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Effect of Chlorination on Microfauna Communities in Activated Sludge Plants

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Communities of microorganisms that colonize activated sludge are composed of bacteria, fungi, protozoa, and small metazoa. Filamentous bacteria are particularly common, and excess growth may result in uncontrolled losses of sludge in effluents due to bulking or foaming. Bulking interferes with the sedimentation, which leads to high sludge volume index (SVI) values. Operational techniques based on the metabolic and kinetic selection of filamentous organisms are now commonly used to control excess growth of filamentous microorganisms (Jenkins et al. 1993; Wanner 1994). However, not all wastewater treatment plants offer sufficient flexibility of operation to prevent bulking and foaming, and so as an emergency measure, disinfectants such as chlorine are sometimes added to reduce the filamentous microorganisms (Jenkins et al. 1993; Wanner 1994).

Filamentous organisms are the main targets of chlorinations of activated sludge. Previous studies on the effect of chlorine on protozoa have focused on disinfected wastewater effluents, and on human parasites. Madoni et al. (1998) and Muela et al. (1998) studied the effect of chlorine on free-living protozoa. Madoni et al. (1998) observed that effects of chlorine on ciliated protozoa in treated wastewater depended on the ecological characteristics of each species, and that polysaprobic ciliates (from highly polluted waters) resisted chlorination better than mesosaprobic or oligosaprobic ciliates (from moderate or slightly polluted waters). Muela et al. (1998) described the effect of disinfected effluents on the bacterivorous ability of protozoa in aquatic systems. However, to our knowledge the effects of chlorination on the microfauna in activated sludge and the repercussions for treatment processes have not been previously studied. In this study we examined the effects of chlorination on the structure of microfauna communities, especially that of ciliated protozoa.

MATERIALS AND METHODS

A conventional activated sludge plant near Barcelona, Spain, which treats urban wastewater was studied over a two-year period. In an attempt to analyze the effects of chlorine on filamentous microorganisms and microfauna, chlorination was initially applied when the abundance of filamentous organisms exceeded 200 m/mL. The chlorine doses are expressed in grams of chlorine per kg of MLVSS and day (g $kg^{-1} d^{-1}$). The chlorine doses ranged from 5 to 20 g $kg^{-1} d^{-1}$. Chlorine was fed continuously into the return activated sludge line with a dose concentration ranging between 15 and 20 mg L^{-1}. The exposure frequency of solids inventory to chlorine was between 3 and 5 times per day. Physical and chemical monitoring included: BOD_5, suspended solids (SS), mixed liquor volatile suspended solids (MLVSS), diluted sludge volume index (DSVI), dissolved oxygen (DO), ammonia-nitrogen, NO_2^-, and NO_3^- were measured in

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accordance with standard methods (APHA 1989). Mean cellular retention time (MCRT) was determined as described in Salvadó (1994).

Weekly samples of mixed liquor from the aeration tank were taken for microscopic observation. Protozoa and small metazoa were counted under an optical microscope, using four replicates of 0.250 µL per sample. The specific diversity of ciliates were calculated using the Shannon-Weaver diversity index and expressed in bits/individual. Ciliates were identified following Foissner et al. (1991), Foissner et al. (1992), and Foissner et al. (1995).

Filamentous organisms were identified following Jenkins et al. (1993). The total extended filamentous microorganism length (TEFL) was calculated using the technique based on the "simplified filament counting technique" described by Jenkins et al. (1993). The method consisted of counting, on consecutive fields, the intersections between filamentous organisms and a line drawn on the microscope eyepiece. Results were expressed in m/mL following Salvadó (1990) equation:

$$\textit{Total length of filaments / volume} = \frac{Ni \quad Ar}{H \quad V}$$

where: *Ni* = number of intersections/number of observed fields, *Ar* = area occupied by the sample (the cover slip) in m^2, $H = (Le)$ (average sine) = (Le) $(2/\pi)$, *Le*= length in meters of a segment drawn on the microscope eyepiece measured with a micrometric slide, and *V* = volume of the sample in mL.

