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Types and Abundance of Filamentous Bacteria, Protozoa and Metazoa in Activated Sludge

Jae-Chun Chung and Nam-Cheon Kim*
Department of Environmental Science, College of Health Science, Yonsei University, Wonju, Korea
**Department of Environmental Science and Technolgy, Seoul College of Public Health*

활성오니에서 발견된 사상세균, 원생동물 및 후생동물의 유형과 우점도 분석

정재춘 · 김남천*
연세대학교 환경과학과, *서울보건전문대학 환경관리과

요 약 — 슬러지 팽화의 원인이 되며 활성오니 공정운영상의 지표생물이 되는 사상세균과 원생동물을 동정하기 위하여 도시하수 활성오니 처리장 6개소와 산업폐수활성오니 처리장 5개소의 폭기조로부터 시료를 채취하여 현미경 경검법과 일부 생리학적 방법을 써서 미생물을 동정하였다. 가장 많이 출현하였던 사상세균은 *Microthrix parvicella*이었으며 다음이 type 0041, type 1701, *Nocardia*의 순이었다. 비록 시료수는 적었지만 이러한 출현빈도는 미국과 네덜란드에서 조사된 것과 비슷한 경향을 보였다. 사상세균의 형태는 이전의 문헌에서 보고된 것과 대체로 일치하였는데 type 1701의 경우에는 전형적인 형태에 비해 사상체(filament)의 직경이 약간 가늘었다. 이러한 사상세균들은 낮은 유기물부하 또는 낮은 DO와 관계가 있는 것들이었으므로 더 많은 조사가 이루어지면 지표생물로서의 활용이 가능할 것이다. 원생동물에 있어서는 *Vorticella*가 가장 많이 출현하였고 다음이 *Aspidisca, Opercularia, Difflugia*의 순이었다. 후생동물에 있어서는 *Philodina*가 유일하게 출현한 속이었다. 이러한 속들은 활성오니성 미생물로서 처리상황이 좋을 때 우점적으로 나타나는 미생물이라고 알려져 있으므로 처리수의 수질과 관련하여 유추해 볼 때 상당한 타당성이 인정되었다.

Bulking is one of the most common problems in activated sludge. Although there are many causes of bulking, the abundance of filamentous bacteria is considered to be main cause of bulking. Until now, approximately 30 different types of filamentous bacteria have been reported in activated sludge (30, 35).

Although their biomass is relatively small in normal operating conditions, their presence could give some important informations to diagnose problems in an activated sludge process. Due to their diagnosic value, some researches have been performed to correlate its proliferation to bulking and other operational conditions.

A careful examination of pertinent environmental engineering literature shows that filmentous bulking relates to specific growth environments in activated sludge, which include ; (a) Low dissolved oxygen (DO) concentration in the aeration basin (1, 4) ; (b) High DO concentration in the aeration basin (5, 6) ; (c) Low organic loading rates (7, 9) ; (d) High organic loading rates (7, 8, 10, 11) ; (e) Completely mixed reactor configurations (12, 16) ; (f) Conventional plug flow reactor configurations (17, 18) ; (g) Inadequate micronutrient concentrations (19, 20) ; (h) Elevated metal concentrations (21) ; (i) Low pH (22) ; (j) elevated sulfide concentrations (23, 25) ; (k) Low relative influent nitrogen and phosphorus content (26, 28) ; and (l) Overgrazing by protozoa (29) ;

Filamentous bacteria are also present in normally operating activated sludge. They serve as a backbone matrix in floc formation.

In Korea, there has been no systematic research concerning filamentous bacteria in spite of more than 2000 activated sludge plants in operation. Since the study of filamentous bacteria is very impor-

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***Corresponding author**

tant in diagnosing activated sludge bulking, it is necessary to identify filamentous bacteria in activated sludge. In the other hand, since filamentous bacteria are common microorganisms normally present in an activated sludge process, the study of their identification and abundance is very important in understanding microbial ecology of activated sludge. Therefore, the purpose of this study was to investigate the types and abundance of the filamentous bacteria in activated sludge in Korea as an initial step.

