Arch. Hydrobiol./Suppl. 90 (Monographische Beiträge)	2	207–251	Stuttgart, Juni 1992

Biology of a high-rate activated sludge plant of a pharmaceutical company

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With 50 figures, 1 diagram and 11 tables in the text

Abstract

The activated sludge organisms of a pharmaceutical sewage plant heavily loaded with organic wastes were studied in a series of 11 samples collected monthly. High population densities of the microflora and microfauna indicate that the waste water contains no toxic compounds beyond tolerable limits. The organic substance on average amounts to 16 g dry mass/l; it consists of 96% prokaryotic (bacterial) and 4% eukaryotic (protozoan) biomass. Related to suspended solids the protozoan biomass constitutes 2%; 4 tons protozoa (wet mass, corresponding to 800 kg dry mass) are produced in 1400 m^3 waste water daily. The high organic load (21 kg $BOD_5/m^3.d$), the short retention time (24 h), the high temperature (30°C) and the lack of distinct flocs favour few well adapted polysaprobic protozoan species. The morphology and ecology of 7 ciliate species found in fresh sludge are described, including *Parastrongylidium oswaldi* n. sp.

Contents

0341–2881/92/0090–0207 $ 11.25

1. Introduction

The pharmaceutical company in Kundl (Tyrol, Austria) has a special sewage treatment equipment consisting of 2 plants, the technology of which was recently described by GREIL (1990). One of these plants is extraordinary because it is continuously fed with a defined waste water (mainly culture medium of the fungus *Penicillium chrysogenum*) having a very high organic load (biochemical oxygen demand about 21000 mg/l, chemical oxygen demand about 38000 mg/l). The excess sludge is processed into an organic fertilizer and must therefore be free or almost free of harmful substances (e.g., heavy metals, antibiotics) which could contaminate the soil. Although this has been assured by chemical analyses, a more reliable long-term integrating indication can be obtained by biological investigations of activated sludge organisms. We focused on the animal populations as these can be more accurately counted than bacteria (CURDS 1973). Moreover, the microfauna enables an improved characterization of the purification technology, such as oxygen economy and plant efficiency (SLÁDEČEK 1958, 1969, 1973; CURDS & HAWKES 1975; FOISSNER 1990). Since no comprehensive data have as yet been published concerning the biology of extraordinarily loaded sewage treatment plants, the present paper is also of general interest.

During the one-year-study we found some interesting species of ciliated protozoa. Their autecology is reviewed and their morphology is described in detail using modern techniques. Generally, the taxonomy of activated sludge ciliates has received little attention, despite their widely acknowledged importance in purifying the effluent (e.g., CURDS 1969; BANINA et al. 1983; MADONI 1981, 1986; MUDRACK & KUNST 1988; FOISSNER 1988a, 1990; AUGUSTIN & FOISSNER 1989).

2. Study site and waste water

The sewage treatment plant investigated (Diagram 1) is in Kundl (Tyrol, Austria). It was constructed by the company's own engineers to purify the waste water of antibiotics production. For a detailed description of the plant technology see GREIL (1990). Our study was restricted to the sewage plant 1 (ARA 1), which is fed exclusively with industrial waste water (1400 m^3/d). This consists mainly of the *Penicillium chrysogenum* culture medium (sugar, soya meal, corn-steep-liquor, essential salts, trace elements). In addition the tanks are fed with wastes of the semisynthetic antibiotics production and the organ preparation (GREIL 1990). Since November 1989 20–25% of the excess sludge of the conventional activated sludge plant ARA2 has been returned to ARA1. No heavy metals or other toxic substances are involved in the above mentioned processes. Wash water, cooling water, rain water and faecal wastes are excluded from ARA1. Prior to purification the pH of the waste

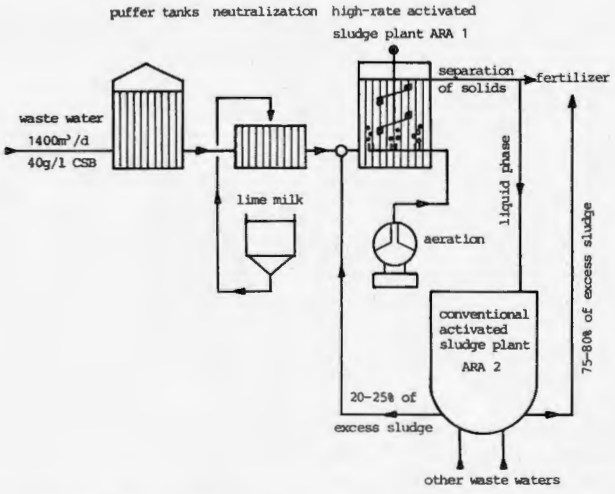


Diagram 1. Waste water treatment during the time of investigation.

water is neutralized with lime (GREIL 1990). Purification takes place in darkness in 7 fermentation tanks, each having about 250 m^3 (total volume 1750 m^3). The strongly exothermic process is regulated to 30°C by a water cooling system. Oxygen is supplied by deep aeration with air. The effluent of the fermentation tanks is centrifugated and the sludge provides the raw material for an organic fertilizer; the residual waste water is discharged into ARA2 (GREIL 1990).

Physico-chemical parameters recorded regularly in ARA1 are summarized in Table 1; for discussion of their variation see chapter 4.1. The biochemical oxygen demand (BOD_5) is irregularly recorded. GREIL (1990) gives a long-term average space loading of 21 $kg/m^3 . d$ which corresponds to 490000 population equivalents. This enormous organic load is purified in 1750 m^3 tanks, while in a conventional activated sludge plant its purification would require a tank volume of 39000 m^3! Such comparison impressively illustrates the extraordinary environmental conditions in this particular activated sludge plant (see also Table 4 for comparison with conventional plants). Sludge loading ranges from 0.7 to 1.0; the ratio COD:BOD is about 1.9, i.e. falls short of the limit 2 indicating heavily digestible waste water (MARTZ 1981; MUDRACK & KUNST 1988). Long-term averages of cleaning efficiency are 90% and 97% with respect to COD and BOD digestion (GREIL 1990).

3. Methods

3.1 Sampling and counting procedures

At approximately monthly intervals a sludge sample was obtained from the joint outlet of the fermentation tanks. Samples were taken on 1.5.1989, 6.6.1989, 4.7.1989, 1.8.1989, 5.9.1989, 3.10.1989, 7.11.1989, 14.12.1989, 9.1.1990, 13.2.1990, 6.4.1990 and were examined at the day of collection (usually within 10 hours).

Free and filamentous bacteria were quantified according to EIKELBOOM & BUIJSEN (1983), i.e. relative abundances of the filamentous bacteria were estimated in 10 µl of sludge stained with Gram- and Neisser solution, respectively. Numbers of amoebae, globular non-

Table 1. Physico-chemical (n = 33; sampling day and 2 days before of each of the 11 sampling occasions) and biological parameters (n = 11) of the activated sludge plant ARA1. Explanation of abbreviations see chapter 3.4.

Parameter	$\bar{x}$	SD	CV	Min	Max
Physico-chemical					
pH influent	5.5	0.4	7	5.1	5.9
pH effluent	7.2	0.3	4	6.9	7.7
Oxygen content (mg/l)	2.2	1.2	55	0.8	3.6
Oxygen saturation (%)	26.9	14.8	55	10	45
Space loading (kg BOD/m^3 . d)	36.8	5.8	16	30.7	43.1
Retention time (h)	25.9	4.6	18	19.9	31.6
COD influent (mg/l)	39200	6100	16	33900	47000
COD effluent (filtrated; mg/l)	3420	1200	35	2070	5230
COD digestion (%)	91.2	2.7	3	86	94.4
Suspended solids (mg/l)	31000	2900	9	27800	35800
Anorganic substance (mg/l)	15400	1700	11	13000	18200
Organic substance (mg/l)	15600	1800	12	12800	19300
Population density					
Mikroflora (semi-quantitative scale after EIKELBOOM & BUIJSEN 1983)					
Filamentous bacteria	3.2	1.3	42	2	5
Non-filamentous bacteria	1.8	1.3	69	1	4
Fungi	1.5	1.1	73	1	4
Mikrofauna (Number/ml)					
Protozoa	3781000	2988000	79	597000	10567000
Metazoa	29	62	214	0	200
Flagellates	2077000	2136000	103	0	5423000
Globules	1309000	1648000	126	385000	6038000
Amoebae	341000	291000	85	0	769000
Ciliates	53500	44700	84	10100	157000
Biomass (mg dry mass/l)					
Prokaryotes	15000	2100	14	11300	18800
Eukaryotes	586	421	71	140	1470
Protozoa	585	418	71	135	1469
Metazoa	1	3	214	0	10
Flagellates	108	110	102	0	276
Globules	138	173	125	40	633
Amoebae	19	16	84	0	42
Ciliates	320	259	81	47	848

motile forms and flagellates were counted in 5 nl of sludge (this small volume was gained by dilution) using interference contrast and a magnification of 1000:1. The efficiency of this counting method has not yet been tested, but the enormous abundance of these organisms demands such high magnification and consequently a small volume. Numbers of ciliates and rotifers were estimated in 5 µl of sludge using a Thoma-counting-chamber and a magnification of 400:1. Recovery experiments showed that this direct counting method yields reliable results (AUGUSTIN et al. 1989). Because of the high abundances of ciliates we took a smaller sample volume than suggested by AUGUSTIN et al. (1989). We moreover applied a higher magnification to count the small forms (10–20 µm), such as *Cyrtolophosis* and peritrich microconjugants,

more accurately. Five replicates were counted at each date (counting error was 18% on average); their arithmetic means were used for subsequent analyses.

3.2 Estimation of biomass

Suspended solids were determined at 105°C (for about 20 hours) using 30 g of sludge (3 replicates). The loss on ignition (volatile solids ~ organic matter) was calculated by subtracting the anorganic matter obtained at 600°C (for half an hour) from the suspended solids. The eukaryotic biomass (wet mass) is a rough estimation obtained by reducing the shape of the animals to simple geometric figures and assuming a specific gravity of 1; the volume-weight ratio is equal to $10^6\ \mu m^3 = 1\ \mu g$ (MADONI et al. 1985). For each species the biomass estimate bases on measurements of 10 living specimens. It is assumed that the dry mass corresponds to 20% of the wet mass (CAPRIULO & CARPENTER 1983). The difference of organic substance and eukaryotic dry mass yields the prokaryotic (bacterial, fungal) dry mass.

3.3 Identification and preparation of sludge organisms

Types of filamentous bacteria were distinguished according to Gram- and Neisser staining as well as the sulphur-test described in EIKELBOOM & BUIJSEN (1983). Flagellates and rotifers were identified according to HÄNEL (1979) and DONNER (1965), respectively.

Ciliates were studied in detail using fresh sludge and bacterized cultures. Small quantities of activated sludge were put into petri-dishes and diluted with approximately 20 ml of bottled spring water (Volvic, France). Sterilized crushed wheat grains, rice grains or egg yolk were added to provide nutrients for the bacteria, which are the major food source for many ciliates. The carnivorous suctor *Prodiscophrya collini* was fed with *Colpoda* spp., *Tetrahymena* spp. and flagellates.

All species were examined in vivo and in silver impregnated slides. Fine details were examined in squeezed ciliates using oil immersion. Body shape was drawn from microscopic observations on live ciliates investigated without coverslip. All drawings of silver impregnated cells were made with the help of a camera lucida. Methods used for preparing ciliates were those described in detail by FOISSNER (1991).

3.4 Physico-chemical parameters and statistics

Physico-chemical data were kindly supplied by Dipl.-Ing. GREIL, the operator of ARA1. All statistical procedures (descriptive statistics, Spearman correlation, Mann-Whitney U-test) follow methods as described in SACHS (1984). In tables, the following abbreviations are used: CV = coefficient of variation; dm = dry mass; Max = maximum; Min = minimum; n = number of individuals or samples investigated; SD = standard deviation; SS = suspended solids; $\bar{x}$ = arithmetic mean; x = $p < 0.1$; * = $p < 0.05$. All measurements in Tables 7–11 refer to µm and data are based on protargol-impregnated specimens, if not stated otherwise.

4. Results and discussion

4.1 Physico-chemical conditions

pH values in the fermentation tanks range from 6.9 to 7.7. Similarly, the temperature does not show significant fluctuations since it is cooled down to 30°C. The efficiency of purification (in terms of COD digestion) is the third parameter

showing a very low variation (Table 1). The last mentioned parameter clearly indicates that the activated sludge flora is fully adapted to the particular waste water and has developed an optimum dissimilatory activity (ADAMSE 1968). The annual variation (expressed as coefficient of variation) in space loading, retention time, COD influent and concentration of suspended solids is mediocre (about 10–20%; Table 1). These quite stable physico-chemical parameters very likely do not have much influence on the variation of activated sludge organisms. The stability results from the uninterrupted production process and the controlled feed of the fermentation tanks mediated by buffer tanks (see Table 4 for comparison with conventional plants).

Much higher fluctuations occur in the COD effluent (about 35%) and the oxygen content (about 55%; Table 1). There is a discrepancy between the stable purifying efficiency and the comparatively high variation of the COD effluent. This may be explained by the so-called diauxy, i.e. that bacteria primarily use the readily degradable nutrients, while the more heavily digestible matter accumulates (e.g., HARTMANN 1983; MUDRACK & KUNST 1988; TOMAN & MEJAČ 1988). The oxygen content shows a variation coefficient of about 55%, which approaches that of the organisms (Table 1). Although the mean of 2.2 mg O_2/l only slightly exceeds the recommended content of 1–2 mg/l (cf. Table 4), the range of 0.4–4 mg/l indicates that the oxygen supply is regulated suboptimally. Ciliates and total protozoa are favoured by a high and stable oxygen content (Table 3), while the bacteria and flagellates seem to be well adapted to lower and varying oxygen values, because their number and biomass correlates negatively with the oxygen content and coefficients of variation (Table 3). The purifying efficiency as well as amoebae and globules (see chapter 4.2.2), however, seem to be unaffected by such fluctuations, i.e. no correlations were found.

The sludge return from ARA2 since November 1989 (5 samples) affected the physico-chemical parameters insignificantly; the mean values of space loading and clarifying efficiency increased, while those of oxygen content, retention time, COD influent and effluent as well as of suspended solids decreased (Table 2).

4.2 Population density

4.2.1 Microflora

The mixed sample from the fermentation tanks was usually lightbrown in colour and had an aromatic odour, except in November and December, when it was yellowish and smelled pungent. The latter very likely reflects the period of ripening in ARA2 since excess sludge return began at this time. Sludge flocs are very small (about 30 µm) and have an open structure. The organisms are largely suspended, hence the resulting sludge is of pulpy consistency and does not settle (sludge volume >600 ml/l). A sludge volume index could thus not be determined.

Filamentous bacteria are the dominant component of this particular activated sludge (Table 1). Their number was increased in months with excess sludge return from ARA2 (Table 2), probably due to bulking frequently occurring there during the ripening period. Bulking does not matter in ARA1 because the excess sludge is centrifugated to produce an organic fertilizer (see chapter 2). Fungal hyphae were usually low in number (Table 1); in December and January a slightly increased density was found.

Non-filamentous, so-called free, bacteria were on average moderately abundant [10–100 per field; estimation according to EIKELBOOM & BUIJSEN (1983)]. Fluctuation was fairly high, however, ranging from few to several hundreds per visual field (Table 1). During the period of sludge return their mean number decreased (Table 2).

