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Reduction rates and growth of two species of Aeolosomatidae on activated sludge

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Abstract

The presence of worms in aerobic wastewater treatment may lead to a substantial sludge reduction, but the practical application of worms for sludge reduction is uncontrollable because of unstable worm growth.

We have tested the survival and reproductive activity of schizogenetic clones of *Aeolosoma hemprichi* and *Aeolosoma viride* in cultures where the medium was made of activated sludge from a wastewater treatment plant. We were able to maintain the survival of worms in cultures for more than three months, using activated sludge from the oxidation stage of the wastewater treatment plant as a medium. The reproductive activity of the worms decreased in the cultures maintained on sludge compared to control cultures; the number of filial zooids per day was reduced to 34% in *Aeolosoma hemprichi* and to 30% in *Aeolosoma viride*. The effect of the worms on volumetric sludge reduction was assessed in small-scale experiments with a minimal concentration of 7 worms per ml. Sludge reduction increased during the first three days and reached approximately 11-12%. Higher concentrations of worms (20, 35 worms per ml) produced similar effects of sludge volumetric reduction.

A cyclical process model of volumetric reduction of sludge in the presence of effective densities of worms was tested; in each cycle the effective density of worms was obtained using part of the sludge from the previous cycle to prompt the reproduction of worms and the process of volumetric reduction in new sludge. The first test, which lasted fifteen days and involved 4 cycles, gave a general volumetric reduction of 22% in the sludge treated with *Aeolosoma hemprichi* and 29% of that treated with *Aeolosoma viride*. The second test, lasting 60 days and involving 15 cycles, produced a volumetric reduction of 27% of the sludge treated with *Aeolosoma hemprichi* and 31% of that treated with *Aeolosoma viride*. A hypothetical model built on the basis of the experimental results foresees on an annual basis 121 cycles and an overall volumetric reduction of 25% of the sludge treated with *Aeolosoma hemprichi* and 45% of that treated with *Aeolosoma viride*.

Introduction

Some authors have shown a positive influence of sessile and free-swimming Oligochaeta (Tubificidae, Aeolosomatidae, Naididae) on the reduction of sludge produced in wastewater treatment plants (Ratsak, 2001; Wei et al., 2003a;Wei & Liu, 2005; Ratsak & Verkuijlen, 2006; Wei & Liu 2006).

To explain how worms reduce sludge it has been proposed that worms, by predating bacteria, produce fecal pellets, which produce mass reduction (Verdonschot, 1989; Ratsak, 2001; Wei et al., 2003b;Wei & Liu, 2005; Ratsak & Verkuijlen, 2006; Wei & Liu 2006).

Vast sludge reduction has been described in association with population blooms of oligochaetes (Wei et al., 2003b;Wei & Liu, 2005; Ratsak & Verkuijlen, 2006; Wei & Liu 2006).

The presence of species belonging to the genus *Aeolosoma* in the sludge is described as a sporadic phenomenon. Their population density is extremely unsteady with spontaneous blooms and drastic reductions resulting in the disappearance of the population. The factors that trigger and regulate these blooms remain unknown (Ratsak, 2001; Wei et al., 2003a; Ratsak & Verkuijlen, 2006). Hence, the practical application of Aeolosomatidae in sludge reduction is hampered by this population instability (Wei et al., 2003a; Ratsak & Verkuijlen, 2006). Wei et al. (2003) recognized the benefits of Oligochaeta for sludge reduction if the problem of population instability can be controlled.

Aeolosoma viride and *Aeolosoma hemprichi* belong to the Aeolosomatidae (Class Aphanoneura). They reach a few millimetres in length and populate fresh water environments.

Generally, aeolosomatids reproduce by paratomy. It begins when the worm reaches a determined number of metameres (depending on the species), which gives rise to the clonal production of a chain of filial zooids that detach themselves from the parental zooid in a few days. This leads rapidly to high population densities (Herlant-Meewis, 1950; 1951; Bunke, 1967; Zaccanti & Falconi, 2002; Falconi et al., 2006).