Materials and methods

For this study, 11 activated sludge plants were investigated. Mixed liquor samples were taken from the aeration basin. One drop of the sample was mounted on the slide after vigorous shaking.

To determine the relative abundance, 10 microscopic fields were randomly chosen and microorganisms were counted. A magnification of 1000x was used for filamentous bacteria and 100x magnification was used for protozoa and metazoa.

Filamentous bacteria were identified using the microscopic method of Eikelboom (30) and Eikelboom and van Buijsen (31) as modified by Strom and Jenkins (35). The presence of sulfur granules was tested by the method shown by Farquhar and Boyle (32). Protozoa were identified to the genus by the key presented by Jahn and Jahn (33) and Lee *et al* (34).

Results and discussion

Table 1 shows operational conditions of the wastewater treatment plant investingated. Influent BOD_5 of the 4 municipal wastewater plants was between 99 ppm and 200 ppm and the concentration of mixed liquor suspended solids (MLSS) was between 1800 ppm and 2550 ppm. Influent BOD_5

Table 1. Operational conditions of the wastewater treatment plant investigated (Feb. 1993)

Plant name	Flow (m^3/d)	Influent			Efluent				Wastewater Treated
		BOD_5 (mg/*l*)	COD (mg/*l*)	SS (mg/*l*)	BOD (mg/*l*)	COD (mg/*l*)	SS (mg/*l*)	MLSS (mg/*l*)	
Seongnam	95,000	180	135	94	18	12	10	1,800	Municipal
Tancheon	715,000	103	62	140	7	—	3	2,200	〃
(Cheongghecheon)									
Plant No.1	250,000	99	43	76	15	9	8	1,960	〃
Plant No.2	226,000	100	55	45	19	14	8	2,170	〃
Plant No.3	1,360,000	123	53	114	15	10	3	2,550	〃
Gwacheon	10,000	200	100	180	15	11	10	2,000	〃
Hyopdong Dyeing Co.	70,000	300	320	96	70	77	17	1,800	Industrial
Jinro Brewing Co.	2,000	800	1,200	650	100	50	200	4,500	〃
Sinwoo Tannery Co.	2,000	300	350	250	50	50	—	2,000	〃
Jungang Tanney Co.	2,700	800	700	100	85	75	45	5,000	〃
Nayom Dyeing Co.	3,000	600	700	250	60	70	20	2,700	〃
Gangnam Hwaseong Co.	—	2,800	3,000	150	20	20	15	5,000	〃

Table 2. Filamentous bacteria found in the activated sludge plants

Plant		Filamentous bacteria	Relative Abundance (No. per field at 1000x)	
			Winter (Feb. 1993)	Summer (Aug. 1993)
Seongnam		*Micorthrix parvicella*	0.9	1.2
		Type 041	0.3	0.2
Tancheon		*M. parvicella*	0.2	0.3
		Type 1701	0.1	2.8
		Nocardia sp.	–	0.3
		Type 0041	–	1.7
Cheongghecheon	Plant No.1	*M. parvicella*	5.3	–
		Type 0041	–	3.0
		Type 1701	–	3.5
	Plant No.2	*M. parvicella*	8.8	8.6
	Plant No.3	*M. parvicella*	5.1	6.9
Gwacheon		Type 1701	0.5	5.1
		Type 0041	1.1	2.3
		M. parvicella	–	4.0
Hyopdong Dyeing Co.		Type 1701	4.6	NI*
		Nocardia sp.	1.4	〃
Jinro Brewing Co.		No dominant forms	–	〃
Sinwoo Tannery Co.		Type 0041	3.7	〃
		H. parvicella	6.2	〃
		Unidentified	2.1	〃
Jungang Tannery Co.		No dominant forms	–	〃
Nayom Dyeing Co.		Type 1701	8.9	9.3
		M. paricella	3.1	9.4
		Type 0041	–	1.3

*Not Investigated

and MLSS of the 6 industrial wastewater treatment plants were higher than those of municipal wastewater treatment plants.