For each sample four replicates of 0.250 µL were counted at 400x magnification, and 15 consecutive fields across the cover slip were observed.

The rise and fall in the sludge inventory and the variation in the chlorine concentration in the chlorine tank meant that it was impossible to maintain the chlorination dosage within a fixed range, although several IO-day periods of stable dosages were recorded. Thus, the samples were grouped by the range of chlorine doses: 5 to 10; 10 to 15; 15 to 20 g kg^{-1} d^{-1}. Each group was divided into two subgroups according to the number of consecutive days of exposure to chlorine before sampling. The samples examined in this study were not exposed to chlorine during the last 20 days before collection.

RESULTS AND DISCUSSION

Dosages of chlorine ranged from 5 to 15 g kg^{-1} d^{-1} had no significant effect on BOD_5, while doses >15 g kg^{-1} d^{-1} led to a clear reduction in the quality of the effluent (Table 1).

The organic loading rate and MCRT remained largely constant for all levels of chlorine. Nitrogen analyses showed very low levels of nitrification or none at all. The affluent ammonia-nitrogen was 28±8 mg/L (mean ±SD). Effluent ammonia-nitrogen, NO_3^-, and NO_2^- were 24±7 , 0.91±0.80 and 0.24±0.13 mg/L, respectively.

The most frequent types of filamentous bacteria observed were Type 021N and Type 1701. Only Type 021N appeared in quantities greater than 100 m/mL and was predominant (>50% of TEFL) in 95% of samples; in 81% of samples it represented >80% of TEFL. Only in three cases was another type of filamentous organism present in a higher proportion. These were Type 1701, *Thiothrix* sp., and Type 1863, with a relative abundance of 72, 43, and 36% respectively, and an absolute abundance of 54, 8, and 11 m/mL, respectively.

Table 1. Operational parameters according to each range and exposure time of chlorination. According to the number of consecutive days of exposure to chlorine there are two groups of samples, each of which was divided into three according to the dosage of chlorine.

Chlorination period	Chlorine dosage g/kg d	no. samples		Chlorine g/kg d	DSVI mL/g	DO mg/L	F/M $gBOD_5/gMLVSS\ d$	MCRT d	BOD_5 effluent mg/L	Removal BOD_5 %
Control	0	18	$\bar{X}$	0	290	1.17	0.440	3.70	8.70	94.8
			SD	0	130	0.14	0.108	0.73	4.42	2.8
From 3 to 6 days	5-10	8	$\bar{X}$	8.2	298	1.00	0.363	4.10	9.00	94.0
			SD	2	179	0.18	0.037	0.65	2.36	3.6
	10-15	7	$\bar{X}$	13.1	252	1.20	0.448	3.24	9.13	93.1
			SD	1.36	165	0.14	0.036	0.73	1.56	1.8
	15-20	8	$\bar{X}$	16.7	419	1.01	0.387	4.68	14.10	92.8
			SD	2.1	126	0.10	0.044	1.20	4.45	2.3
From 7 to 10 days	5-10	8	$\bar{X}$	8.4	315	1.01	0.394	3.49	9.33	93.1
			SD	2.05	150	0.02	0.049	0.68	4.02	3.3
	10-15	9	$\bar{X}$	11.92	229	1.07	0.440	3.77	9.17	95.2
			SD	0.82	111	0.12	0.078	0.62	4.44	2.3
	15-20	6	$\bar{X}$	16.25	292	0.99	0.374	4.30	16.70	91.0
			SD	1.18	79	0.02	0.084	0.93	5.45	2.6

($\bar{X}$ = average, SD = standard deviation, DSVI = diluted sludge volume index, DO = dissolved oxygen in aeration tank, F/M = organic loading rate, MCRT = mean cellular retention time, Removal BOD_5 = percentage of BOD_5 removed by the biological treatment)

