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BIOLOGICAL WASTE WATER TREATMENT: 1. MONITORING METABOLIC ACTIVITY OF ACTIVATED SLUDGE AND THE CHEMICAL PARAMETERS OF WASTE WATER TREATMENT.

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Abstract

In this paper there are present the results concerning chemical parameters and the rate of metabolic activity of activated sludge in Constanta Nord waste water plant. Special emphasis is focused on time evolution of nitrates, ammonium, phosphorus concentrations and chemical oxygen demand in inlet, waste water and outlet, purified water. These results are discussed in correlation with metabolic activity of active sludge microbiota measured as the rate of resazurine reduction, and microscopic images obtained both in bright field and in epifluorescence microscopy. The results open *inter alia* the possibility to use the rapid method of resazurine reduction (15-20 minutes) to assess the activity of activated sludge microbiota and to correlate it with standardized, but time-consuming (hours to 5 days) methods, thus increasing the possibility to take good operational decisions in shorter time.

Key words: *waste water, chemical oxygen demand, activated sludge, resazurine reduction, Gram and Neisser stains, SYTOX Green.*

INTRODUCTION

There is an increase in water shortage around the world so great attention is paid to the reuse of wastewater; spent municipal and industrial wastewaters being treated in plants often by an activated sludge process. (Ardern and Lockett,1923; Grady and Lim, 1980; Vaicum, 1981;Negulescu, 1985; Arceivala, 1988; Bitton, 1999; Cheremisino, 2002; Burton and Stensel, 2003).Wastewater treatment is a complex biotechnological activity comprising mechanical processes (screening, sedimentation, filtration, or flotation), chemical processes (disinfection, adsorption, or precipitation) and biological processes involving microbial activity which are responsible for organic matter degradation and removal of nutrients. (Hanel, 1988; Metcvaff and Eddy, 1997;Vaicum, 1981; Templeton and Butler, 2001). Waste water treatment plants are very complex ecological systems built up by mankind, based on the knowledge concerning degradation processes occurring both in natural ecosystems and in natural ecosystems with strong anthropic impact (Godeanu 1973; Curds and Hawkes, 1983; Guterstam, 1996; Mitsch, 1997; Todd, 1997; Gray, 2002; Godeanu, 2013). The aim of this paper is to monitor sludge with respect to both its rate of metabolic activity (rapidly measured as resazurine reduction) and to the outcome of this activity, the decrease in chemical oxygen demand and removal of nutrients during waste water treatment at Constanța North waste water treatment plant.

MATERIALS AND METHODS

Samples were taken form Constanta Nord waste water plant at the following dates: the 13th of June 2014 (I), the 16th of June 2014 (II), the 21st of June 2014 (III), the 28th of June 2014 (IV), the 7th of July 2014, the 11th of July 2014 (VI), the 25th of July 2014 (VII), the 1st of August 2014 (VIII), the 29th of

January 2015 (IX), the 5th of February 2015 (X), and the 14th of February 2015 (XI). Constanța North waste water treatment plant was built in 1959 and further developed, the new plant complying with the EU directives and the Romanian standards (Presură et al., 2014).

Chemical analysis were performed according to standard procedures: chemical oxygen demand (STASS SR ISO 6060), ammonium (STASS SR ISO 7150-1), phosphorus (STASS SR EN 1189) nitrate (STASS SR ISO 7890-1) and dry solids in activated sludge (STASS SR EN 12880).

Quantitative determination of resazurin reduction

Resazurin (10 -oxide7-hydroxy-3H-phenoxazin-3-one) is a blue non-fluorescent dye; it can be reduced to resorfine (pink and highly fluorescent), which is further reduced to hydroresorufina (colorless and nonfluorescent). Resazurine was used primarily as an indicator of oxidation-reduction reactions in cell viability tests. The test is used from many years for monitoring bacterial contamination of milk (Ramsdell et al,1935; Noyer and Cambell, 1963). Recently resazurine became very popular as a simple and versatile method for measuring cell proliferation and cytotoxicity both in prokaryotes and eukaryotes, in different types of samples (Perony and Rossi,1986; Larson, 1997; Al-Nasiry et al., 2007;Duarte et al., 2009; Guerin et al., 2001). Conversion of resazurin to fluorescent resorufin is proportional to the number of metabolically active and viable cells present in a population. Calculation of reduced resazurin was done following standard protocol as previously shown (Ghiță et al., 2013).

