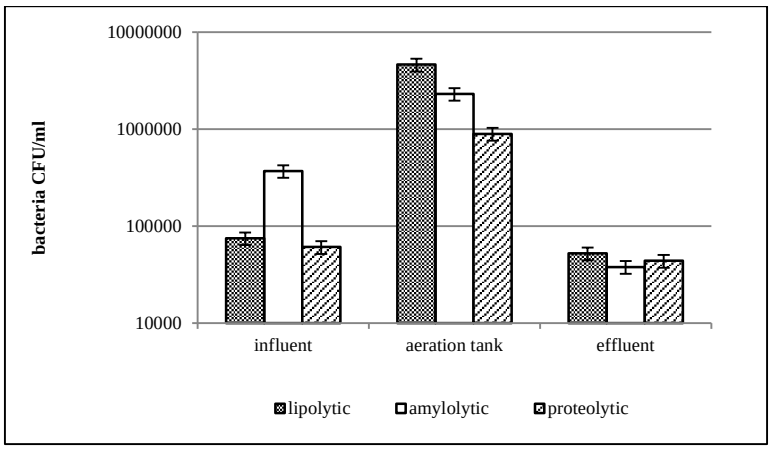


Fig. 7. Composition of exoenzymes-producing bacteria in influent, aeration tank, and effluent
(the number of bacteria represents the average rounded values calculated for the studied period (July- November))

Although temperature negatively affected some bacterial groups abundance, fluctuation of density might be attributed also to the organic matter quality and quantity in the influent, volume discharge of wastewaters and other environmental parameters. Composition of exoenzyme-producing populations was different and variable in influent, aeration tank, and effluent.

Thus, in the influent major abundance belonged to amylolytic bacteria while in the aeration tank it was a strong shift towards a high proportion of lipolytic bacteria. In the effluent, proportion of different exoenzyme-producing populations was more balanced, but with numerical predominance of lipolytic and proteolytic bacteria.

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