

## **DOCTORAL THESIS - SUMMARY**

### **MICROORGANISMS INVOLVED IN OXYBIOTIC PROCESSES IN THE WASTE WATER TREATMENT PLANT CONSTANTA NORD: FUNDAMENTAL ASPECTS AND APPLICATIONS**

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Wastewater effluent from treatment systems is one of the biggest sources of pollution. Among the negative effects of these wastewaters on aquatic ecosystems and humans, we find the death of aquatic creatures, algal blooms, habitat destruction due to sedimentation of household waste, short and long-term toxicity due to chemical contaminants. The issue is of great scientific and social importance, having the appropriate legislative treatment at both national and European level.

Council Directive 91/271 / EEC of 21 May 1991 on Urban Wastewater Treatment, as amended and supplemented by Commission Directive 98/15 / EC on 27 February 1998, is the legal basis for Community waste legislation, it has been fully transposed in the Romanian legislation by GD no.188 / 2002 for the approval of the norms regarding the discharge conditions of the waters in the aquatic environment, modified and supplemented by GD no. 352 / 2005. The central objective of the Directive is to protect the environment from the negative effects of discharges of urban waste water and waste water from certain industrial sectors.

It is in everyone's advantage that a community is able to treat its residual water in the most economical way. The activated sludge treatment process has the advantage of producing a high quality effluent with a reasonable operating cost and maintenance.

In order to comply with the legal requirements for water treatment, plant operators should carefully control the treatment process so they can intervene promptly to prevent exceedances of the limit values imposed by water management permits. In addition to chemical and physical methods, wastewater treatment is primarily based on biological treatment with active sludge. Knowledge of the required nutrients and the composition of the activated sludge are therefore necessary for efficient exploitation.

The main chemical pollutants in wastewater are nitrogen, phosphorus, heavy metals, detergents, pesticides and hydrocarbons. Of these chemicals, nitrogen and phosphorus are

limiting factors. The presence of nitrogen in waste water evacuation may be undesirable because it has ecological effects and also affects public health.

The main forms of nitrogen are organic nitrogen, ammonium ( $\text{NH}_4^+$  or  $\text{NH}_3$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ). The source of these nutrients is generally associated with household waste water and fertilizers applied to agricultural crops. Nitrogen from untreated wastewater is mainly present in the form of ammonium and organic nitrogen. In the activated sludge treatment, ammonium is converted to the nitrate form in the nitrification process.

Surface water also contains phosphorus, found in various compounds, which is an essential component of living organisms. Under natural conditions, the concentration of phosphorus in water is balanced, but when additional phosphorus intake, which aquatic organisms can not use, eutrophication occurs. Control of phosphorus discharge from wastewater treatment plants is a key factor in preventing surface water eutrophication.

In order to prevent the negative effects of discharging properly unpurified wastewater, a good understanding of the water treatment processes that can be applied, the pollutants found in water and the repercussions resulting from the non-fulfillment of the final objective - the cleaning of waste water, is necessary. In this regard, there is a great deal of research on both purification processes (physical, chemical and biological) as well as on more and more elaborate technologies that aim at water cleaning at minimal cost and maximum yield.

**The main objectives of the thesis are represented by:**

- I. Simultaneous determination of the active sludge purification function and its metabolic activity / dehydrogenase activity in batch systems or sequential bioreactor system (SBR) at laboratory level;
- II. Studies on the use of synthetic sludge and synthetic waste water in sequential bioreactor (SBR) at laboratory level;
- III. Studies on microbiological activity of activated sludge immobilized on synthetic supports for the treatment of synthetic waste water at the laboratory level;
- IV. Enrichment of microbiota in denitrifying microorganisms in the form of activated sludge or immobilized on SAM (mobile artificial supports).

Chapter 4 presents the data referring to the identification and application of new methods of quantitative and qualitative analysis of the active sludge, considering that in our country the

monitoring of the active sludge treatment is mostly for quantitative monitoring of the active sludge nutrient concentrations in the effluent and effluent of a treatment plant.