The very small flocs observed are typical for heavily loaded and/or young sludge where the food concentration is high enough to promote dispersed sewage bacteria (EIKELBOOM & BUIJSEN 1983; WAGNER 1984). The dominance of non-flocculating bacteria can be explained by their much higher area-to-volume ratio compared to that of bacteria clumped in flocs. Moreover, it has been observed that simple, soluble organic compounds which are readily metabolized by the majority of microorganisms favour the growth of filamentous bacteria while complex, insoluble compounds which have to be hydrolyzed before being metabolized favour the growth of organisms forming sludges with good settling properties (PIPES 1967; CHUDOBA 1985). Free and filamentous bacteria, however, very effectively use the dispersed nutrients (WAGNER 1984; MUDRACK & KUNST 1988), as confirmed by the good efficiency of purification (Table 1).

4.2.2 Microfauna

The microfauna is almost entirely constituted by protozoa (Table 1). Few rotifers occurred in August, September and October; consequently the metazoan number and biomass show the maximum coefficients of variation (Table 1). Nematodes were never observed during counting, but single specimens could be found in large amounts of activated sludge. It is understandable that metazoa are almost absent, because of the unfavourable ratio between their long doubling time and the short sewage retention time (e.g., WOOMBS & LAYBOURN-PARRY 1987).

Flagellates are the numerically dominant protozoan group ($\bar{x} = 2$ million/ml; Table 1). Their mean population density significantly increased since November, the beginning of excess sludge return from ARA2 (Table 2). The reasons for their absence in May and October, which causes the high coefficient of variation, are unclear; methodical problems can be excluded since the examination of larger quantities of sludge confirmed their absence. The usually high number of flagellates in this special sludge is reasonable since its retention time is extremely short (about 1 day); thus the sewage plant is constantly in a starting phase and flagellates are,

Table 2. Physico-chemical (n = 18, 15) and biological (n = 6, 5) parameters of ARA1 without and with excess sludge return from ARA2. Means were compared by the U-test. ns = non significant; x = significant at 10%; * = significant at 5%. Explanation of abbreviations see chapter 3.4.

	without excess sludge			with excess sludge			
Parameter	$\bar{x}$	SD	CV	$\bar{x}$	SD	CV	Test
Physico-chemical							
Oxygen content (mg/l)	1.9	1.1	61.3	2.5	1.2	45.7	ns
Oxygen saturation (%)	23	14	62.0	31	14	46.0	ns
Space loading (kg BOD/m^3.d)	35.4	5.6	15.8	38.4	5.8	15.2	ns
Retention time (h)	27.6	4.0	14.3	23.9	4.7	19.8	ns
COD influent (mg/l)	40300	6200	15.3	37800	5900	15.5	ns
COD effluent (mg/l)	4000	1100	28.0	2700	800	28.7	ns
COD digestion (%)	89.9	2.7	3.0	92.9	1.7	1.8	ns
Suspended solids (mg/l)	32300	3000	9.3	29400	2000	6.9	ns
Anorganic substance (mg/l)	15900	1800	11.4	14800	1500	10.0	ns
Organic substance (mg/l)	16400	1800	11.1	14600	1400	9.4	x
Population density							
Mikroflora (semi-quantitative scale after EIKELBOOM & BUIJSEN 1983)							
Filamentous bacteria	2.3	1.2	51.9	4.2	0.4	10.6	
Non-filamentous bacteria	2.2	1.5	67.9	1.4	0.9	63.9	
Fungi	1.2	0.8	64.5	2.0	1.4	70.7	
Mikrofauna (Number/ml)							
Protozoa	2500000	1975000	79.0	5356000	3424000	63.9	x
Metazoa	53	79	147.5	0	0	0.0	
Flagellates	1109000	1973000	178.0	3238000	1857000	57.3	*
%	27	33	121.7	61	21	33.9	
Globules	823000	700000	85.1	1915000	2321000	121.2	x
%	46	33	71.4	36	23	63.9	
Amoebae	530000	269000	50.8	131000	111000	84.7	*
%	25	17	69.5	3	2	84.9	
Ciliates	38900	26300	67.6	71000	58500	82.4	ns
%	2	1	67.2	1	1	55.2	
Biomass (mg dry mass/l)							
Prokaryotes	16000	1900	11.9	13800	1800	13.3	*
%	97	2	1.7	94	4	4.3	
Eukaryotes	416	252	60.6	791	511	64.6	ns
%	3	2	64.0	6	4	71.7	
Protozoa	413	249	60.2	791	511	64.6	ns
Metazoa	3	4	147.5	0	0	0.0	ns
Flagellates	56	101	179.2	169	94	55.4	*
Globules	86	73	85.1	201	243	121.2	ns
Amoebae	29	15	50.8	7	6	84.7	*
Ciliates	242	211	87.2	414	302	73.1	ns

beside the free-swimming bacterivorous ciliates, the "pioneer group" in raw sewage (e.g., MADONI 1982; CURDS 1982). This results from their short generation time, for instance 1.7 h in *Monas termo*, and their ability to feed on soluble organic substances and on free bacteria (HÄNEL 1979). Moreover, flagellate predators such as *Parastrongylidium* and *Prodiscophrya* occurred only infrequently and in low numbers (Table 5). HÄNEL (1979) investigated about 80 different waste water plants and reported mean values between 200000 and 2 million flagellates/ml. He observed top values of 3–17 million/ml at high temperatures (32 °C) and under well-aerated conditions. The former situation is similar to that in the aeration tanks in Kundl, while flagellates in ARA1 tolerate an unstable oxygen supply (Table 3). However, we counted a maximum number of "only" 5.4 million/ml, very probably because of the short retention time. This assumption is supported by HÄNEL's (1979) observation that at 32 °C the maximum population density is reached later, i.e. after 48 hours.

Globular, non-motile forms (10 µm in diameter with a nucleus and a thin cell membrane) are a regular and a numerically important component of ARA1 sludge; they increased during the period of sludge return (Table 1, 2). Whether the globules are cysts of flagellates or naked amoebae or another kind of organism could not be clarified; they are certainly not ciliate cysts because a micronucleus is absent. Unfortunately, no data are available regarding inactive protozoans in activated sludge (cf. KLIMOWICZ 1972). BARK (1972) noticed that the majority of flabellulid amoebae were inactive during counting; however, he neither specified their number nor their morphology.

Data concerning the abundance of naked amoebae in activated sludge range from 60 to 18600 individuals/ml (BARKER 1942; BARK 1972; KLIMOWICZ 1972). Except in December, when they were absent, we found 31000–769000, on average 341000 cells/ml (Table 1). Possibly, our much higher values result from the improved counting method (interference contrast etc.) and the particular kind of sludge. KLIMOWICZ (1970) concluded from laboratory experiments that the number of amoebae (and similarly of flagellates) is determined mainly by the composition of the influent waste water and not by the pollution load or oxygen content. This seems to be supported by their independence of oxygen content and their significantly decreased numbers since excess sludge return from ARA2 (Table 2, 3).

Ciliates occurred with a frequency of 100%; however, compared to the abundances of the smaller-sized protozoans their density is low, ranging from 10000 to 157000 individuals/ml (Fig. 1; Table 1). Numbers of 10000–50000 correspond very well to those found in communal treatment plants (BAINES et al. 1953; MINISTRY 1968; BARK 1972; AUGUSTIN et al. 1989). MADONI (1986) considers a mean abundance of 1000 ciliates/ml as usual in normally functioning plants, while higher numbers (>10000) indicate optimum plant performance. Ciliate density in ARA1 correlates slightly (at 10% level) with a high and stable oxygen content

Table 3. Spearman rank correlation of oxygen saturation and biological parameters (n = 11) in ARA1. About 50 additional pairs of variables were tested, but insignificant. The hypothesis of independence was tested. R = Spearman correlation coefficient; x = significant at 10%; * = significant at 5%.

Variable x	versus	Variable y	R	Test
Oxygen saturation (3-day-coefficient of variation)		Organic substance	0.6545	*
		Ciliate number and biomass	−0.5091	x
		Flagellate number and biomass	0.4455	x
		Eukaryotic biomass	−0.5000	x
		Prokaryotic biomass	0.6182	*
		Protozoan number and biomass	−0.4455	x
Oxygen saturation (%) (3-day-mean)		Prokaryotic biomass	−0.5455	*
(3-day-mean)		Ciliate number	0.4182	x
(day before sampling)		Prokaryotic biomass	−0.5856	*

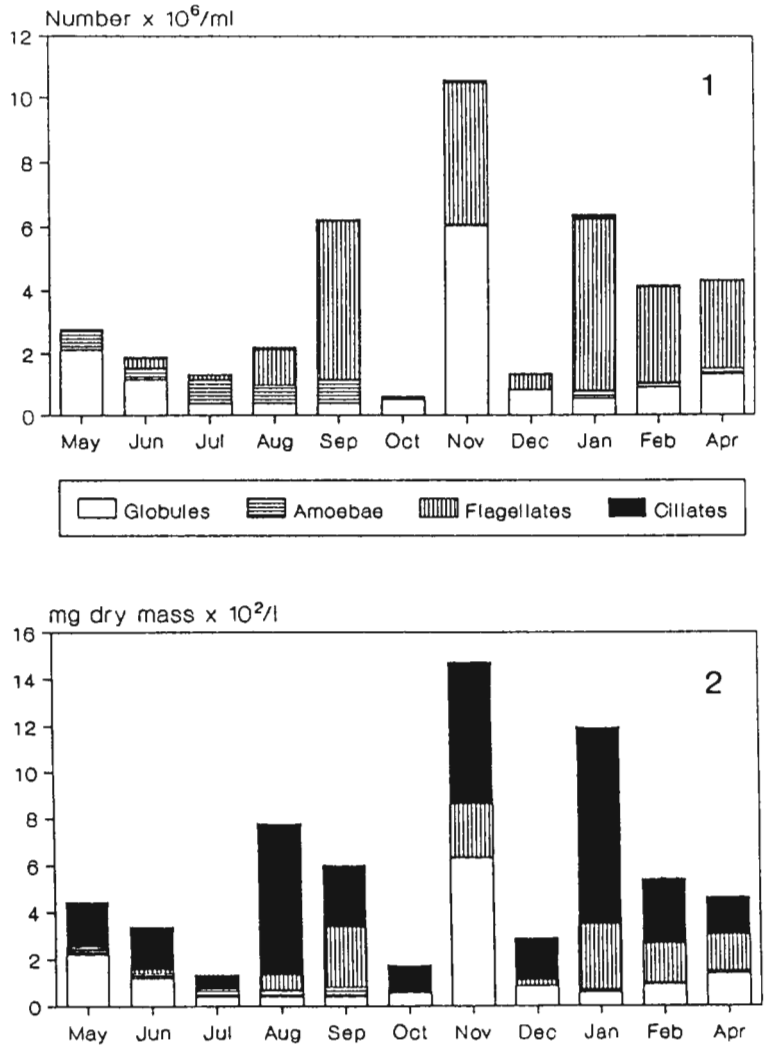
(Table 3); probably due to the improved oxygen supply, their mean number almost doubled during the period of sludge return (Table 2).

4.3 Biomass

The organic substance in ARA1 is composed of prokaryotic and eukaryotic biomass, i.e. bacteria and protozoa, respectively. As evident from the community structure, the prokaryotic biomass consists largely of filamentous bacteria. Compared to the prokaryotic proportion of 89–99%, the eukaryotic biomass is low (1–11%; for absolute values see Tables 1, 2). Based on the effluent quantity from all tanks a yield of about 22 tons organic dry mass/day has been calculated (eukaryotic measured, prokaryotic derived from organic substance). This agrees with the actual quantity of fertilizer produced daily (GREIL pers. comm.). Related to wet mass, the daily increment of organisms in ARA1 is about 100 tons bacteria and about 4 tons protozoa in a volume of 1400 m^3 of sludge!

As far as we know, these are the first definite data concerning the ratio prokaryotes/eukaryotes in activated sludge. In conventional sludge plants the ciliate biomass was estimated very roughly, i.e. without any solid data basis, to constitute about 5% of the suspended solids (MINISTRY 1968; CURDS 1973; FOISSNER 1990). In ARA1 the total protozoan biomass constitutes on average 3.9% of the organic substance and 1.9% of the suspended solids, indicating that the earlier values were very likely overestimated. Our ratio corresponds well to those found in other environments, e.g., in soils (FOISSNER 1987).

The eukaryotic biomass can be subdivided into metazoan and protozoan organic substance. Despite the comparatively large size of the rotifers constituting the sole metazoan group in the sludge investigated, their biomass is negligible due



Figs. 1, 2. Number (1) and biomass (2) of activated sludge protozoa in the sewage treatment plant ARA1 1989/90. The arithmetic means of 5 subsamples are shown. Relative proportions illustrate that flagellates are numerically dominant, while ciliates have the highest biomass values.

to their low frequency and number (Table 1; cp. above). The protozoa thus constitute 98.7–100% of the eukaryotic biomass. Based on 50 weekly samples from 2 activated sludge plants clarifying communal wastes, SYDENHAM (1971) concludes that the eukaryotic biomass (determined volumetrically) is composed of 2% flagellates, 11.5% rotifers, 15.8% naked amoebae, 32.3% testate amoebae and 38.4% ciliates. Unfortunately, absolute values are lacking. The large proportion of

testaceans and rotifers indicates a high sludge age, thus these values are not very representative. SLÁDEČEK (1958) gives "200–500 mm^3/l optimum protozoan biomass"; this corresponds to 200–500 mg/l (very probably wet mass) which is about 15 times less than we estimated in ARA1.

Within the protozoa, the ciliates have the highest biomass values due to their larger sizes compared to those of flagellates and amoebae; they constitute more than 50% of the protozoan biomass (Fig. 2; Table 1). MADONI & ANTONIETTI (1984) studied the ciliate biomass in the municipal sewage plant of Parma (Italy) during 120 days from the start of the plant until its stabilization. They calculated a minimum of 1.2 mg wet mass/l and a maximum of 587 mg/l; converted to dry mass these are 0.2 and 117 mg/l, respectively, corresponding to about one third of the

Table 4. Comparison of the high-rate waste water treatment plant ARA1 with conventional activated sludge plants. (Synopsis of data available in the literature, particularly in MARTZ 1981, HARTMANN 1983, MUDRACK & KUNST 1988.) Explanation of abbreviations see chapter 3.4.

	ARA1			Conventional plants		
Parameter	Min	Max	$\bar{x}$	Min	Max	$\bar{x}$
Temperature (°C)	–	–	30	5	25	–
pH	6.5	7.7	7	4	9	7
Oxygen content (mg/l)	0.4	4.0	2.2	1	2	–
Oxygen saturation (%)	5	50	26	–	–	–
Space loading (kg BOD/m^3.d)	–	–	21	0.25	1.5	1
Sludge age (d)	–	–	1	13	35	–
BOD influent (mg/l)	–	–	21000	–	–	300
BOD effluent (mg/l)	–	–	3000	–	–	20
COD influent (mg/l)	–	–	38000	500	600	550
COD effluent (mg/l)	–	–	3400	–	–	100
COD:BOD	–	–	1.9	–	–	<1.7
COD digestion (%)	–	–	90	–	–	90
Sludge loading (kg BOD/kg SS.d)	0.7	1.0	0.8	0.05	0.3	–
Sludge volume (ml/l)	–	–	>600	300	600	–
Sludge index (ml/g SS)	–	–	>200	80	120	–
Floc size (µm)	–	–	<30	150	500	–
Suspended solids (%)	–	–	3	–	–	<1
Anorganic substance (%)	45	56	50	15	65	40
Organic substance (%)	44	55	50	35	85	60
Prokaryotic biomass (mg dm/l)	11000	18800	15000	–	–	–
Eukaryotic biomass (mg dm/l)	140	1470	590	–	–	–
Dominant bacteria		filamentous			flocculating	
Dominant protozoa		flagellated			ciliated	
Amoebae (Number × 1000/ml)	0	770	340	0	19	–
Flagellates (Number × 1000/ml)	0	5400	2000	0	17000	2000
Globules (Number × 1000/ml)	385	6000	1000	–	–	–
Ciliates (Number × 1000/ml)	10	157	54	1	50	–
Rotifers (Number/ml)	0	200	29	0	2300	–
Nematodes (Number/ml)	0	0	0	–	–	1

mean value in ARA1. The globular, non-motile forms and the flagellates have biomass values in the same order of magnitude (about 20% each), while the naked amoebae contribute on average only 3% (Fig. 2; Table 1).

