

## Microbiological investigation of activated sludge in sequencing batch bioreactor for urban wastewater treatment

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This study presents measurement of containment parameters of wastewater obtained from influent and effluent of sequencing batch bioreactor (SBR). *Arcella* Sp. dominantly encountered in Sample I from Karaman-Adapazari-Sakarya Urban Wastewater Treatment Plant in Turkey. Amount of cells belonging to *Diffugia* Sp., however, had increased for Sample II. In Sample III, *Arcella* Sp., and *Diffugia* Sp. lost their dominance, whereas *Didinium* Sp., *Aspidisca* Sp., *Targidrade* Sp., and *Peranema* Sp., were encountered. This finding indicates that protozoa and metazoan population in activated sludge enable daily control of wastewater treatment efficiency.

**Keywords:** Activated sludge, Metazoa, Protozoa, Sequencing batch bioreactor (SBR)

### Introduction

Sequencing batch bioreactor (SBR) has been widely used to treat municipal wastewater, landfill leachates and various industrial wastewaters<sup>1-5</sup>. Protozoan taxa in wastewater treatment plants (WWTP) can be classified as flagellates, amoeba and, in particular, ciliates. Majority of samples of activated sludge biomass reveal presence of larger organisms' small metazoa (nematodes, rotifers and oligochaete worms) with generation times shorter than sludge age<sup>6</sup>. A limited number of studies have focused on significance of protozoa and metazoa in WWTP as key organisms for improving plant's final effluent quality. Moreover, plant's protozoa community structure rapidly changes as a response to different operating conditions. Thus, regular plant monitoring is significant for predicting day-to-day performance<sup>7</sup>. This paper examines efficiency of wastewater treatment of SBR and variety of protozoa and metazoan that affect efficiency in activated sludge.

### Experimental Section

#### Sequencing Batch Bioreactor (SBR) Operation

Experimental studies were conducted in a SBR (total vol, 55 l; operation vol, 40 l; base sides, 35 cm; and height, 45 cm) at laboratory scale. SBR (Fig. 1) was

manufactured with glass material. Aeration was realized by air stones located in reactor using double effluent aquarium pumping (capacity, 2500 cm<sup>3</sup> air/ min) so that dissolved oxygen concentration is kept above 2 mg l<sup>-1</sup>. Mechanical mixer (120 cycles min<sup>-1</sup>) was utilized in reactor. Wastewater feeding and discharge of reactor were implemented by peristaltic pumps. SBR (active vol, 40 l) was put into use by feeding with activated sludge (34 l) and wastewater (6 l). One cycle SBR was set as 4 h (filling, 0.5; reaction, 1.5; settlement, 1.5; and decant phase, 0.5 h). Upper phase (6 l) was sucked in via peristaltic pump following settlement duration of 1.5 h. Activated sludge samples were collected during aerobic/mixing phase and settlement phase of SBR for further microbial community analyses.

#### Characterization of Wastewater

Wastewater was obtained from effluent of Karaman-Adapazari-Sakarya-Turkey Urban Wastewater Treatment Plant. Activated sludge was taken from activated sludge effluent of same plant. Chemical oxygen demand (COD), biochemical oxygen demand (BOD), total nitrogen (TN), total phosphorus (TP), solids in suspension (SS), pH, conductivity, color, turbidity, and temperature were measured at influent-effluent of SBR to determine system performance. Measurements were conducted by applying standard methods. Microorganism types and floc structure of composed sludge in SBR were