Figure 4.3 shows the reduction rate of resazurine. The sensitivity of the resazurin reduction test to changes in nutrient or toxic levels recommends it as a possible method for both routine monitoring of treatment plants and activated sludge research studies.

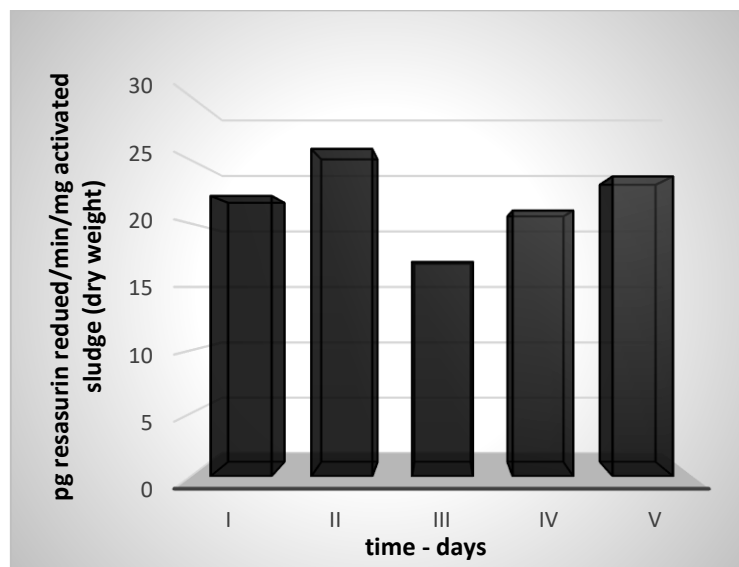


Figure 4.3 Reduction of resazurin expressed in pg resazurin reduced / min / mg activated sludge (dry weight)

Chapter 5 presents data on active sludge activity previously formed in a sewage treatment plant in the two essential processes in wastewater treatment, namely nitrification and denitrification (in a sequential bioreactor at the laboratory level). Figure 5.1 shows the evolution of ammonium and nitrate concentration during the first experiment (190 g wet sludge mass / 3L). Simultaneously with the monitoring of nitrification and denitrification we determined the rate of reduction of resazurin in the two important stages of the purification process, the aerobic phase and the anoxic phase (nitrification / denitrification).

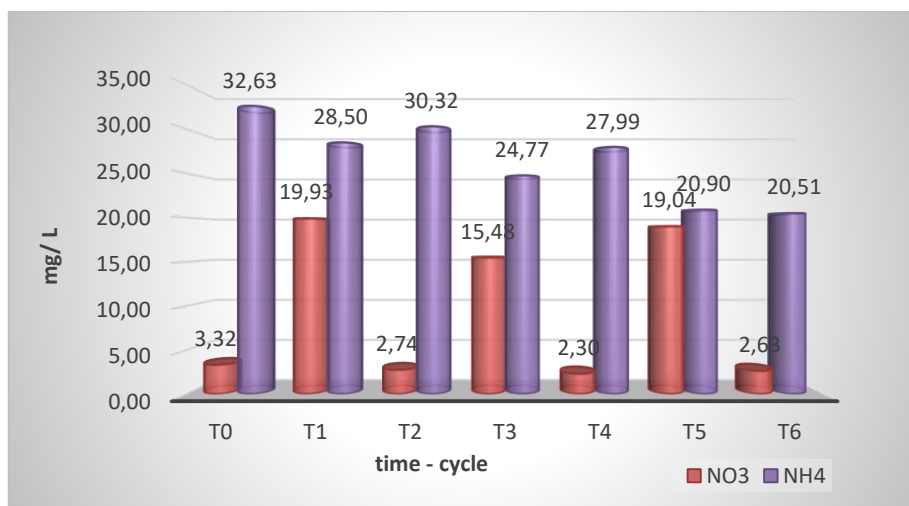


Figure 5.1 Evolution of ammonium and nitrate concentration during the first experiment (190 g wet active sludge / 3L)