4.4 Species number

4.4.1 Microflora

The types of filamentous organisms are mentioned with some reservation because of the still great taxonomic problems (EIKELBOOM & BUIJSEN 1983); therefore we cannot rule out that some special types occur in ARA1. At each sampling date 7–11 different types were found (Table 5). In most samples gram positive bacteria, especially type 1851, dominated. In January, the gram negative type 021N, was particularly conspicuous; this form showed an increased mean relative abundance since the beginning of sludge return. Similarly, in this period type 1851 tended to form large bundles which were less prominent before November, though it was the dominating filamentous bacterium (on average 31%) throughout the investigation period. Type 1851 scarcely dominates in conventional activated sludge plants (EICKELBOOM & BUIJSEN 1983; BLACKBEARD et al. 1988). The second dominant species in ARA1 is type 021N, which is a well known bacterium in bulking incidents (NIEKERK et al. 1986; WAGNER 1984). This may indicate that the ARA1 sludge is some sort of bulking sludge. The remaining types observed in ARA1 are also quite common in conventional waste water treatment systems. Types usually indicating oxygen deficiency, such as spirills, *Thiotrix* sp., *Sphaerotilus natans* and *Beggiatoa* sp., were not observed.

4.4.2 Microfauna

During the 1-year-study about 4 flagellate, about 2 amoebae, 8 ciliate and 1 rotatorian species were observed in fresh sludge (Table 5). Additional species (about 6 flagellates, some amoebae, 6 ciliates) occurred in samples kept for some weeks or longer. Such low species numbers are in accordance with observations in other heavily loaded activated sludge plants (HÄNEL 1979; CURDS & COCKBURN 1970b). We found the same dominant flagellate genera, *Bodo* and *Monas*, as HÄNEL (1979). Similarly, the mean number of 3 ciliate species corresponds well to the 2 species found by CURDS & COCKBURN (1970b) in plants with high organic load (0.6–0.9 BOD/g SS.d). In ARA1, a further factor limiting species diversity is the short retention time (24 h). *Habrotrocha bidens*, the single metazoan species observed, was also found by KLIMOWICZ (1970, 1972) in conventional activated sludge at varying loadings.

The peritrich *Opercularia asymmetrica* (Figs. 22–35) and the holotrich *Colpoda ecaudata* (Fig. 3) were the only ciliate species being a regular component of the sludge (frequency 91–100%). *Opercularia asymmetrica* was eudominant

Table 5. Species composition, species number and relative abundance (dominance) of activated sludge organisms in ARA1. Taxa are arranged according to decreasing dominance. – = no organism found (filamentous bacteria have not been investigated in July); 0 = dominance <1. Explanation of abbreviations see chapter 3.4.

Group, species or type	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Apr	x̄	SD
Filamentous bacteria													
Species/Type number	7	11	–	10	11	8	8	11	11	11	7	10	2
T 1851	40	–	–	25	10	40	65	50	–	40	40	31	22
T 021N	10	10	–	15	10	–	5	2	60	20	25	16	17
Nocarida sp.	10	15	–	10	15	10	5	10	2	2	10	9	5
Haliscomenobacter hydrossis	10	20	–	5	15	10	5	2	2	10	10	9	6
T 1702	10	10	–	10	10	5	5	2	2	2	5	6	4
T 1863	10	10	–	5	10	–	–	10	4	5	–	5	4
Nostocoida limnicola II	–	5	–	5	5	10	5	10	2	10	–	5	4
Streptococcus sp.	–	–	–	15	–	10	–	2	20	–	–	5	8
T 0411	–	5	–	5	5	10	5	5	2	2	–	4	3
T 1701	10	5	–	–	5	5	–	–	–	–	5	3	3
Nostocoida limnicola I	–	10	–	–	10	–	5	–	2	2	–	3	4
Microthrix parvicella	–	5	–	–	5	–	–	5	2	5	5	3	2
T 0041	–	5	–	5	–	–	–	2	2	2	–	2	2
Protozoa													
Flagellates	–	16	12	52	81	–	41	38	85	74	65	42	32
Globules	76	62	29	17	6	86	57	61	8	21	30	41	28
Amoebae	22	21	58	26	12	11	0	–	4	4	4	15	17
Ciliates	2	2	1	4	1	3	1	2	2	1	1	2	1
Flagellates													
Species number	–	2	1	2	3	–	2	2	3	3	1	2	1
Bodo sp.	–	49	–	87	71	–	80	85	77	93	100	58	40
Monas sp.	–	51	100	13	28	–	20	15	21	6	–	23	30
Scytomonas sp.	–	–	–	–	–	–	–	–	3	1	–	0	1
Trepomonas sp.	–	–	–	–	1	–	–	–	–	–	–	0	0
Ciliates													
Species number	3	2	3	6	4	2	4	5	3	3	3	3	1
Opercularia asymmetrica	57	100	63	81	94	95	86	50	85	81	89	80	16
Colpoda ecaudata	4	1	34	1	2	–	5	15	7	11	10	8	10
Epistylis sp.	–	–	–	17	4	5	–	24	8	9	–	6	8
Cyrtolophosis elongata	39	–	3	1	–	–	–	–	–	–	–	4	12
Pseudocohnilembus pusillus	–	–	–	–	–	–	8	–	–	–	1	1	2
Pseudochilodonopsis fluviatilis	–	–	–	1	–	–	–	6	–	–	–	1	2
Parastrongylidium oswaldi	–	–	–	–	0	–	1	5	–	–	–	1	1
Prodiscophrya collini	–	–	–	0	–	–	–	–	–	–	–	0	0
Opercularia asymmetrica													
Trophonts	57	100	63	24	94	75	84	50	63	79	88	70	22
Precysts	–	–	–	57	–	6	–	–	–	–	–	6	17
Swarmers	–	–	–	–	–	0	0	–	0	–	1	0	0
Microconjugants	–	–	–	–	–	14	2	–	22	1	0	4	7

contributing 50–99% of individuals (Table 5). In August it occurred largely in a precystic form and in December its dominance decreased to 50%. On both occasions the oxygen regime was disturbed, i.e. very low or strongly varying. *Colpoda ecaudata*, the next "dominant" species, contributes on average only 8% of specimens. The other species occurred rarely having a frequency of 18–27% (Tables 5, 6). A new species, *Parastrongylidium oswaldi*, was also found (Figs. 7–21). The suctor *Prodiscophrya collini* (Figs. 36–50) was only recorded in August, when the maximum number of 5 ciliate species occurred.

The ciliates observed may be divided into 3 groups on the basis of their habit (FOISSNER 1990): (i) free-swimming forms which move actively through the liquid *(Colpoda, Cyrtolophosis, Pseudocohnilembus);* (ii) non-attached crawling forms, inhabiting the surface of sludge flocs *(Parastrongylidium, Pseudochilodonopsis);* (iii) forms attached with a stalk to the substrate *(Epistylis, Opercularia, Prodiscophrya)*. With respect to their nutrition, most species feed on the bacteria suspended in the liquid; they are not equipped to ingest filamentous bacteria. The suctor *Prodiscophrya collini* is the single predatory species recorded. Free-swimming ciliates constitute about 17% of individuals, a proportion which is fairly high compared to conventional sewage plants, probably due to the short sewage retention time (MADONI 1986). The proportion is in fact even higher (more than 90%) since the "attached" filter feeder *Opercularia* was almost exclusively found solitary and unattached, viz. stalkless. This morphological peculiarity can be interpreted as adaptation to the peculiar sludge structure, i.e. a pulpy bacterial mass rather than flocs suitable for attachment. The almost inexistent flocs likewise cause crawling ciliates to be negligible in ARA1 (relative abundance 1%). In conventional sewage plants, however, attached and crawling species, such as *Vorticella* spp., *Opercularia coarctata, Aspidisca costata* and *Euplotes affinis*, are most common in terms of abundance and frequency (MINISTRY 1968; CURDS & COCKBURN 1970a; KLIMOWICZ 1972; ALONSO et al. 1981; MADONI & GHETTI 1981; MADONI 1986).

Besides different life form types and different dominant species, it is remarkable that species characteristic for the starting phase in conventional plants, e.g., *Colpidium campylum, Glaucoma* spp., *Cyclidium* spp., are lacking in ARA1 (MADONI 1982, 1986; MADONI & ANTONIETTI 1984). Likewise, the occurrence of *Colpoda ecaudata* is unusual, since it has never been recorded from conventional plants (although it might have sometimes been misidentified). Colpodids (*Colpoda* spp., *Cyrtolophosis* spp.) are more r- than K-selected (LÜFTENEGGER et al. 1985), i.e. have a short generation time (cp. short retention time of sludge!), are weak competitors (cp. low number of species and absence of predators) and are colonizers of extreme habitats (cp. very high organic load, high temperature).

Evidently this particular industrial waste water favours a special, probably r-selected ciliate community. Main factors are presumably the extraordinarily saprobic conditions, the high temperature, the very small flocs and the short retention time.

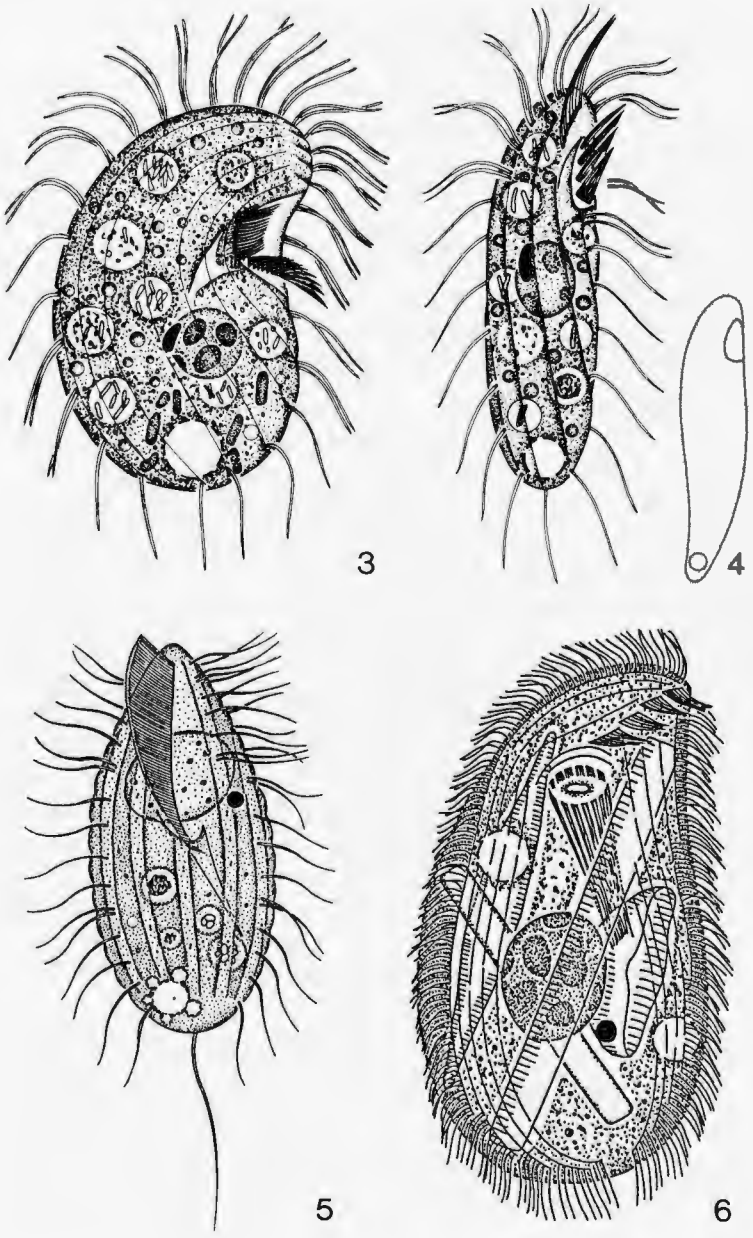
Table 6. Ecograms of ciliated protozoa found in an activated sludge plant heavily loaded with pharmaceutical wastes (n = 11). Long-term averages: temperature 30 °C, BOD_5 21000 mg/l, total nitrogen 2000 mg/l, chlorid 1500 mg/l, sulphat 8000 mg/l. Physico-chemical parameters represent arithmetic means of 3 days (sampling day and 2 days before). Taxa are arranged in alphabetical order. Upper line = minimum; lower line = maximum. Ceca = *Colpoda ecaudata*, Celo = *Cyrtolophosis elongata*, Oasy = *Opercularia asymmetrica*, Posw = *Parastrongylidium oswaldi*, Pcol = *Prodiscophrya collini*, Pflu = *Pseudochilodonopsis fluviatilis*, Ppus = *Pseudocohnilembus pusillus*.

Parameter	Ceca	Celo	Oasy	Posw	Pcol	Pflu	Ppus
Biomass of 10^6 individuals (mg)	9	0.4	32	73	78	12	6
Frequency (%)	91	27	100	27	9	18	18
Number/ml	140	320	6320	160	40	400	360
	12300	19800	98200	1160	40	1360	8640
pH	6.5	6.5	6.5	6.8	6.9	6.8	6.9
	7.7	7.5	7.7	7.4	7.2	7.2	7.4
Oxygen (mg/l)	0.4	0.4	0.4	0.4	0.8	0.4	0.6
	4.0	2.0	4.0	4.0	2.0	4.0	3.6
Oxygen saturation (%)	5	5	5	5	10	5	8
	50	25	50	50	25	50	45
Space loading (kg COD/m^3.d)	25	32	25	26	35	35	32
	49	42	49	49	42	49	46
COD effluent (g/l)	1.9	2.4	1.9	2.0	2.4	2.0	2.0
	5.5	5.5	6.8	5.0	4.5	4.5	4.5
COD digestion (%)	85	89	85	87	89	89	90
	96	95	96	96	95	95	96
Prokaryotic dry mass (g/l)	11	15	11	11	15	15	11
	19	19	19	16	15	16	14
Free bacteria	1	1	1	1	2	1	1
(semi-quantitative scale)	4	2	4	3	2	2	1
Filamentous bacteria	2	2	2	3	2	2	4
(semi-quantitative scale)	5	3	5	5	2	5	4

4.5 Taxonomy and ecology of ciliated protozoa recorded

Few activated sludge ciliates have been studied using modern techniques, particularly silver staining, which is usually required for their correct identification (AUGUSTIN et al. 1987; FOISSNER 1988a, 1990; AUGUSTIN & FOISSNER 1989). Although the species number found is comparatively low, a new hypotrich was discovered. The descriptions are arranged according to life form types, i.e. free-swimming, crawling and attached (cp. chapter 4.4.2). Terminology is largely according to KAHL (1930) and CORLISS & LOM (1985). Morphometric data and ecograms are summarized in Tables 6–11 and are not repeated.

Evidently all species are well adapted to live in sludge with an exceptionally high organic load; thus they indicate poly- to isosaprobic conditions according to SLÁDEČEK (1961). Moreover, the mean value of 1500 mg chloride/l shows that they are oligo- to mesohaline (ALBRECHT 1984).



Figs. 3–6. Life appearance of ciliates recorded in the fresh sludge. 3: *Colpoda ecaudata*, 30 µm (from FOISSNER 1992). 4: *Cyrtolophosis elongata*, 26 µm; the shape variant shown on the right was most frequently found in ARA1 sludge (from FOISSNER 1992). 5: *Pseudocohnilembus pusillus*, 30 µm (original). 6: *Pseudochilodonopsis fluviatilis*, 62 µm (from FOISSNER 1988 b).

