ECOLOGIA BALKANICA

2014, Vol. 5, Special Edition April 2014 pp. 41-47

Hydrobiological Investigation of the Activated Sludge from Sequencing Batch Reactors of WWTP –Hisarya

Dasha Mihaylova[1*], Tsvetko Prokopov[2], Nikolay Mihalkov[3]

1 - Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, BULGARIA

2 - Department of Environmental Engineering, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, BULGARIA

3 - Wastewater treatment plant, Hisarya, BULGARIA

*Corresponding author: dashamihaylova@yahoo.com

Abstract. The operations of the relatively new wastewater treatment plant of Hisarya, Bulgaria were evaluated in the present paper. The bacterial diversity of activated sludge from aeration basins of cyclic type (SBR-method) during the seasons was studied. The Cyclic Activated Sludge System is one of the most popular sequencing batch reactor (SBR) processes. The hydrobiological characteristic of the activated sludge was performed with regular observations by using light microscopic examinations, but also by evaluation of the flocculation ability and settleability. The presence of positive bioindicators - *Aspidisca* and *Epistyllis* species during the investigated period of time (2012 Year) was established, which confirmed the gut purification of the wastewater and the carrying out of the nitrification process. Moreover, the results obtained demonstrated that the performance of the SBR maintained high level, and the SBR system remained stable during this study.

Keywords: wastewater treatment, activated sludge, SBR-method

Introduction

Biological treatment is an important and integral part of any wastewater treatment plant that treats wastewater from either municipality or industry having soluble organic impurities or a mix of the two types of wastewater sources. The obvious economic advantage, both in terms of capital investment and operating costs, of biological treatment over other treatment processes like chemical oxidation; thermal oxidation etc. has cemented its place in any integrated wastewater treatment plant.

Cyclic Activated Sludge System as the name suggests is one of the most popular sequencing batch reactor (SBR) processes employed to treat municipal wastewater and wastewater from a variety of industries including refineries and petrochemical plants. For the first time sequencing batch reactors-technology has been used in 1914. Later in 20th century it is becoming more and more popular due to the excellent opportunities for adaptation to seasonal changes without limitation of the required optimal treatment capacity at each load. The technology offers several operational and performance advantages - great flexibility in terms of the implementation and control of different phases of the biological treatment process, such as biological phosphorus removal, aerobic oxidation of nitrogen (nitrification) and anoxic elimination of nitrate (denitrification). Several studies

Union of Scientists in Bulgaria – Plovdiv
University of Plovdiv Publishing House

demonstrated the effectiveness of SBR-technology and its application as an alternative to conventional flow system with respect to the treatment of municipal and industrial wastewater, especially for smaller flow (JANCZUKOWICZ *et al.*, 2001; MACE & MATA-ALVAREZ, 2002).

Communities of prokaryotic micro-organisms present in activated-sludge or biofilm reactors are responsible for most of the carbon and nutrient removal from sewage and thus represent the core component of every biological wastewater treatment plant (WWTP). By contrast, mass occurrence of certain bacterial species can also be detrimental for sewage treatment by negatively influencing the settling properties of activated-sludge in the secondary clarifiers, by contributing to the formation of foam or by out competing microorganisms required for nutrient removal. Consequently, a thorough knowledge of the ecology of the microbial communities is required to reveal factors influencing the efficiency and stability of biological WWTPs and to develop promising strategies for improved process performance.

The characteristic of the activated sludge flocks are important not only for researcher but also in the everyday operations of the wastewater treatment plants. Many different living beings live in sewage water. Bacteria constitute the major component of activated sludge flocks and they are responsible for the biological oxidation of organic substrates, nitrification of ammonia, denitrification of nitrate and accumulation of phosphorous (BITTON, 1994). Some bacteria form conglomerates or clusters, others float as individual cells or threads in the interconglomeratic fluid. Typically bacteria clusters include every group of protozoa (*Protozoa*): flagellates (*Zooflagellata*), rhizopods (*Rhizopoda*), heliozoans (*Heliozoa*), ciliates, sectarians (*Suctoria*). Metazoans are represented mostly by worms: nematodes (*Nematoidea*), rotifers (*Rotatoria*), and oligochaetes (*Oligochaeta*). *Protozoa* are predators of bacteria or consume dissolved substrate. So the composition of protozoa fauna of an activated sludge not only depends on environmental conditions (temperature, pH, dissolved oxygen) but the available dissolved substrate and the particular bacteria present in the sludge. That is why *Protozoa* can be used as indicator of plant performance and technological parameters (SANGJIN *et al.*, 2004). Protozoa can be classified practically in three groups: ciliates, flagellates, rhizopoda. More than 200 ciliate species have already been determined in activated sludge samples, but only a few of them can be found in a particular activated sludge. Most of the ciliates graze on bacterial cells, but some of them feed on other ciliates. Generally, diverse and high abundance of ciliate population indicate good plant behavior and excellent effluent parameters (SEVIOUR & BLACKALL, 1999).