The obtained results give us the opportunity to use the resasurin rate reduction test in optimizing the processes that take place both in the sequential type bioreactor (the one used in the experiment) and in the other types of biological basins using active sludge. The active sludge activity in the anaerobiosis phase was more intense compared to the aerobic phase, which may be correlated with the absence of molecular oxygen; aerobically bound microorganisms lose electrons only by resasurin reduction, while in the presence of molecular oxygen, electrons can reduce either the oxygen molecule or the resasurin.

In Chapter 6 are presented the data referring to the use of a bioreactor of the sequential type, fed with synthetic wastewater, having different nutrient concentrations.

Referring to the ammonium concentration, the best removal yield was obtained in the second experimental variant, C: N: P 195: 3.22: 1, the reduction being 92%.

In the case of chemical oxygen demand, the same removal efficiency was obtained both in the first experimental variant, C: N: P 154: 5.3: 1, and in the third experimental variant, C: N: P 103 : 4.45: 1, the reduction being 30%.

We can conclude that a C: N: P ratio of 195: 3.22: 1 is more beneficial for removing ammonia from wastewater, so more carbon, while chemical oxygen demand (organic matter) is reduced more efficiently when there is a balance (found in literature) between the three elements, carbon, nitrogen, phosphorus.

Figure 6.1 shows the evolution over time of ammonium, nitrate, nitrite concentrations during the first experiment (CCO: N: P 154: 5.3: 1).

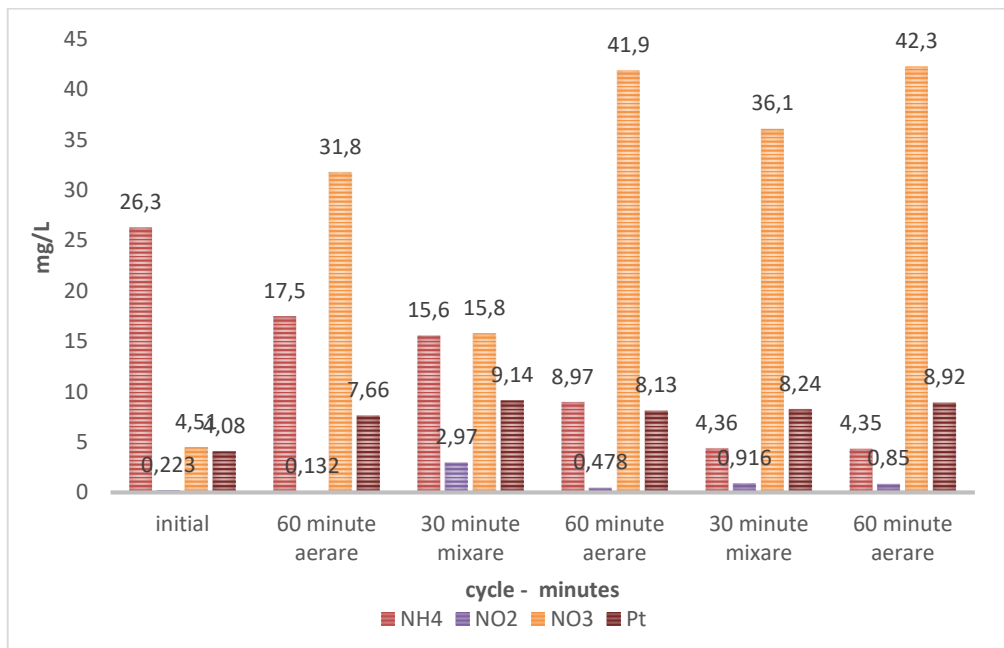


Figure 6.1 Time evolution of concentrations of ammonium, nitrate, nitrite during the first experiment (CCO: N: P 154: 5.3: 1)

In Chapter 7 are presented the data referring to the quantification of biomass cleaning efficiency on artificial substrate in sequential bioreactor at the laboratory level.

