COMPARISON OF ACTIVATED SLUDGGE FLOCK STRUCTURE AND MICROBIAL FAUNA OF TWO HUNGARIAN WASTEWATER TREATMENT PLANTS

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Abstract

Activated sludge consists of bacteria, protozoa and metazoa species, but the major components of sludge flocks are active bacterial cells. The bacteria are responsible for the main biodegradation processes, such as carbon oxidation, nitrification, denitrification, phosphorous accumulation etc. The protozoa and metazoa community of the wastewater do not take part in the basic pollutant removal processes, but they live in close relationship with the bacteria and influence flock formation. Because of this interaction the protozoa species can be used as bioindicators of wastewater treatment plant performance. Activated sludge of two Hungarian waster treatment plant was examined, and the main flock properties and protozoa community of the sludge were determined. Although the capacity, the efficiency and the technology of the two wastewater treatment plants were very similar, the characteristic of the two activated sludge was found to be quite different. The diversity of the protozoa community and the number of found species were significantly dissimilar in the sludge samples of the two plants. No clear correlation could be made between the found indicative protozoa species and the treatment performance. The conclusion was that every wastewater treatment plant has a specific microorganism community, which is not directly determined by the characteristics of the wastewater or the treatment technology. Therefore long term microscopic tracking of activated sludge flock structure and microorganisms at a particular plant can provide more information about treatment performance, then the comparison of microbial fauna of different plants.

1 Introduction

1.1 Bacteria in flock formation

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 [[2]].

Bacteria can be grouped upon their role in flock formation. The free swimming bacteria are floating in the wastewater among activated sludge flocks, if they are present in huge number they may contribute to high effluent BOD_5. The flock forming bacteria are attached to each other and constitute well settling flock-like biomass, which can be separated easily from the liquid phase in the final clarifier. Filamentous bacteria can be useful, because they can be work as skeleton for flocks. On the other hand, if lots of filamentous bacteria are present in the sludge they often cause bulking and foaming problems [[7]].

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 produce a transparent effluent with little suspended solids content.

Light microscopic examination of activated sludge flocks can provide information about settling properties, but uninformative about treatment performance (e.g.: nitrification, denitrification) and other technological parameters (dissolved oxygen, sludge resident time).

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1.2 Protozoa as indicators

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 [[8]]. 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 (5000-1000 ind./l) of ciliate population indicate good plant behavior and excellent effluent parameters [[9]].

The crawling or creeping ciliates (Aspidisca, Euplotes) feed on bacteria on the surface of sludge flocks. Aspidisca sp., Euplotes affinis are well known bio-indicators of nitrification [[3]]. Free ciliates(eg.: Chilodonella, Paramecium, Lionotus) graze on free-swimming bacteria, so they clean the liquid phase of the activated sludge. Chillodonella sp. can be correlated with low F/M ratio and long SRT, while Lionotus lamella related with deficiently settling sludge [[8],[10]]. 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.:Voticella microstoma,) [[5]].

Flagellates can take up dissolved substrate by absorption, so they can live without bacterial cells. Some important flagellates: Bodo sp., Hexamitus sp., Monosiga sp.. Flagellates are indicators of high substrate concentration and low dissolved oxygen concentration [[3]].

Rhizopoda (amobae) subdivided into amoeba (e.g., Amoeba proteus) and shell covered thecamoeba. High number of thecamoeba in the activated sludge indicate 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[[7]].

1.3 Objective of the study

The aim of this project was to determine the microbial fauna composition and flock structure of two modern Hungarian wastewater treatment plants (WWTP) with similar treatment technology and evaluate practical usefulness of microscopic examinations. We were seeking for correlation between the treatment technology and the microbial community and wanted to verify the internationally published indication mechanisms on Hungarian activated sludge.