The crawling or creeping ciliates (*Aspidisca*, *Euplotes*) feed on bacteria on the surface of sludge flocks. *Aspidisca* sp., *Euplotes affinis* are well known bioindicators of nitrification (GULÝAS, 1990). Free ciliates (eg.: *Chilodonella*, *Paramecium*, *Lionotus*) graze on free-swimming bacteria, so they clean the liquid phase of the activated sludge (MARTIN-CRECEA *et al.*, 1996; SANGJIN *et al.*, 2004). Stalked ciliates (eg.:*Vorticella*, *Carhesium*, *Epistylis*) are attached to flocks by their stalks. An ideal activated sludge contains stalked and crawling ciliates in abundance, but some of them can indicate bad effluent quality (e.g.:*Vorticella microstoma*) (MADONI *et al.*, 1993).

Flagellates can take up dissolved substrate by absorption, so they can live without bacterial cells. Flagellates are indicators of high substrate concentration and low dissolved oxygen concentration (GULÝAS, 1990). Rhizopoda (amobae) subdivided into amoeba (e.g., *Amoeba proteus*) and shell covered the camoeba. High number of the camoeba in the activated sludge indicates underloaded system and long sludge age. The generation time of small metazoa (eg.: *Rotifier* sp., *Tradigrada* sp., *Nematoidea* sp.) is long, they can be washed out in case of short solid residence time. They indicate stabile, old sludge with good flock structure (PÁSZTOR & SZENTGYÖRGYI, 2004).

The Bulgarian experience in wastewater treatment plants with a total biomass for complete removal of BOD_5, nitrogen and phosphorus is relatively new and limited. Recent projects for new wastewater treatment plants are designed by mathematical models and programs. (KUZMANOVA, 2011). One of the recently build WWTP is located in Hisarya, which is one of the famous resorts in Bulgaria with its famous mineral springs and spa, attracting thousands of tourists especially in summer. The installation was developed with the finance help of the EU Project by collaboration with Germany.

The purpose of the present paper was to evaluate the performance of the WWTP - Hisarya, which includes biological stage in aeration basins of cyclic type (SBR-method) in terms of presence of bioindicators, which evaluate the purification process itself.

Materials and Methods

Object of the present study was WWTP - Hisarya, Bulgaria. The presented results are for the period of one year (January to December, 2012). The station was put into operation in 2011 and is relatively new. Design values of the performance of the plant are: load 10000-25000 PE, wastewater dry weather flow 7250 m^3/d, wastewater wet weather flow up to 2000 m^3/h, daily treatment volume in wet weather 1080 m^3/h, organic load as BOD_5 up to 1500 kg/d, total nitrogen load 275 kg/d, total phosphorus load 45 kg/d, three aeration basins (SBRs, Fig.1), aerobic stabilization of sludge, dewatering machine (centrifuge) and conditioning with lime, installed capacity of about 430 kW, daily consumption of electricity at full load about 2000 kWh/d, specific consumption of electric energy per unit volume of wastewater 0.27 kWh/m^3 and specific electricity consumption equivalent per capita per year 29 kWh/PE.

The operation of the WWTP was evaluated by determination of the values of the following standard indicators: dissolved oxygen and temperature of the water. For characterization of the activated sludge the parameters settled sludge volume and sludge volume index were determined and the dry matter and the humidity of the waste activated sludge were also established. The evaluation of all parameters was performed by standard methods in triplicates and the characteristic of the activated sludge was performed with regular observations by using light microscopic examinations.

Fig. 1. SBR aeration basins

Results and Discussion

Since one of the critical elements in designing an aeration system are the estimation oxygen demand requirements by the microorganisms and the estimation the waste activated sludge production in the present study the values of those parameters were evaluated.

