ACTIVATED SLUDGE BULKING EPISODES AND DOMINANT FILAMENTOUS BACTERIA AT WASTE WATER TREATMENT PLANT CONSTANȚA SUD (ROMANIA)

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The study has been carried out during September 2006–January 2007 at Wastewater Treatment Plant Constanța Sud and it had the aim to identify main microorganisms responsible for bulking upsets. A comparative survey of abundance and size of floc-forming and filamentous bacteria has revealed that proportion of these bacterial groups could significantly change at different sludge volume index (SVI) values. Dominant filamentous bacteria have been identified as Eikelboom type 0041, *Nostocoida limicola*, and *Sphaerotilus natans*. It seemed that their proliferation would be supported by oxygen fluctuations and fully mixed operational mode of the treatment plant.

Key words: Activated sludge; Bulking; Filamentous bacteria; Zoogloeal organisms.

INTRODUCTION

The activated sludge consists of a complex biological community including viruses, bacteria, protozoa fungi and metazoa that achieve the degradation of organics in waste water[1]. Quantitatively, bacteria represent around 95% of the total microbial population[2] and are essential for biological removal of organic carbon, ammonium and phosphate in the aeration tank[3]. The next step consists of settling and compaction of the activated sludge in the secondary clarifier and it is critical to guarantee a good effluent quality. The activated sludge technology is based on the ability of microorganisms to form flocs when wastewater is aerated[4]. Within flocs, living and dead cells, organic and inorganic particles, fibres are held together by a slime matrix produced by bacteria. Optimal formation of flocs is essential for sludge quality and clean water leaving the final clarifier. In fact, architecture of the flocs is complex enough, both zoogloeal (among which most common is *Zoogloea ramigera*) and filamentous bacteria are participating in the floc development[5]. In first stage, filaments act as a "backbone" of the floc and as attachment site for zoogloeal organisms. Fingered zoogloeal colonies attract and hold small organic and inorganic particles. Alteration of flocs development due to dispersed or excesive growth of bacteria may lead to specific operational problems of activated sludge plants. In both cases biomass does not settle resulting in very turbid effluent. Most problems are associated with excessive growth of filamentous bacteria condition called filamentous bulking[6]. Bulking of activated sludge have become a common problem of many plants[7, 8]. It may be the main cause of poor effluent quality, environmental damages, and in severe cases sludge loss to the effluent. During last three decades microbiological investigations have lead to description and characterization of 25 different filamentous bacteria commonly found in activated sludge[9, 10, 11, 12, 13]. Since each filament can lead to specific problems in operating of wastewater plants identification of organisms is the basic approach to determine the cause and find solutions to control the dominant bacterial species[13, 14]. Following the morphological-physiological method our paper analyzes the relationships between abundance, size of flocs, oxygen dynamics, and dominant filamentous bacteria during several bulking episodes at Wastewater Treatment Plant Constanta Sud.

MATERIAL AND METHODS

At Constanța Sud aeration unit includes two tanks (F1, F2) equal as volume eacch of them receiveing both domestic and industrial wastewaters. Samples were taken from three points: a) waste water before entering the aeration tank; b) activated sludge from the aeration tank; c) water samples downstream the secondary clarifier. Oxygen concentration was determined according SR EN 25813 ISO 5813/2000[15]. Microscopic examination was carried out several hours after sampling with or without dilution. Abundance of flocs was determined by direct microscopic count using a high resolution digital camera (Euromex) connected to a Novex microscope. Image analysis was done with ImageFocus v.1.2.1.3. and Image Tool 1.2. software package. Gram and Neisser staining were performed according to usual bacteriological procedures[16]. Filamentous bacteria have been identified using Eikelboom and Jenkins system[10, 11, 12, 13].