2 Materials and method

2.1 Description of the plants

Two similar wastewater treatment plant were chosen for the comparative examination, both plants have A2/O technology with anaerobic sludge stabilization (figure 1.). The two plants are the Veszprém and Székesfehérvár WWTP. The hydraulic loading rate of Veszprém WWTP is $14000\text{m}^3/d$ and $23000\text{m}^3/d$ of the one in Székesfehérvár WWTP, they receive mainly communal wastewater. As the examined plants have similar technology, similar influent wastewater characteristics and similar treatment efficiency, we assumed that the activated sludge microbial composition and flock structure would be analogous, too. The actual technological and operational parameters of the plants were colleted from the staff of the plants. The influent and effluent concentrations were provided by the wastewater laboratories which take the routine measurements of the plants. The main technological properties and wastewater characteristics are shown on table 1.

2.2 Sampling and examination

Activated sludge samples were taken from the end of the aerated basin (final biological step) of both plants once a week for 5 weeks. The samples were examined in 1-2 hours without storing or cooling. The activated sludge samples were investigated at various magnifications (100, 400, 1000X) using phases contrast and normal lenses. The size of flocks and protozoa were measured with micro scale lenses.

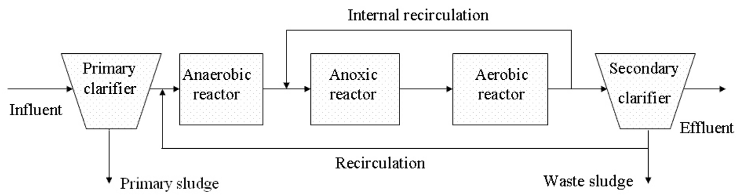

Figure 1: Simplified configuration of the two WWTPs

Table 1: Technological parameters and wastewater characteristics at the two examined WWTP

Characteristics:	Székesfehérvár WWTP			Veszprém WWTP		
Hydraulic load (m3/d)	23000			14000		
Technology	A2/O			A2/O		
Polyelectrolyte dose to aer. basin	Yes			No		
Liquid temperature (oC)	14-16			11-12		
Concentrations:	Influent	Effluent	Efficiency	Influent	Effluent	Efficiency
COD (mgO2/l)	727	60	91.75%	667	41.8	93.73%
BOD (mgO2/l)	360	10.8	97.00%	307	6.8	97.79%
Total Suspended Solids (mg/l)	364	44.5	87.77%	412	65	84.22%
Ammonium-N (mg/l)	40.3	1.8	95.53%	56.7	2.5	95.59%
pH	7.8	7.6		8	7.7	

Each examination was started with the macroscopic characterization of the samples in glass Erlenmeyer vessel. The main macroscopic properties, such as: color, transparency, smell etc. were first recorded on laboratory worksheets. The worksheets were constituted according to forms used by Seviour and Blackall [[9]].

After that pre-weighed two drops of mixed sample were investigated separately. The whole surface of the drops of sample was scanned by the microscope and all the found species were determined and counted. To ease identification work, short video files were taken by digital camera, and international scientific literature was used [[1],[4],[6]].

3 Results

3.1 Macroscopic and flock properties

The main macroscopic and flock structure properties of the two activated sludge is shown in table 2. The activated sludge of Veszprém WWTP was lighter color, consisted of smaller flocks(flock diameter $< 300\mu$m) and had less compact structure with low bridging effect of filamentous bacteria. The main difference was that the sludge of Székesfehérvár plant contained less Microthrix Parvicella filamentous bacteria in the liquid phase between flocks. The filaments were stuck to the surface of flocks, there were no or only a few floating free filaments among flocks and no bridging effect was observed. This difference may have been caused by the regular polyelectrolyte addition to the aerated basin at Székesfehérvár WWTP to avoid sludge bulking. The cause of the other macroscopic and flock structure differences of the samples of the two WWTPs was not found out.