It is known that the concentration of oxygen must be enough in the aerobic zone for microorganisms that have accumulated phosphorus for complete or near complete nitrification. The concentration of oxygen must be 2–6 mg/l, no less than 1 mg/l in the aerobic zone, because otherwise the phosphates will split from the micro-organisms to wastewater (HENZE *et al.*, 1997; DROSTE, 1996). In our case, the oxygen concentrations are in the appropriate range during the whole 2012 year. The average of dissolved oxygen content in aeration basins SBR_1 and SBR_3 were 2.68 ± 0.04 and 2.69 ± 0.02 mg/l, respectively, which means sufficient aeration (Table 1). The temperatures were in range 22.1 ± 4.7 and 22.1 ± 4.6 °C, respectively.

An ideal activated sludge flock is dense, big, has spherical shape and contains only a few filamentous bacteria. An activated sludge with ideal flocks is easy to settle, filters the wastewater during the clarifying process, and produces a transparent effluent with little suspended solids content. In table 2 are represented the values of the activated sludge in the aeration basins in terms of settled sludge volume (SSV), dry matter (DM), and sludge volume index (SVI). In table 3 are represented the values for excess sludge.

Table 1. Characteristic of wastewater effluent from aeration basins SBR_1 and SBR_3

Months 2012	Aeration basin SBR_1				Aeration basin SBR_3			
	O_2, mg/l		t, °C		O_2, mg/l		t, °C	
	Av	*STD*	Av	*STD*	Av	*STD*	Av	*STD*
January	2.68	*0.15*	16.6	*1.5*	2.70	*0.10*	17.0	*1.1*
February	2.65	*0.08*	14.2	*1.0*	2.65	*0.23*	14.4	*0.8*
March	2.66	*0.16*	18.2	*1.8*	2.69	*0.14*	18.2	*1.6*
April	2.70	*0.20*	21.5	*1.2*	2.70	*0.18*	21.5	*1.2*
May	2.70	*0.21*	22.3	*2.1*	2.67	*0.16*	22.5	*2.1*
June	2.72	*0.11*	26.1	*1.5*	2.72	*0.11*	26.1	*1.5*
July	2.73	*0.10*	28.0	*0.6*	2.68	*0.13*	27.9	*0.7*
August	2.68	*0.15*	27.4	*0.9*	2.65	*0.10*	27.3	*0.8*
September	2.72	*0.13*	26.3	*0.5*	2.69	*0.09*	26.4	*0.7*
October	2.66	*0.15*	24.9	*1.4*	2.70	*0.13*	25.1	*1.2*
November	2.64	*0.14*	22.7	*0.8*	2.72	*0.13*	22.3	*0.8*
December	2.60	*0.15*	17.0	*1.2*	2.66	*0.14*	17.0	*1.1*
Average	**2.68**	***0.04***	**22.1**	***4.7***	**2.69**	***0.02***	**22.1**	***4.6***

Av – Average value; STD – Standard deviation

The results represented in both tables 2 and 3 confirmed the high quality of the technological process of water purification and the very good sedimentation properties of the sediment. All values of sludge index are in the recommended range.

The purity level of water, the current relevant properties of water quality can be determined in a fast, efficient, and cost effective way using bioindicators. Bioindicators indicate the presence and condition of the different stages of sewage treatment, also indicating the absence or excessive level of an entity. Observing the bioindicators, the quality of water, and the condition and operations of the treatment equipment can be continuously checked and controlled in a cost effective way. Thus, the study of bioindicators is justifiable. The optimal conditions in the aeration basins are indicated not only by the sufficient aeration but also by the presence of certain ciliate protozoan: *Aspidisca* species. They signal the process of nitrification, decreased ammonia level, and favorable aerobic (i.e. pertaining to the amount of oxygen) conditions. *Epistylis* ciliate protozoa are present in large numbers when the efficiency of sewage treatment is above 65%.

In table 4 are represented the presented indicator microorganisms found in the examined activated sludge.