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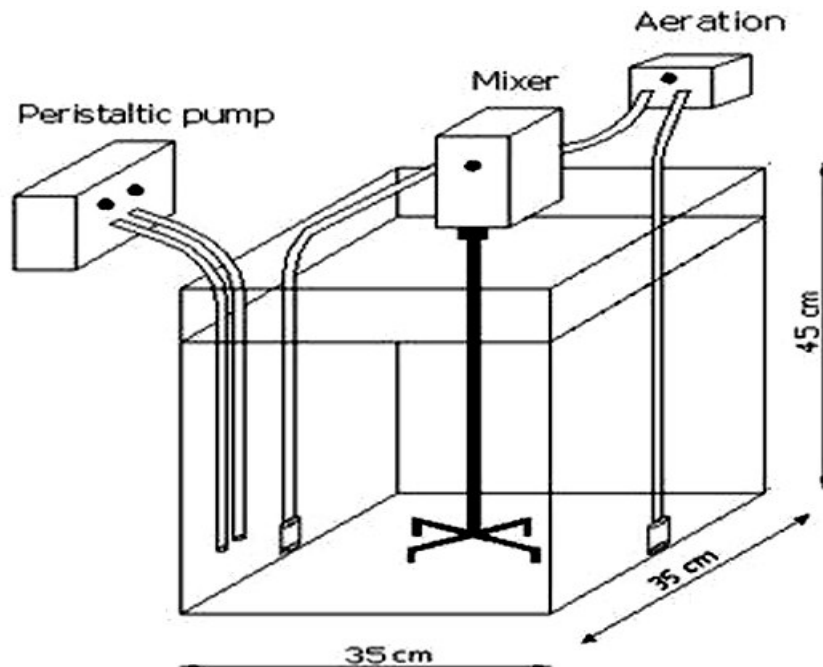


Fig. 1—Sequencing batch bioreactor in laboratory scale

examined in microscope. Samples of activated sludge, taken during mixing phase, and base sludge, taken during settlement phase, from reactor were examined. OLYMPUS BX51T phase contrast light microscope (100x, 400x, and 1000x magnifications) for obtained images and 400x, and 1000x magnifications were used for identification of protozoa and metazoa.

### Results and Discussion

Sample I, II and III were taken in optimum operation conditions for urban wastewater treatment in SBR from same lab-scale wastewater treatment plant (total vol, 40 l) at same operational times in different weeks. Total time for one cycle was 4 h and SBR was operated at 6 cycles per day.

#### Sample I

Sludge volume index (SVI) in Sample I was measured as 76 mlgr<sup>-1</sup>. Influent value of COD decreased from 206 mg l<sup>-1</sup> to 35 mg l<sup>-1</sup>; and an efficiency of 83% was established (Table 1). BOD influent value decreased from 101 mg l<sup>-1</sup> to 9 mg l<sup>-1</sup>, and removal efficiency occurred as 91%. SS influent-effluent concentrations were 277 mg l<sup>-1</sup> and 13 mg l<sup>-1</sup>, respectively, and efficiency occurred as 95%. TN influent concentration, however, was 152 mg l<sup>-1</sup> whereas effluent concentration was 7.9

mg l<sup>-1</sup>, with a high level of removal efficiency (95%). Concentration for TP decreased from 4.3 mg l<sup>-1</sup> to 2.73 mg l<sup>-1</sup>, with considerably low removal efficiency (36%). During microbiological investigation, SBR was operated at 1.5 h reaction and 1.5 h sedimentation time. Floc structure was tight during mixing and settlement phases for Sample I. Considering these specifications, sludge fits to be Floc Type 1, which is a characteristic sludge type of treatment plants with a sludge load < ca. 0.2 kg BOD/MLSS day using surface aerators for oxygen supply. Floc size was 25-250 μm and flocs were tight.

*Arcella* sp. was frequently encountered in low charged activated sludge systems and especially under circumstances of nitrifications. Most important feature of *Arcella* sp. was browning due to settlement amount of ions in sludge. *Arcella* sp. (2100 ind./ml), *Epistylis* sp. (1750 ind./ml) and *Carchesium* sp. (1230 ind./ml), as indicators of good quality sludge, were encountered in activated sludge images at mixing phase for Sample I. Moreover, *Scyphidia* sp. (550 ind./ml), *Philodina* sp. (920 ind./ml), *Ulothrix* sp. (100 ind./ml), and *Diffugia* sp. (430 ind./ml) were observed in activated sludge. *Epistylis* sp. (1820 ind./ml), *Carchesium* sp. (1500 ind./ml), *Acineta* sp. (200 ind./ml), *Arcella* sp. (2500 ind./ml), *Ulothrix* sp., and Rotifers (1045 ind./ml), on the other hand, were seen in base sludge images at the

