

Sludge reduction by predatory activity of aquatic oligochaetes in wastewater treatment plants: science or fiction? A review

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Abstract

Biological aerobic wastewater treatment plants (WWTPs) produce a lot of excess sludge. The costs for handling this residual product are increasing, so the search for alternative techniques to reduce the amount of sludge has to be continued. Activated sludge consists of inorganic and organic substances, bacteria, protozoa and metazoa. Due to incomplete biomass conversion, sludge consumption yields less oligochaete biomass. From a technological point of view, the application of aquatic oligochaetes to reduce the sludge production offers interesting perspectives. This paper aims to review the feasibility for the reduction of activated sludge in WWTPs by means of aquatic oligochaetes. Also the current techniques concerning sludge reduction are taken into account. Several of the WWTPs relevant parameters, which may influence predatory activity of aquatic oligochaetes, are discussed: particle size, organic content of substrate, bacteria preference, life cycle and population dynamics of aquatic oligochaetes, temperature, pH, oxygen and process conditions. From the literature it appeared that most research has been performed on laboratory scale. Only a few authors mention a significant reduction of the sludge production by 'sessile' species such as *Lumbriculus*. Vermicultures for the reduction of activated sludge are rather common in developing countries. Incidentally large annelid blooms have been noticed in WWTPs. It remains obscure which factors trigger the initiation of annelid blooms in WWTPs and which are of importance to maintain a stable annelid population in WWTPs. The influence of a considerable worm bloom on the waste sludge production is still under investigation.

Introduction

Oligochaete worms are found in both fresh and saline continental water. Many river and lake biotopes are often dominated by oligochaetes, which may comprise 50–60% and even 100% of the benthic biomass.

Oligochaete worms dwelling in the profundal zone of lakes play an important role in the exchange of matter and are responsible to a notable degree for the rate of mud formation and

mineralization of bottom sediments. Additionally, the presence of a large population of saprophytic species of oligochaetes is an important factor in the self-purification of polluted waters.

Many environmental variables including water temperature, oxygen concentration, presence of competitors or predators and substrate properties influence habitat selection and selective feeding by oligochaetes. A number of species of oligochaete worms are able to feed on waste organic materials such as sewage sludge and cattle excrement. These

worms have subsequently been used as a feed for fish or farm animals, for example, 60–70% of the dry weight of *Eisenia foetida* is protein, high in essential amino acids. Other studies also indicate that enchytraeid worms are a highly suitable fish food (Kirk & Howell, 1972; Kirk, 1973, Bouguenec, 1992). In the tropical regions of India systems of fishes grown in treated domestic sewage has been investigated (Inakollu & Wanganeo, 2002).

Many different types of sessile (benthic species which are attached to carrier material in WWTPs) and free-swimming oligochaetes have frequently been found in low loaded (0.04–0.07 kg BOD/kg DW d) WWTPs (Ratsak, 1994, 2001; Janssen et al., 1998; Elissen et al., submitted for publication). A WWTP may be considered as an artificial ecosystem (Hawkes, 1963) that is determined by its influent characteristics, its design and treatment management. The concentration of activated sludge is controlled by the sludge wastage rate. As a result of high sludge wastage rate the sludge concentration will decrease.

Excess sludge production is affected by the sludge loading rate, temperature and the oxygen concentration. Raising the concentration of dissolved oxygen in the sludge and thus stimulating the microbial activity responsible for mineralization of the sludge enhances the reduction of the excess sludge production (Abbassi et al., 1999). The number of parameters affecting the bacteria in biological wastewater treatment plants is immense and is continuously varying in time. Occasionally, the appearance of oligochaetes seems to coincide with a considerable reduction of the waste sludge (Ratsak, 1994; Wei et al., 2003).

Since the introduction of legislation prohibiting the use of sludge as fertilizer in agriculture in the Netherlands, disposal of activated sludge from wastewater treatment plants has become very expensive. Generally, large amounts of bio-sludge are formed in biological wastewater treatment processes and the separation, dewatering, treatment and disposal of this sludge require large investments and form a major percentage of the operating costs for treatment. The disposal of excess sludge from WWTPs represents a rising challenge in activated sludge processes for modification in design and operation to minimize sludge.

Sludge minimization may be a result of reduced production of sludge and/or disintegration pro-

cesses both in the wastewater treatment stage and in the sludge stage. In biological wastewater treatment processes there are principally three main strategies by which sludge reduction can be achieved, (a) by enhanced pre-treatment (reduced load), (b) by yield reduction and (c) by sludge disintegration processes by which a greater part of the sludge becomes more biodegradable so that more can be mineralized and assimilated by the biomass.

In the literature, various sludge disintegration technologies for sludge minimization are discussed (see Fig. 1), including mechanical methods (focusing on stirred ball-mill, high pressure homogenizer, ultrasonic disintegrator), electrical methods, chemical methods (focusing on the use of ozone and hydrogen peroxide), physical methods (focusing on thermal and thermal/chemical hydrolysis) and biological methods (focusing on enzymatic processes such as lysocryptic growth, decay, uncoupling metabolism, maintenance metabolism and anaerobic treatment). Finally, an alternative strategy is sludge reduction by predation. At each transfer to a higher trophic level of the food chain, a large proportion of the potential energy is lost as heat, due to maintenance processes, respiration and reproduction. Ultimately, a lower biomass remains. Vermiculture is considered as post-treatment of dewatered or thickened sludge and sludge reduction by aquatic oligochaetes is comprehensively dealt with in this review. Excellent reviews including pros and contras of the above mentioned methods are outlined by Ødegaard (1993), Mayhew & Stephenson (1997), van Loosdrecht &

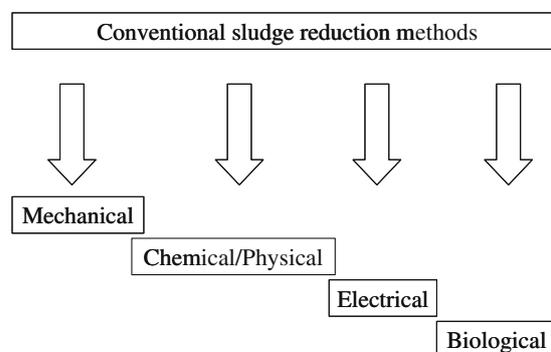


Figure 1. Sludge disintegration methods used for activated sludge minimization.