In standard breeding conditions and at constant volumes we observed that a population of *Aeolosoma viride* grows until it reaches a maximum; then a drastic reduction in numbers occurs resulting in the total disappearance of the culture; by contrast, at constant density conditions of 2 zooids/ml we observed that the Aeolosomatidae population keeps growing and can be maintained for an indefinite time (Zaccanti & Falconi, 2002).

Our aim was to maintain *Aeolosoma hemprichi* and *Aeolosoma viride* population density constant in order to be effective for sludge reduction.

Materials and methods

Experimental cultures of *Aeolosoma viride* and *Aeolosoma hemprichi* were obtained from stocks bred for a long time in the zoological laboratory of the Department of Biology of the University of Bologna.

The activated sludge for the reduction tests was from oxidative phases of a slaughter wastewater treatment (I.N.A.L.C.A. Castelvetro, Modena, Italy).

Our tests were carried out in a conditioned room at a temperature of 23±1°C with 12 hours of light alternated with 12 hours of dark. Control sample cultures are maintained in dechlorinate tapewater and fed with chopped spinach. Both control and sludge cultures were maintained in cylindrical containers with a diameter/height ratio of approximately 1:5 to improve the capability of appreciating volume variation.

For the two species of aeolosomids observations were made to establish the following parameters.

Reproductive rate in a sludge environment.

Three replicate units of sludge and control cultures were placed in 2-ml containers with an initial density of 5 worms/ml. Daily observations were made to count the number of worms and the cultural media were replaced. The cultures were maintained for 10 days. The reproductive activity was assessed as reproductive index (r) and duplication time (t_2), the time needed to double the initial number of specimens. The "r" value was obtained by the formula:

$$r=(\ln(N_t)-\ln(N_0))/t$$

derived from the logarithmic transformation of the equation

$$N_t=N_0*e^{rt}$$

where "N_t" is the number of specimens at time "t" (days); "N_0" is the initial number of specimens (t=0); "e" is the Nepero number. The "t_2" value was obtained by the formula:

$$t_2=\ln(2)/r.$$

Worm densities useful for volumetric reduction of sludge

Three replicate units of sludge cultures were placed in 2-ml containers with an initial worms/ml density of 0 (control), 2, 4, 7, 20, and 35. The cultures were maintained for 6 days, and observed daily to assess sludge volume variations. The sludge volumes (V) were assessed by measuring the length (h) of the segment between the container base and the sludge-supernatant interface:

$$V=Ab*h$$

where "Ab" is the base area.

Continuous sludge reduction by the control of worm densities

Continuous sludge cultures were made to maintain worm densities effective for the volumetric reduction of the sludge, by the following steps: the worms are seeded in a specific volume of sludge (1); the worms multiply and produce sludge volume reduction (2); when the volume reduction reaches maximum values (3) and after the elimination of the supernatant, a part of the compressed sludge is extracted from the container and eliminated (4); the remaining part is stirred into the new sludge (5) for further multiplication of the worms and consequent sludge volume reduction (2). Volume variations are evaluated daily. The first series of three replicate units of sludge cultures is performed in 20-ml containers for 15 days with 4 cycles of reduction and the addition of sludge. The second series of two replicate units is performed in 200-ml containers for 2 months with 15 cycles of reduction and the addition of sludge.

A model of sludge volumetric reduction plants is constructed using the experimental data obtained.

Results

Reproductive rate in a sludge environment

The multiple cultures of aeolosomatids maintained in a sludge environment showed significant reproductive activity, albeit lower than that of the control animals (Table 1). The reduction in reproductive rate and the increase in the duplication time were greater in *Aeolosoma viride* than they were in *Aeolosoma hemprichi*.

Table 1. The reproductive rate of two species of Aeolosomatidae under standard conditions and in sludge.“r”: reproductive index; “sd”: standard deviation; “t2”: duplication time in days; *A.h.*: *Aeolosoma hemprichi*; *A.v.*: *Aeolosoma viride*

	Control			sludge		
A. h.	r	Sd	t2 (days)	r	sd	t2 (days)
A. v.	0.667	0.194	1.0	0.282	0.135	2.0
	0.346	0.168	2.5	0.116	0.042	6.0

The ability of Aeolosomatids to compress the sludge

The worm’s ability to compress the sludge was proven by the observation that in a Petri dish with 0.02 ml circa of sludge a single worm compresses a remarkable amount of the sludge after a few hours (Figure 1). As soon as the sludge was introduced into the container it was widely distributed on the bottom. Subsequently, it gathered into a sub-spherical mass with a volumetric size of at least three orders of magnitude more than that of the worms. The effect of sludge compression, which occurred in a short time, seemed to be attributable to a prevailing mechanical-type causal action exerted by the worms. In fact, the quantitative disproportion between the sludge and the worms seems to reduce the importance of the feeding effect.