Microscopic investigation were done with Zeiss microscope, Gram and Neisser staining being done as shown in Spellman (1999).

RESULTS AND DISCUSSIONS

In figure 1 there are presented some chemical parameters (phosphorus, ammonium, nitrates concentrations and chemical oxygen demand) in inlet, waste water and in outlet, purified water.

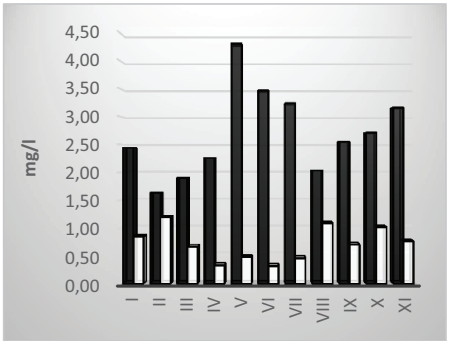


Figure 1A Time evolution of phosphorus concentration in waste water (dark columns) and in purified water (light columns). Roman numbers indicate different times of sampling (see material and methods)

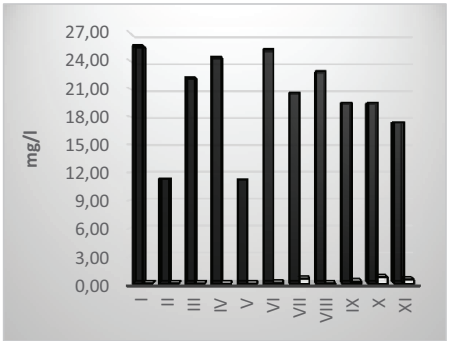


Figure 1 B Time evolution of ammonium concentration in input waste water (dark columns) and in effluent, purified water (light columns). Roman numbers indicate different times of sampling (see material and methods)

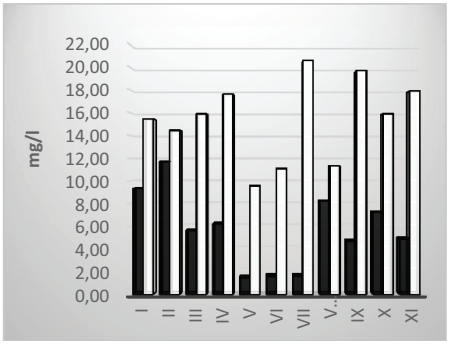


Figure 1 C Time evolution of nitrate concentration in input waste water (dark columns) and in effluent, purified water (light columns)

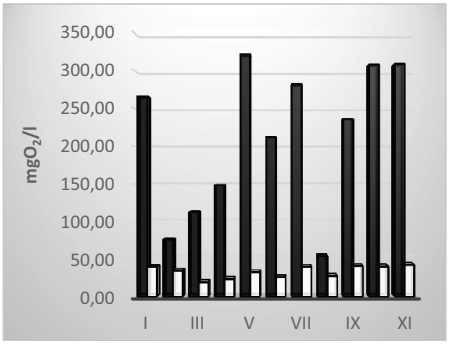
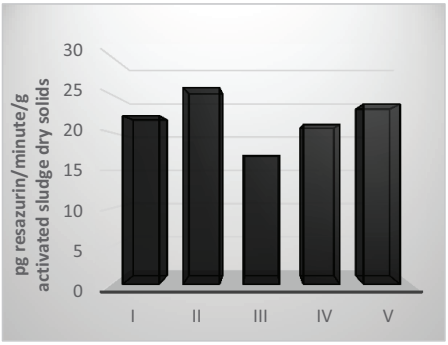


Figure 1 D Time evolution of COD in input waste water (dark columns) and in purified water (white columns).

As one can see in figure 1 A, B and D there is a strong decrease in concentrations of pollutants in effluent as compared with input, waste water; the only exception is the concentration of nitrate (Fig. 1 C) which is under increase, as a consequence of biological oxidations during waste water treatment.