Exposure to concentrations of chlorine between 5 and 10 g kg^{-1} d^{-1} had not significant effect on filamentous organisms, indeed with increased exposure time a slight mean increase was observed in the abundance of Type 021N and the DSVI (Table 2). Within this range, microscopical observations revealed gonidia formation (dispersion cells) in Type 021N bacteria. Exposure to chlorine at doses above than 10 g kg^{-1} d^{-1} had a marked effect on filamentous organisms. Thus, large quantities of broken filamentous bacteria were observed and gonidia formation was not observed in Type 021N. Exposure to chlorine concentrations between 15 and 20 g kg^{-1} d^{-1} produced a 50% reduction in filamentous organisms between 7 and 10 days; the DSVI also fell slightly.

The effects of chlorination on the microorganisms are reflected in the loss of individuals and species (Table 3). As a result of the high activity and interspecies competition the populations of microorganisms in activated sludge are subjected to large variations in abundance over time. This is reflected in the high standard deviations. Nevertheless the total abundance and diversity of ciliated protozoa remain fairly constant, with low standard deviations, but are reduced as the period and dose of chlorination increase. This these two parameters may be considered as indicators of toxicity, as observed by Gracia et al. (1994).

Table 2. Effect of chlorine on filamentous organisms and DSVI, according to dosage and exposure time. From the three ranges of chlorine dosage the chlorination periods were divided in two groups according to a given number of consecutive days exposed.

Chlorine g/ kg d	Period of chlorination (days)	number of periods	Statistics	Final values / initial values TEFL Total	TEFL Type 021N	DSVI
5-10	3-6	8	$\bar{X}$			1.16
			SD			0.29
	7-10	7	$\bar{X}$	0.86	1.10	1.35
			SD	0.65	1.09	0.69
10-15	3-6	5	$\bar{X}$			0.83
			SD			0.42
	7-10	5	$\bar{X}$	0.54	0.53	0.69
			SD	0.32	0.32	0.43
15-20	3-6	7	$\bar{X}$			0.81
			SD			0.29
	7-10	4	$\bar{X}$	0.42	0.41	0.61
			SD	0.31	0.33	0.35

($\bar{X}$= average, SD =standard deviation, DSVI = diluted sludge volume index, TEFL = Total extended filamentous organism length)

Chlorine doses between 5 and 10 g kg^{-1} d^{-1} for periods between 3 and 6 days had only a slight effect on protozoa. However, some common species of cirthophorid ciliates, including *Chilodonella uncinata* and *Trochilia minuta,* were not observed. Moreover, species commonly found in activated sludge, such as *Acineria uncinata* and *Aspidisca cicada,* suffered considerable reductions in number. With exposure time between 7 and 10 days, *Aspidisca cicada, Litonotus lamella,* and *Pseudochilodonopsis fluviatilis* disappeared, and the abundance of *Uronema nigricans* and *Vorticella convallaria* were markedly reduced. In contrast, the densities of a few species such as *Vorticella microstoma, Vorticella infusionum,* and *Epistylis plicatilis* increased considerably.

At doses of chlorine between 10 and 15 g kg^{-1} d^{-1} the total amount and diversity of ciliates decreased considerably, but the number of less sensitive species of protozoa increased, these included some species of small gymnamoebae and peritrichous ciliates, such as *Opercularia* spp, *Zoothamnium* sp, *Vorticella microstoma, V.* sp, and *V. infusionum.* These effects were stronger for longer exposure times. Finally, doses of chlorination between 15 and 20 g kg^{-1} d^{-1} for periods between 3 and 6 days resulted in mortality of large amounts of microorganisms in sludge, yet some peritrich species of the genera *Vorticella, Opercularia, Zoothamnium,* and *Epistylis* were still recorded at levels of more than 100 ind/ml. The mortality rates of all ciliates increased with exposure time. Apart from *Acineta tuberosa,* which was occasionally found all other ciliates observed belonged to the following genera: *Vorticella, Opercularia,* or *Zoothamnium; Opercularia* underwent the greatest relative increase compared to the periods without chlorination.