Colpoda ecaudata (LIEBMANN, 1936) FOISSNER, BLATTERER, BERGER & KOHMANN, 1991

Fig. 3

This free-swimming species belongs to the order Colpodida, family Colpodidae. Our specimens measure in vivo 27–48 × 15–23 µm ($\bar{x}$ = 39.5, SD = 5.6, CV = 14.2, n = 10; $\bar{x}$ = 19.2, SD = 2.3, CV = 11.8, n = 10) and largely correspond to those found by FOISSNER (1992) in soil.

Colpoda ecaudata strongly resembles *C. steinii* and *C. aspera*. The most suitable character for distinguishing these species is the shape of the left oral ciliary field: rectangular in *C. ecaudata*, spoon-like in *C. steinii* and with a notch in *C. aspera* (FOISSNER 1992). An additional indication for in vivo separation is the morphology of the macronucleus: few large nucleoli at periphery in *C. ecaudata*, 1 large, central nucleolus in *C. steinii* and reticulate chromatine in *C. aspera* (FOISSNER 1992).

Ecology (Table 6; see also chapter 4.4.2). *Colpoda ecaudata* was originally found in the sewer system of Leipzig. The autecology of this species is reviewed in FOISSNER (1992). Thus only main points are summarized: distributed worldwide in polysaprobic freshwaters, bacteria-rich soils and in faeces (coprophilic); feeds exclusively on bacteria; reproduces between 5 °C and 27 °C (the high frequency of *C. ecaudata* at 30 °C in ARA1 shows that this range has to be extended), optimum temperature between 13 °C and 16 °C, upper lethal temperature about 60 °C; optimum of pH 6.5, range pH 4.6–9.2, lethal values pH 3.0 and 12.0; resistant to hypertonic solutions (up to 2% NaCl); can move in an almost amoeboid fashion under certain experimental conditions; tolerates anoxic conditions at least 3 weeks, but does not reproduce; survives complete desiccation by forming resting cysts or (rarely) in a state of anabiosis. Based on these and our data FOISSNER et al. (1991) classified *C. ecaudata* as polysaprobic indicator organism: p-i; a = 1, p = 9, I = 5, SI = 3.9E.

Cyrtolophosis elongata (SCHEWIAKOFF, 1892) KAHL, 1931

Fig. 4

This free-swimming species belongs to the order Cyrtolophosidida, family Cyrtolophosididae. Our specimens measure in vivo 16–25 × 4–11 µm ($\bar{x}$ = 19.2, SD = 2.5, CV = 12.9, n = 10; $\bar{x}$ = 6.3, SD = 1.5, CV = 23.6, n = 10) and largely correspond to those found by FOISSNER (1992) in soils.

It differs from *C. mucicola* in the terminal location of the contractile vacuole, the absence of a dwelling tube, the number of somatic kineties, the movement and the lateral flattening.

Ecology (Table 6). *Cyrtolophosis elongata* is frequently found in soils (FOISSNER 1992). Our record is the first from activated sludge. Little is known about its autecology; it is probably rather similar to that of *C. mucicola*, which is a pioneer species, i.e. reaches high abundances when most other species still occur in low numbers. Such a relationship is, however, not evident from our data. The strong

population found in ARA1 suggests that *C. elongata* prefers a polysaprobic environment; this is also substantiated by DETCHEVA (1983), who found this species in an alpha-mesosaprobic river. Feeds on bacteria and algae.

Pseudocohnilembus pusillus (QUENNERSTEDT, 1869) FOISSNER & WILBERT, 1981

Fig. 5; Table 7

This free-swimming species belongs to the order Scuticociliatida, family Pseudocohnilembidae. In vivo it measures 27–35 × 14–18 µm ($\bar{x}$ = 29.9, SD = 1.9, CV = 6.4, n = 10; $\bar{x}$ = 15.6, SD = 0.5, CV = 3.1, n = 10).

The identification bases on the meridional course of the somatic kineties and the absence of scattered basal bodies near the end of the oral apparatus. Compared to the populations morphometrically characterized by FOISSNER & WILBERT (1981), our organisms are slightly smaller and show a wider range of somatic kineties (Table 7). Thus, the distinction from *P. putrinus* is weakened.

Ecology (Table 6). *Pseudocohnilembus* species are ecologically rather similar, i.e. bacterivorous, euryhaline and world-wide distributed (e.g., HOARE 1927; FOISSNER & WILBERT 1981; POMP & WILBERT 1988; FENCHEL 1990). Thus they

Table 7. Morphometric characterization of *Pseudocohnilembus pusillus*. 1st line = activated sludge population from ARA1; 2nd line = population from a potatio infusion with dung-heap liquid, dry silver impregnation (FOISSNER & WILBERT 1981); 3rd line = population from heavily polluted tidal pool in Banyuls-sur-Mer, wet silver impregnation (FOISSNER & WILBERT 1981). Explanation of abbreviations see chapter 3.4.

Character	$\bar{x}$	SD	CV	Min	Max	n
Body, length	26.4	3.9	14.9	18	32	28
	34.5	0.6	9.4	29	42	29
	31.3	0.6	9.8	25	39	29
Body, width	13.9	1.7	12.5	9	16	28
	20.8	0.5	12.6	15	26	29
	15.4	0.4	14.7	12	20	29
Distance anterior end to end of oral apparatus	15.9	1.9	11.8	12	20	28
	17.2	0.3	9.6	15	22	29
	15.7	0.2	8.3	13	18	29
Macronucleus, length	8.1	1.4	17.6	6	13	28
	6.5	0.1	11.2	5	8	29
	6.9	0.1	10.0	5	8	29
Macronucleus, width	6.5	1.1	16.9	4	9	28
	6.1	0.1	8.0	5	8	29
	6.3	0.1	9.8	5	8	29
Somatic kineties, number	10.6	1.5	14.0	9	12	28
	11.0	0.0	0.0	11	11	29
	10.0	0.0	0.0	10	10	29
Pairs of basal bodies in dorsal kinety, number	16.3	2.0	12.5	12	20	28
	19.1	0.3	8.0	16	22	29
	17.8	0.4	11.5	15	23	29

inhabit not only freshwater but also tidal pools and saline soils; the latter are characterized by the eco-order Pseudocohnilembetalia (FOISSNER 1987). Moreover, *P. pusillus* is known to be coprophilic; it is unable to grow in normally formed faeces and when it is admixed with urine, but develops when faeces is slightly diluted with water (HOARE 1927).

Already 1927, HOARE published some interesting autecological data on *P. pusillus:* it can be easily cultured at 24°C, but does not tolerate a sudden increase of temperature to 37°C; reproduces between pH 5 and 10, optimum pH 7.4–8.0, killed at pH 4.5; requires 8 days for adaptation to pure sea water; a freshwater strain was maintained in sea water for 163 days without exhibiting any morphological change. Recently, FENCHEL (1990) studied different phenotypes of a marine population of *P. pusillus* (salinity 32‰): trophonts do not divide when starved, instead they form motile swarmers (theronts) having half of trophont cell volume. These small forms can resume cell divisions quickly when food is available. About half of the swarmers are capable of forming resting cysts 10–30 h after the onset of starvation; cysts depend on episodic food supply; they do not survive the passage through mice intestines (HOARE 1927). Young (0–10-h-old) cysts excyst within 30 min after exposure to a bacterial suspension and the total lag time for cell division is ~2.5 h, corresponding to the lag of equally old (10–20 h) theronts; 60-day-old cysts need on average 30 h (range: 5–70 h) for excystation and the total lag time prior to fission is on average 55 h (range 25–90 h; FENCHEL 1990).

Pseudocohnilembus pusillus has been frequently recorded from polysaprobic running waters. Our data and that above support the saprobic classification suggested by SLÁDEČEK (1973) and FOISSNER (1988a): a–p; a = 5, p = 5, I = 3, SI = 3.5.

Parastrongylidium oswaldi nov. spec.

Figs. 7–21; Table 8

This crawling species belongs to the order Hypotrichida, family Spirofilidae.

Diagnosis. In vivo 50–80 × 30–50 µm, oval to fusiform. Cortical granules (extrusomes) colourless, spherical, 0.5–1.5 µm in diameter, mainly between cirral rows. 13 cirral rows and 19 adoral membranelles on average. 1 enlarged frontal cirrus. 1 dorsal kinety.

Type location. Activated sludge of a heavily loaded industrial sewage plant in Kundl, Tyrol, Austria, (E12°, N47°47′).

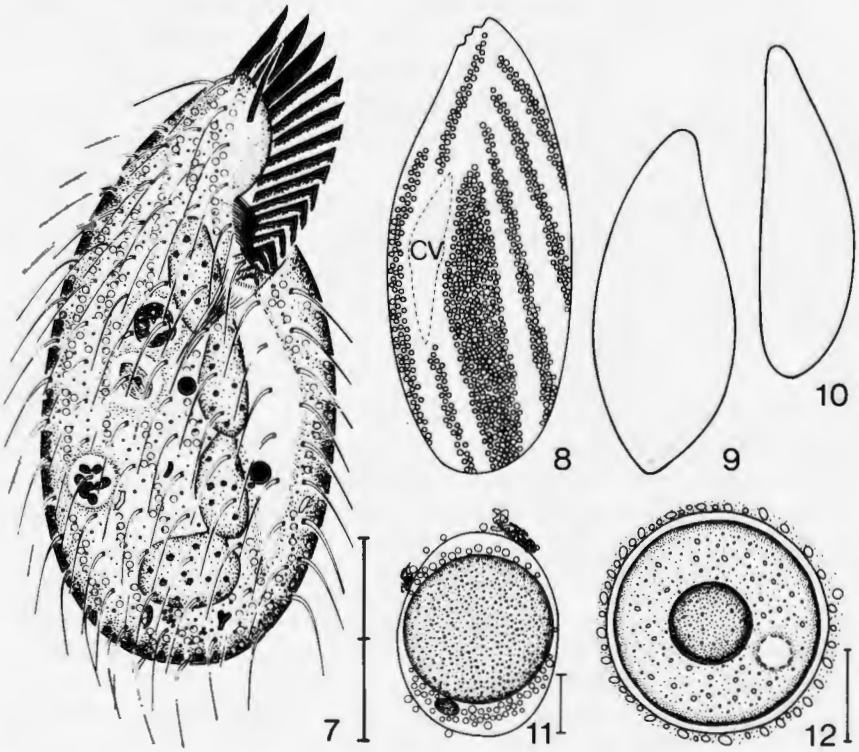
Type material. A slide of holotype specimens and 1 slide of paratype specimens are deposited in the "Sammlung der mikroskopischen Präparate" in the Oberösterreichisches Landesmuseum in Linz (Austria).

Dedication. The species is named in honour of Dr. JÖRG-DIETER OSWALD, who stimulated this study.

Description. Size in vivo on average 65 × 42 µm (SD = 10.7, CV = 16.5, n = 10; SD = 8.1, CV = 19.3, n = 10). Brownish at low magnification (40×). Right and left

Table 8. Morphometric characterization of *Parastrongylidium oswaldi*. Explanation of abbreviations see chapter 3.4.

Character	$\bar{x}$	SD	CV	Min	Max	n
Body, length	62.3	8.1	13.0	50	76	15
Body, width	28.0	2.8	10.1	22	33	15
Adoral zone of membranelles, length	23.3	1.8	7.7	20	26	15
Dorsal unciliated field, maximum width	12.5	1.7	13.4	10	16	15
Macronucleus figure, length	46.9	6.6	14.1	38	56	15
Macronucleus figure, maximum width	6.5	1.7	26.1	4	10	15
Macronucleus figure, minimum width	0.5	0.6	131.0	0	2	15
Micronucleus, diameter	1.4	0.3	18.7	1	2	15
Micronuclei, number	3.8	1.1	28.5	2	6	15
Adoral membranelles, number	19.5	1.4	6.9	18	22	15
Frontal cirri, number	1.0	0.0	0.0	1	1	15
Cirral rows, number	13.1	0.9	7.0	11	14	15
Dorsal kineties, number	1.2	0.4	34.5	1	2	15
Pairs of dorsal bristles, number	19.4	2.6	13.6	12	22	15



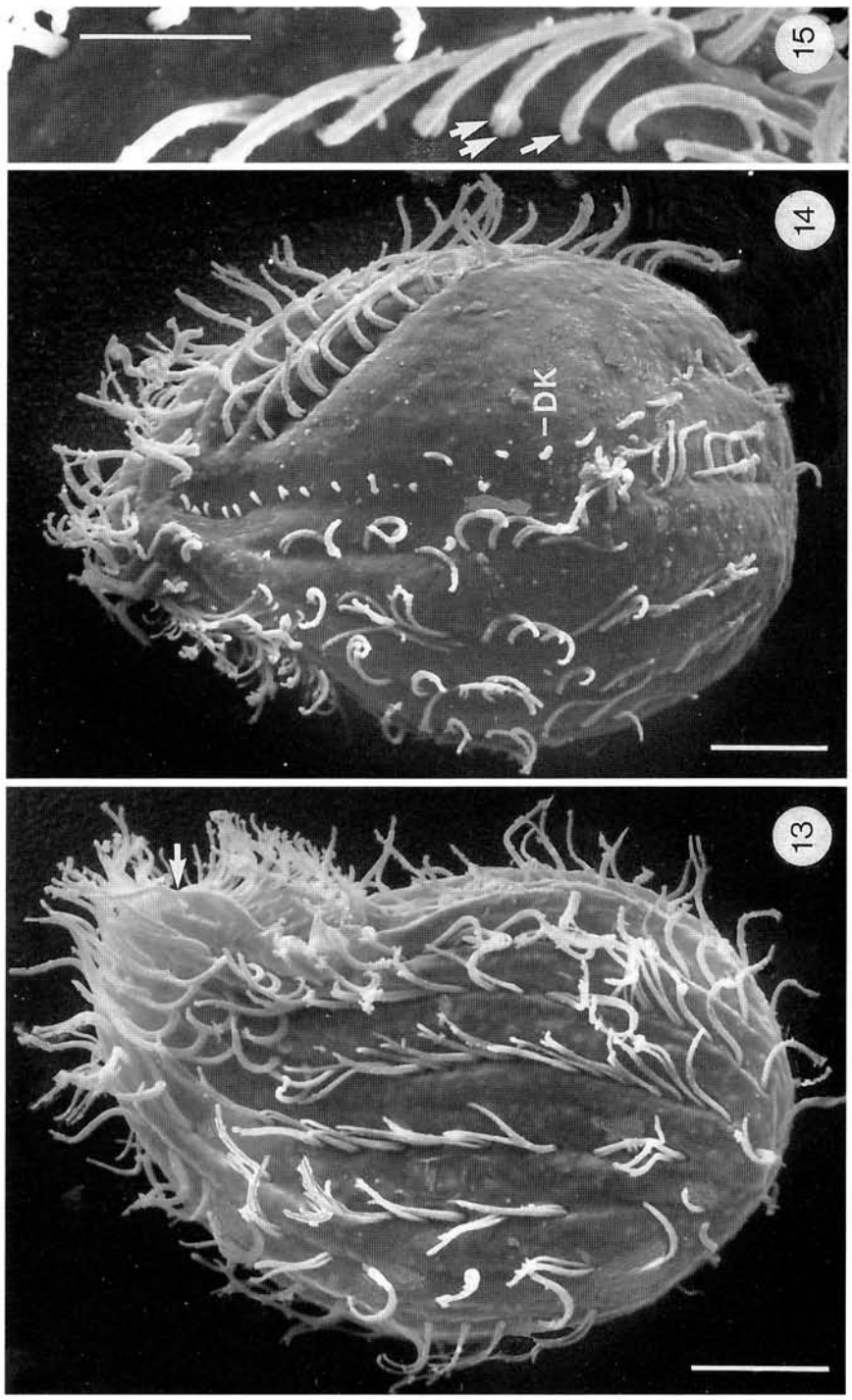
Figs. 7–12. *Parastrongylidium oswaldi* in vivo. 7: Ventral view. 8: Arrangement of cortical granules. CV = contractile vacuole. 9: Fusiform shape variant. 10: Lateral view. 11, 12: Young and fully differentiated resting cyst. Scale bar divisions = 10 µm.

margin convex, usually both ends rounded. About 10% of the specimens show pointed ends and are thus fusiform (Fig. 9). Flattened approximately 2:1. Flexible, acontractile. Macronucleus in median of cell or slightly left of it, composed of 3–4 roughly ellipsoid, lobed segments connected by bridges resulting in moniliform pattern; chromatine bodies small and moderately numerous. Micronuclei spherical, in vivo hardly recognizable. Contractile vacuole in mid-body on left margin, during diastole with distinct collecting lacunae (Figs. 7, 8). Pellicle soft, flexible, slightly crenelated along cirral rows, underlain by cortical granules and numerous mitochondria. Cortical granules largely in strings between cirral rows (heterotrich-like; Figs. 7, 8), stain slightly red with methyl green-pyronin. Their size varies with culture conditions, i.e. in aged individuals they are fairly small (about 0.5 µm), while they are very conspicuous (up to 1.5 µm) in specimens from well-flourishing cultures. Cytoplasm colourless, with moderately numerous cytoplasmic crystals, 2–5 × 1–2 µm in size, and 8 µm sized food vacuoles, containing heterotrophic flagellates and bacteria. Movement moderately fast, usually creeping, sometimes rotating about main body axis.

