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MiniReview

Monitoring the community structure of wastewater treatment plants: a comparison of old and new techniques

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Abstract

Wastewater treatment is a process of increasing importance in a world with an ever growing human population. Today, most wastewater treatment processes make use of the natural self-purification capacity of aquatic environments which is the result of the presence and action of microbial communities. Consequently, wastewater treatment facilities are designed to maintain high densities and activities of those microorganisms that satisfy the various purification demands. The performance, at least of large plants, has to be constantly monitored and is subject to strict regulation. Nevertheless, malfunctions resulting in decreased purification efficacy are frequent. This has, over the decades, prompted many microbiologists to compare the structure, dynamics and function of these 'good' or 'bad', but always complex, microbial communities. Part of these studies was targeted to a basic understanding of the various processes, another part, however, deals with the monitoring of community structure as a means to direct the plant operation towards higher elimination rates and overall stability. Even though the last decade has seen a molecular revolution in microbiology, the standard methods for monitoring wastewater treatment plants still rely on the tools available to the researcher at the beginning of this century, the microscope and agar plates. It is the goal of this MiniReview to compare the potential of the newly available molecular methods with the old monitoring techniques.

Keywords: Wastewater treatment; Monitoring; Community structure; Microbial ecology; Nucleic acid probe; Microscopy

1. General remarks

In today's world with its increasing human population and the concomitant pollution problems there is an increasing awareness of the necessity for environmental protection. Our ability to control the pollution caused by human activities is, in the long run, crucial for the further development of mankind. Wastewater treatment is one of the basic processes in this regard. In former times, the natural ecosystems were, by the so-called self-purification, able to deal with the much lower levels of pollution. Microorganisms mineralized the waste and made it available again for primary production. Interestingly enough, most modern wastewater treatment processes still rely on the action of complex microbial communities. And even today these communities

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are not deliberately assembled from individual species with known functions, but they remain the result of natural selection. The art of building an efficient wastewater treatment facility is still to make the best use of the natural processes, by condensing them in space and time, or as Curds and Hawkes wrote in 1975 [1]: ''Used water treatment by biological oxidation plants may be regarded as the environmental control of the activity of the appropriate organisms''.

The group of organisms most directly involved in wastewater treatment are the bacteria. They dominate, both in numbers and biomass, all other groups and dominate the processes of mineralization and elimination of organic and inorganic nutrients. They are favored, in traditional high load plants that operate with short sludge retention times, by their low generation times. Modern low load systems have high retention times and also allow for the presence of more slowly growing bacteria and of organisms with a more complex organization such as flagellates, amoebae, ciliates or even worms and insect larvae. The protozoa and metazoa are able to feed on particulates, such as those coming in with the sewage or bacterial flocs. It is generally assumed that their primary role in the wastewater treatment is the clarification of the effluent.

The microbial communities catalyzing wastewater treatment have long been viewed as 'black boxes'. Today, even engineers agree that a thorough knowledge of the structure and function of these complex communities would be a good starting point for future plant optimization. However, with classical techniques a detailed analysis of the bacterial community structure has not been possible. Therefore, studies were not performed on those cells that catalyzed reactions of interest, but on so-called indicator organisms that were morphologically or phenotypically conspicuous and that could be linked, by their presence, to a good or bad plant performance.

2. Classical techniques for monitoring the microbial communities of wastewater treatment plants

The study of Smit in 1934 [2] in which he connects problems in sludge settling to the presence of high numbers of filamentous bacteria in the sludge is an early example of a type of wastewater treatment monitoring that has not changed a lot in the last six decades. Here, light microscopy was used in an attempt to visualize the cause of a particular problem. This is, in the case of sludge bulking, straightforward which is in most cases a phenomenon directly linked to the occurrence of filamentous microorganisms. Since then, the principle approach has not changed, but it has been considerably refined. Often filamentous bacteria have enough morphological detail like cell dimension, shape or length, form and branching of filaments that they can be classified in situ (for details see [3]). This enabled comparative studies on the presence or absence of specific filaments with regard to sewage composition and plant operation (e.g. [4–6]). Keys are now available that help in the identification of filaments by the combination of light microscopy and simple staining techniques such as those according to Gram and Neisser. However, it should always be kept in mind that morphology of all bacteria (including the filamentous types) is a quite unstable character and consequently is not a good foundation for a sound identification.