Table 3. Microorganisms in the aeration tank according to each range and exposure time of chlorination.

Chlorination period (days)	-		3 to 6			7 to 10	
Range of Chlorine in g /kg d	0	5-10	10-15	15-20	5-10	10-15	15-20
No. of samples	18	8	7	8	8	9	6
Statistics	X̄ (SD)	X̄ (SD)	X̄ (SD)	X̄ (SD)	X̄ (SD)	X̄ (SD)	X̄ (SD)
Ciliates ind/ml							
Acineria uncinata	2391 (5166)	312 (179)	48 (85)	19 (57)	147 (197)	66 (108)	0 (0)
Litonotus lamella	5 (21)	4 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litonotus crystallinus	4 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Chilodonella uncinata	23 (90)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudochilodonopsis fluviatilis	69 (148)	13 (18)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Trochilia minuta	90 (227)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pseudomicrothorax agilis	7 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tokophrya ciclopum	14 (29)	12 (16)	5 (13)	4 (11)	3 (7)	0 (0)	0 (0)
Podophrya/Prodiscophrya	4 (12)	5 (11)	3 (8)	0 (0)	4 (9)	0 (0)	0 (0)
Acineta tuberosa	11 (27)	11 (19)	0 (0)	0 (0)	0 (0)	49 (47)	4 (8)
Uronema nigricans	109 (245)	57 (114)	12 (14)	1 (34)	6 (14)	18 (34)	0 (0)
Vorticella convallaria	625 (842)	784 (1010)	17 (20)	8 (18)	188 (402)	20 (28)	0 (0)
Vorticella microstoma	185 (256)	308 (381)	368 (504)	124 (193)	663 (1223)	587 (753)	83 (155)
Vorticella infusionum	359 (874)	902 (573)	259 (511)	754 (739)	703 (822)	189 (321)	229 (391)
Opercularia asymmetrica	96 (227)	91 (182)	134 (166)	293 (478)	87 (159)	83 (173)	84 (168)
Opercularia minima	20 (78)	0 (0)	0 (0)	33 (100)	4 (9)	394 (1035)	0 (0)
Opercularia microdiscum	697 (1634)	315 (375)	242 (305)	644 (820)	178 (290)	686 (989)	275 (295)
Opercularia articulata	90 (184)	167 (152)	570 (1396)	1423 (1973)	49 (110)	17 (44)	619 (356)
Epistylis plicatilis	276 (362)	840 (1275)	389 (678)	41 (124)	750 (1203)	48 (101)	0 (0)
Epistylis chrysemydis	341(528)	161 (202)	180 (199)	196 (585)	309 (678)	100 (158)	0 (0)
Epistylis coronata	256 (645)	245 (323)	100 (150)	72 (216)	83 (150)	30 (78)	0 (0)
Epistylis entzii	47 (125)	31 (62)	0 (0)	0 (0)	44 (109)	0 (0)	0 (0)
Zoothamnium sp	51(177)	36 (73)	22 (54)	71 (156)	219 (999)	217 (824)	124 (165)
Euplotes aediculatus	20 (55)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aspidisca cicada	545 (781)	24 (48)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ciliates total	6498 (5647)	4951 (922)	2396 (1455)	4247 (1725)	3443 (1559)	2747 (1768)	1505 (671)
Diversity index (bits/ind)	2.08 (0.75)	2.10 (0.32)	1.37 (0.49)	1.38 (0.48)	1.74 (0.67)	1.70 (0.59)	1.33 (0.63)
No. of species in each sample	8.90 (2.14)	6.33 (1.85)	5.64 (0.90)	5.11 (0.77)	5.71 (1.48)	5.88 (2.03)	4.20 (1.17)
Other protozoa ind/ml							
Flagellates <20 µm (x 1000)	417 (822)	632 (687)	73 (52)	272 (306)	381 (569)	301 (378)	91 (116)
Flagellates >20 –50< µm	18039 (70210)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Flagellates >50 µm	8 (16)	16 (19)	0 (0)	0 (0)	6 (14)	0 (0)	0 (0)
Gymnamoebae <50 µm (x 100)	76 (87)	80 (79)	152 (206)	86 (94)	182 (306)	229 (294)	79 (113)
Gymnamoebae >50 µm	149 (351)	40 (43)	28 (69)	34 (56)	37 (42)	25 (45)	3 (5)
Testate amoebae	41 (90)	8 (16)	0 (0)	2 (5)	0 (0)	4 (11)	0 (0)
Metazoa ind/ml							
Nematodes	19 (31)	13 (24)	79 (169)	5 (15)	38 (58)	10 (20)	0 (0)
Rotifers	93 (119)	353 (513)	89 (103)	126 (108)	151 (230)	180 (142)	108 (73)
Lecanidae	69 (104)	340 (551)	65 (101)	70 (77)	72 (108)	85 (54)	70 (81)
Philodinidae	10 (16)	71 (61)	23 (22)	80 (76)	8 (16)	58 (61)	39 (48)
TEFL m/ml							
Type 021N	306 (373)	302 (369)	319 (146)	368 (358)	249 (166)	185 (94)	151 (48)
Type 1701	9 (10)	8 (4)	5 (5)	18 (22)	17 (19)	6 (4)	18 (13)
Type 1863	1 (3)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Nocardioform actynomicetes	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Haliscomenobacter hydrossis	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0.6 (0.4)	0 (0)
Thiothrix sp	4 (8)	1 (1)	0 (0)	1 (1)	0.7 (0.9)	0.9 (0.7)	3 (5)
Filamentous organisms	328 (390)	311 (372)	327 (149)	399 (360)	268 (164)	194 (97)	175 (47)