Henze (1999), Wei et al. (2003), WIRES (2004). The potential to manipulate the process to reduce yield is increasing and each individual methodology has its advantages depending on the process configuration.

The present discharge standards on nitrogen have lead to a decrease in both total amount of sludge and organic content of excess sludge, due to increase of retention time and therefore an ongoing mineralization. However, due to strengthened environmental standards, total number of WWTPs is increasing.

Especially in small WWTP (<100 000 population equivalents) without an anaerobic digester, it is interesting to find ways of designing and operating biological treatment to reduce biomass production. Oligochaetes may be introduced to the aeration process or the waste sludge may be separately treated by oligochaetes.

The oligochaetes used for treatment of excess activated sludge can be divided into two groups, firstly the large aquatic worms such as the Tubificidae, Lumbriculidae and the semi-aquatic or terrestrial Enchytraeidae and, secondly the small aquatic worms such as Naidids and Aeolosomatids. The usability of these groups is quite different because of the different niches they occupy. Whereas the former needs a substratum in which it can swirl around, the latter occurs in the mixed liquor or in the biofilm attached to a carrier material. Because it is difficult to create favorable conditions for their maturation and successful sexual reproduction, these small aquatic species are not suitable for stable long-term cultivation so far. This important difference has to be taken into account for the development of a predation stage in the wastewater treatment process.

The objective of this review is to provide insight in the feasibility of sludge reduction in WWTPs by using worms. To reach this insight a comparison was made between the information available on oligochaetes in their natural habitat, and in a laboratory environment, with the information available on worms in WWTPs. Their role in the latter is becoming more and more interesting from both sustainable and economical point of view. The review is not covering all the literature published about the subjects listed below. This would be quite a laborious exercise.

In the subsequent sections several important parameters, which are relevant for wastewater treatment plants and may influence the predatory activity of aquatic oligochaetes are discussed: particle size, organic content of substrate, bacteria preference, life cycle and population dynamics, temperature, pH, oxygen and process conditions.

Particle size

Particle size is supposed to play a major role in the distribution and abundance of tubificids. This section deals with two distinct aspects of particle size selectivity: the influence of particle size on distribution (habitat selection) and selective feeding.

Sauter & Güde (1996) found the grain size fraction less than 63 μm was an important environmental factor affecting the presence of many of the tubificid species of Lake Constance. In substrates with a heterogeneous grain size where tubificids can be selective many species occur almost exclusively in their particle size preference range, which is highly species specific. Five species showed a preference for substrates with a high clay and silt content. These are *Tubifex tubifex*, *Potamothenrix heuscheri*, *Potamothenrix bedoti*, *Ilyodrilus templetoni* and *Aulodrilus plurisetus*. Three species *Potamothenrix moldaviensis*, *Limnodrilus claparedeianus* and *Tubifex ignotus* are more likely to occur in sandy substrates, having clay and silt content of less than 10% (see Table 1).

Juget (1979) reported by microscopic analysis, the maximum diameter of the biggest particle observed in the gut of the tubificid worm *Tubifex tubifex* was 130 μm . *Tubifex tubifex* mainly ingested particles <63 μm .

Rodriguez et al. (2001) measured the particle size distribution of fecal pellets produced by *T. tubifex*. The fecal material was composed of particles with a mean diameter under 63 μm . This suggested that this species actively selected the silt-clay fraction, avoiding the larger sand particles. A more detailed analysis showed that about 75%, by volume, was composed of particles with a mean diameter <25 μm , and the mode was <10 μm .

Only one reference has been found with respect to small aquatic oligochaetes and size selective feeding. Bowker et al. (1983) indicated the shape

Table 1. Substrate preference of species in relation to the clay and silt content of the sediment (after Sauter & Güde, 1996)

Species	Clay and silt content (%)
<i>Tubifex tubifex</i>	>60
<i>Potamothenrix heuscheri</i>	>60
<i>Potamothenrix bedoti</i>	>60
<i>Ilyodrilus templetoni</i>	>60
<i>Aulodrilus plurisetia</i>	>60
<i>Stylogrilus heringianus</i>	20–30
<i>Limnodrilus claparedeianus</i>	<10
<i>Potamothenrix moldaviensis</i>	<10
<i>Tubifex ignotus</i>	<10
<i>Psammoryctides barbatus</i>	<10
<i>Limnodrilus udekemianus</i>	50–70
<i>Spirosperma ferox</i>	50–70
<i>Limnodrilus hoffmeisteri</i>	No preference
<i>Limnodrilus profundicola</i>	No preference
<i>Potamothenrix hammoniensis</i>	No preference

of the pharynx from the study of *Nais elinguis* possibly determined the maximum size of food. The rate at which algae were ingested was negatively correlated with the lengths of the algae (Bowker et al., 1985a). Also unicellular algae were ingested at a greater rate than colonial and filamentous algae.