Furthermore, eukaryotic organisms are used to classify activated sludge. Ciliates, rotifers, nematodes and oligochaetes have enough morphological detail to be reliably identified (e.g. [7–9]). Even though these organisms are not responsible for the primary degradation activity of the wastewater communities they contribute to elimination of particulate matter. Moreover, they are often useful indicators for environmental conditions of the treatment process, e.g. ammonia-tolerant ciliates such as *Tetrahymena* indicate high load conditions with preliminary degradation efficacy, metazoa such as rotifers and nematodes indicate long sludge retention times, as found in sludge deposits or floated sludge fractions. Based on the presence or absence of indicator organisms countermeasures can be suggested in attempts to change the community structure and function and thereby the overall plant performance.

It has, however, to be noted that methods based on simple light microscopic observations do not allow the monitoring of those groups of bacteria that are responsible for processes such as nitrification, denitrification or enhanced biological phosphorus removal. Bacteria usually have very limited morphological diversity and this also applies to many of the

most abundant protozoa, e.g. the nanoflagellates and the small amoebae. Nevertheless, the examination of sludge by microscopy and with reference to classic works such as the Microscopic Sludge Investigation Manual by Eikelboom and van Buijsen [10] or the Manual on the Causes and Control of Activated Sludge Bulking and Foaming [11] is today a standard monitoring technique in many plants.

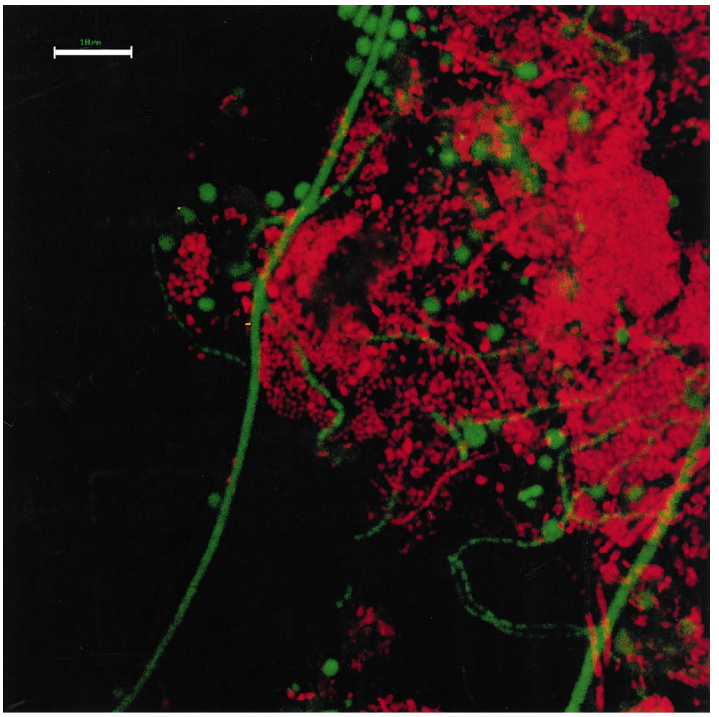
Another classical approach is based on the isolation of bacterial pure cultures, mostly by the agar plate technique, followed by the identification and, if necessary, physiological characterization of the respective strains. For those bacteria that lack a conspicuous morphology this has long been the only approach to assess their presence and number. Many of these cultivation studies have been performed, among some well known are those of Prakasam and Dondero [12], Benedict and Carlson [13] and Kavanaugh and Randall [14]. In the early studies [12,13], media with high carbon concentrations (>1 g C l^{-1}) were applied which select for fast growing bacteria. Typical examples of such r-strategy saprophytes are the aeromonads. It has long been realized [12] that by the use of one medium, even if it is called non-selective, no reasonably complete insight into the community structure can be obtained. Activated sludge contains a tremendous diversity of bacteria ranging from extremely slow growing ones (e.g. the chemolithoautotrophic nitrifiers) to those with a generation time of less than 30 min (e.g. *Aeromonas* sp.) and encompassing aerobes as well as facultative or obligate anaerobes. Many of these different bacteria can neither be grown nor enumerated on a rich medium like a plate count agar. Various studies have now shown that, even on optimized media, always less than 20% and usually somewhere between 10 and 1% of those bacteria visualized by microscopic methods form colonies [15,16]. This is due in part to the use of the 'wrong' media and cultivation conditions and in part to the clustering of cells in the activated sludge flocs. Even today there is a lack of good methods for converting activated sludge flocs or biofilms to suspensions of single cells prior to plating. Therefore, cultivation based methods are not suitable for exact quantification of the populations in wastewater treatment.