(X̄ = average, SD = standard deviation, TEFL = total extended filamentous organisms length)

In general, chlorination decreased the number of microorganism species and effluent quality, but slightly increased the number of species with low sensivity. This can be seen as an acclimatization of the sludge to chlorine. Among the ciliates, the selection of the fixed species was clearly observed. Exposure to high doses of chlorine for long periods led to the survival of only three genera of peritrich ciliates that serve as indicators of poor biological performance and are associated with a high final effluent BOD_5 according to other authors (Curds and Cockbum 1970; Esteban et al. 1990; Madoni 1994; Salvadó et al. 1995). *Opercularia* was the genus with the greatest number of individuals and it is one of the most resistant to toxic substances like Cu^{2+}, Cr^{6+}, and Zn^{2+} (Sudo and Aiba 1973; Gracia et al. 1994; Madoni et al. 1996). This genus is also the most abundant in those plants that receive industrial waste water containing toxic substrates (Esteban et al. 1990; Bécares 1991; Aescht and Foissner 1992). The selection of attached ciliated species versus free-swimming or crawling species as a toxic effect of chlorination might also be due to a reduction of cell division and locomotion capacity. Thus, sessile species are transported by flocs and require a lower velocity of division than mobile species, which therefore escape with the effluent (Curds and Vandyke 1966). Moreover, it has been demonstrated that toxicity leads to a loss of locomotion capacity (Bergquist and Bovee 1976; Salvadó et al 1997a), making it more difficult for crawling ciliates to move onto flocs.

The effects of chlorination on flagellates varied greatly from group to group. Thus, while the number of small flagellates belonging primarily to the Bodonidae family varied considerably for all concentrations and exposure times, average abundance was high in periods of lower chlorine concentrations. In contrast, large flagellates (Euglenidae) were not observed at chlorine doses between 5 and 10 g kg^{-1} d^{-1}. Among the smaller gymnamoebae, the average abundance was constant, increasing slightly during chlorination; whereas increased doses and exposure time gradually reduced populations of the larger gymnamoebae. When chlorine is applied at the doses tested here, small protozoa (flagellates <20 µm and gymnamoebae <50 pm) can maintain high numbers or increase their abundance. This may be a competitive response to the decrease of ciliates; thus, flagellates and gymnamoebae would acquire a major role grazing a higher amount of bacteria. On the other hand, the decrease in number of *Acineria uncinata,* which is a predator of flagellates that control small-flagellated populations (Salvadó et al. 1997b), could also permit an increase in their number.