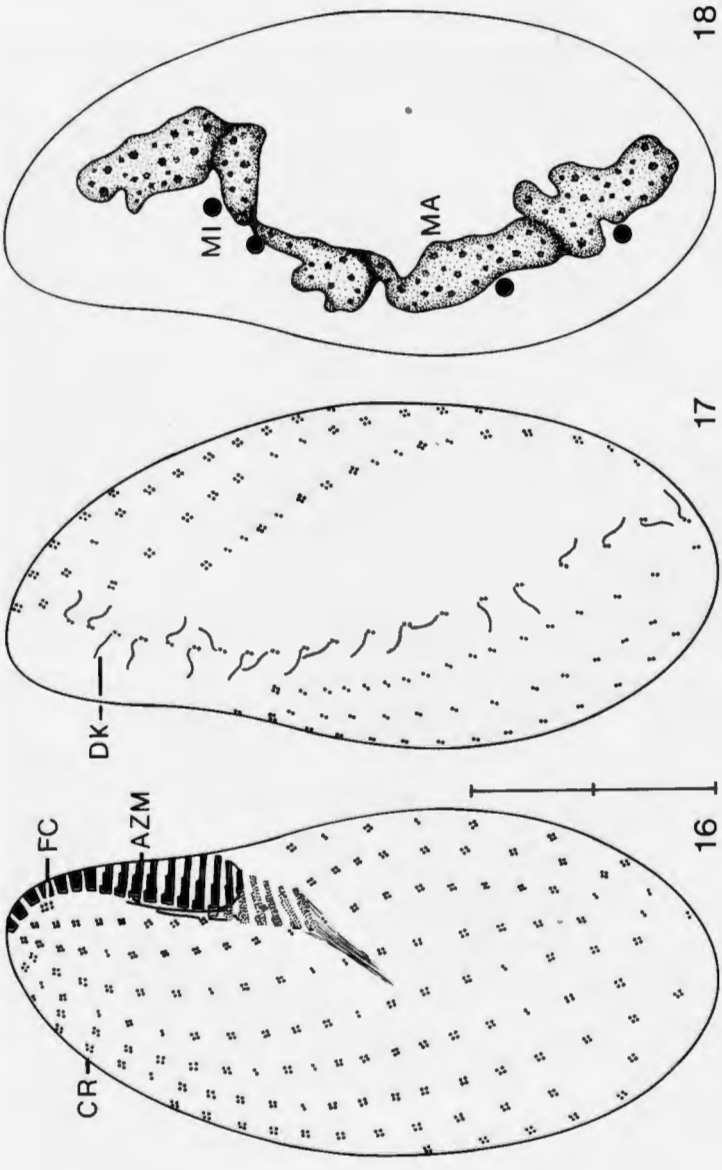
Adoral zone of membranelles about 1/3 of body length, buccal area flat and very narrow. Paroral and endoral membrane almost straight, about the same length, superimposed or side by side. Cirri in vivo 13–15 µm, beat cilia-like. Single frontal cirrus consists of 4–6 cilia, while all other cirri comprise 1 or 2 pairs of basal bodies (Figs. 13–17). Cirral rows somewhat spiralized, extend near posterior end. Lateral cirral rows anteriorly slightly to 1/3 shortened, cirral rows on left dorsal side merge into long dorsal kinety (Figs. 13–17). Dorsal cilia motile, in vivo 3–4 µm long. A second short dorsal kinety (probably a residue from last morphogenesis) occasionally observed in posterior half. Buccal, posterior frontal cirri, transverse and caudal cirri absent (proved by study of morphogenesis).

Morphology of cysts. Outline of 4-week-old cyst markedly ellipsoid, 25–37 × 21–30 µm ($\bar{x}$ = 30.9, SD = 4.0, CV = 12.9, n = 10; $\bar{x}$ = 25.5, SD = 2.7, CV = 10.6, n = 10), whereas the densely granulated content is spherical, 20–26 µm in diameter ($\bar{x}$ = 22.5, SD = 2.0, CV = 8.9, n = 10; Fig. 11). Fully differentiated resting cysts spherical, 22–26 µm in diameter ($\bar{x}$ = 24.1, SD = 1.37, CV = 5.7), covered by thin, mucous layer, about 2 µm thick, with released cortical granules and adherent bacterial endospores and detritus (Fig. 12). Cyst wall about 1 µm thick. Cytoplasm contains some yellow, opaque inclusions and a single, spherical macronucleus, about 8 µm in diameter, coated by a layer of highly refractive granules, 0.5 µm in size. 77% of cysts have a non-contractile vacuole (n = 122; Fig. 12). The peripheral cytoplasmic zone of the fully differentiated resting cyst lacks cortical granules, indicating that all have been discharged.

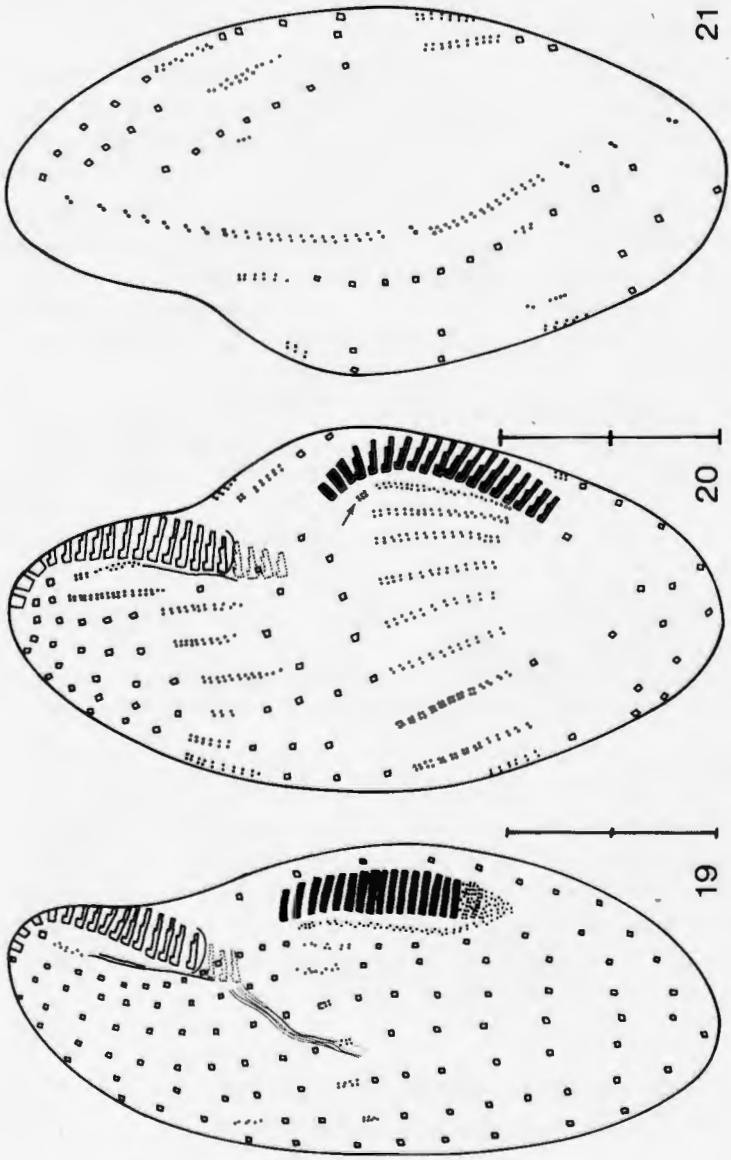
Morphogenetic processes. The processes during cell division largely correspond to those described by FLEURY & FRYD-VERSAVEL (1984) in *Parastrongylidium martini*. We can confirm the absence of reorganization bands in the macronucleus. A major difference, however, concerns the number of frontal cirri originating from



Figs. 13–15. *Parastrongylidium oswaldi*, scanning electron micrographs. 13, 14: Ventral and dorsal view. Note the single frontal cirrus (arrow). DK = dorsal kinety. Bars = 10 µm. 15: Cirri consist of 2 (arrow) or 4 (two arrows) cilia. Bar = 5 µm.



Figs. 16–18. *Parastrongylidium oswaldi* after protargol impregnation. 16, 17: Infraciliature of ventral and dorsal side. AZM = adoral zone of membranelles; CR = cirral row; DK = dorsal kinety; FC = frontal cirrus. 18: Nuclear apparatus. MA = macronucleus; MI = micronucleus. Scale bar divisions = 10 µm.



Figs. 19–21. *Parastrongylidium oswaldi*, protargol impregnation of dividing cells. 19: Formation of anlagen in the anterior and posterior daughter cell. 20, 21: Fully developed anlagen of ventral and dorsal side. The single frontal cirrus (arrow) detaches from the primordium of the undulating membranes. Scale bar divisions = 10 µm.

the oral primordium. In *Parastrongylidium oswaldi* only a single frontal cirrus arises from the primordium of the undulating membranes (Figs. 19, 20), whereas in *P. martini* 3 or 4 enlarged frontal cirri originate from several anlagen (FLEURY & FRYD-VERSAVEL 1984).

Hologamic conjugation, i.e. total resorption of one member of the pair, occurred excessively in an ageing culture.

Comparison with related species. *Parastrongylidium oswaldi* closely resembles *P. martini* FLEURY & FRYD-VERSAVEL, 1984 in size, the nuclear apparatus, the single dorsal kinety and the highly polluted habitat (activated sludge/dung-heap). However, *P. martini* is markedly pointed at both ends and has no cortical granules FLEURY & FRYD-VERSAVEL 1984; AUGUSTIN pers. comm.). Two further characters concern the infraciliature: *P. martini* has 3–4 enlarged frontal cirri, while *P. oswaldi* has only 1. 87% of *P. martini* specimens have eleven and 13% twelve cirral rows of which only the leftmost row is shortened (n = 23; FRYD-VERSAVEL pers. comm.); *P. oswaldi* shows 3 or 4 such shortened rows resulting in an average of 13 cirral rows.

Ecology (Table 6). The population found in ARA1 suggests that *P. oswaldi* prefers a polysaprobic environment; feeds on bacteria and heterotrophic flagellates.

Pseudochilodonopsis fluviatilis FOISSNER, 1988

Fig. 6; Table 9

This crawling species belongs to the order Cyrtophorida, family Chilodonellidae. Our specimens measure in vivo 41–51 × 17–30 µm ($\bar{x}$ = 45.2, SD = 3.7, CV = 8.2, n = 10; $\bar{x}$ = 22.2, SD = 3.1, CV = 14.1, n = 10) and largely correspond to those found by FOISSNER (1988 b) in a river; the dorsal brush is, however, on average composed of fewer basal bodies (Table 9).

Ecology (Table 6). *Pseudochilodonopsis fluviatilis* is common in alpha- to betamesosaprobic rivers (FOISSNER et al. 1991). Our record is the first from activated sludge, although it might previously have been confused with *Chilodonella uncinata*, which is rather similar in vivo (cp. FOISSNER et al. 1991); *Pseudochilodonopsis*, however, has a fragmented preoral kinety (Fig. 6). *Pseudochilodonopsis fluviatilis* feeds on diatoms (FOISSNER 1988 b) and bacteria (present study). FOISSNER et al. (1991) proposed the following saprobic classification for *P. fluviatilis:* b–a; b = 5, a = 3, p = 2, I = 2, SI = 2.7.

Opercularia asymmetrica (BICZOK, 1956) nov. comb.
(Basionym: *Pyxidium asymmetricum*)

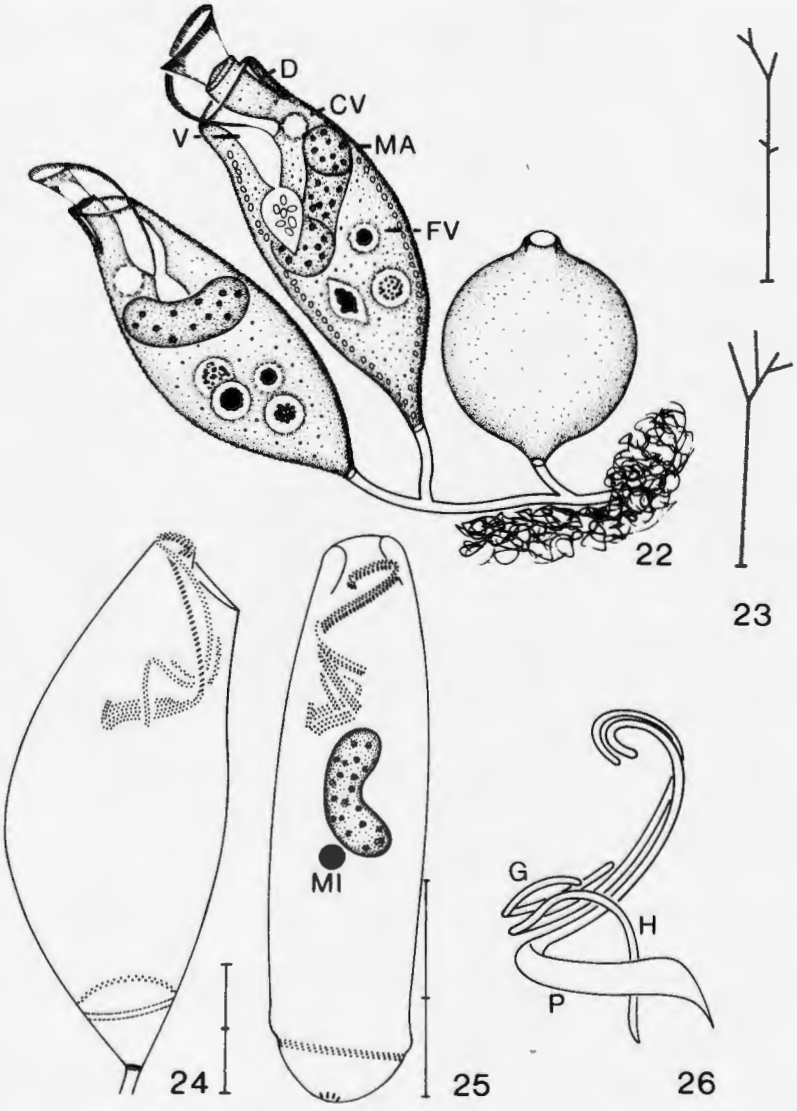
Figs. 22–35; Table 10

This attached species belongs to the order Peritrichida, family Epistylididae.