Organic content of substrate

The aspects distribution (habitat selection) and selective feeding also applies to the relationship between tubificid abundances and organic matter. Most available literature concerns the (semi-) aquatic worms Lumbriculidae and Tubificidae, especially *Tubifex tubifex*.

Substrate selection is a taxis as opposed to kinesis. It appears to be a taxis response to dispersing microbiota or molecules with molecular weight greater than 12,000–14,000 (McMurtry et al., 1983). This means that worms must be able to detect the presence and direction of a sediment component dispersing through the water. However individual variation in habitat selection behavior exists due to the considerable behavioral heterogeneity of individuals.

Studies of the influence of substrate properties on habitat selection by tubificids have pro-

duced conflicting results. Wachs (1967) found that *T. tubifex* preferred nutrient-rich sediment (i.e. high organic C and total N) regardless of particle size while Zahner (1967) found that this species chose coarse-grained nutrient-poor sediment (i.e. low in organic C, organic N, and organic P) to fine-grained nutrient-rich sediment.

Brinkhurst & Jamieson (1971) pointed out that few correlations between the variations of total organic matter and the distribution and abundance of worms have been demonstrated. The quality of organic matter in the sediment is therefore more important in determining tubificid preference than the quantity of organic matter. However, when the correlation of abundance of tubificids with total organic matter in sediments was investigated, the results were contradictory. Sometimes positive correlation has been observed (Brinkhurst, 1970), but in many instances tubificid abundance was not correlated or was even negatively correlated with the organic content of sediments (McMurtry et al., 1983; Robbins et al., 1979, 1989).

Total organic carbon is an unsatisfactory measure of the nutrients available to tubificids. Poor correlations occur because total organic carbon includes not only valuable food sources (bacteria or algae) but also substances resistant to decomposition and presumably unsuitable as a food source (lignin and humic materials) (Pasteris et al., 1994). All in all it can be concluded that the results are not always unambiguous.

According to Rodriguez et al. (2001), *T. tubifex* exhibits two levels of selectivity in its feeding behavior. Firstly, particle size and secondly the organic content of the particles. *T. tubifex* selectively fed the organic rich particles and this feeding was independent of particle size (within the fine (silt-clay) fraction) of the sediment.

Also other studies have shown selective feeding by oligochaetes on the organic component (living or non-living) of the sediment (Brinkhurst & Chua, 1969; Wavre & Brinkhurst, 1971; Brinkhurst et al., 1972; Brinkhurst & Austin, 1979; Matisoff et al., 1999). *Branchiura sowerbyi* was able to grow on activated sludge but only when this was mixed with sand or river mud (Aston & Milner, 1982; Aston et al., 1982).

Finogenova & Lobasheva (1987) studied the growth of *T. tubifex* on highly caloric activated

sludge and poor in organic matter natural silt in glass vessels (10 cm in diameter). Selectivity in feeding of oligochaetes did not only depend on the type of ground inhabited by the worms but also on their age. The specific growth rate of worms on activated sludge was twice as high as that on natural silt. Furthermore the worms reached maturity more quickly. The number of eggs produced and values of fecundity also were obviously attributed to specific characteristics of the food involved. The average number of eggs/ind/100 days was respectively 2.5 and 5 times higher of the worms grown on activated sludge compared with the worms cultured on natural silt or fainted lettuce. The extreme tolerance to pollution and ability to accelerate growth and development allow *T. tubifex* and *L. hoffmeisteri* (ecologically closely related) to be predominant under conditions of great quantities of easily assimilated organic material, i.e. in waters affected by human activity such as sewage sludge.

Sewage sludge (50–70% organic carbon) originating from a low loaded conventional activated sludge plant can be used as a food source for oligochaetes (Rensink & Rulkens, 1997; Klapwijk et al., 2000). Pilot plant experiments have been carried out to study the mineralization of sludge with and without addition of tubificids in trickling filters filled with lava slag or synthetic carrier material. Sludge reduction through predation varied between 18 and 67%. Unfortunately however, it was not possible to reproduce the experiments. This could be ascribed to the fact that an unknown amount of sludge accumulates within the filters.

Buijs et al. (submitted for publication) investigated the effect of predation by *Lumbriculus variegatus* (Family Lumbriculidae) on the stabilization of activated sludge and found an enhanced stabilization of activated sludge by predation of the sludge in aerobic/anoxic batch cultures. An additional sludge reduction up to 30% was found compared with a reference setup without *L. variegatus*.

There is not much literature available about the small aquatic worms and activated sludge with respect to this item. Ratsak et al. (1993) developed a mathematical model for the growth of *Nais elinguis* on activated sludge.

Bacteria preference

Coler et al. (1968) demonstrated selective feeding on bacterial communities for the first time. The importance of bacteria in the diet of tubificids is now well established (Brinkhurst & Chua, 1969; Wavre & Brinkhurst, 1971; Brinkhurst et al., 1972). Further, Coler et al. (1968) have shown that mixed populations of tubificids can discriminate between different species of bacteria. It is assumed that while tubificids can respond to many different environmental characteristics, the relative importance of these differs and only a few of them exert primary influence on the preference response in the natural environment (Cummins & Lauff, 1968).

Also Brinkhurst (1974) and Milbrink (1993) investigated the existence of selective feeding on bacterial communities associated with sediment particles or with organic matter in the sediment. The role of potential food sources such as anaerobic bacteria, protozoa and large organic molecules in substrate selection is largely unknown. McMurtry et al. (1983) concluded that the microbial constituents of the sediment are more important than its physical and chemical properties in influencing substrate selection by Tubificids. There was a significant correlation between the abundance of heterotrophic aerobic bacteria in sediment and tubificid preference.