RESULTS AND DISCUSSIONS

Physicochemical parameters

During analyses temperature of wastewaters varied from 12°C to 19°C and pH value showed only minimal fluctuations between 7.2–7.6. Dissolved oxygen (DO) concentration ranged from 1.2 to 2.2 mg l^{-1} (Fig. 1). These fluctuations could affect the diversity of microbial consortium and its metabolism as well as the activated sludge physical properties.

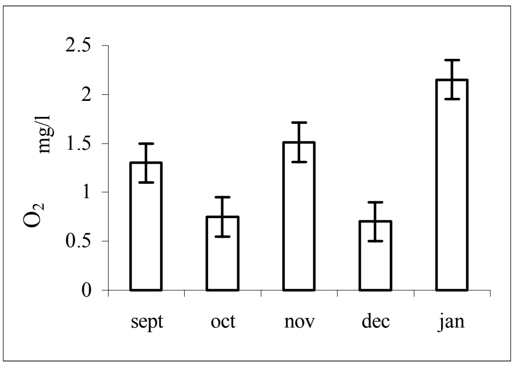

Fig. 1. Dynamics of oxygen concentration.

Abundance and morphology of flocs

Seize of flocs is an important parameter that influences of activated sludge settling. Generally, it is believed that the activated sludge settles well when the dominant size class of the flocs is around 50 µm. Larger or smaller flocs have tendency to settle slowly in the final clarifier and the effluent is turbid and of low quality. The purpose of this study was to analyze the relationship between abundance and size of flocs and settling qualities of sludge. Another purpose was to determine how accurate could be the microscopic examination in order to diagnose and predict the bulking upsets. During our observations the abundance of flocs and their sieze were variable. Flocs within 100–200 µm class size dominated the initial period of observation with average density of 30×10^3 flocs/ml activated sludge. At the end of observation period flocs within 50–100 µm size class were more abundant with everage density of about 27×10^3 flocs/ml sludge. As size of flocs has decreased progresively so the sludge volume index lowered too. In general, our observations have indicated that increased abundance of flocs larger than 100 µm in diameter occurred at increased SVI values. Since the optimal characteristics of sludge are the result of balanced developement of both filmentous and floc-former bacteria, another purpose was to relate specific-group abundance with sludge index. *Zoogloea ramigera* is the main floc-former organism with characteristic fingered colonies of extracellular slime enclosing individual cells in a common mass. (Figure 3c). Most theories[12, 13] consider these bacteria the key organism in genesis of flocs because they act as primary microsite for colonization of another microorganims and for entraping particles existing in waste water.

Enumeration of zoogloeal masses has been made after negative staining with nigrosine (Fig. 3b) when a higher number has been counted in comparison with phase contrast examination. Abundance of zoogloeal organisms was relatively inconstant, ranging from 10×10^3 to 90×10^3 colonies/ml sludge (Fig. 2a, b). Their size class changed slightly from 10-50 µm in the intial period to 50–100 µm at the end of period (Figure 2a, b) and, in general, a lower abundance was observed for colonies >100 µm in diameter. They were equal or higher than abundance of flocs, but their dimension was in most cases more reduced than that of flocs.