In activated sludge with a mean cellular retention time between 3 and 4.5 days, the metazoa were never found in high abundance. Nematodes were observed at all ranges of chlorination, but their abundance in the samples without chlorine was low (3 1%) compared with that of rotifers (70%). Although rotifers are sometimes absent in sludge, their average abundance is greater during chlorination. Rotifers occupy many ciliate niches in freshwater habitats (Fenchel 1987), and are probably more resistant than ciliates to the effects of chlorine.

After chlorination, the recovery of activated sludge depends on the state of microorganism communities. We observed that the recovery of a sludge with a mean abundance of 500 to 1500 ciliates/mL and a Shannon-Weaver diversity index between 1 and 1.5 bits/individual ranged from 8 to 14 days.

Among the protozoa, the flagellates had considerable oscillations in density. Three to eight days after chlorination, the mean abundance of small flagellates increased from 0.05 to 0.3 million cells/mL to 0.5-2 million cells/mL. One or two weeks later, their abundance

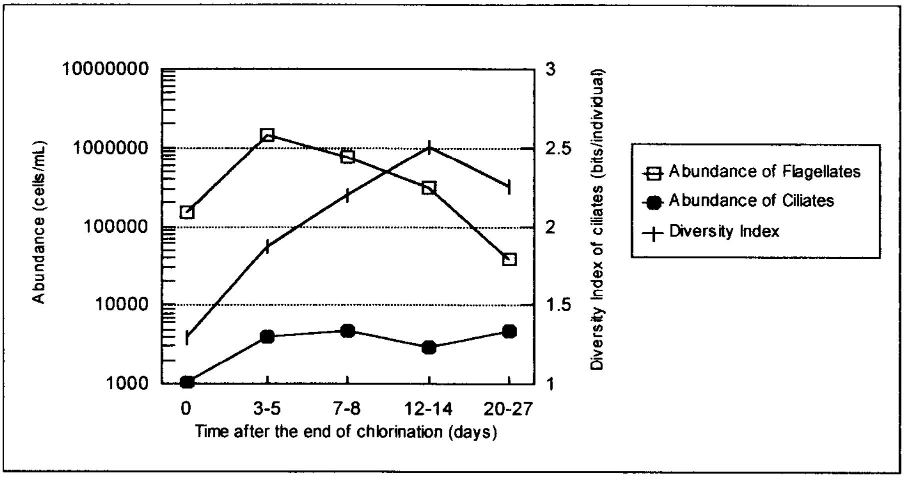

Figure 1. Changes in ciliate and flagellate abundances and the diversity index of ciliates after the end of chlorination. Time is expressed in ranges of days: for 0 days n=3; for 3-5 days n=5; for 7-8 days n=7; for 12-14 days n=3 and for 20-27 days n=4. Time 0 was the last day of chlorination (ranged 15-20 g kg^{-1} d^{-1}).

had decreased to below 0.5 million cells/mL. During the same period, the ciliates grew in abundance to levels 2500 ind/mL, while the number of species reached diversity index values of 2 to 2.8 bits/ind (see Fig. 1). The succession observed between ciliates and flagellates corresponds to the succession observed in the first stages of colonization of the activated sludge according to Curds and Cockburn (1970) Madoni (1994) and Salvadó (1994).

This study reports that the doses of chlorine needed to reduce the number of filamentous organisms found also affected the structure of the activated sludge protozoan communities, which compromised the reliability of process. The effect of chlorine is reflected in the loss of abundance and diversity of ciliated protozoa.

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