The substrate preference can be caused by selective colonization of different bacteria/algae species in the substrate. However, the occurrence of a certain species is not determined by a single environmental factor. If individual tubificid worms were exposed to more heterogeneous substrates, worms were more selective in the range of grain size in which they occurred.

Oligochaete species have been shown to differ in their ability to digest different bacterial species. Wavre & Brinkhurst (1971) found most of the heterotrophic bacteria (about 70%) were killed on passage through the gut, and the freshly produced feces became rich in monospecific bacteria. Different theories about the origin of bacterial concentrations on feces say that bacteria surviving gut passage may multiply rapidly due to relaxed competition, factors in the gut itself may stimulate bacterial growth, or selection may favor rapidly growing strains of microorganisms (Juniper, 1981).

According to Brinkhurst (1974) tubificids as a group ingest particles selectively and three tubificid species fed the same food source egests different proportions of at least one representative fraction of the bacterial flora present, the heterotrophic aerobes. The feces of one worm species contain a high proportion of bacteria that are the preferred food of another species in the association and thus worms may profit from each other (mutualism). These findings are supported by the research of Milbrink (1987). He found an increased growth rate of 50% and sexual maturity was reached earlier in mixed cultures due to each species selectively grazing concentrations of bacteria associated with the feces of another species in the community.

Selective feeding therefore permits more tubificid species to coexist, thereby lowering the respiration rate and increasing growth due to higher assimilation efficiency by feeding on available food. Solitary species may exhaust their preferred food and may actively burrow in search of preferred food hence raising their respiration loss, reducing time available for feeding and lowering their assimilation efficiency by feeding on available food.

There is ample evidence that polychaetes, oligochaetes, chironomid larvae, crustaceans, etc., are selectively attracted to colonies of bacteria, and it has been argued 'microbial stripping' of particles like fecal pellets can enhance microbial turnover and biomass of ingested particles (Milbrink, 1993). Mermillod-Blondin et al. (2003) found the combination of *Limnodrilus* and *Tubifex* worms produced higher sediment redistribution and a higher stimulation of bacterial numbers in the top 5 cm of the sediment than tested separately. Furthermore, the combination of the two genera produced lower oxygen consumption than the two genera tested separately in the deep layers of the sediment.

The fact that many deposit-feeders produce fecal pellets enriched in organic matter relative to available sediment indicates selective ingestion (Hargrave, 1976; Juniper, 1981; Cammen, 1982). Brinkhurst et al. (1971) found oligochaete feces had a higher percentage of organic matter, energy and nitrogen than the surrounding sediments. It has been argued, however, that the degree of enrichment is often too low to meet energy demands. Therefore most deposit feeders would

also require nonliving food for subsistence, i.e. microorganisms largely for proteins, and undifferentiated detritus for carbohydrates (Lopez & Levington, 1987).

Enchytraeids had no detectable effect on microbial biomass, but the microbial respiration increased by 35% in the surface horizon. Because of the enhanced microbial activity in this horizon, Cole et al. (2000) suggested enchytraeids indirectly drive processes of decomposition and nutrient mineralization in organic upland soils. There were no effects on the release of inorganic N or P, although the release of ammonium and phosphate was correlated with the number of enchytraeids. Enchytraeids almost doubled the availability of organic carbon.

Selective feeding by *Nais elinguis* and *Nais variabilis* is investigated by Harper et al. (1981a, b), Bowker et al. (1983, 1985a, b). No conclusive evidence was obtained indicating a preferential ingestion of certain kinds of bacteria, that the gut environment was selectively hostile to the particular kinds of bacteria studied or that worms had a specialized gut micro-flora. On the contrary, *N. elinguis* actively selected diatoms in preference to other algae species (Bowker et al., 1985a, b). The worms were able to discriminate between filamentous algae and/or diatoms and unicellular or colonial chlorophycean algae. Diatoms were selected in preference to latex beads but the worms could not discriminate between living filaments and nylon monofilaments. A thigmotactic response to filaments and a chemo tactic response to diatoms may be implicated.

The (mostly small) metazoa frequently found in the activated sludge and biofilm systems are the oligochaetes *Aeolosoma hemprichi*, *Pristina longiseta*, *N. variabilis* and *N. elinguis*. Additionally, Inamori et al. (1983) also encountered *Dero* sp. (especially on biofilm), *Chaetogaster* sp., *Paranais* sp., *Limnodrilus* sp. and *Tubifex* sp.

Only two authors investigated food selectivity in small aquatic oligochaetes (Inamori et al., 1983, 1987; Kuniyasu et al., 1997). *Nais* sp., *Aeolosoma hemprichi*, *Pristina* sp. and *Dero* sp. were cultured on sterilized activated sludge in order to investigate maximum specific growth rates of the worms fed different diets and at different temperatures. *A. hemprichi* was able to monoxenically grow on *Pseudomonas putida*, *Escherichia coli*, *Acinetobacter*

calcoaceticus, *Klebsiella pneumoniae*, *Flavobacterium luteus*, *Flavobacterium suaveolens* en *Streptococcus acidominus*. *P. longiseta* and *N. variabilis* could also grow on these species and on *Micrococcus luteus*. Neither of the tested oligochaetes was able to grow on *Bacillus cereus*, *Bacillus subtilis* and *Achromobacter cycloclastus* (Kuniyasu et al., 1997; Inamori et al., 1987, 1990). Perhaps the worms had a preference for Gram-negative bacteria. This aspect however, is not mentioned in the discussion. Especially the bacterial species was an important factor for the growth of *A. hemprichi* and a still unknown sludge component was important for the growth of *Nais* sp. (Inamori et al., 1983).