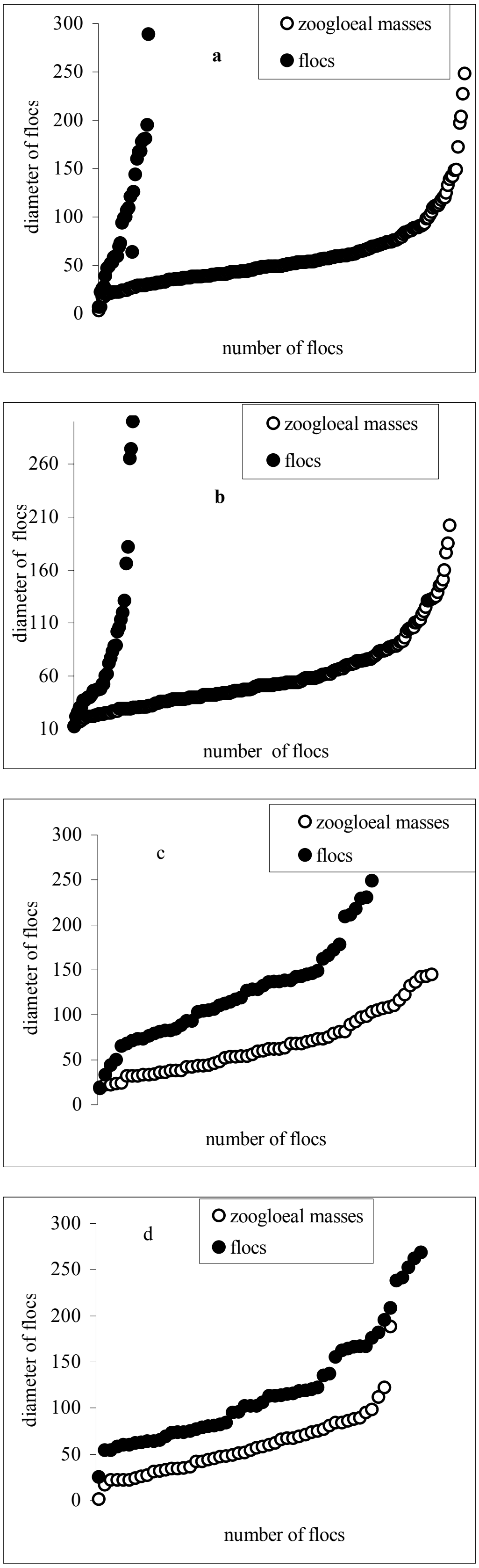

Fig. 2. Abundance and size distribution of floc formers and filamentous bacteria: a – October tank F1: b – October tank F2: c – January tank F1: d – January tank F2.

Zoogloea provides the intial site, but flocs become larger on the expense of filamentous bacteria protruding outside. Regarding the relative abundance of the two bacterial groups we have noted two different situations during several bulking episodes. An intial bulking event was characterized by SVI value of 299–331 and by an obvious disproportion between floc formers and filamentous bacteria (Fig. 2a, b). Apparently the cause of bulking was determined by excessive proliferation of zoogloea like organisms.

The microscopic examination showed large flocs with voids, and extended filaments interbridging and connecting different flocs (Fig. 3a). Zoogloeal masses were numerous but small and dispersed within less compact structure of the flocs. During the second bulking event when the sludge had lower SVI values ranging from 170 to 225 both abundance and size of the two groups were closer and more balanced (Fig. 2c, d). Microscopic exmination showed more compact flocs with less but larger zoogloeal masses.

It is believed that high nutrient loading of wastewater might favour *Zoogloea* growth[17]. Bulking (slime bulking) often occurs at high F/M (Food to Microorganim) ratio when these organisms proliferate faster than filamentous bacteria. We assume that in a first stage these organisms have grown and depleted the nutrients. Subsequently, filamentous bacteria have gradually replaced them since they were able to grow at low nutrient concentration due to their particular metabolism.

Dominant filamentous bacteria

Eikelboom type 0041. Trichomes produce a sheath often heavily colonized by epiphytic bacteria. It is a Gram variable filament, reaction to Gram staining depending on the presence of attached growth. When attached growth is massive the trichomes appear as Gram negative (Fig. 4a). Filaments are long and grow both in the flocs and in the bulk liquid. Type 0041 is a Neisser negative organism, but sometimes the presence of a slime layer around the trichome may react Neisser positive. Senescent filaments provide the sckeleton for epiphytic bacteria and act as initial microsite for floc development. Eilkelboom type 0041 is one of the most widespread organism involved in bulking events in South Africa[18], Asia[19], Europe[20, 21], North America[22], Australia[23], and South America[24].