Improved diagnosis. Total size range in vivo 28–92 × 13–40 µm (BICZOK's and our data). Asymmetrically fusiform, anterior end obliquely truncated. Macronucleus horseshoe-

Table 9. Morphometric characterization of *Pseudochilodonopsis fluviatilis*. Upper line = activated sludge population from ARA1; lower line = population from a river (FOISSNER 1988 b). Explanation of abbreviations see chapter 3.4.

Character	$\bar{x}$	SD	CV	Min	Max	n
Body, length	44.6	5.5	12.2	40	55	10
	53.2	3.6	6.9	46	58	10
Body, width	27.1	3.6	13.2	22	35	10
	26.7	2.2	8.1	22	29	10
Distance anterior end to innermost circumoral kinety	7.8	0.9	11.8	6	9	10
	8.7	2.0	23.0	7	13	10
Distance anterior end to dorsal brush	3.4	1.0	28.4	2	5	10
	4.3	0.5	11.2	4	5	10
Distance anterior end to macronucleus	23.9	5.9	24.7	18	38	10
	25.9	4.3	16.7	18	31	10
Distance anterior end to anterior excretory pore	19.4	3.4	17.5	16	23	9
	22.3	3.5	15.7	18	28	10
Distance anterior end to posterior excretory pore	26.7	6.5	24.5	19	37	6
	37.7	2.9	7.8	34	42	10
Basket, length	8.3	1.1	13.4	7	10	7
	18.8	1.6	8.6	16	21	10
Basket, maximum width	4.4	0.7	15.4	3	5	10
	5.6	0.0	0.0	5.6	5.6	10
Postoral unciliated field, maximum width	13.9	2.5	17.8	10	17	10
	13.6	0.8	6.2	12	15	10
Dorsal brush, length	1.9	0.2	11.1	1.5	2.0	10
	4.4	0.7	16.6	3	6	10
Internal kinety of left ciliary field, length	12.1	6.7	55.3	4	23	9
	22.9	8.2	35.7	8	35	18
External kinety of left ciliary field, length	6.6	2.7	40.5	4	11	10
	15.3	3.5	22.7	7	21	18
Macronucleus, length	13.7	2.6	18.9	10	17	10
	14.2	1.3	8.0	13	17	10
Macronucleus, width	9.5	1.0	10.2	8	11	10
	10.2	1.3	12.9	8	13	10
Somatic kineties of right ciliary field, number	5.1	0.3	6.2	5	6	10
	5.0	0.0	0.0	5	5	10
Somatic kineties of left ciliary field, number	5.9	0.3	5.4	5	6	10
	6.0	0.0	0.0	6	6	10
Circumoral kineties, number	2.0	0.0	0.0	2	2	10
	2.0	0.0	0.0	2	2	10
Praeoral kineties, number	4.0	0.0	0.0	4	4	10
	4.0	0.0	0.0	4	4	10
Basket nematodesmata, number	17.6	1.3	7.6	15	20	9
	16.0	1.4	8.8	15	18	10



Figs. 22–26. *Opercularia asymmetrica* in vivo (22, 23) and after protargol impregnation (24–26). 22: Extended and contracted zooids. CV = contractile vacuole; D = discus; FV = food vacuole; MA = macronucleus; V = vestibulum. 23: Branching patterns of colonies. 24, 25: Infraciliature of the zooid and the swarmer. MI = micronucleus. 26: Scheme of the oral ciliature. G = germinal kinety; H = haplokinety; P = polykinety. Scale bar divisions = 10 μm.

shaped, in transverse or longitudinal axis of cell. One large, spherical micronucleus. Contractile vacuole at dorsal vestibular wall. Haplo- and polykinety describe 3/4 to 1 turn around peristomial disc. In field often solitary.

Neotype material. 2 slides of neotype specimens are deposited in the "Sammlung der mikroskopischen Präparate" in the Oberösterreichisches Landesmuseum in Linz (Austria).

Redescription. Size of zooids in vivo 28–75 × 13–40 µm ($\bar{x}$ = 44, SD = 6.9, CV = 15.5, n = 10; $\bar{x}$ = 27, SD = 5.1, CV = 19.1, n = 10). About twice as long as broad, maximal width in mid-body. Peristomial width about one half of body width. In field samples aged from a few hours to 1 day specimens are ellipsoid and stalkless and solitary or aggregated in pseudocolonies, viz. attached to substrate with the scopula (Fig. 27). Most have the oral apparatus retracted (Figs. 28–30). These forms probably correspond to the "temporary-resting-stage" described by GUHL (1979). Individuals from well-flourishing cultures are more or less fusiform, ventral shorter and less convex than dorsal producing obliquely truncated anterior end. Peristomial margin thin, not bulged. Peristomial disc narrow, slightly bulged by ciliary bands, projects slightly, rarely distinctly, beyond peristomial margin. Contracted zooids about half as long as extended ones, anterior and posterior end snout-like, latter not overlapping stalk (Fig. 22). Contractility remarkably weak, in spite of distinct myoneme system (Fig. 34), which corresponds to that described by FOISSNER (1981) in *O. arboricolum*. Macronucleus horseshoe-shaped, rarely reniform or ellipsoid, in vivo 5–9 µm broad, often both ends thickened; numerous small nucleoli (Figs. 22, 29). Micronucleus in vivo 3–4 µm in diameter. Contractile

Table 10. Morphometric characterization of *Opercularia asymmetrica*. Explanation of abbreviations see chapter 3.4.

Character	$\bar{x}$	SD	CV	Min	Max	n
Body, length (BL)	32.1	3.6	11.2	28	38	15
Body, maximum width	16.1	2.5	15.7	13	21	15
Vestibulum, length (VL)	13.5	1.6	12.1	10	15	15
BL/VL	2.4	0.3	12.2	2	3	15
Peristome, width (PW)	7.7	1.1	14.2	5	9	15
BW/PW	2.1	0.4	17.6	1	3	15
Truncation[a]	1.9	1.2	63.6	0	4	15
Discus, length[b]	3.9	1.6	41.4	0	6	12
Discus, width (DW)	4.0	0.9	22.7	2	6	15
PW/DW	2.0	0.4	22.0	1	3	15
Macronucleus, width	4.8	0.8	17.1	4	6	14
Distance aboral ciliary band to stalk	5.6	0.7	13.1	4	7	15

[a] Difference in length of dorsal and ventral side, i. e. measures oblique truncation of anterior end.

[b] Measured from distal end to attachment site in cell.

vacuole in anterior third of cell, no collecting vesicles (Figs. 22, 27). Pellicle very narrowly striated, smooth even at high magnification (1000×), underlain by numerous mitochondria. Cytoplasm colourless, few to many spherical food vacuoles. Feeds exclusively on bacteria.

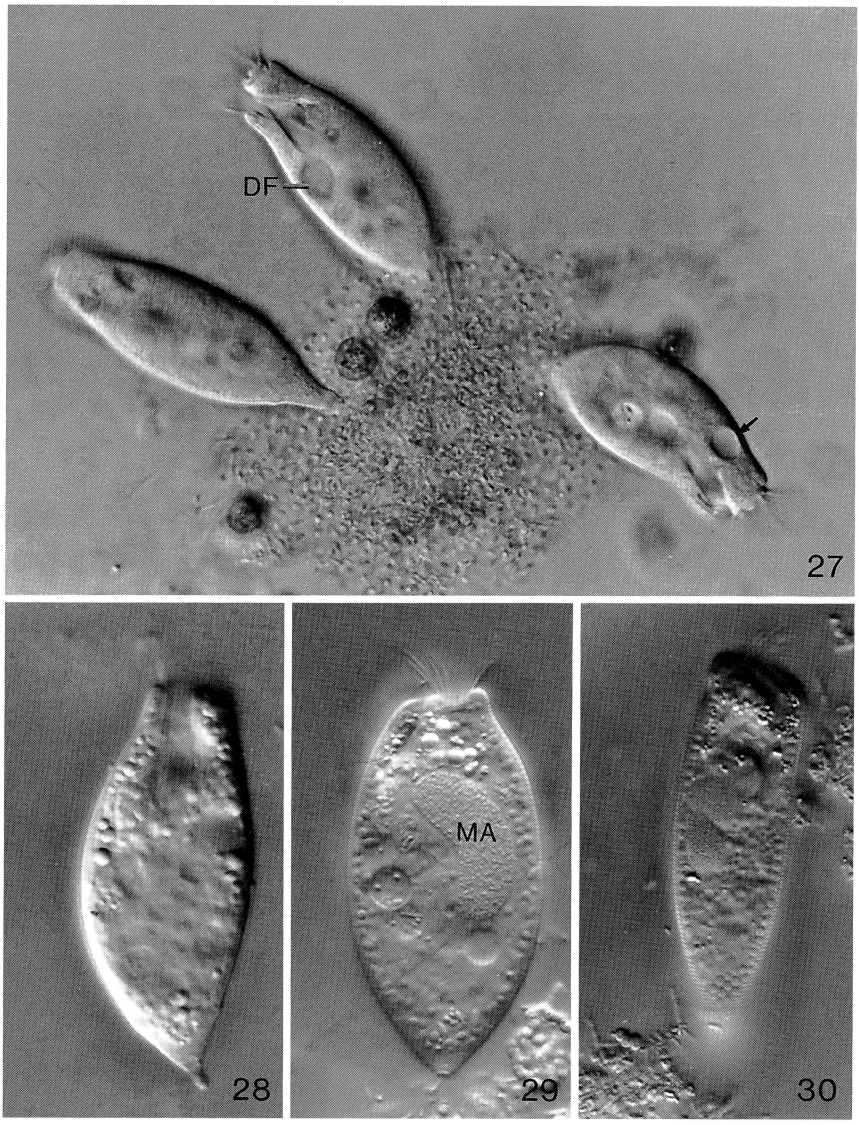
Zooids frequently stalkless, if present stalk is thin, i.e. 1.5–2 µm, short and usually unbranched. Colonies were formed in about 1% of several hundred observations during 1 year of culture, mostly consisting of 2 specimens. A maximum number of 14 individuals has been observed on irregularly branching stalks. Main stalk 25–55 µm in length, branches 2–14 µm, basal plate 4–8 µm in diameter (Fig. 23).

Cilia in vivo 9–12 µm. Vestibulum relatively large, extends almost vertically about 1/3 of body length into cell. Haplo- and polykinety turn 3/4 to 1 time around peristomial disc before plunging into vestibulum where polykinety forms 3 closely adjacent peniculi (Figs. 24, 26, 32). 1st and 2nd peniculus with 3 rows of cilia each, 3rd peniculus with 6–8 pairs or triads of basal bodies. Germinal (stomatogenic) kinety short, in middle region of haplokinety, consists of irregularly arranged basal bodies. Primordium of aboral ciliary band in posterior fourth of body, comprises paired basal bodies. Scopula consists of about 12 argyrophilic granules (probably basal bodies; Fig. 33).

Silverline system narrowly striated, i.e. distance between individual silberlines 0.5–0.6 µm (Fig. 35). 52–97 ($\bar{x} = 75.1$, SD = 11.7, CV = 15.5, n = 13) silverlines from oral apparatus to scopula; 62–103 ($\bar{x} = 81.5$, SD = 11.5, CV = 14.1, n = 13) pellicular pores per 100 µm^2.

Swarmers (telotrochs) in vivo long-ellipsoid, 20–47 × 12–27 µm ($\bar{x} = 32.0$, SD = 7.4, CV = 23.1, n = 10; $\bar{x} = 14.8$, SD = 1.7, CV = 35.4, n = 10). Swim very rapidly by rotation about longitudinal body axis. Microconjugants in vivo almost spherical, 10–15 × 6–11 µm ($\bar{x} = 12.8$, SD = 1.4, CV = 10.9, n = 10; $\bar{x} = 9.5$, SD = 1.6, CV = 16.8, n = 10), affix to zooid in mid-body. Apart from aboral ciliary band, which consists of triads of basal bodies, infraciliary structures of swarmers and microconjugants correspond to those of zooids (Fig. 25). Epistomial membrane absent. Encystment could not be induced.

Identification and taxonomic position. Our population matches *Pyxidium asymmetricum* BICZOK, 1956 well by the asymmetrical zooids, the position of the contractile vacuole at the dorsal vestibular wall and the ellipsoid shape of the swarmer. BICZOK's population is slightly larger (zooids 56–92 µm, swarmers 40–86 µm, n = ?) and shows a longer peristomial disc. However, these characters vary with culture conditions. Unfortunately, BICZOK did not substantiate the unusual reproductive mode of longitudinally dividing swarmers; perhaps he observed transforming zooids (see his Fig. f). Since he only found solitary individuals with very short stalks, he assigned the species to the genus *Pyxidium*. This is also the usual appearance of our population. In cultures, however, colonies are formed; *P. asymmetricum* thus has to be transferred to the genus *Opercularia*.



Figs. 27–30. *Opercularia asymmetrica*, light micrographs of living cells. 27: Solitary individuals attached to a bacterial floc by a very short stalk or the scopula. Arrow marks contractile vacuole. DF = descending food vacuole. 28–30: Shape varies with culture conditions; forms with retracted oral apparatus (29) usually occurred in fresh sludge. MA = macronucleus.

GUHL (1979) synonymized *Pyxidium asymmetricum* with *Opercularia coarctata*. *Opercularia asymmetrica* indeed resembles GUHL's ecotype I of *O. coarctata* which may be asymmetrically ovoid and has a peristomial width of 9–13 µm and a disc width of 3–6 µm. We consider *O. asymmetrica* as valid species because its contractile vacuole is at the dorsal vestibular wall, whereas the original figure of *O. coarctata* definitely shows its ventral location (CLAPARÈDE & LACHMANN 1858). In addition, the haplo- and polykinety surround the peristomial disc only once in *O. asymmetrica*, whereas there are 11/4 to 11/2 turns in *O. coarctata* (RUIZ & ANADON 1986; FERNÁNDEZ-GALIANO et al. 1988). The latter character distinguishes *O. asymmetrica* also from *O. arboricolum*, which is rather similar concerning the myoneme and silverline system (FOISSNER 1981).

Ecology (Table 6: see also chapter 4.4.2). BICZOK (1956) found *O. asymmetrica* in a grass-root infusion. In hay infusions, it grew well with *Colpoda fastigata*, but perished with *Paramecium caudatum* and the flagellate *Chilomonas paramecium*. *Opercularia asymmetrica* ingests 2000–3000 bacteria per hour (BICZOK 1956). Other records are not known. AUGUSTIN (pers. comm.) has, however, recently found it in a conventional sewage plant indicating that it might have sometimes been confused with *O. coarctata*.