Life cycle and population dynamics

The structure and persistence of biological communities is strongly influenced by biological interactions between component species: few would deny the importance of competition and degradation in ecological theory development and in natural communities. The available literature about this item is innumerable. Only some remarks are placed with respect to wastewater treatment in advance of the discussion section.

The reproduction modes of the oligochaetes are very important for manipulation of the population growth. The formation of paratomic budding is genetically determined and limited (Hämmerling, 1924). A great variety of asexual reproductive modes are known among aquatic oligochaetes.

Several intrinsic density regulation mechanisms exist; for example, a decrease in population abundance (e.g. by predation) causes both the percentage maturation and the specific fecundity to increase, so leading to an overall numerical growth. This new situation in its turn will bring out, through the decreased maturation and fecundity, a decline in the population size (Adreani et al., 1984). From population dynamics modeling, density dependence appeared to be extremely important as far as regulation of population abundance of the tubificids was concerned (Bonacina et al., 1989b). Generation time and fecundity were lower in 'eutrophic' than in 'oligotrophic' species. Also a positive correlation between minimum generation time and the embryonic devel-

opmental time was shown. Maturation time is revealed as density dependent and depends on the individual growth rate. Density affects negatively the percentage of the population that reaches maturity and the specific fecundity (Adreani et al., 1984).

Population density controls the fecundity, growth, maturation and ovigeration rates of *Tubifex tubifex* (Bonacina et al., 1989a, b). Growth rates of *T. tubifex* and mean number of eggs laid/ovigerous are inversely related to density, but generation time appears to be directly related. Very low or very high initial densities display efficient density controls.

Some associations are the results of species-specific feeding on bacteria colonizing the fecal pellets of the component species (Milbrink, 1987, 1993). Each species is supposed to grow significantly better when fed upon the fecal pellets of the other species (see also previous section). The results suggest facultative mutualism may be more common than generally believed. The mutualistic interaction is likely to be 'indirect' via the species-specific intake by the worms of bacteria associated with the fecal pellets of the component species.

Christensen found in dense mass cultures (with densities above 400), *Enchytraeus bigeminus*, reproduced solely by fragmentation and subsequent regeneration. At densities less than 300–350 (in 200 ml glass jars and 75 cm³ wet coarse sand substratum), sexual reproduction was not suppressed. The ecological importance of this reproductive behavior in dense populations of *E. bigeminus* is based upon suppression of sexual reproduction in some potential competitors that are obligatory sexual breeders (Christensen, 1973).

Although some authors claim Naididae and Aeolosomatidae are characterized by a regular, mostly seasonal alternating of sexual and asexual reproduction (Christensen, 1984; Timm, 1984), we did not find any evidence for sexual reproduction of Aeolosomatidae and Naididae in wastewater treatment systems. The sexual generation appears in natural waters, as a rule, before winter or drought and survive by diapausing cocoons only. Old worms always die after the egg-laying period.

According to Loden (1981), asexual reproduction is employed by species of Naididae during favorable conditions. Sexual reproduction occurs rarely and sporadically. His observations indicated

a pattern to the occurrence of sexual and asexual phases in Naididae of the eastern and Midwestern United States. These occurrences are related to habitat, geographic distribution and certain species adaptations. In general, there has been conflicting evidence concerning where, why, by what stimulus, and how sexual maturity occurs among species of Naididae. The cocoon is a device to survive adverse conditions. Species of Naididae in unstable situations appear adapted for rapid breeding and cocoon deposition. Loden found four patterns to the abundance of naidids in unstable habitats: those that thrive in winter and spring, the ('winter species'); those present in summer and autumn, ('summer species'); those present throughout the year, but with a population maximum in spring; and those present year-round, but with a late summer or autumnal abundance.

Temperature, pH and oxygen

Indications from field data suggest that both dissolved oxygen and temperature regimes of lakes are critical and highly species-specific variables for freshwater invertebrates and specifically aquatic oligochaetes thereby affecting species composition and abundance. Lakes liable to develop anoxic conditions had fewer species (Reynoldson, 1987).

The optimal temperatures for both somatic growth and reproduction for *Tubifex tubifex* usually is in the range between 20 and 25 °C (Reynoldson et al., 1996). However, genotypic or phenotypic variations exist. *T. tubifex* and *Limnodrilus hoffmeisteri* in the English Lake District have similar narrow temperature ranges over which growth occurs, from 10 to 13 °C and both show a considerable tolerance to extended periods of anoxia, of at least 16 and 10 weeks respectively. However no growth was observed under anoxic conditions. A third common species *Ilyodrilus templetoni* is sensitive to anoxia and could not survive more than 4 weeks. There was no evidence of differences in substrate quality affecting growth rates of either *T. tubifex* or *L. hoffmeisteri* (Reynoldson, 1987). *L. hoffmeisteri* has a higher egg production at higher temperatures (20–25 °C) and *T. tubifex* at lower temperatures 8 °C. Famme & Knudsen (1985) however, have been found evidence for anoxic survival, growth and reproduc-

tion by *Tubifex* sp. in an anoxic chemostat. *Tubifex* was able to survive more than 10 months of anoxia and they concluded that adult *Tubifex* might be considered as obligate anaerobe.

Aston et al. (1982) studied the effect of temperature on the culture of *Branchiura sowerbyi* (Oligochaeta, Tubificidae) on activated sludge. Highest reproduction and growth rates occurred between 21 and 29 °C with a population doubling time of 1.48 week at 25 °C (the optimum temperature). In an other study he found the optimum temperature for cocoon laying in mature worms, also growth in sexually immature worms, was near 25 °C. The optimum temperature for growth in sexually mature worms, however, was lower (10 °C in the Avon population and 15 °C in the Thames population). Probably the growth of sexually mature worms at 20–24 °C was depressed by the high rate of cocoon production (Aston, 1968).