Nostocoida limicola may be found both inside and outside the flocs in the bulk liquid as long and coiled filaments. Individual cells inside the trichom are oval. *N. limicola* is a Gram variable and Neisser positive organism. Its reaction to Gram staining depends on the composition of wastewaters. We have found only the Gram negative type as usually reported for domestic wastewaters. It is a facultatively anaerobic organism able to use carbohydrates, alkohols and organic acids. It seems that increased proliferation of this species is favoured by high organic and oxygen content of waste waters.

Growth of this filaments might be prevented by reducing of the sludge age and by maintaining the wastewater in anaerobiosis a short time before they are introduced in the aeration tank. During our study *N. limicola* (Figure 4b) had a relatively high frequency, but its overall abundance was rather low. *N. limicola* has been found mainly in Europe[25].

Sphaerotilus natans is chemoorganotrophic, aerobic organism able to degrade low molecular organic substrates. Trichomes are surrounded by a sheath with iron deposits. *S. natans* is a Gram negative (Figure 4c), Neisser negative organism, easily identifiable due to the dichotomous false branching. Excessive growth of *S. natans* leads to altered settling capacity of sludge. Interfloc bridging is the main mechanism causing bulking and plants are difficult to operate when *S. natans* is the dominant organism. *S. natans* has been described in Japan[19] and Argentina[24]. Identified filaments can be physiologically divided[26] into three groups: a) low dissolved oxygen aerobic zone growers (*S. natans*)[27]; b) aerobic zone growers (*N. limicola*); c) variable aerobic and anaerobic zone growers (type 0041). Consequently, dominant species are physiologically diverse and sludge bulking can occur therefore to a wide range of dissolved oxygen. This fact suggest that among another factors large fluctuation of oxygen content in the aeration tanks would allow the proliferation of diverse and distinct filamentous bacteria in respect of their oxygen requirements. Thus, control of filamentous bacteria needs in the first stage a careful monitoring of oxygen supplied to the activated sludge. On the other hand, bacterial growth depends not only on the oxygen content but also on level and availability of nutrient in the wastewaters. At low substrate concentration filamentous bacteria and floc-forming bacteria are competing for nutrients[28, 29]. Floc-former bacteria are fast-growing having growth rates and affinity constant for substrates (k-strategists) higher than filamentous bacteria (r-strategists)[30, 31]. In systems where substrate concentration is low filamentous bacteria win the competition since they can uptake the nutrients found at low level[32, 33]. The competitive ability of filamentous bactreria over floc formers is also enhaced by their particular morphology[34]. When the substrate become limiting they grow predominantly out of the flocs and gain easier nutrients from the bulk liquid than floc formers. At lower nutrient concentration the growth of floc formers is supressed due to difficult diffusion of nutrients inside the flocs[35] but filaments are favoured since they can grow and extend out of the flocs. This is the preffered growth pattern of filamentous bacteria in continuously fed completely mixed activated sludge systems. Continuous-flow systems with completely mixed mode such is that of Constanta Sud are wide spread in the world. To limit bulking it have been proposed several solutions such as intermitently fed systems (sequencing batch reactor) or compartmentalised aeration tanks (plug-flow reactor). Taking into account the above observations, we assume that main factors favouring at this plant the bulking episodes would be low oxygen content and design of reactor.

CONCLUSIONS

We have recorded a significant discrepancy between abundance of filamentous and floc forming bacteria on the one hand and size of flocs on the other during bulking events. Filamentous bacteria accounted for the major biomass proportion of flocs and explained the increase of SVI value during bulking episodes. When both groups were closer as abundance and class size distribution the properties of the activated sludge have been improved. Dominant filamentous bacteria belonged to *S. natans, N. limicola,* and Eikelboom type 0041. They are physiologically heterogenous as oxygen requirement and are able to grow to a wide range of dissolved oxygen. Therefore, bulking of activated sludge can occur to a wide range of oxygen content and the main short-term tool to control the excessive growth of filamentous bacteria lies on the careful monitoring of oxygen supply in the aeration tanks. This will allow long-term and more appropriate measures to limit bulking.

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