Table 2 shows filamentous bacteria found in the plants investigated. Among the filamentous bateria found in the plants, *Microthrix parvicella* (Fig. 1) was present most frequently followed by type 0041 (Fig. 2), type 1701 (Fig. 3) and *Nocardia* sp. (Fig. 4).

M. parvicella presented in the aeration basin had a typical morphology reported in other literatures (30, 35). Its filament diameter was 0.4~0.6 μm with coiled filament up to 1,000 μm long. It had no crosswall and no inclusions. It was Gram-positive and Neisser-negative.

Type 1701 showed also a typical morphology reported in the previous literatures except somewhat slender diameter. It had no branching. It was Gram-negative and Neisser-negative. Type 1701 is typically chains of cylindrical cells, enclosed by a sheath. False branching occurs incidentally. The length of the filaments are normally exceeds 200 μm. The septa are clearly visible. The cells, 0.5~0.9 μm in diameter and 2.5~3.5 μm long, may contain PHB-g sanules. The arrangement of the cells, usually uniseriately, may be irregular.

Type 0041 showed also a typical morphology. Its filament diameter was approximetely 1.4 μm with

Fig. 1. ***Microthrix parvicella*****, wet mount, 1000x (Cheongghecheon wastewater treatment plant sludge sample).**

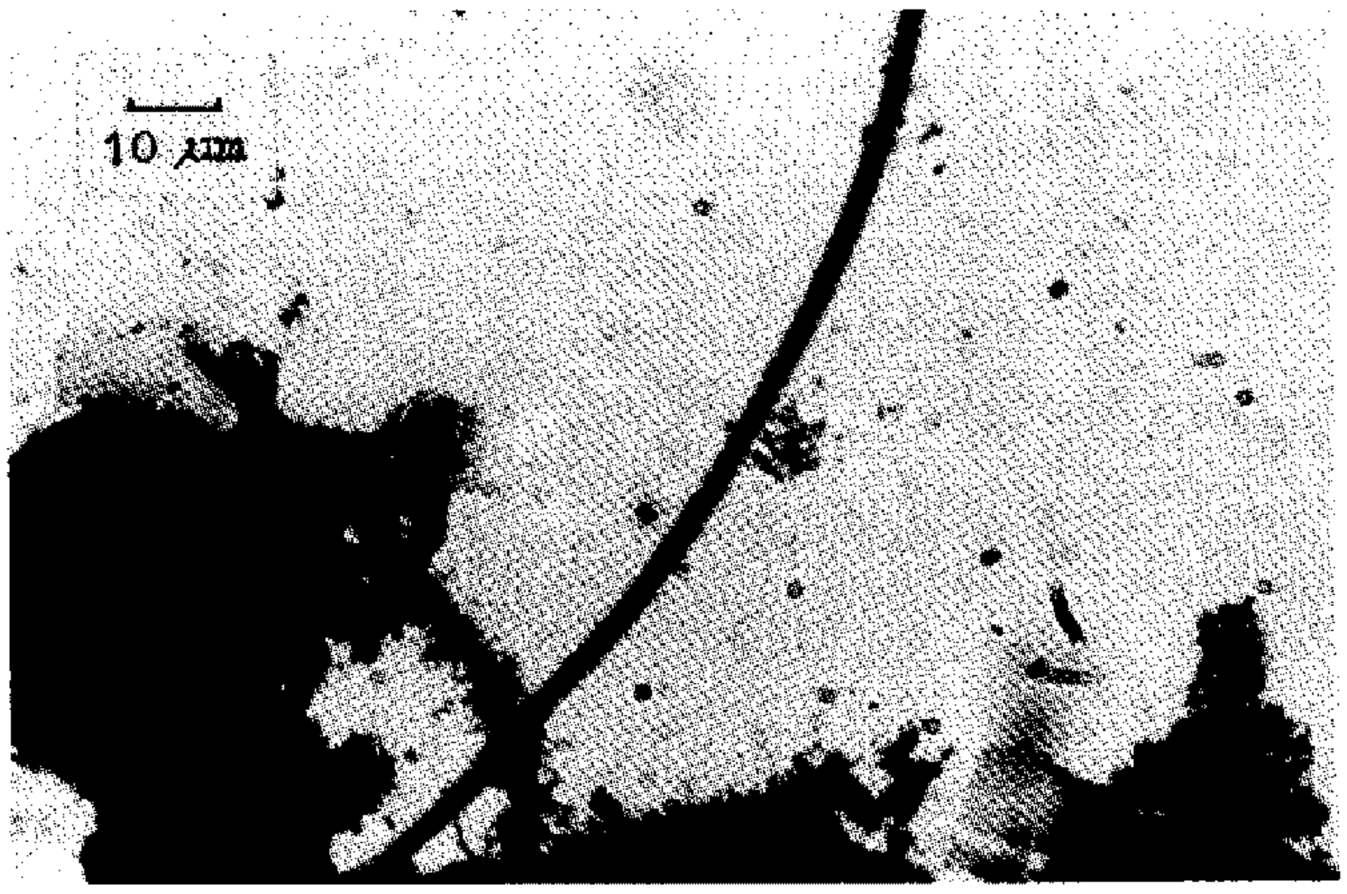

Fig. 2. Type 0041, wet mount, 1000x (Sinwoo Tannery Co. wastewater treatment plant sludge sample).