Scientists interested in quantification may today use direct, cultivation-independent methods. These direct methods have also the potential to alleviate one of the major problems in quantitative population analysis in activated sludge: with cultivation-based methods, even in their improved modern form (e.g. [17]). This problem is the definitive identification of a statistically significant number of colonies, which is very laborious and time-consuming. This does not mean, though, that there is no place for pure culture studies in future wastewater microbiology. The characterization of those bacteria, identified with other methods as important catalysts in the purification process, for their physiological potential, can only be performed on pure cultures, even in the age of molecular biology.

The old cultivation studies have shown that even when the early scientists isolated the right, important bacteria for a given plant, they often had problems in classification. This was the time of artificial, not evolutionarily based classification, and not the enlightened era that we have reached today, largely based on rRNA-based systematics. As a consequence, recent years have seen major changes in taxonomy, a process that will likely continue. Strains that were earlier lumped in the polyphyletic genus *Pseudomonas* are now recognized to be distributed over many species in several subclasses of the class Proteobacteria and most of the pseudomonads from environmental sources have been renamed (for a review see [18]).

3. Direct monitoring approaches based on chemotaxonomy

In the last decade, several attempts have been made to analyze the bacterial community structure of activated sludge by direct chemotaxonomic methods. Among these approaches were profiles of respiratory quinones [19], polyamine patterns [20] and phospholipid fatty acid patterns [21]. We will discuss the principles and potential pitfalls of these approaches by taking as an example the study of Auling and colleagues [20]. After prior analysis of reference strains, diaminopropane (DAP), the main polyamine compound produced by *Acinetobacter* spp., was used as a biomarker for this genus. High DAP content was found in low load plants with enhanced biological phosphorus elimination



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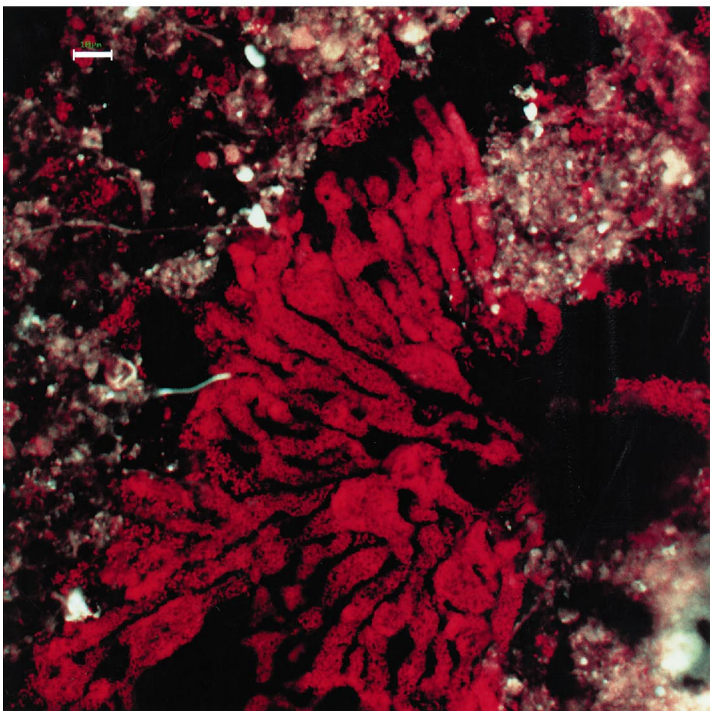