In figure 2 there are presented the rate of resazurine reduction within the experimental period. When resazurine is reduced it is transformed in resurfine which has a strong red fluorescence this fluorescence can be used as a label to see metabolically active bacteria in microscopic preparations (Fig. 2 B). These microscopic pictures of activated sludge could be further correlated with image analysis as already shown for other experimental systems (Armaselu et al., 2011; Sarchizian et al., 2011). Image analysis opens the great opportunity to quantify the metabolic activity at the level of each individual microorganism, either unicelular or filamentous, which can be further correlated with photometric measurements of resazurine reduction by suspension of cells.



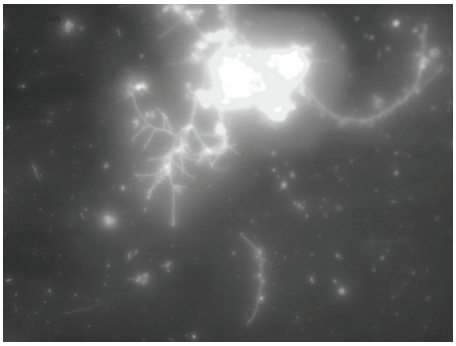


Figure 2 Resazurine reduction by activated sludge. A. The rate of resazurine reduction expressed as pg reduced resazurine/min/mg active sludge (dry weight).B microscopic images (epifluorescence) of cells and filaments containing resourfine produced by reduction of resazurine.

As one can see in figure 2 there are relatively large differences in the ability of active sludge to reduce resazurine when expressed on dry biomass, showing that the metabolic rate of activated sludge is not constant from one sampling time to another, besides its concentrations are maintained relatively constant in time (figure 3).

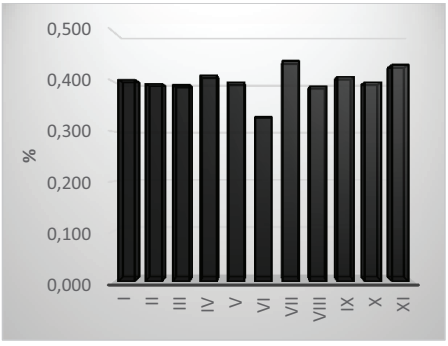


Figure 3 Time evolution of activated sludge dry weight concentration.

These experiments were initiated because the study of the ability of activated sludge to reduce artificial electron acceptors, including resazurine, is a method to a measure metabolic activity (Liu D. 1983; Bensaid et al 2000; McCluskey, et al., 2005; McNicholl et al., 2007). Liu (1983) showed that, the use of resazurine to measure dehydrogenase activity of activated sludge gave good correlations with important parameters such as oxygen consumption and heterotrophic bacterial count, both in the pilot plant and in the laboratory simulations.

Furthermore, the sensitivity of resazurine reduction against changes in nutrient levels or toxic substances recommed it as a tool both for routine plant monitoring and for research in activated sludge activity, as well (Liu, 1983). Bensaid et al (2000) showed that XTT, a tetrazolium salt can be used as a tool for estimating the activity of the electron transport system in activated sludge They found that the production of formazan by reduction of XTT is depended on the initial concentration of the XTT and is proportional to live cell biomass; furthermore, oxygen uptake rate and XTT reduction rate were highly correlated and indicated significant variations depending on the growth conditions (Bensaid et al., 2000). McNicholl and colab (2007) developed a rapid, robust and cost-effective method of assaying the metabolic activity of the biomass of activated sludge plants based on the redox dye resazurin, in which levels of reduction of the dye are proportional to cell biomass and respiration rate.

In figure 4 there are presented microscopic investigations of activated sludge in aerobic and anaerobic bioreactor concerning Gram reaction and Neisser reaction , which are the most used staining procedures for activated sludge Grady and Lim, 1980; Bitton, 1999; Cheremisino, 2002; Burton and Stensel, 2003). For the sick of simplicity, in this paper there are presented microscopic images concerning only one sampling time; qualitatively speaking, there are no microscopic differences between the samples collected at different times.