Table 2. Characteristics of the activated sludge in the aeration basins SBR_1 and SBR_3

Month-2012	Aeration basin SBR_1						Aeration basin SBR_3					
	SSV, cm^3/l		DM, g/l		SVI, cm^3/g		SSV, cm^3/l		DM, g/l		SVI, cm^3/g	
	Av	*STD*	Av	*STD*	Av	*STD*	Av	*STD*	Av	*STD*	Av	*STD*
January	484	*61*	5.5	*0.3*	88	*6*	468	*42*	5.6	*0.3*	84	*4*
February	552	*105*	5.4	*0.5*	103	*13*	543	*63*	5.3	*0.5*	102	*7*
March	451	*88*	4.6	*0.5*	97	*11*	383	*46*	4.4	*0.3*	87	*15*
April	453	*79*	4.6	*0.5*	99	*11*	453	*36*	4.5	*0.4*	101	*9*
May	489	*89*	5.1	*0.6*	95	*8*	395	*79*	4.3	*0.4*	88	*13*
June	365	*84*	4.5	*0.7*	84	*5*	388	*64*	4.3	*0.8*	91	*8*
July	486	*77*	5.2	*0.6*	93	*7*	389	*81*	4.4	*0.9*	89	*10*
August	569	*72*	5.5	*0.6*	102	*7*	321	*37*	3.4	*0.7*	97	*12*
September	379	*49*	4.0	*0.3*	94	*8*	438	*67*	4.7	*0.6*	94	*3*
October	417	*90*	4.4	*0.5*	94	*10*	349	*41*	4.0	*0.2*	86	*6*
November	516	*53*	5.0	*0.5*	104	*3*	389	*83*	4.3	*0.6*	90	*8*
December	448	*139*	4.6	*0.9*	95	*12*	607	*55*	5.6	*0.3*	108	*5*
Average	**467**	***62***	**4.9**	***0.5***	**95**	***6***	**427**	***81***	**4.6**	***0.6***	**93**	***7***

Av – Average value; STD – Standard deviation

Table 3. Characteristics of the waste activated sludge

Month - 2012	Humidity,%		DM, g/l	
	Av	*STD*	Av	*STD*
January	97.8	*0.2*	29.5	*1.2*
September	98.0	*0.1*	30.0	*6.0*
Average	**97.9**	***0.14***	**29.75**	***0.35***

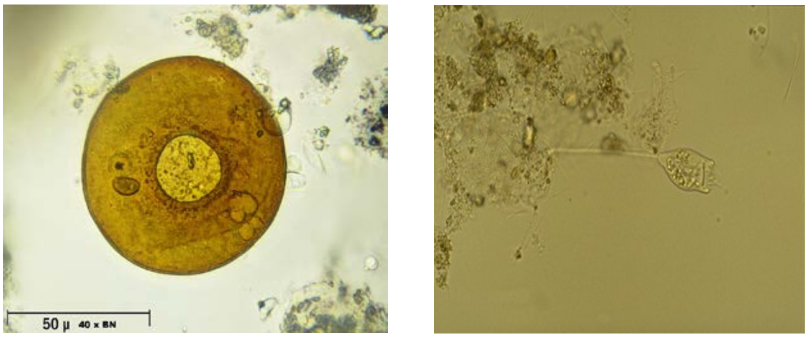
Av – Average value; STD – Standard deviation

Table 4. Presence of bioindicators in the activated sludge

Bioindicator	Month - 2012											
	01	02	03	04	05	06	07	08	09	10	11	12
Flagellatae sp.	*2*	*2*	*2*	*4*	*3*	*2*	*3*	*2*	*4*	*1*	*2*	*2*
Euglypha laevis	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*4*	*1*	*1*	*1*
Arcella vulgaris	*1*	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*4*	*1*	*2*	*1*
Epistyllis plicatilis	*4*	*2*	*2*	*4*	*2*	*3*	*3*	*4*	*2*	*4*	*2*	*4*
Turricolla similis	*1*	*2*	*3*	*2*	*2*	*1*	*2*	*4*	*4*	*4*	*2*	*2*
Arcella discoides	*2*	*2*	*2*	*2*	*3*	*1*	*1*	*2*	*4*	*1*	*2*	*2*
Vorticella microstoma	*4*	*2*	*2*	*4*	*4*	*4*	*2*	*2*	*2*	*3*	*2*	*4*
Rotatoria	*3*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*3*	*3*
Hyalodiscus linax	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
Aspidisca sp.	*2/3*	*4*	*4*	*2*	*3*	*3/2*	*3*	*3*	*2*	*2*	*4*	*2*
Vorticella convallaria	*2*	*4*	*3*	*1*	*2*	*1*	*4*	*4*	*1*	*3*	*2*	*2*
Opercularia coarctata	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*3*	*4*	*2*	*4*	*4*
Hydracarina sp.	*4*	*4*	*4*	*4*	*4*	*4*	*3*	*4*	*4*	*3*	*3*	*4*
Aspidisca costata	*4*	*4*	*3*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
Nematoda	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*3*
Philodina roseola	*4*	*4*	*4*	*4*	*4*	*4*	*3*	*4*	*4*	*4*	*4*	*4*

1- commonly represented
2- mid represented
3- poorly represented
4- not represented

Many different living beings live in sewage water. Bacteria represent the most populous group. The protozoa communities of the activated sludge were considerably different. In the sludge of WWTP-Hisarya several genera of protozoa were found. The most abundant genera in sludge were the stalked ciliates *Vorticella*. The dominant group of protozoa was ciliates.