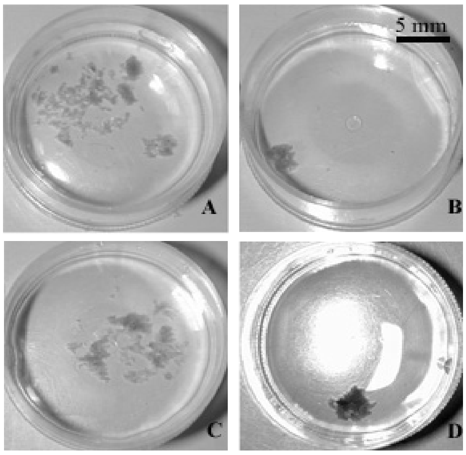

Figure 1. A single worm is put inside a Petri dish in the presence of a certain amount of sludge (left). After a few hours the sludge is compressed (right). A, B: *Aeolosoma hemprichi*; C,D: *Aeolosoma viride*

Worm densities useful for sludge volumetric reduction

A test of sludge reduction activity of *Aeolosoma hemprichi* and *Aeolosoma viride* at different densities is summarized in Figure 2. In control cultures (density of 0 worms/ml) the volume reduction after the first day was very small (approximately 0.1%) and stayed constant over the following days. In the cultures with added worms the sludge reduction was significant and progressive and correlated with worm density. At densities of 7, 20, and 35 worms/ml the sludge volume reduction reached, on the third day, a similar maximum of about the 12% for *Aeolosoma hemprichi* and 11% for *Aeolosoma viride*; then the volume reduction maintained the values reached.

Continuous sludge reduction through the control of worm densities

The first sludge reduction test consisted of 4 cycles over an overall time of 15 days (Figure 3). 20-ml containers were used with an initial density of 7 worms per ml and incubation times for each cycle of 3-5 days. At the end of each cycle, the elimina- ted sludge (step 4) and the sludge used to start the processes of multiplication of the worms and reduction of the new sludge (step 5) was 50% of the volume of compressed sludge (step 3). For *Aeolosoma hemprichi* 55 ml of sludge was introduced in the system, which at the end of the four cycles reduced by 22±3%; the mean reduction per cycle was 15±3%; for *Aeolosoma viride* 57±6 ml of sludge was introduced, which reduced by 29±11%; the mean reduction per cycle was 21±12%.