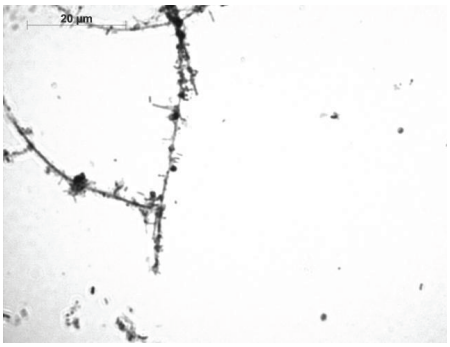


Figure 4 A Gram staining of activated sludge from aerobic tank.

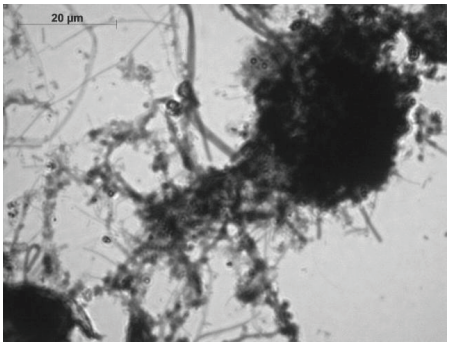


Figure 4 B Neisser staining of activated sludge from aerobic tank

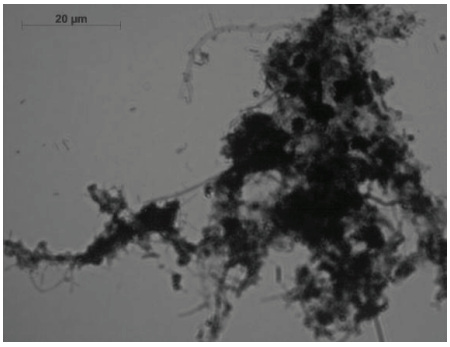


Figure 4 C Gram staining of activated sludge from anaerobic tank

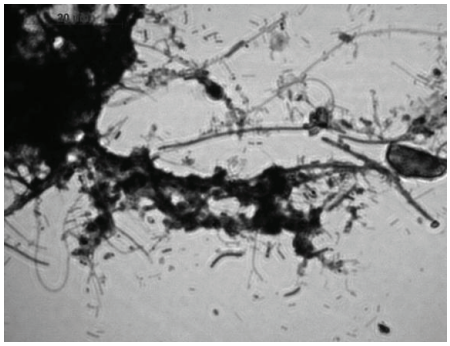


Figure 4 D Neisser staining of activated sludge from anaerobic tank

As one can see in figure 4, qualitatively speacking, there is a dominance of Gram-negative filamentous bacteria both in the aerobic tank and in the anaerobic one (A and C, respectively) and a dominance of Neisser pozitive filamentous bacteria (B and D, respectively). These qualitative results are in agreement with reports in the literature concerning the microbiota of activated sludge under normal operational parameters (Grady and Lim, 1980; Eikelboom and van Buijsen,1981; Arceivala, 1988; Droste, 1997; Bitton, 1999; Cheremisino, 2002; Burton and Stensel, 2003).

The visualization of living cells is an important task in biotechnology. When it comes to activated sludge, the use of already classical cocktail of SYBR Green and propidium iodide is not useful because the activated sludge has a strong red fluorescence (results not shown) that interfere with the fluorescence of propidium iodide, thus making impossible the specific label of dead cells by this fluorochrome. However, dead cells can be labeled by SYTOX green (Roth et al., 1997) which has a green fluorescence. In figure 5 and 6 there are presented microscopic images in bright field (A) and in epifluorescence (B) showing the same microscopic field for aerobic and anaerobic activated sludge, respectively

As one can see in figure 5 and 6 there are some microorganisms (either isolated cells and filamentous bacteria) which are permeable to SYTOX Green, thus considered as dead cells.