Arcella vulgaris *Vorticella convallaria*

Fig. 2. Bioindicators presented in the activated sludge

The most important bioindicative processes could be observed e.g.: abundance of *Aspidisca* species indicated good nitrification and dominancy of *Ciliates* sowed excellent treatment performance. The presence of *Vorticella convallaria* and *Arcella vulgaris* (Fig. 2) was a positive indicator for the wastewater treatment process whereas the presence of *Vorticella microstoma* was undesirable and indicates bad effluent quality. The numerous flagellates in the activated sludge were unexpected, because they generally indicate low oxygen concentration or overloading. However, the appearance of activated sludge is good, is fast settling of dense flocks supernatant is clear.

Conclusions

Analysis of the results of WWTP-Hisarya operation shows that it is designed and built a future oriented, modern wastewater treatment plant with all conditions to work reliably for many years, which in terms of equipment, structure and economical effectiveness set new accents.

The massive presence of positive bioindicators and the rare presence or absence of undesirable bioindicators indicates that the aeration basins work well and microorganisms are able to purify to a sufficient degree the incoming wastewater.

Acknowledgments

The authors greatly appreciate the kind assistance of the Municipality and Mayor of Hisarya and the permission to access the WWTP of Hisarya.

References

BITTON G. 1994. *Wastewater Microbiology,* Wiley-Liss Publication, New York, USA, p. 150-155.

DROSTE R.L. 1996. *Theory and Practice of Water and Wastewater Treatment.* 1st edition. John Wiley & Sons, ISBN: 978-0471124443. 816 p.

GULÝAS P. 1990. *Activated sludge under microscope,* Innotrade kft, Budapeest, Hungary (in Hungarian).

HENZE M., P. HARREMOES. J.I.C. JANSEN. E. ARVIN. 1997. *Wastewater Treatment, Biological and chemical process.* 2nd edition. Springer/Sci-Tech/Trade, ISBN: 978-3540627029. 383 p.

JANCZUKOWICZ W., M. SZEWCZYK. M. KRZEMIENIEWSKI. J. PESTA. 2001. Settling Properties of Activated Sludge from a Sequencing Batch Reactor (SBR). - *Polish Journal of Environmental Studies*, 10(1): 15-20.

KUZMANOVA K. 2011. [Problems with newly WWTP with suspended biomass-sized "sensitive areas", with complete removal of organic pollution, nitrogen and phosphorus]. – In: *Proceedings, reports Fourth International Conference "Bulaqua."* Bulgarian Water Association, Sofia, (In Bulgarian).

MACE S., J. MATA-ALVAREZ. 2002. Utilization of SBR technology for Wastewater Treatment: An overview. - *Industrial and Engineering Chemistry Research*, 41: 5539-5553.

MADONI P., D. DAVOLI. E. CHIERICI. 1993. Comparative analysis of the activated sludge microfauna in several sewage treatment works. - *Water Research*, 27(9): 1485-1491.

MARTIN-CRECEA M., S. SERRANO. A. GUINEA. 1996. A comparative study of ciliated protozoa communities in activated-sludge plants. - *FEMS Microbiology Ecology,* 21: 267-276.

PÁSZTOR I., H. SZENTGYÖRGYI. 2004. Bioindicators of activated sludge. –In: *XVII. National Environmental Conference an Exhibitions,* 21-23 September, Siófok, Hungary,\ (In Hungarian).

SANGJIN L., B. SOMMATH. W.T. CHARLES. W.W. IRVINE. 2004. Ciliate populations as bio-indicators at Deer Treatment Plant. - *Advances in Environmental Research*, 8: 371-378.

SEVIOUR R.J., L.L BLACKALL. 1999. *The microbiology of activated sludge,* Kluwer Academic Publishers, London, UK.

Received: 17.10.2013
Accepted: 02.03.2014