Prodiscophrya collini (ROOT, 1914) KORMOS, 1935

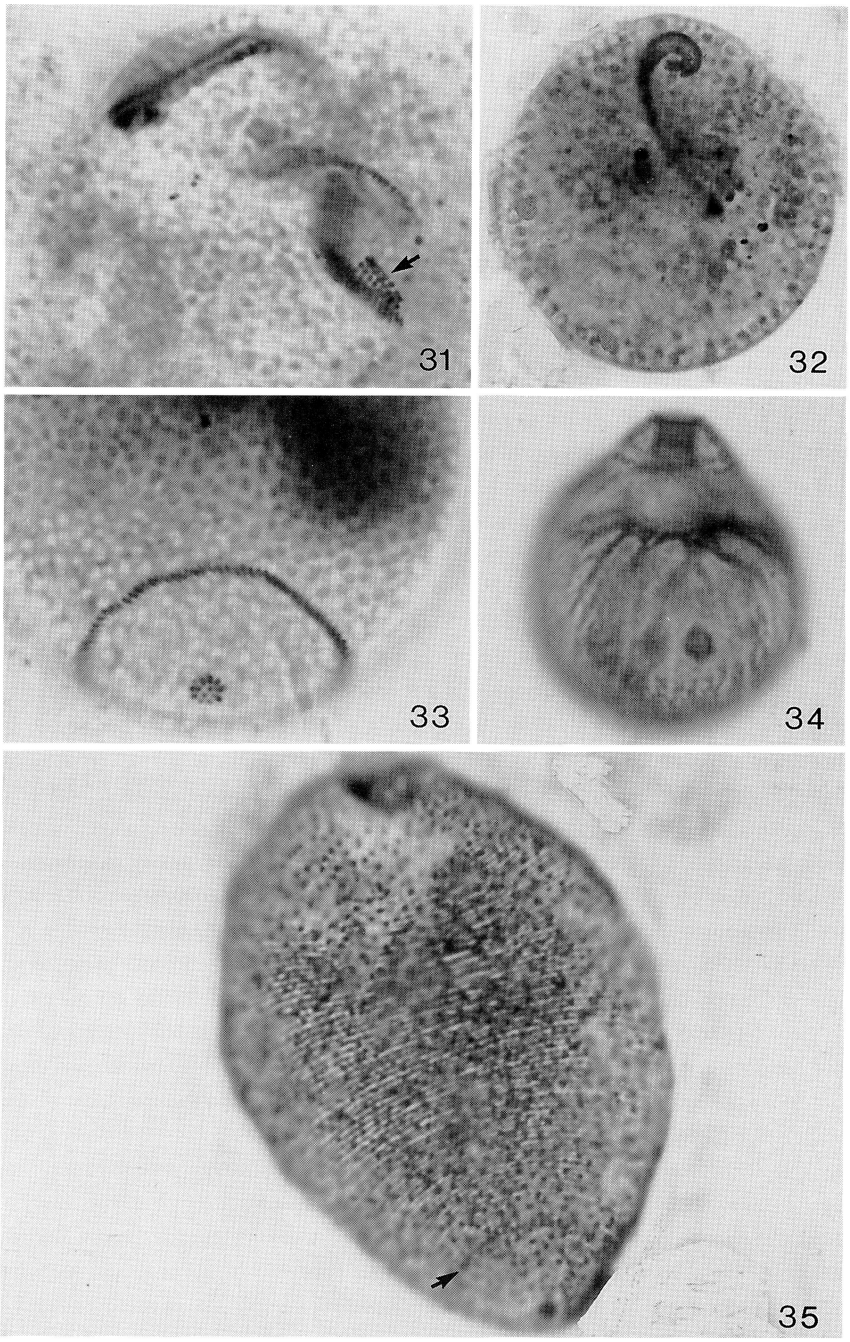
Figs. 36–50; Table 11

This attached species belongs to the order Suctorida, family Discophryidae. It has a complicated synonymy.

1914 *Podophrya collini* ROOT, Arch. Protistenk. 35: 164.
1935 *Prodiscophrya Collini* (ROOT) – KORMOS, Allatt. Közl. 32: 152 (first new combination).
1957 *Discophrya Collini* (ROOT) – CANELLA, Monitore zool. ital. 65: 180 (second new combination).
1959 *Podophrya collini* ROOT – LAIRD, Ecology 40: 213 (substantiated record on mosquito larvae).
1977 *Discophrya collini* (ROOT) – HENK & PAULIN, J. Protozool. 24: 134 (substantiated record in activated sludge).
1978 *Prodiscophrya collini* (ROOT, 1914) – JANKOWSKI, Dokl. Akad. Nauk SSSR 242: 528 (improved genus diagnosis).
1981 *Suctorella collini* (ROOT, 1914) – JANKOWSKI, Proc. Acad. Sci. USSR 107: 111 (third new combination).
1988 *Suctorella collini* (ROOT, 1914) – FOISSNER, Hydrobiologia 166: 30 (saprobiological revision).
1988 *Discophrya collini* (ROOT 1914) HULL 1954 – MATTHES, Protozoenfauna 7/1: (last revision).

Neotype material. 2 slides of neotype specimens are deposited in the "Sammlung der mikroskopischen Präparate" in the Oberösterreichisches Landesmuseum in Linz (Austria).

Redescription. Adult, sessile field stages in vivo 28–93 × 20–68 µm ($\bar{x}$ = 65.3, SD = 8.5, CV = 13.0, n = 12; $\bar{x}$ = 47.8, SD = 4.7, CV = 9.7, n = 12). Usually obovoid,



Figs. 31–35. *Opercularia asymmetrica* after silver carbonate (31–33), protargol (34) and dry silver nitrate impregnation (35). 31: Oral ciliature. Note the short 3rd peniculus (arrow). 32: Haplo- and polykinety usually describe less than one turn around peristomial disc. 33: Primordium of the aboral ciliary band and scopula of a zooid. 34: Myoneme system. 35: The silverline system is narrowly striated; pellicular pores are visible as dots. Arrow marks anlage of aboral ciliary band.

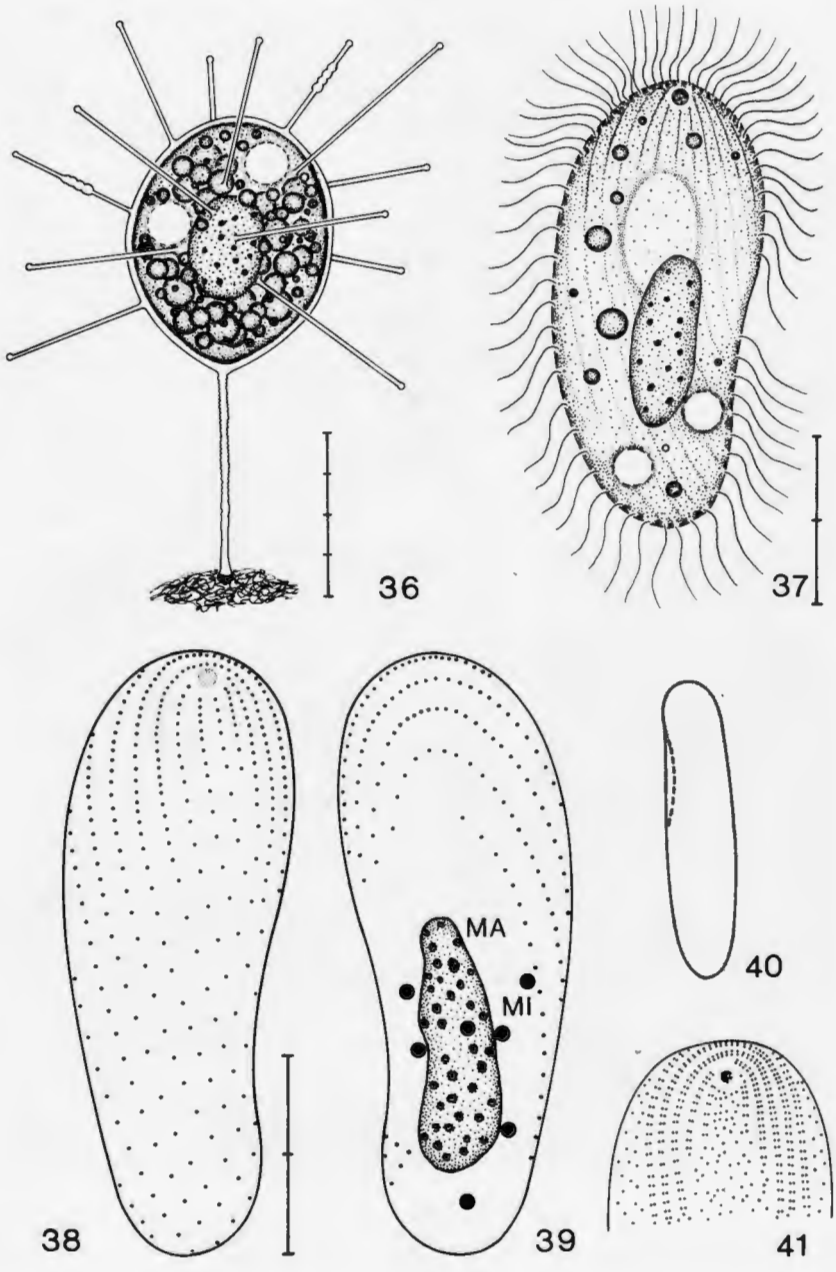
well-fed specimens almost spherical (Fig. 36). About 20 tentacles, in vivo 5–70 µm long and about 1 µm in diameter, scattered over entire cell surface, except near stalk (Figs. 42, 46). Specimens with 2 tentacles occurred in aged cultures. Tentacles distally knobbed (Fig. 47, inset), about 1.4 µm in diameter, impregnate heavily with protargol. Stalk always present, 18–210 µm long, usually as long as body, about 2 µm width, surface often wrinkled (Fig. 48), at least in anterior portion, terminates in small, argyrophilic attachment (basal) disc. Macronucleus in centre of cell, ellipsoid, in vivo 15–35 × 10–25 µm. Micronuclei recognizable neither in vivo nor after various staining procedures. Usually 2 (perhaps sometimes only 1) contractile vacuoles in somewhat variable position: one at anterior end, more or less out of median, the other in mid-body on margin. Epiplasm rather distinctly gelatinous, appears as 1–2 µm thick, bright layer (Fig. 42). Cytoplasm colourless, more or less packed with spherical (food) vacuoles 3–6 µm in diameter and small greasily shining droplets. Silverline system narrowly meshed, numerous pellicular pores also impregnate.

Swarmer from young cultures in vivo 25–105 × 10–50 µm ($\bar{x} = 78.3$, SD = 25.4, CV = 32.5, n = 6; $\bar{x} = 35.0$, SD = 13.0, CV = 37.1, n = 6). Reniform, anteriorly usually more broadly rounded than posteriorly, occasionally vice versa (Figs. 37–39, 45). Distinctly (>2:1) flattened, less ciliated side depressed in centre of anterior third (Fig. 40); usually both sides depressed in a population from a small pond in the town of Salzburg. Tentacles lacking. Macronucleus ellipsoid, in vivo 15–30 × 8–10 µm, in posterior half. 3–11 micronuclei, 1–2 µm in diameter, scattered around macronucleus (Fig. 39). 2 contractile vacuoles in posterior quarter of cell, obliquely arranged, viz. that on left concave side is more distant from posterior end than that on right convex side (Figs. 37, 45; Table 11). Movement moderately rapid, rotating about longitudinal axis, with scopula region ahead.

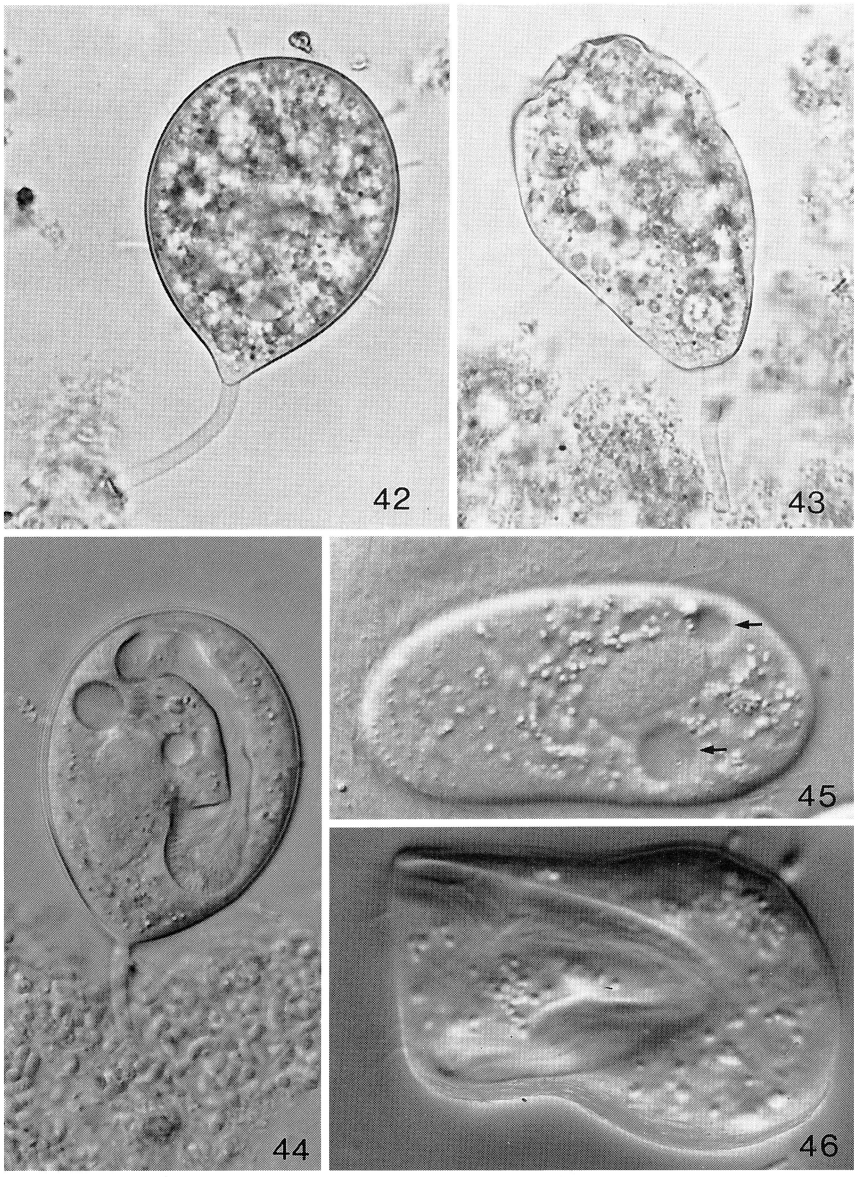
Cilia 10–12 µm long, form 7–9 somatic kineties which commence at posterior end and extend obliquely to scopula, where they curve back and terminate in anterior left lateral and dorsal third; 4–6 kineties commence at left quarter of cell and terminate at scopula. All kineties composed of monokinetids which are more closely spaced anteriorly than posteriorly. Silverline system as in adults, also basal bodies of cilia impregnate (Fig. 50).

Swarmer formed by evaginative budding (Fig. 44), leaves parent in anterior third; adults rarely transformed into swarmers (reactive budding; Fig. 46). Encystment experiments usually resulted in sessile forms without tentacles and a slightly thickened epiplasm; no special cyst wall was formed. In some cases, peculiar degenerating stages of tentacles were noticed, viz. knob-like structures with a spiralized central microtubular core (Fig. 49). This stage persisted for some days.