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Fig. 1. In situ hybridization of activated sludge from a large municipal plant (Munich-Großlappen; basin I) with fluorescently labeled, rRNA-targeted oligonucleotide probes targeting the beta- (Cy3-labeled probe BET42a; red) and gamma-subclass of Proteobacteria (fluorescein-labeled probe GAM42a; green). A fully equipped confocal laser scanning microscope (Zeiss LSM 410; Carl Zeiss, Jena, Germany) with a 100× oil immersion objective (NA 1.3) was used to create this all-in-focus image. Bar = 10 μm.

Fig. 2. In situ identification of the typical zoogloeal fingers in the high load plant of Kraftisried (for details see [33]) by hybridization with the Cy3-labeled probe ZRA targeting the neotype strain, *Z. ramigera* ATCC 19544. The all-in-focus confocal laser scanning image was recorded on a Zeiss LSM 410 with a 40× oil immersion objective (NA 1.25). Bar = 10 μm.
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(EBPR) and low DAP content in plants with high organic loading. Assuming that DAP is the main polyamine not just of the strains investigated, but also of all members of the genus *Acinetobacter*, a low DAP concentration in the sludge indeed demonstrates that *Acinetobacter* spp. can not be a major component of the total community. In contrast, the interpretation of high DAP levels is not straightforward. DAP might be present in various other bacterial species which have not so far been studied for their polyamine patterns, e.g. due to problems in cultivation. Recently, DAP could, for example, be detected in aeromonads (Kämpfer, personal communication) and *Aquaspirillum dispar* [22]. This might explain the high DAP content present in EBPR plants for which a relatively low in situ abundance of *Acinetobacter* spp. could be demonstrated by immunofluorescence [23] and rRNA-targeted probes [24].

Another general disadvantage of chemotaxonomy-based monitoring approaches is the fact that they are often compromised by varying cellular concentrations of the indicator compounds during the cell cycle or due to changes in the environment. Therefore, it will be almost impossible to calculate absolute cell numbers from compound concentrations. It has, however, been pointed out that this type of monitoring is quick, inexpensive and applicable to large numbers of samples. It might be a good tool to follow populations in environments that are well understood in terms of overall diversity.

4. Identification of non-conspicuous bacteria by immunofluorescence

Early attempts for specific in situ identification of individual microbial cells in wastewater treatment plants were based on immunofluorescence. This technique was successfully used on various wastewater bacteria, a study on ammonia-oxidizing bacteria is discussed here [25]. Detection of this group of slow-growing bacteria by standard cultivation methods requires incubation times of at least one month. Detection and enumeration of two serotypes of *Nitrosomonas* sp. by immunofluorescent labeling followed by flow cytometric analysis required just several hours. However, even though this approach can be directly applied to the complex sample, it still requires prior isolation of the organism of interest to produce the antibodies. Other problems are limited diffusion of the antibody probes through EPS layers [26] and the potential for antigenic variance in the target groups.

5. rRNA-based monitoring techniques

Today, the rRNA approach is a powerful tool to analyze, in a truly cultivation-independent way, the structure of microbial communities [27]. There have been two types of rRNA-based studies in wastewater treatment in the last few years. Group- and genus-specific oligonucleotide probes were used to analyze directly the community structure of activated sludge by in situ hybridization ([16,28]; see also Figs. 1 and 2). Usually more than 75% of the bacteria stained with the general DNA stain DAPI also hybridized with a bacterial probe. This also proves a high cellular rRNA content which strongly suggests viability and activity of the majority of cells in wastewater treatment plants. The use of phylum- and subclass-specific probes, as shown in Fig. 1, for simultaneous hybridization with probes for the beta- and gamma-subclass of Proteobacteria, generally indicated dominance of the beta-subclass Proteobacteria [16].