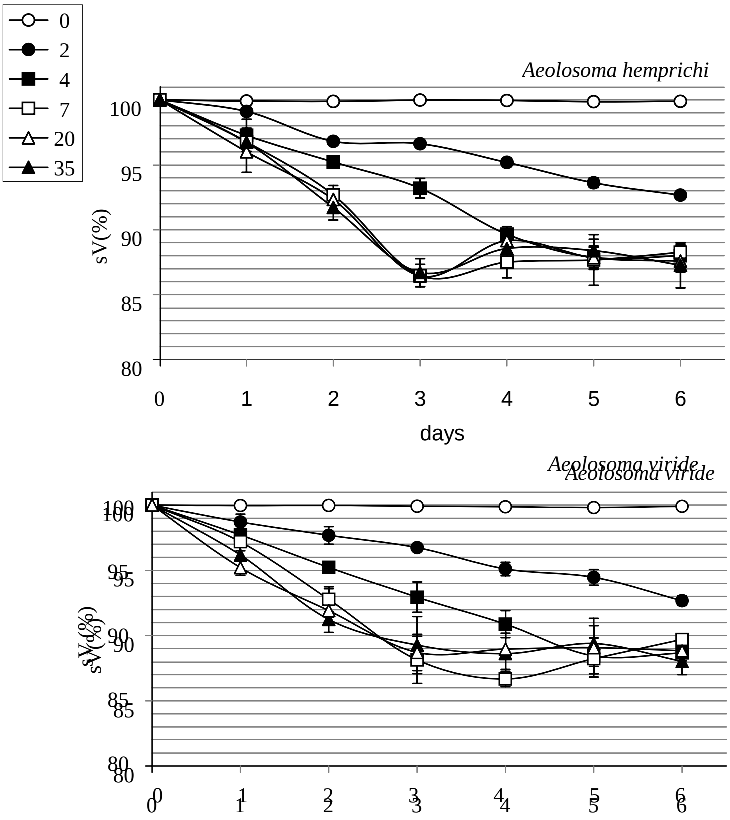

Figure 2. Volumetric reduction of the sludge with different densities of worms; "SV (%)" variations the sludge rate in comparison with the initial volume; "0, 2, 4, 7, 20, 35": initial density of worms in number per ml; Bar: standard deviation

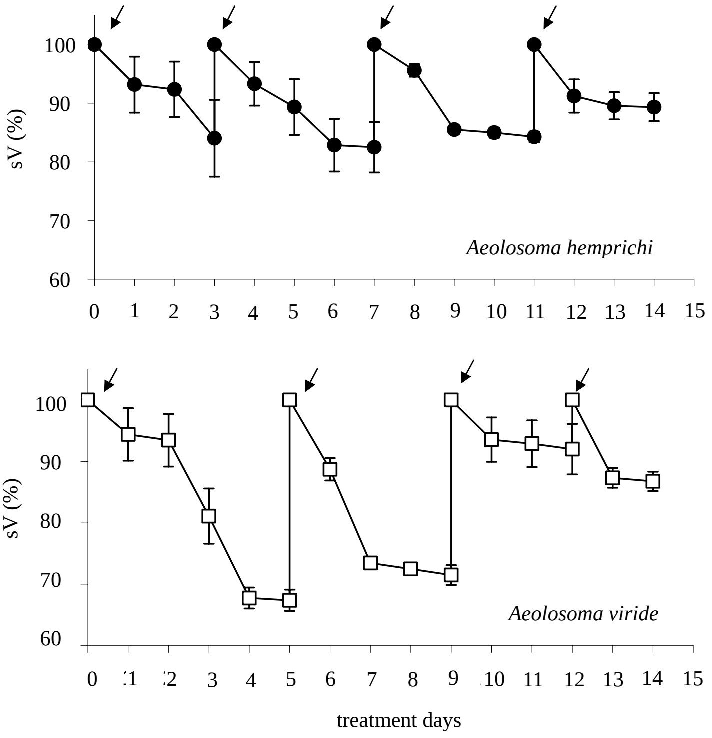

Figure 3. Four cycles of sludge volumetric reduction with a initial worm density of 7 zooids/ml. At the end of each cycle half of the compressed sludge is removed and the other half is mixed with the new mud to prime the processes of multiplication of the worms and volumetric reduction of the sludge. "SV (%) "variations the sludge percentage in comparison with the initial volume. "Arrow heads": start of the cycles

The second cyclical reduction test was performed in 200 ml containers and lasted 60 days. The initial density was again 7 worms per ml and the duration of each cycle was 3-5 days. The number of cycles performed was 15. As in the first test, 50% of the sludge obtained in step 3 was used for step 4 and step 5. For *Aeolosoma hemprichi* 1,833 ml of sludge was introduced in the system, which at the end of the fourteen cycles reduced by 27±4%; the mean reduction per cycle was 16±5%. For *Aeolosoma viride* 1,880 ml of sludge was introduced, which reduced by 31±8%; the mean reduction per cycle was 20±9%.

The results obtained in the two tests seem to show that it is possible to produce and maintain an effective density of worms in the sludge for volumetric cyclical reduction of the sludge, with both the species of aeolosomatids used.

Proposed plant model

On the basis of our results, we propose a model based on a series of three-day cycles, over a one-year period (Table 2).