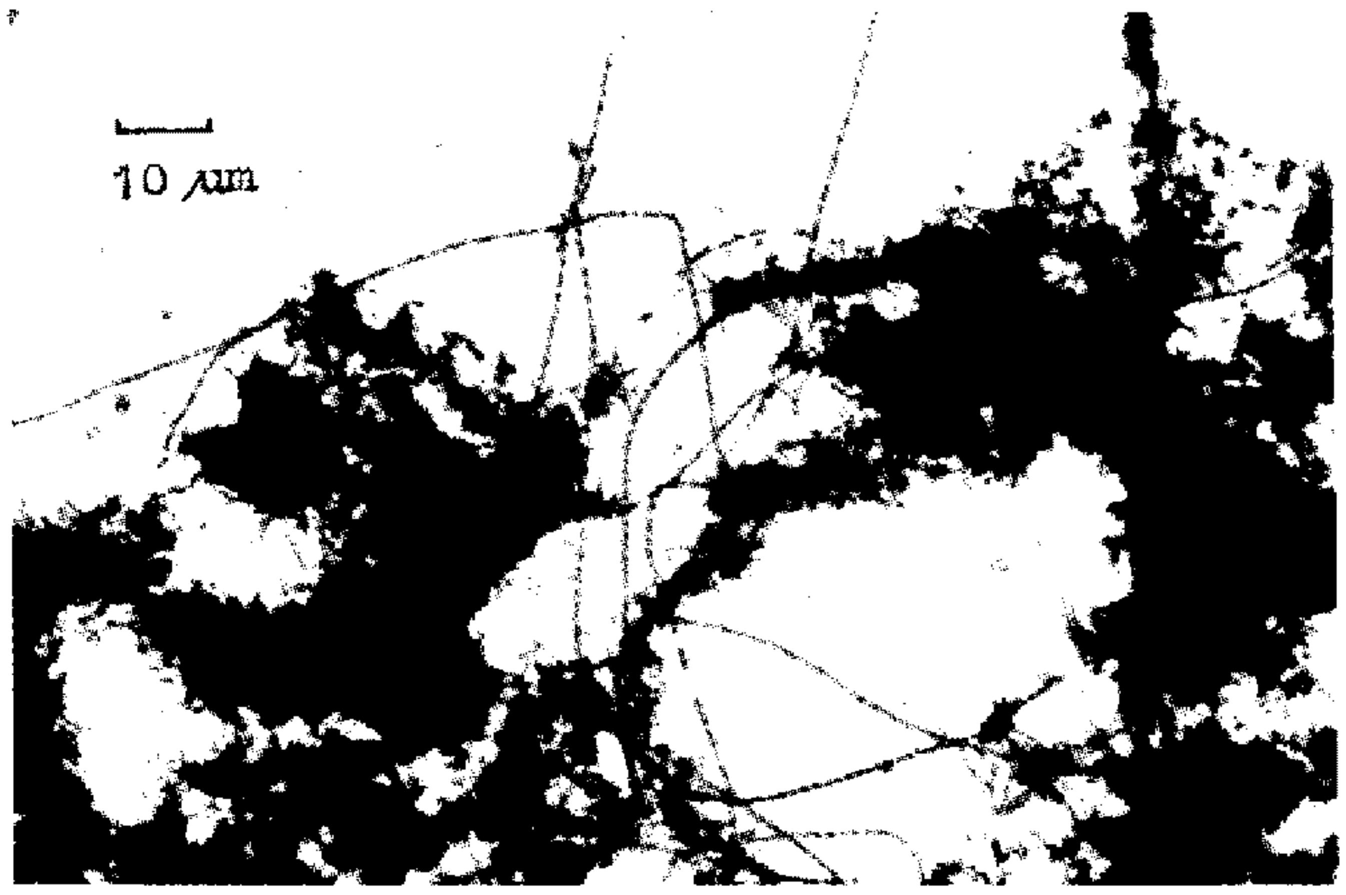

Fig. 3. Type 1701, wet mount, 1000x (Hyopdong Dyeing Co. wastewater treatment plant sludge sample).