3.2 Protozoa and metazoa found

The classification of protozoa is unstable, because the theory of evolution is constantly changing. During the examination we followed the classification and taxonomy of Patterson, which is intended to be a simple

Table 2: Macroscopic and flock structure properties of activated sludge of the two examined plants

Property	Székesfehérvár WWTP	Veszprém WWTP
Color of activated sludge	Brown, grayish brown	Light brown
Smell of activated sludge	Marshy	Ground
Flock diameter	Few small ($< 150\mu$m) Lot of medium ($150 - 500\mu$m) Some large ($> 500\mu$m)	Lot of small ($< 150\mu$m) Few medium ($150 - 500\mu$m)
Flock morphology	Round and compact	Round and diffuse
Filament effect on flock structure	Little	Bridging
Filament abundance	Common, mainly on the surface of flocks	Very common
Free swimming bacteria	Few	Few, some

filing system, evolutionary relationships are not implied [[6]]. The found protozoa and metazoa groups are shown in table 3. Individuals of different genera were counted in every sample and after averaging, they were put in four categories according to their abundance: scarce = 5-10 ind./ml, moderate = 10-100 ind./ml, abundant = 100-1000 ind./ml, more abundant = 1000 ind./ml.

The protozoa communities of the two activated sludge were considerably different. In the sludge of Székesfehérvár WWTP 23 genera of protozoa were found, while in the sludge of the other plant only 13 groups were present. The dominant group of protozoa was cilliates at both WWTP, but the samples of Veszprém contained less genera. Other big difference was that many falagellates were found in the sludge of Székesfehérvár, while flagellates were very scare in Veszprém.

The highest frequencies of occurrence were of genera Vorticella and Amoeba in the Székesfehérvár WWTP samples. The most abundant genera in sludge of Veszprém WWTP was Vorticella and Aspidisca. Numerous genera was found only in one of the WWTP e.g.: Opercularia, Euplotes, Carchesium, etc.

Legends:

Table 3: Name and quantity of protozoa end metazoa found in activated sludge of the two examined WWTP

	Abundance			Abundance	
Name	Székesfehérvár WWTP	Veszprém WWTP	Name	Székesfehérvár WWTP	Veszprém WWTP
Ciliates			Rhizopoda		
Vorticella	√√√√	√√√√	Amoeba	√√√√	√
Opercularia	√√√		Arcella	√	√√√
Epistylis	√√√	√√	Flagellates		
Aspidisca	√√√	√√√√	Peranema	√√√	√√
Thuricola	√√√		Petalomonas	√√√	
Colpidium	√√√		Monas	√√	
Trachelophyllum	√√√	√√	Entosiphon	√√√	
Prorodon	√√√	√√	Bodo	√√	√
Litonotus	√√		Suctoria	√√	
Chilodonella	√√√		Metazoa		
Calyptotricha	√√		Rotifer	√√√	√
unknown	√		Tradigrade	√√	
Uronema	√		Nematode		√
Euplotes		√√√	Free swimming bacteria	◊	◊
Carchesium		√			

◊ = 5-15 ind./ wiew (magnification 1000X)
√ = 5-10 ind./ml
√√ = 10-100 ind./ml
√√√ = 100-1000 ind./ml
√√√√ > 1000 ind./ml

4 Conclusions

Despite of the similar influent wastewater, the same treatment technology and treatment performance, the activated sludge of the two examined WWTPs were very different. The sludge of Veszprém WWTP had not only lighter color, contained more filamentous bacteria and had poorer flock structure, but its protozoa community was less diverse and less abundant. The flocks of Székesfehérvár WWTP must have been larger and more compact, as there was polyelectrolyte addition to the aeration tank. Cause of other macroscopic differences of the sludge was not revealed.

The most important bio-indicative processes could be observed e.g.: abundance of Aspidisca species indicated good nitrification, metazoan species were found because of long sludge age, dominancy of Ciliates sowed excellent treatment performance, etc. The numerous flagellates in the activated sludge of Székesfehérvár were unexpected, because they generally indicate low oxygen concentration or overloading. In this case the flagellates must have been growing in the anaerobic reactor and did not indicate insufficient aeration.

The main conclusion of the investigation was that the pollutant removal efficiency and settling characteristics of an activated sludge can not be evaluated by using only light microscopic examinations. Every wastewater treatment plant has a specific microorganism community and flock structure, which is not directly determined by the characteristics of the wastewater or the treatment technology.

Therefore, comparison of two activated sludge is not recommended on the basis of only microfauna community or macroscopic characteristics. Nevertheless long term and regular microscopic tracing of a particular activated sludge can provide useful information about developing flock structure problems, unrealized operational deficiencies or immediate changes in wastewater quality.

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