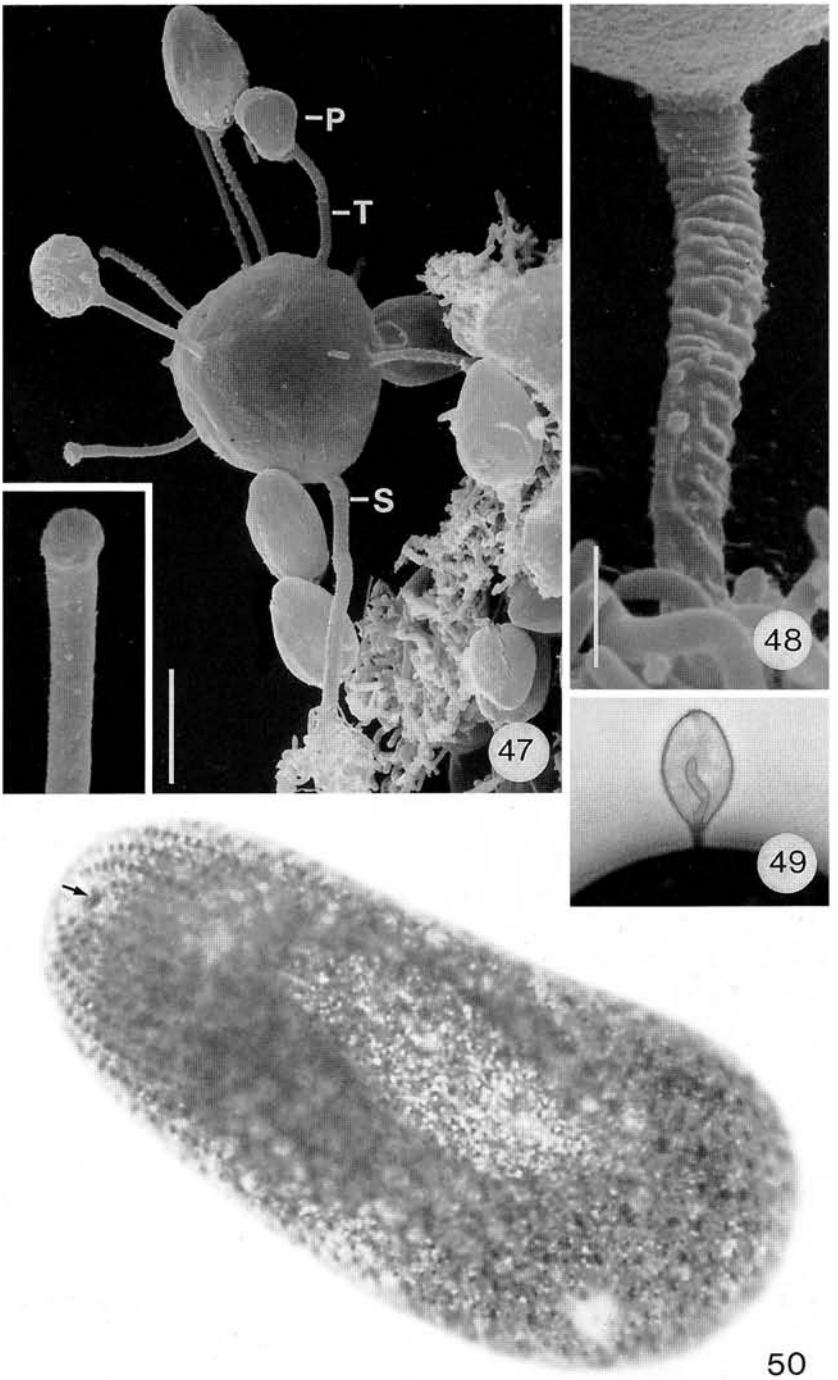
Taxonomic position and comparison with other species. This species does not belong to *Podophrya* because its swarmer is not formed by exogenous budding (HENK & PAULIN 1977; MATTHES 1988). The combination with *Discophrya* is frequently dedicated to HULL (1954), although this author merely suggested that



Figs. 36–41. *Prodiscophrya collini* in vivo (36, 37, 40) and after protargol (38, 39) and dry silver nitrate impregnation (41). 36: Adult, sessile form. 37: Swarmer. 38, 39: Infraciliature of the swarmer. MA = macronucleus; MI = micronucleus. 40: Lateral view of swarmer. Note anterior depression. 41: Anterior end of swarmer showing scopula, basal bodies and pellicular pores (cp. Fig. 50). Scale bar divisions = 10 μm.



Figs. 42–46. *Prodiscophrya collini*, light micrographs of living cells. 42, 43: Sessile adults. 44: Swarmer is produced by evaginative budding. 45: Swarmer showing its less ciliated side and its 2 obliquely arranged contractile vacuoles (arrows). 46: Adult transforming into swarmer (reactive budding).



Figs. 47–50. *Prodiscophrya collini* in the scanning electron microscope (47–48) and after silver carbonate (49) and dry silver nitrate impregnation (50). 47: Sessile form feeding on flagellates. Inset shows distal tentacle knob. P = prey; S = stalk, T = tentacle. Bar = 10 μm and 2 μm, respectively. 48: The stalk is frequently wrinkled. Bar = 2 μm. 49: Degenerating tentacle with spiralized central microtubular core. 50: Silverline system of swarmer on the denser ciliated side. The scopula (arrow) marks the physiological anterior end.

Table 11. Morphometric characterization of *Prodiscophrya collini*. Explanation of abbreviations see chapter 3.4.

Character	$\bar{x}$	SD	CV	Min	Max	n
Sessile forms						
Body, length	41.3	11.8	28.5	25	63	25
Body, maximum width	34.2	12.2	35.6	19	53	25
Macronucleus, length	18.7	5.1	27.4	11	30	25
Macronucleus, width	12.5	2.6	20.5	8	19	25
Stalk, length	34.3	15.6	45.6	10	80	25
Swarmers						
Body, length	57.5	5.9	10.2	47	71	15
Body, width (anterior quarter)	22.5	2.5	11.3	18	28	15
Body, width (mid-body)	19.3	2.1	11.0	15	23	15
Body, width (posterior quarter)	16.9	2.2	13.1	13	21	15
Distance right excretory pore to posterior end	7.3	2.2	30.4	10	18	7
Distance left excretory pore to posterior end	14.1	3.3	23.6	10	18	7
Macronucleus, length	22.5	4.7	21.1	15	35	15
Macronucleus, width	7.1	0.7	10.0	6	8	15
Micronucleus, diameter	1.5	0.4	28.3	1	2	14
Micronucleus, number	6.8	2.3	33.8	3	11	14
Somatic kineties, number	11.8	0.7	5.7	11	13	15

Discophrya piriformis is synonymous with *P. collini*. The swarmer of *D. piriformis* has an anterior and a posterior ciliary field (GUILCHER 1947), while in *P. collini* the ventral surface is completely ciliated and the dorsal side shows a single anterior field (KORMOS & KORMOS 1957; SUAREZ et al. 1987; Figs. 38, 39). We thus disregard the synonymy suggested by HULL (1954). Based on observations on *P. collini*, KORMOS (1935) established the genus *Prodiscophrya* due to its distinct sexual dimorphism, viz. a drop-shaped unciliated microgamete invades a macrogamete. Because this is rather unreliable, the genus was not widely accepted (see list of synonyms). *Prodiscophrya collini* is not "disc"-shaped and has no tentacle bundles like typical *Discophrya* species. Thus, we acknowledge the rediagnosis by JANKOWSKI (1978, 1981). FOISSNER (1988a) listed a *Suctorella collini* following a remark by JANKOWSKI (1981) that *Suctorella* is possibly the correct genus assignment. Contrary to *P. collini*, however, the type species, *Suctorella ciliata* FRENZEL, has conical tentacle tips. In the same paper, JANKOWSKI (1981) synonymized *P. collini* with *P. solaris* STEIN. This species was, however, already synonymized with *Podophrya fixa* by CLAPARÈDE & LACHMANN (1859) due to some figures by STEIN indicating exogenous budding.

The size and shape of the sessile and mobile stage of our population corresponds fairly well to that of *P. collini* described by ROOT (1914). However, he gives

a range of 30 to 60 tentacles, whereas the specimens of our population usually had about 20 tentacles; in starved specimens there were even only 2, the maximum number observed was about 40. The stalk length varied greatly in our cultures, i.e. in starved individuals it may be up to 4 times the body length, while ROOT emphasized that the stalk length, unlike the body, does not vary with nutritive conditions. The size and number of micronuclei of the swarmer are very similar in both populations (1–8 in ROOT 1914; Table 11). ROOT (1914) described resting cysts with a thickened gelatinous sheath perforated by knob-like, degenerated tentacles which persisted for at least 4 months. We observed similar stages, but no special cyst wall; we therefore assume that this species does not form resting cysts. This is supported by many other authors who never observed this peculiar "tentacle-stage" in *P. collini* (e.g., HENK 1979; HACKNEY & BUTTLER 1981; HACKNEY et al. 1982; AL-KHAZZAR et al. 1984).

In aged cultures, very small (about 20×10 µm), mobile specimens occur, which are reminiscent of the so-called microgametes mentioned by KORMOS & KORMOS (1956) in *P. endogama*. However, we never observed conjugation during the 11 months of culturing *P. collini*.

The ciliary pattern of the swarmer is similar to that described by KORMOS & KORMOS (1957) and SUAREZ et al. (1987). The number of kineties is, however, about 16 (sample size and range not given) in the populations investigated by these authors, while we found a range of 11 to 13 kineties in normally sized swarmers, in aged cultures their number decreased markedly.

The sessile stage of *P. collini* is almost indistinguishable from *Podophrya maupasii* and *P. fixa*, which have been frequently recorded from activated sludge. A reliable identification is only possible by the swarmers: with tentacles and formed by exogenous budding in *Podophrya* (MATTHES 1988); without tentacles and formed by evaginative budding in *Prodiscophrya*.

Ecology (Table 6). The autecology of *Prodiscophrya collini* will remain obscure until further reliably determined populations have been studied. We found it only on one sampling occasion and in low numbers, indicating that at least highly loaded sludges are not its preferred biotope. HENK & PAULIN (1977) recorded it from a conventional activated sludge plant. FOISSNER (1988a) gives the following saprobic classification: a–b; $a = 7$, $p = 2$, $I = 3$, $SI = 3.1$.

5. Summary

(1) The number, biomass and species composition of activated sludge organisms were studied in 11 samples, collected monthly, from a very highly loaded (BOD_5 21 kg/m³.d; COD 38 kg/m³.d) bioreactor purifying wastes of a pharmaceutic company. The waste water consists mainly of medium used to culture *Penicillium chrysogenum*. The investigation period covered 6 months without and 5 months with excess sludge return from a conventional sewage plant. The morphology and ecology of 7 ciliate species found in fresh sludge are described, including *Parastrongylidium oswaldi* n. sp. (Figs. 3–50; Table 6–11).

(2) The organic substance on average amounts to 16 g dry mass/l; it consists of 96% prokaryotic (~bacterial) and 4% eukaryotic (~protozoan) biomass (Table 1). Related to suspended solids the protozoan biomass constitutes about 2%. In the 1400 m^3 sludge standing crop about 4 tons protozoa (wet mass, corresponding to about 800 kg dry mass) are produced daily.

(3) On average 2 million flagellates, 1.3 million globular non-motile organisms, 340000 naked amoebae, 53000 ciliates and 29 rotifers occurred per ml activated sludge (Table 1). The fluctuations in abundances and biomasses were considerable and can be partially explained by the variation of abiotic factors, especially by the oxygen economy (Figs. 1, 2; Table 3). A high and stable oxygen supply slightly enhanced ciliates, while bacteria were decreased. Flagellates reacted more strongly to the variation than to the content of oxygen.

(4) The extreme sludge conditions [heavy load, short retention time (24 h), high temperature (30°C), lack of distinct flocs] cause a peculiar community structure, viz. a low mean species number (2 flagellate, 1 amoebaean and 3 ciliate species) and a dominance of free-swimming ciliates, including some rare, r-selected colpodids, which occur infrequently in conventional sewage plants (Table 5).

(5) No significant differences of physico-chemical parameters were found comparing mean values before and after excess sludge return. In contrast, protozoan numbers were doubled by the sludge return, whereas the proportion of prokaryotic biomass decreased from 97% to 94% (Table 2). However, sample size is too small for serious conclusions concerning advantages and disadvantages of excess sludge return.

(6) The biological investigations revealed that the activated sludge process is well regulated, though oxygen supply could be economized. High individual numbers and biomasses of sludge organisms indicate that the wastes are easily digestible and very likely do not contain toxic substances (e.g., heavy metals, antibiotics) beyond tolerable limits. The particular sludge community works well as shown by the mean purification efficiency of 90% COD-digestion.

6. Zusammenfassung

(1) In 11 monatlich gezogenen Proben aus einem extrem hoch belasteten (BSB_5 21 kg/m^3.d; CSB 38 kg/m^3.d) Bioreaktor eines pharmazeutischen Betriebes wurden die Anzahl, die Biomasse und der Artenbestand der Mikroorganismen untersucht. Das Abwasser besteht vorwiegend aus dem Kulturmedium von *Penicillium chrysogenum*. Der Untersuchungszeitraum umfaßte 6 Monate ohne und 5 Monate mit Schlammrückführung aus einer konventionellen Kläranlage. Die Morphologie und Ökologie von 7 Ciliatenspecies werden beschrieben, einschließlich der neuen Art *Parastrongylidium oswaldi* (Abb. 3–50; Tab. 6–11).

(2) Die organische Substanz beträgt durchschnittlich 16 g Trockenmasse/l; sie besteht zu 96% aus prokaryontischer (~Bakterien) und zu 4% aus eukaryontischer (~Protozoen) Biomasse (Tab. 1). Bezogen auf die gelösten Stoffe ist der Anteil der Protozoenbiomasse etwa 2%. In 1400 m^3 Abwasser entstehen täglich etwa 4 Tonnen Protozoen-Feuchtmasse, entsprechend etwa 800 kg Trockenmasse.

(3) In 1 ml Belebtschlamm leben im Jahresdurchschnitt etwa 2 Millionen Geißeltiere (Flagellaten), 1,3 Millionen globuläre Organismen, 340000 Nacktamöben, 53000 Wimpertiere (Ciliaten) und 29 Rädertiere (Rotatorien) (Tab. 1). Die Abundanz- und Biomasseschwankungen sind beträchtlich und können z.T. auf die Variation der abiotischen Faktoren, besonders des Sauerstoffgehaltes, zurückgeführt werden (Abb. 1, 2; Tab. 3). Hohe und stabile O_2-Werte fördern die Ciliaten, während sich die Bakterien vermindern. Flagellaten reagieren empfindlicher auf O_2-Schwankungen als auf die absoluten O_2-Mengen.

(4) Die außergewöhnlichen Umweltbedingungen [hoch konzentriertes Abwasser, kurze Aufenthaltszeit (24 h), hohe Temperatur (30°C), geringe Flockenbildung] verursachen eine besondere Gemeinschaftsstruktur, nämlich eine niedrige mittlere Artenzahl (2 Flagellaten-, 1 Amöben- und 3 Ciliatenspecies) und eine Dominanz freischwimmender Ciliaten, einschließlich einiger r-selektierter colpodider Ciliaten, die man in konventionellen Kläranlagen selten findet (Tab. 5).

(5) Der Vergleich der Durchschnittswerte der Monate mit und ohne Schlammrückführung ergab bezüglich der physikalisch-chemischen Parameter keine statistisch absicherbaren Unterschiede. Die Protozoenabundanzen waren in den Monaten mit Schlammrückführung doppelt so hoch wie in jenen ohne; der Anteil bakterieller Biomasse verringerte sich hingegen von 97% auf 94% (Tab. 2). Die geringe Stichprobenanzahl ermöglicht jedoch keine generelle Aussage über die Vor- und Nachteile dieser Maßnahme.

(6) Die biologischen Befunde deuten auf eine sehr gute Wartung des Bioreaktors; bei der Sauerstoffversorgung wären jedoch Einsparungen mög'ich. Die hohen Individuenzahlen und Biomassen belegen, daß das Abwasser für die Organismen gut verwertbar ist und mit hoher Wahrscheinlichkeit keine über der Norm liegenden Mengen an toxischen Substanzen (z. B. Schwermetalle, Antibiotika) enthält. Die Biozönose ist sehr gut an das spezielle Abwasser angepaßt, da der durchschnittliche CSB-Abbau 90% beträgt.

7. Acknowledgements

We thank Prof. H. Adam, the head of the Institute of Zoology, University of Salzburg, for institutional support. Many thanks to Dipl.-Ing. K. H. Greil, the operator of ARA1, for providing the physico-chemical data. The photographic assistance of Mrs. K. Bernatzky and Mr. A. Zankl is greatly acknowledged. Thanks to Mr. E. Strobl for improving the English. This study was supported financially by the Biochemie Ltd. Co. in Kundl (Tyrol, Austria).

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