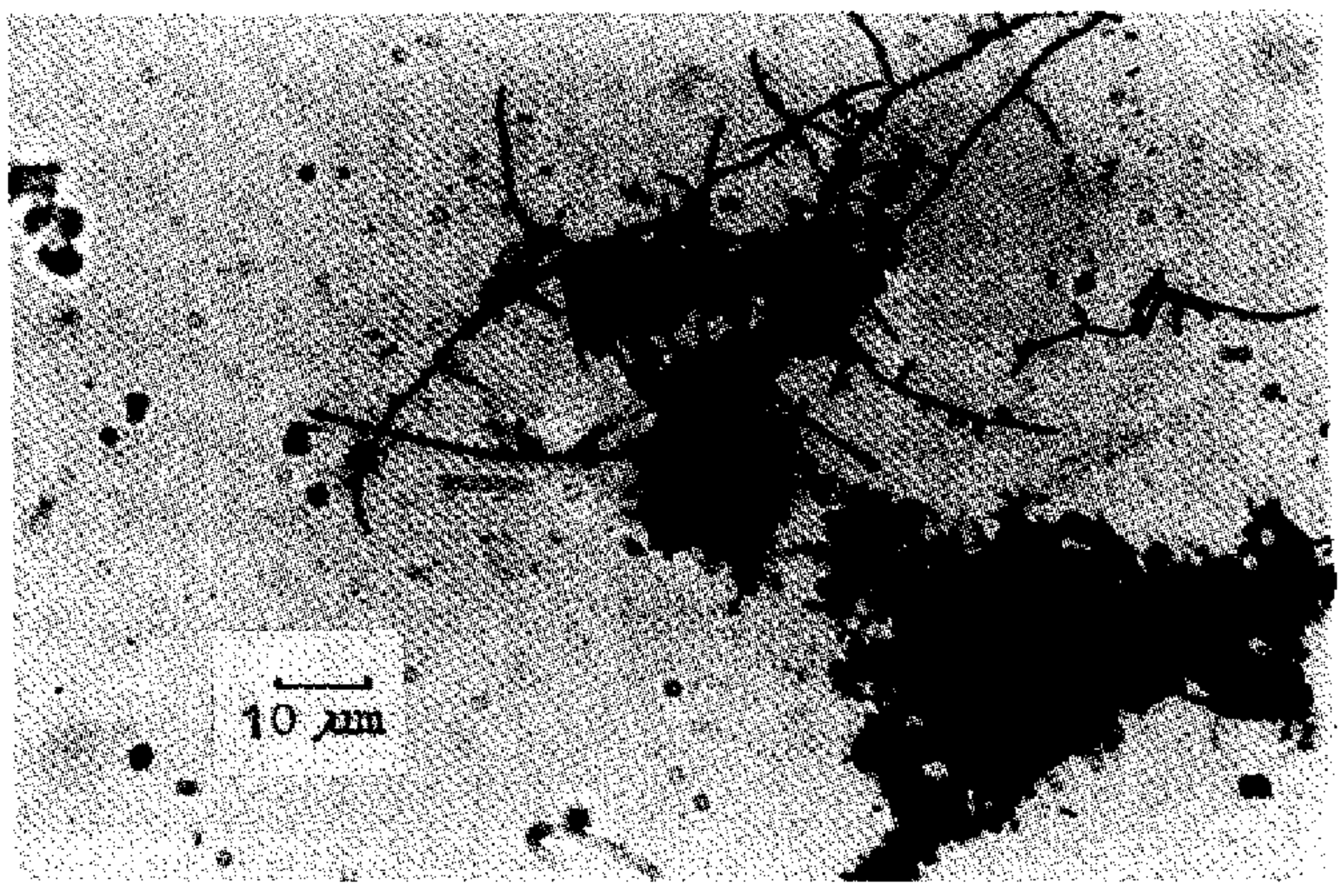

Fig. 4. ***Norcardia*** **sp., wet mount, 1000x (Hyopdong Dyeing Co. wastewater treatment plant sludge sample).**

attached growth. It had crosswall and no inclusions. It was Gram-positive and Neisser-negative. Type 0041 is typically straight or slightly bent multicellular organisms up to several hundred microns long and surrounded by a sheath of uniform with. False branching was incidentally observed. Septation is clearly visible. The sheaths are mostly completely filled with short cylindrical to barrel-shaped cells. 0.7～2.3 µm long and 1.0～1.4 µm wide. The sheath may be strongly electron dense but near the tip of the trichomes the sheath is hardly visible and the end cells are not enclosed at all by a sheath. Formation of cell units resembling hormogonia is restricted to the end of the trichomes. Type 0041 is Gram-positive but parts of the trichomes, where many bacteria are found adhering to the sheath may give only a slightly positive reaction.

Nocardia showed also typical morphology. It had true branching. It was Gram-positive and Neisser-negative.

The ranking of filamentous types by frequency of occurrence in this study was very similar to those of Strom and Jenkins (35), and Eikelboom (30).

In the USA, the most frequently occurring filamentous bacteria was type 1701, followed by *Nocardia* spp., type 0041 type 021N, type 0092, *Haliscomenobacter hydrossis* ; *Sphaerotilus natans* and *Microthrix parvicella*.

In the Netherlands, *Microthrix parvicella* was most common followed by type 021N, *Haliscomenobacter hydrossis*, type 0092, type 1701 and type 0041. *Nocardia* spp. placed the 14th in the Netherlands.

Microthrix parvicella and type 0041 were 0041 were suggested to be associated with low organic load in activated sludge (9). This seems to be consistent with the results of this study although organic loading was not calculated.