Table 1—Characterization of Karaman-Adapazari-Sakarya-Turkey urban wastewater (Optimum time: filling, 0.5 h; reaction, 1.5 h; settlement, 1.5 h; decant, 0.5 h)

Parameters	Sample I			Sample II			Sample III		
	SBR	SBR	E	SBR	SBR	E	SBR	SBR	E
	Inf.	Eff.	%	Inf.	Eff.	%	Inf.	Eff.	%
Temperature, °C	21.4	22	-	20.6	21	-	22	22.4	-
pH	9.4	7.68	-	7.2	7.7	-	7.8	7.6	-
BOD, mg <sup>l</sup> <sup>-1</sup>	101	9	91	119	9	92	94	12	87
COD, mg <sup>l</sup> <sup>-1</sup>	206	35	83	226	35	85	197	38	81
Total nitrogen (TN), mg <sup>l</sup> <sup>-1</sup>	152	7.9	95	174	7.3	96	148	7.6	95
Total phosphor (TP), mg <sup>l</sup> <sup>-1</sup>	4.3	2.73	36	5.3	0.65	88	4.1	0.93	77
Suspended solids (SS), mg <sup>l</sup> <sup>-1</sup>	277	13	95	138	20	85	127	18	86
Conductivity, mS	4.2	4	5	1.3	1.5	-	3.5	3.1	11
Turbidity, NTU	260	17	95	106	12	89	104	13	88
Color, m <sup>-1</sup>	46.7	10.7	77	36.2	14.6	60	34.8	14.9	57

settlement phase for Sample I. *Ulothrix* sp. encountered at both mixing and settlement phases for Sample I. Sludge in Sample I was containing microorganisms for a proper treatment while considering efficiency of wastewater containment characteristics. Low efficiency for TP is conspicuous since it can not be considered as negligible.

**Sample II**

SVI in Sample II was measured as 75 mlgr<sup>-1</sup>. Influent value of COD decreased from 226 mg<sup>l</sup><sup>-1</sup> to 35 mg<sup>l</sup><sup>-1</sup>, and an efficiency of 85% was established (Table 1). BOD decreased from 119 mg<sup>l</sup><sup>-1</sup> to 9 mg<sup>l</sup><sup>-1</sup> and removal efficiency occurred as 92%. SS influent and outlet flow concentrations were 138 mg<sup>l</sup><sup>-1</sup> and 20 mg<sup>l</sup><sup>-1</sup>, respectively, while efficiency occurred as 85%. TN concentration decreased from 174 mg<sup>l</sup><sup>-1</sup> to 7.3 mg<sup>l</sup><sup>-1</sup>, with a high level of removal efficiency (96%). Concentration of TP decreased from 5.3 mg<sup>l</sup><sup>-1</sup> to 0.65 mg<sup>l</sup><sup>-1</sup>, with a considerable increase in removal efficiency (88%). Floc structure was tight during both mixing and settlement phases for Sample II. Considering these specifications, sludge fits to be Floc Type 1.

*Arcella* Sp. (1852 ind./ml), *Cocconeis* sp. (180 ind./ml), *Carchesium* sp. (1100 ind./ml), *Diffflugia* sp. (1652 ind./ml), and *Vorticella* sp. (1380 ind./ml) encountered in activated sludge images at mixing phase for Sample II. Amount of *Diffflugia* sp. increased in this sample. *Diffflugia* sp. (2250 ind./ml), Rotifers (950 ind./ml), *Volvox* sp. (83 ind./ml), *Carchesium* sp. (1104 ind./ml), and *Arcella* sp. (1245 ind./ml) were seen dominantly in base sludge images at the settlement phase for Sample

II. amount of *Diffflugia* sp. increased in this sample. *Cocconeis* sp. is a diatom and *Volvox* sp. is an algae. Species of *Diffflugia* sp. were seen frequently in low charged activated sludge systems. Species were observed in base sludge at both mixing and settlement phase for Sample II. While considering efficiency of wastewater containment characteristics, sludge in Sample II was containing microorganisms for a good treatment.