Dumnicka & Pasternak (1978) found the occurrence of *N. elinguis* was positively correlated with temperature in the range from 0.3 to 20.6 °C. This species finds the best conditions of development in moderately oxygenated and fertile waters, but not in strongly polluted ones ($BOD_5 > 9.24$ mg/l). They were more numerous in water when the BOD_5 values of the water were lower and the oxygen content is higher. Thus *N. elinguis* is a very sensitive indicator of any slight pollution or even eutrophication of water. This species did not show any allegiance to specific types of habitat, since it is equally numerous in stony, sandy, and slimy bottoms.

Lochhead & Learner (1983) and Juget et al. (1989) investigated the effect of temperature on asexual population growth of *N. variabilis*, *N. elinguis* and *P. equisetata*. The mean population doubling time was about 3 days for *N. variabilis* and *P. aequiseta* at 20 °C and the lowest was 22 days for *P. aequiseta* at 8 °C. The values for *N. elinguis* were somewhat lower than expected probably due to less suitable experimental conditions.

Research on the growth of small aquatic oligochaetes such as *Aeolosoma hemprichi*, *Pristina longiseta* and *Nais variabilis* on activated sludge has especially been investigated by Japanese researchers. Temperature, pH and oxygen concentration in conventional domestic wastewater treatment systems are summarized in Table 2.

There has been very little recent research published by other authors. Kuniyasu et al. (1997) and Inamori et al. (1983) measured the effects of temperature, pH, phosphate buffer concentration, shake stress endurance and food concentration on the specific population growth rate of *A. hemprichi*, *P. longiseta* and *N. variabilis* (Table 3) and *Nais* sp., *A. hemprichi*, *Pristina* sp. and *Dero* sp. (Table 4), respectively, monoxenic cultivated on sterilized activated sludge. The relationship between the specific population growth rate (μ) and feed concentration was described according to the Monod formula, $\mu = \mu_{\max} \{S/(K_s + S)\}$. μ_{\max} = maximum specific population growth rate, K_s is the

saturation constant where $\mu = 0.5\mu_{\max}$ and S is the feed concentration. The activation energy was estimated with the Arrhenius formula, $\log \mu = -\Delta E/2.303RT + b$; where ΔE is the activation energy (cal/mole), R is the gas constant, T is the absolute temperature and b is a constant. The Arrhenius formula describes how physiological processes depend on temperature. The activation energy of *A. hemprichi* was lowest. This means that *A. hemprichi* was most uninfluenced by temperature. It was concluded that the feed concentration affected growth of the oligochaetes very much (Kuniyasu et al. 1997).

Table 2. Some abiotic parameters in conventional wastewater treatment systems

Zones in WWTPs	T (°C)	pH	Oxygen (mg/l)
Anaerobic	10–20	7–8	<0
Anoxic	10–20	7–8	0 < O ₂ <0.5
Oxic	10–20	7–8	0.5 < O ₂ <2

Table 3. Optimum growth parameters of *A. hemprichi*, *P. longiseta* and *N. variabilis* (after Kuniyasu et al., 1997)

Parameter	<i>A. hemprichi</i>	<i>P. longiseta</i>	<i>N. variabilis</i>
Phosphate buffer (M)	1/375	1/150	1/150
pH	5–9	5–9	5–9
Shake stress	High	Low	Low
K_s^* (mg/l)	120	100	120
Activation energy (cal mol ⁻¹)	12 500	18 500	18 000

*Growth on sterilized activated sludge.

Process conditions

In wastewater treatment plants the following parameters are involved: process design, organic loading rate, sludge and hydraulic retention time, type of electron acceptor (for instance oxygen, nitrate/nitrite or sulfur compounds), wastewater composition, sludge volume index (svi), oxygen concentration, pH and temperature. The svi is the volume in milliliters occupied by 1 g of a suspension after 30 min settling. SVI typically is used to monitor settling characteristics of activated sludge and other biological suspensions. Although svi is not supported theoretically, it is an integral part of routine process control.

The abiotic factors pH, temperature and oxygen concentration already are discussed in the previous section. This section focuses on the remaining parameters mentioned above.

The occurrence of the free-swimming species strongly depends on the process conditions of the

Table 4. Optimum growth parameters of *A. hemprichi*, *Nais* sp., *Pristina* sp. and *Dero* sp. (after Inamori et al., 1983)

Parameter	<i>A. hemprichi</i>	<i>Nais</i> sp.	<i>Pristina</i> sp.	<i>Dero</i> sp.
Temperature (°C)	33	25	30	n.d.
Specific population growth rate ¹ (μ) (d ⁻¹)	0.35–0.43	0.10–0.13	n.d.	n.d.
Phosphate buffer (M)	1/375	1/150	1/150	1/7500
pH	6–8	6–8	6–8	6–8
Shake stress	High	Low	Low	Nil
Max. pop. Density ² (number/ml) (batch culture)	100	60	60	n.d.
Max. pop. density (continuous culture)	1000	200	200	n.d.

¹Sludge concentration >500 mg/l.

²The maximal density is measured under favorable growth conditions.

plant. Elissen et al. (submitted for publication) monitored the presence of several free-swimming oligochaetes in 4 wastewater treatment plants and investigated the relationship between the amount of worms in the plant and the prevailing process conditions. There was no relationship between the temperature and the presence of the worms. The worms seemed to influence the sludge volume index positively. A statistical analysis to quantify the relationship between some selected operational parameters and the presence of the worms is now in progress.