Table 3. Protozoa and Metazoa found in activated sludge

Plant		Protozoa/Metazoa	Relative Abundance (No. per field at 1000x)	
			Winter (Feb. 1993)	Summer (Aug. 1993)
Seongnam		*Vorticella* sp.	2.5	–
		Aspidisca sp.	0.5	1.3
		Amoeba sp.	0.3	–
		Philodina sp.	0.1	–
Tancheon		*Volticella* sp.	3.4	0.7
		Aspidisca sp.	1.2	3.2
		Difflugia sp.	–	5.2
Cheongghecheon	Plant No.1	*Vorticella* sp.	3.0	–
		Aspidisca sp.	6.0	0.2
		Tokophrya sp.	0.1	–
		unidentified ciliates	–	0.2
	Plant No.2	*Vorticella* sp.	4.5	–
		Aspidisca sp.	2.2	–
	Plant No.3	*Vorticella* sp.	2.0	–
		Aspidisca sp.	4.2	0.8
		Arcella sp.	0.2	0.2
		Difflugia sp.	–	0.3
		Philodina sp.	–	0.1
Gwacheon		*Aspicisca* sp.	2.8	1.7
		Arcella sp.	0.2	0.4
		Difflugia sp.	0.1	–
		Opercularia sp.	0.4	–
		Vorticella sp.	–	0.6
		Philodina sp.	0.8	–
Hyopdong Dyeing Co.		*Aspicisca* sp.	0.4	–
		Vorticella sp.	1.0	2.0
		flagellated protozoa	0.2	–
Jinro Brewing Co.		*Vorticella* sp.	0.1	NI*
		flagellated protozoa	0.2	
Sinwoo Tannery Co.		*Tertrahymena* sp.	2.4	NI
		Opercularia sp.	0.3	NI
Jungang Tannery Co.		*Opercularia* sp.	0.4	NI
Nayom Dyeing Co.		*Opercularia* sp.	0.2	NI
		Epistylis sp.	0.4	NI
		Vorticella sp.		1.0

*Not Investigated

Type 1701 is usually believed to be present with low DO in activated sludge. In this study, Hyopdong Dyeing Co. (Summer) and Nayom Dyeing Co., had dominant Type 1701 populations in the aeration basin. Although the DO concentration was not measured, it seems that the aeration basin had low DO condition considering the high influent concentration of the two plants.

Considerable work remains to be done to characterize most of the filamentous types. The eventual goal should be isolation, biochemical and related testing, and identifcation of each type, with creation of new species and genera as necessary.

Table 3 shows potozoa and metazoa found in activated sludge. *Vorticella* spp. were most fequently observed, followed by *Aspidisca* spp, *Opercularia* spp., and *Diffugia*. For the metazoa, *Philodia* sp. was the only genus obseved. Sudo and Aiba (1984) classified *Vorticella, Opercularia, Aspidisca* and *Philodina* and associated with good working activated sludge when they are dominant in the system. Taking into account his classification, municipal wastewater plants with dominant populations of the above genus seems to be working in a good operational condition. This is consistent with their low BOD_5 concentration of the effluent.

Abstract

Filamentous bacteria and other large microorganisms, which are useful indicators of bulking and operation, were identified by microscopic observation. Activated sludge samples were taken from the aeration basin of 5 municipal wastewater plants and 6 industrial ones. Among the filamentous bacteria found, *Microthrix parvicella* was most frequently present, followed by type 0041, type 1701 and *Nocardia*. This frequency of occurrence was similar to those reported in USA and the Netherland. The morphology of filamentous bacteria observed were generally identical with those previously reported, except type 1701 which had slender filament diameter. Among protozoa, *Vorticella* was most frequently present, followed by *Aspidisca, Opercularia* and *Difflugia*. *Philodina* was the only metazoa observed. Both filamentous bacteria and protozoa would be useful indicator organisms. The potential for these organisms as indicators were discussed.

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