Table 2. Mean values of parameters used for a model of volumetric sludge reduction.*"A.h."*: *Aeolosoma hemprichi*; *"A.v."*: *Aeolosoma viride*; "tC": duration of each cycle; "r": reproductive index; "iSV": initial sludge volume; "iWd": initial worm density; "nZ/ml": number of zooids per ml; "SVr": sludge volume reduction at the end of each cycle; "fSV": final sludge volume at the end of each cycle; "SVout": sludge volume eliminated at the end of each cycle; "SVc": sludge volume conserved and blended with new sludge for the start of the following cycle; "SVin": new sludge volume introduced at the start of the following cycle; "step 1, 2, 3, 4, 5": see text in the Material and Methods

	A. h.	*A. v.*	
tC(days)	3	3	
r	0.282	0.116	
isV	1	1	step 1,2
iwd(Nz/ml)	7	7	
SVr	0.163	0.197	step 3
fSV=iSV-SVr	0.837	0.803	
Svout=Fsv-SVc	0.478	0.236	step 4
SVc=Fsv/(e×p(tC*r))	0.359	0.567	step 5
Svin=iSV-SVc	0.641	0.433	

Based on our evaluations of the reproductive rate of the two species, we modified the quantities of sludge of step 5 used in the previous experimental protocols, so as to have an initial density of worms of 7/ml for each cycle. Evaluations of the reproductive rate "r" for the two species show that the quantities of incubated sludge needed to get the initial density of worms in each cycle must be higher for *Aeolosoma viride* than for *Aeolosoma hemprichi*. In fact, in previous experiments the implants with *Aeolosoma viride* showed a high variability of the yields, with a tendency towards a reduction in the succession of cycles. We believe that this variation is due to the progressive diminution of the densities of *Aeolosoma viride* in the following seedings.

According to our model, using *Aeolosoma hemprichi*, over a year, 79 sludge starting volumes can be treated with a final reduction of 25%, corresponding to 20 starting volumes. With *Aeolosoma viride* 54 sludge volumes can be treated with a final reduction of 45%, corresponding to 24 starting volumes.

Aeolosoma viride has a greater capability to reduce the volume of sludge but a lower reproductive activity in comparison with *Aeolosoma hemprichi*. In other words, to treat the same quantity of sludge the apparatus with *Aeolosoma viride* should have a capability of around one and a half times that of the apparatus with *Aeolosoma hemprichi* and would produce an 80% higher reduction effect.

Discussion

Sludge reduction by Aeolosomatidae has been attributed to the consumption of bacteria (Ratsak, 2001; Kooijman, 1993; Wei et al., 2003a; Ratsak & Verkuijlen, 2006; Wei & Liu 2006). We think that, in addition to the sludge mass reduction due to the eating process, there is a further volume reduction due to the mechanical compression of the sludge mass by aeolosomatid movement. This hypothesis comes from the observation referred to in paragraph 2 of the results.

Our results demonstrate that, in an environment of sludge from a wastewater treatment plant, it is possible to maintain densities of free-swimming worms, resulting in volumetric sludge reduction, at least on a small scale.

In sludge, worms survive and reproduce, but their reproductive rates decrease compared to standard conditions (Falconi et al., 2006). In our laboratory tests, with a starting density of 7 worms per millilitre in both species, we obtained a volumetric sludge reduction of about 15% after 3 days. On the basis of the worm's agametic reproduction, during the processes of sludge volume reduction the number of worms increases and it is possible to use part of the reduced sludge to seed in new sludge worm densities, which is efficacious to trigger the process of volu- metric reduction in the new amount of sludge. We have therefore a cyclical process that can be extended indefinitely.

The two species have yielded different quantitative results. *Aeolosoma hemprichi*, with a high reproductive rate, allows the treatment of greater sludge amounts, but with less volumetric reduction with respect to the other species. *Aeolosoma viride*, with a low reproductive rate, allows the treatment of smaller amounts of sludge, but greater efficiency in terms of volumetric reduction. In the proposed model the volumetric sludge reduction is assessed as 25-45% on an annual basis. Of course, the effectiveness of the model needs to be verified in a wastewater treatment plant.

A large-scale test of the proposed model based on free-swimming worms could have interesting applications in wastewater treatment plants. Important volumetric reductions have been obtained with sessile worms (Wei & Liu, 2005; 2006), and the use of integrated systems, involving both free-swimming and sessile worms is another possibility.

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