Generally, free-swimming worms only occur in (ultra) low loaded systems with enough oxygen available for growth and reproduction. The sludge age has to be not too short in order to prevent wash out of the worms. Also other still obscure factors can determine whether or not worms are present in WWTPs (see Elissen et al., submitted for publication).

Another new appearance in wastewater treatment is the membrane bioreactor (MBR). In the MBR the separation of biomass and the liquor takes place in the membrane module instead of a settling tank. Boran Zhang and Kazuo Yamamoto studied the role of predators on sludge mineralization in a MBR (Thesis, 1997). Wei et al. (2003) compared the performances of a submerged MBR and a conventional activated sludge process (CAS) on sludge reduction induced by oligochaetes. Only two types of worms were found in the MBR, *Aeolosoma hemprichi* and *Nais elinguis*. Worm presence and absence alternated in the MBR. The worms in the CAS reactor maintained throughout the operating period. In the CAS reactor besides *A. hemprichi* and *N. elinguis*, also *Pristina aequisetata* was found. The latter only occasionally appeared in the reactor. The worm growth in the MBR did not lead to a lower sludge production but worm bloom in the CAS reactor greatly decreased sludge yield and improved sludge settling characteristics at high density. It appeared that *Nais* had more potential for sludge reduction than *Aeolosoma*.

A final type of WWTP which will 'become extinct' by tightened regulations, is the trickling filter, a low loaded activated sludge systems with carrier material to encourage bacterial and metazoan growth. Trickling filters are the oldest WWTPs. In 1893–1894, Joseph Corbett in Salford,

England developed the first trickling filter (Koot, 1980). Nowadays the trickling filters are more and more replaced by conventional activated sludge systems. Trickling filters examined in the United Kingdom contained *Eisenia foetida*. *E. foetida* is capable of rapidly converting large quantities of activated sludge in earthworm biomass (Hartenstein et al., 1984).

Vermiculture or vermicomposting

Vermiculture or vermicomposting involves the use of semi-aquatic earthworms as novel bioreactors for bioprocessing of agricultural and non-toxic city and industrial wastes for the production of biofertilizers and primary proteins. During the process organic material is fed to a variety of worm species for the purpose of converting the organic material into increased worm biomass and vermicast. The worm bodies represent a valuable source of protein that could be utilized for livestock (pig, fish and chickens) or even human consumption. Vermiculture is the latest aspect of technology, fast developing in advanced countries like the UK, the USA and Japan.

The use of vermiculture to stabilize sewage sludge and other organic wastes has been mooted for the past 50 years. Considerable work has been done on the stabilization process and value of the vermicast byproduct (Lotzof, 1999). Vermicast is worm excreta that can be used as a plant growth medium and soil conditioner. The biomass of the worms themselves has been sold for bait, animal feed and domestic and small composting systems.

For application of the worms several critical elements are mentioned (Lotzof, 1999):

- Preparation of the sludge before feeding it to the worms.
- Controlled application of the feed to the worm beds.
- Raised cage bed structures.
- Environmental control systems for managing moisture, temperature and wind.
- Worm biomass management.
- Harvesting of the vermicast.
- Post-processing of the vermicast to sale.
- Leachate control.

- Sampling and analyses systems for sludge and vermicast.
- Information management systems.

A variety of vermicomposting systems have been investigated in recent decades, treating both cakes and liquid sludge and results have proved promising. For maximum potential to be realized the worm beds should be carefully designed and operated (i.e. with due consideration to the temperature, moisture content, drainage and other physiochemical requirements of the worms). Careful selection of the worm species is also required. The epigeic worm *Eisenia foetida* has been the focus of much of the research done on using worms to treat sewage sludges (Clark, 1997).

According to Vigueros & Camperos (2002), a determinant factor in the survival of *Eisenia foetida* is humidity. The most adequate value for this parameter was in the range of 70–88%, which should be maintained throughout the process. The mixture with the greatest biomass production measured in numbers of cocoons per kilogram of vermicompost was that of 70% sewage sludge + 30% water hyacinth, for which its use is recommended, with the option of adding backyard waste. The reduction in the TVS/TS ratio pointed out the important role that worms play in the degradation of waste. Also Kaplan et al. (1980) mentioned a humidity percentage between 70 and 80% the sludge-conditioning material mixture should have to assure an adequate process and this humidity should be maintained during the entire process.

Athanasopoulou (1993) treated anaerobically stabilized effluents of dried vine fruit industry in earthworm filters. He used the species *Lumbricus rubellus*. COD removal was 95% for loadings of 0.10 and 0.15 kg COD/m²·d. The earthworm biomass seems to be in its upper bearing capacity of approximately 2 kg/m² and did not increase seriously with time.

Elvira et al. (1996) investigated the bioconversion of solid paper-pulp mill sludge by the epigeic earthworm *Eisenia andrei*. Regardless of the presence of earthworms, degradation occurred during the bioconversion period, but the presence of earthworms accelerated the mineralization of organic matter, favored the breakdown of structural polysaccharides and increased the humification

rate. Consequently, the C/N ration and the degree of extractability of heavy metals were lower in the worm-worked end product. The Bioverm worms are of terrestrial origin, but with some relatively small adaptations of the culture operation design, the positive experiences with vermiculture opens new perspectives for application of aquatic oligochaetes to be of usage for aerobic sludge stabilization.