**Sample III**

SVI in Sample III was measured as 92.30 mlgr<sup>-1</sup>. Influent value of COD decreased from 197 mg<sup>l</sup><sup>-1</sup> to 38 mg<sup>l</sup><sup>-1</sup> (Table 1), and an efficiency of 81% was established. BOD influent decreased from 94 mg<sup>l</sup><sup>-1</sup> to 12 mg<sup>l</sup><sup>-1</sup>, and removal efficiency occurred as 87%. SS influent and effluent concentrations were 127 mg<sup>l</sup><sup>-1</sup> and 18 mg<sup>l</sup><sup>-1</sup>, respectively, with efficiency as 86%. TN concentration decreased from 148 mg<sup>l</sup><sup>-1</sup>, to 7.6 mg<sup>l</sup><sup>-1</sup>, and a high removal efficiency (95%) was established. Concentration for TP was decreased from 4.1 mg<sup>l</sup><sup>-1</sup> to 0.93 mg<sup>l</sup><sup>-1</sup>, and an increase in removal efficiency (77%). Floc structure was tight both during mixing and settlement phases. Sludge fits to be Floc Type 1 when these specifications are considered.

*Habrotrocha* sp. (850 ind./ml), *Aspidisca* sp. (620 ind./ml), *Arcella* sp. (1140 ind./ml), *Carchesium* sp., *Epistylis* sp. (1240 ind./ml), *Tardigrade* sp. (300 ind./100ml), and *Peranema* sp. (420 ind./ml) were encountered in activated sludge images at mixing phase for Sample III. *Didinium* sp. (940 ind./ml), *Vorticella* sp. (1300 ind./ml), *Acineta* sp. (740 ind./ml), *Peranema*

sp. (500 ind./ml), *Tardigrade* sp. (340 ind./ml), *Scyphidia* sp. (250 ind./ml), and *Epistylis* sp. (1100 ind./ml) were seen in base sludge images at settlement phase for Sample III. *Didinium* sp., *Aspidisca* sp., *Tardigrade* sp., and *Peranema* sp. were seen at both phases, unlike Sample I and II, for Sample III. These species were frequently observed in low charged activated sludge systems. Number of filaments, on the other hand, was limited. An increase in the population usually caused a decrease in settling velocity of flocs; causing SVI to deteriorate. Bulking sludge occurs if SVI exceeds 150 mlgr<sup>-1</sup>. SVI value was measured to be less than 150 mlgr<sup>-1</sup> in examined samples of activated sludge. *Flexibacter* sp., *Type 021 N*, *N. limicola III*, *Thiothrix* sp., *M. parvicella*, *N. limicola II*, *Actinomyces* sp., *Type 0092*, *Type 0914*, *S. natans*, *Beggiotoa* sp., *N. limicola I*, *Type 1851*, and *Type 0803* can be listed as filamentous bacteria types observed in activated sludge sampled in this study.

### Conclusions

Efficiencies of COD, BOD, SS, TN, and TP displayed similar changes for all samples. Quite low efficiency of TP was observed in Sample I. *Arcella* sp. was dominantly encountered in Sample I containing sludge taken from Karaman-Adapazari-Sakarya-Turkey urban wastewater treatment facility. *Diffflugia* sp. increased for Sample II. *Arcella* sp. (dominant in Sample

I) and *Diffflugia* sp. (dominant in Sample II) lost their dominance, whereas *Didinium* sp., *Aspidisca* sp., *Tardigrade* sp., and *Peranema* sp., were not observed in Samples I and II, unlike in Sample III. Limited numbers of rotifers and tardigrades species were encountered in this study. *Epistylis* sp., *Carchesium* sp., and *Vorticella* sp. were frequently observed in each sample. Thus protozoa and metazoa population in activated sludge had required characteristics for good quality activated sludge.

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