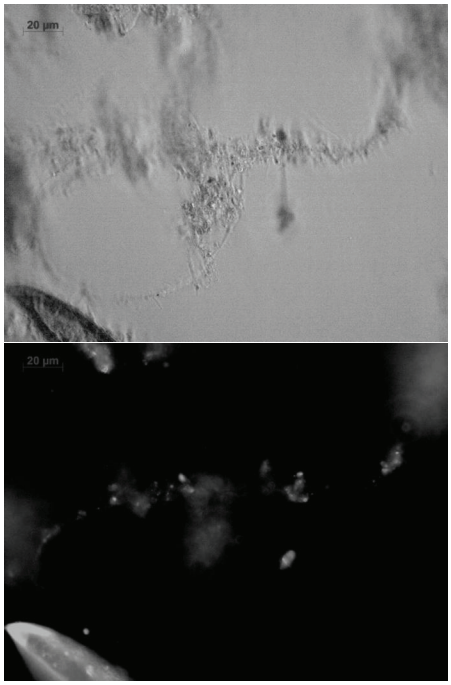


Figure 5 Microscopic images of aerobic activated sludge: A) bright field and B) fluorescence signal of dead cells labeled by SYTOX green.

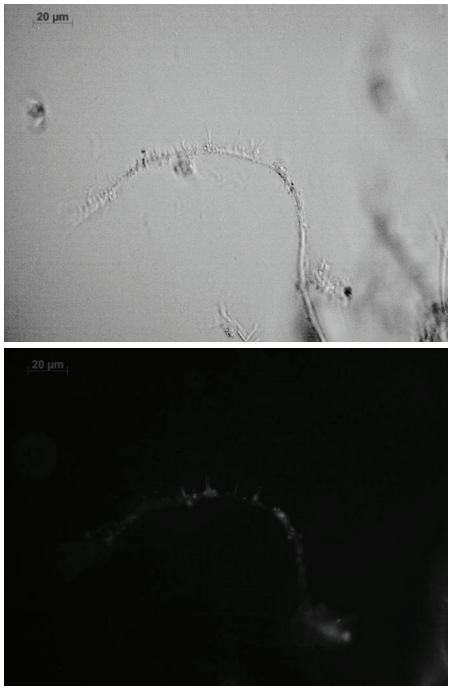


Figure 6 Microscopic images of anaerobic activated sludge: a) bright field and b) fluorescence signal of dead cells labeled by SYTOX green.

CONCLUSIONS

Chemical parameters of the waste water plant are in agreement with National and EU legislation. Qualitative microscope inspection (Gram character and Neisser reaction) show a healthy activated sludge and quantitative determination of the metabolic activity show different intensities of resazurine reduction, suggesting that the activity of activated sludge is variable in time. The qualitative presence of dead cells is seen using the fluorochrome SYTOX Green.

FUTURE PROSPECTS

The use of quantitative image analysis a powerful tool in microbiology to quantify mainly the occurrence of Gram-positive and Gram–negative bacteria, the presence of volutine-containing bacteria and the characterization of flocs (Eikelboom and van Buijsen,1981; Droste, 1997).

A better understanding of the correlation bewteen metabolic activity of activated sludge measured with resazurine or other artificial electron acceptor and/or by other methods e.g respirometry (Walker and Davis,1977) and both operational chemical parameters and microscopic parameters of the activated sludge from aerobic tank, anaerobic tank and re-circulating tank.

A deeper understanding of the overall process would open the possibility for sludge population optimization : i) selecting the most desirable species for a specific function; ii) controlling the growth of unwanted or undesirable microorganisms in the system and iii) optimizing microbial properties, as originally proposed by Yuan and Blackall (2002).

Furthermore, understanding waste water treatment plant not only as an (bio)industrial unit but also as a very complex ecological system in which microbiota strongly interacts with multicellular macroorgansims (Godeanu 1973; Grady and Lim, 1980; Vaicum, 1981; Curds and Hawkes,1983; Arceivala, 1988; Guterstam, 1996; Mitsch, 1997; Todd , 1997; Bitton, 1999; Cheremisino, 2002; Gray, 2002; Burton and Stensel, 2003; Godeanu, 2013) could help the biotechnological professionals to increase the performances of the overal process, friendly for the environment.

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XXX-Determinarea continutului de fosfor STASS SR EN 1189/1996
XXX-Determinarea reziduului uscat si a continutului din namoluri STASS SR EN 12880.