The quantification of the fixed biomass purity was determined by determining the initial concentrations during the experiment every 24 hours and the final concentrations of nitrate, ammonium, total nitrogen and total phosphorus. In this regard we used several experimental variants in which either the aeration / mixing time or the amount of biomass used changed.

Thus, we could conclude that SAM is a purification method with which significant removal efficiencies for the parameters analyzed (ammonium, total nitrogen, phosphorus) are obtained.

Setting the optimal aeration time (nitrification) and mixing (denitrification) depends on both the waste water load and the amount of biomass, so we obtained higher removal rates in experiments where the aeration time was longer (45 minutes and 60 minutes). The best yield for the removal of total nitrogen was obtained in the first experimental version, 500 ml of waste water and 500 ml of biomass, with a 45 minute aeration time and a mixing time of 135 minutes.

Figure 7.1 shows the evolution of ammonium concentration in the second experiment, variant A (60 minutes aeration phase) and B (30 minutes aeration phase).

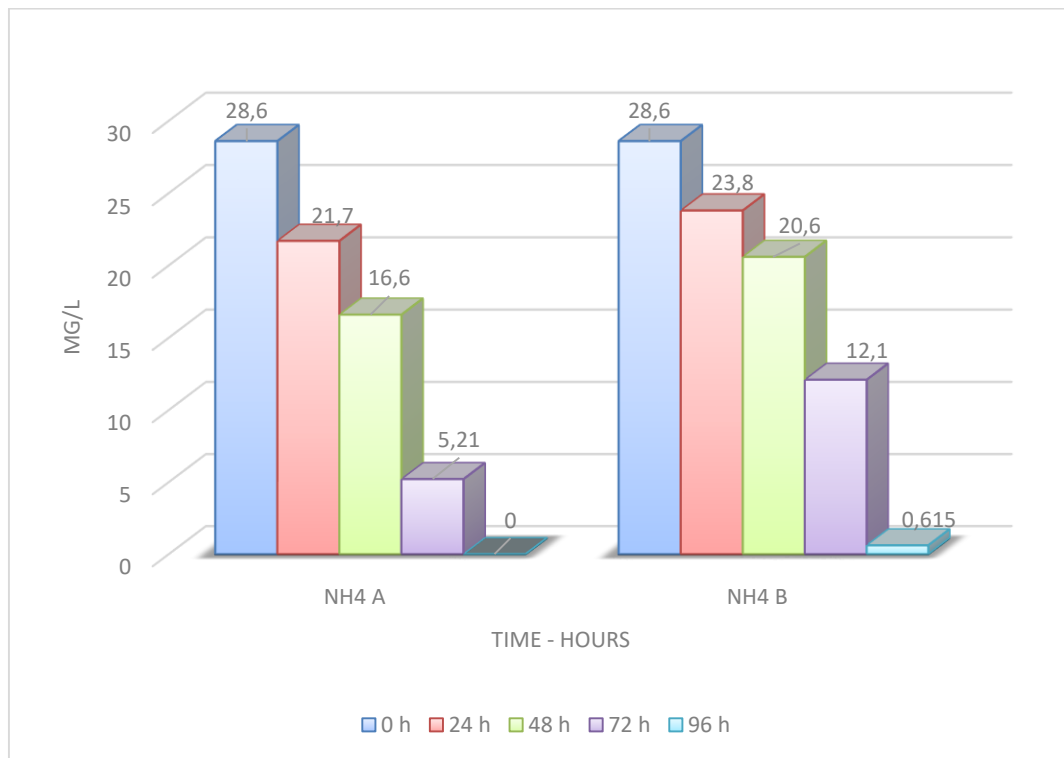


Figure 7.1 Evolution of ammonium concentration in the second experiment, variant A (60 minutes aeration phase) and B (30 minutes aeration phase)

Chapter 8 presents data on population enrichment of denitrifying bacteria so that the concentration of nitrate is reduced as much as possible. Enhanced sludge enrichment strategies for denitrifying sludge have focused on in situ enrichment of active sludge by additionally adding a source of carbon (ethanol) and nitrogen (nitrate) to the liquid phase to stimulate the proliferation of microbial clusters of interest.