Worm species such as *Allobophora chloritica* and *Dendrobaena venteta* also offer potential in sewage sludge systems (Clark, 1997). The African nightcrawler *Eudrilus eugeniae* can grow twice as fast as *E. foetida*. Once fully populated worms can eat between one half and their own body weight a day. To prevent deterioration of the worm activity composting of the material or anaerobic sites has to be circumvented. It is therefore essential that the quantity fed matches the daily quantity consumed by the worms.

Discussion and conclusion

This paper reviews the possibility to put forward a new sustainable technique for handling and reduction of the excess sludge through predation of the sludge by aquatic oligochaetes. Another benefit of this approach is the application of annelid biomass as a resource for protein production, fish feed, etc.

Only four scientific research groups have been performed research on oligochaetes in activated sludge: two Japanese groups (MBR), a Chinese group (MBR) and the Dutch group (sludge reduction). Incidentally other researchers are interested in this item but until now no articles have been published. There is evidence that also commercial groups are working on this item, but for policy reasons access to their results is not possible. The limited data available so far are sometimes incomplete, contradictory or difficult to interpret. Furthermore both the experimental conditions and the reactor configurations differ. Therefore future laboratory experiments should be set up according a general concept, which facilitates comparison between other experiments and scale up from laboratory to applied research.

Until now, the experiments have mainly been performed on small laboratory scale and relevant

data about oligochaetes is limited. Therefore oligochaete knowledge collected from field data or small-scale laboratory experiments is necessary in order to understand and to be able to predict the behavior of oligochaetes in WWTPs. Also deducible measures can be taken in order to guarantee optimal growth conditions for the oligochaetes.

In general, interpretation of data concerning oxygen demand at different sludge concentrations and temperatures is difficult because other factors like micro-turbulence and mechanical aeration determine the prevailing oxygen concentration during the experiments.

There is a wide variation in the estimates of specific sludge production in aerobic wastewater treatment processes, varying from 35 to 85 g dry solids per population equivalent per day. The total European sludge production is put at 6 million dry solid tonnes per annum (EC, 1991) and is expected to rise by the year 2006 to 10 million tonnes or even 17 million tonnes according to Saabye & Schwinning (1994). About 60% of the total operation costs is accounted for by the cost of sludge storage, transport, digestion and disposal. It is clear therefore that the major part of the cost in aerobic wastewater treatment is associated with the treatment and disposal of sewage sludge.

The benefits of sludge application in agriculture is limited by its potential health hazards, i.e., transmission of pathogenic organisms, accumulation of nitrates in the groundwater, soil and water contamination by eventual highly toxic organic constituents of the sludge, and accumulation of toxic heavy metals in soil, water and crops. The danger of transmission of heavy metals and pathogenic organisms appears to be the major element of concern.

Preliminary short time (7 days) experiments (unpublished data) with oligochaetes fed contaminated sludge have been showed the heavy metals remained in the sludge although the organic part of the sludge was completely digested by the worms. Whether or not heavy metals will accumulate in worm tissue depends on the heavy metal concentration in the sludge, the exposure time, the organic content of sludge and the worm species themselves.

Most annelid worms are able to grow on bacterial biomass. The worms tolerate temperatures between 2 and 8 °C. The optimum temperature for growth and reproduction ranges between 20 and

25 °C. The prevailing operating conditions in conventional wastewater treatment plants are thus suitable for the worms. However one has to prevent that species out compete themselves and that the food source does not become depleted.

Since small free-swimming oligochaetes are able to survive under conditions of less than 5 °C these oligochaetes would contribute to wastewater treatment in biological wastewater treatment plants except at extremely low temperatures. The application of these worms for reduction of bacterial biomass is, however, despite all research on this topic, hampered by the fact that this process is difficult to control.

Within Oligochaeta, asexual reproduction is a regular phenomenon in the lifecycles of Naidids and Aeolosomatids whereas it is of sporadic occurrence or is unknown within the other families. Only five species of Enchytraeids are known to have adopted this reproductive method (Christensen, 1973). In some species sexual reproduction was not correlated with density but with temperature. Sexually mature individuals are mainly recorded in the colder season, which coincides with the period when enchytraeid populations usually attain maximal densities. Finally one has to keep in mind that the factors responsible for sexual reproduction in naidids are different from those for example in *E. bigeminus*. Since reproductive data available in the literature still show conflicting results, the predation suitability of a certain type of sludge has to be measured under standardized conditions. Only if the worms are able to grow and reproduce (either sexually and/or asexually) on the sludge, application of these worms in sludge treatment may be technically feasible.

From an economical point of view the main item to solve is how to reduce the required area for the application of oligochaetes in WWTPs. Also it is necessary to design a reliable process that can cope with changes in food supply and environmental factors. Before using the worms for fish food, an environmental risk assessment for the public health should be carried out in order to allow consumption of the fish.

In conclusion, it is clear that the total amount of sludge can be reduced by the introduction of oligochaetes in WWTPs. For the free-swimming oligochaetes future research should focus on the factors that trigger the initiation of annelid blooms

in WWTPs and how to maintain a stable annelid population in WWTPs. Use of these worm species is not an option unless knowledge about these subjects becomes available.

Over recent years there has been a growing realization that the treatment and disposal of sludge presents a major challenge in wastewater management. Consequently rising sludge production and costs due to strengthened environmental standards demand a new approach of sludge treatment. Now, the challenge is to introduce a sustainable technology, which also yields a product that will satisfy defined quality standards. These facilities require a large investment of capital and to be cost effective they need to have long and useful lives. There is therefore great pressure in the wastewater management to maximize the returns on existing investments by improving and refurbishing existing capital plants to meet the new demands. The search after new sustainable technologies justifies an accelerated acceptance for the application of sludge predation by aquatic oligochaetes in the wastewater treatment process.

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