To carry out this type of experiment, we had several experimental variants in which we modified either the ratio of the carbon and nitrogen source or the amount of activated sludge / fixed biomass volume (SAM).

After the experiment, we noticed a significant elimination of the nitrate concentration in all proposed experimental variants, the removal efficiency being maximal when using a relatively small amount of active sludge (50 ml) and a ratio of nutrients of 10-1 and 5-1 respectively, carbon-nitrate. Also, a significant amount of nitrate (66.75 mg / 24h) is eliminated by biomass formed on the Artificial Artificial Support (SAM).

From the point of view of the removal efficiency, it increases significantly in the case of SAM, as the culture of denitrifying microorganisms increases; the initial yield was 33.78%, ending at 99.26%.

## GENERAL CONCLUSIONS

1. The obtained results confirm the capacity of the activated sludge, both as a biomass in the form of flocs and as an artificial biomass, to purge municipal or synthetic wastewater, both at the laboratory level and in a treatment plant municipal.
2. The obtained results confirm the ability of the sequential reactor (SBR) to purify wastewater from different loads.
3. The capacity of activated sludge to remove pollutants from wastewater depends on its physiological state, quantity (active sludge concentration), operating conditions (nitrification / denitrification time) and waste water load. Thus, we obtained better removal efficiencies in experiments where the amount of active sludge was higher, 31% NO<sub>3</sub> + removed, 41% NH<sub>4</sub>-removed, 78% CCO removed, using 242 grams of active sludge versus 21% NO<sub>3</sub> + 37% NH<sub>4</sub>-removed, 54% CCO removed, using 190 grams of active sludge.
4. Determination of the optimal time of aeration (nitrification) and mixing (denitrification) depends on both the waste water load and the amount of biomass, so we have obtained yields to remove higher ammonium concentrations in experiments where the aeration time was longer (45 minutes and 60 minutes).
5. Creating an imbalance between nutrient sources has the consequence of slowing down the nitrate elimination process. Thus, the best ammonium removal yield was obtained when the ratio of carbon, nitrogen, phosphorus was C: N: P 195: 3.22: 1 (92% ammonium removed in 4 hours (chemical reaction), while chemical oxygen demand (organic matter) decreases more effectively when there is a balance (found in literature) between the three elements carbon, nitrogen, phosphorus (C: N: P 154: 1/103: 4.45: 1).
6. Quantitative analysis of metabolic activity shows different intensities of resazurin reduction rate, even at the same concentration of active sludge (expressed as dry mass), indicating that the metabolic rate of the active sludge is not constant from one sample to the next.
7. By measuring the resazurin reduction rate, higher values are recorded in the anaerobiosis phase compared to the aerobic phase, which may be correlated with the

absence of molecular oxygen; aerobically bound microorganisms lose electrons only by resasurin reduction, while in the presence of free molecular oxygen, the electrons are distributed both to the oxygen molecule and the resasurin.

8. Qualitative microscopic analyzes (Gram staining and Neisser staining) highlighted a healthy active sludge with good sewage and sedimentation capacity.
9. Enrichment of activated sludge (SAM) in denitrification significantly increases the yield of nitrate removal, which could help operators maintain the nitrate concentration at the evacuation below the authorization limit. For this purpose, nitrate removal yields have increased after enrichment of the sludge in denitrification, from 33.78% to 99.26%



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