Table 1
Occurrence of protozoa and metazoa in activated sludge plants in Germany

Group	Species	Frequency
Protozoa		
Flagellates	*Anthophysa* sp.	common
	Bodo sp.	frequent
	Cercomonas sp.	frequent
	Euglena sp.	common
	Hexamita sp.	widespread
	Peranema sp.	frequent
	Trepomonas agilis	widespread
	Trepomonas rotans	widespread
	Trigonomonas compressa	rare
	Rhynchomonas nasuta	common
Amoebae		frequent
testate amoebae	*Arcella* sp.	frequent
	Euglypha sp.	frequent
	Centropyxis sp.	common
Ciliates/Suctoria		
Cyrtophorida	*Chilodonella uncinata*	frequent
	Odontochlamys alpestris	frequent
	Pseudochilodonopsis fluviatilis	common
	Trithigmostoma cucullulus	frequent
	Trithigmostoma steini	widespread
	Thigmogaster oppositevacuolatus	frequent
	Trochilia minuta	common
Gymnostomatida	*Paradileptus* sp.	rare
	Spathidium sp.	rare
Heterotrichida	*Metopus* sp.	rare
	Spirostomum caudatum	rare
	Spirostomum teres	rare
	Spirostomum minus	rare
	Stentor coeruleus	rare
Hymenostomata	*Cyclidium glaucoma*	frequent
	Cyclidium heptatrichum	rare
	Calyptotricha lanuginosa	rare
	Cinetochilum margaritaceum	common
	Pseudocohnilembus pusillus	widespread
	Dexiostoma campylum	rare
	Glaucoma scintillans	rare
	Paramecium bursaria	widespread
	Paramecium aurelia complex	rare
	Paramecium caudatum	rare
	Uronema nigricans	rare
Hypotrichia	*Aspidisca cicada*	frequent
	Aspidisca lynceus	frequent
	Aspidisca turrita	widespread
	Euplotes affinis	frequent
	Euplotes aediculatus	common
	Tachysoma pellionellum	common
	Oxytricha sp.	common
Nassulida	*Drepanomonas revoluta*	widespread
	Microthorax pusillus	rare
Peritrichia	*Carchesium polypinum*	frequent
	Epistylis chrysemydis	widespread
	Epistylis entzii	frequent
	Epistylis plicatilis	frequent
	Opercularia articulata	frequent
	Opercularia coarctata	common
	Opercularia nutans	widespread
	Pseudovorticella monilata	widespread
	Thuricola kellicottiana	rare
	Vorticella convallaria complex	frequent
	Vorticella aquadulcis complex	frequent
	Vorticella campanula	widespread
	Vorticella infusionum complex	widespread
	Vorticella octava complex	widespread
	Vorticella microstoma complex	rare
	Vorticella picta	rare
Pleurostomatida	*Amphileptus claparedii*	widespread
	Amphileptus punctatus	common
	Amphileptus pleurosigma	rare
	Acineria incurvata	rare
	Acineria uncinata	frequent
	Litonotus alpestris	widespread
	Litonotus cygnus	rare
	Litonotus fusidens	common
	Litonotus lamella	common
	Litonotus crystallinus	widespread
Prostomatida	*Coleps hirtus*	common
	Holophrya discolor	common
	Plagiocampa rouxi	widespread
Suctoria	*Acineta tuberosa*	common
	Tokophrya infusionum	rare
	Tokophrya lemnarum	common
	Tokophrya quadripartita	common
	Podophrya sp.	widespread
	Dendrosoma sp.	rare
	Trichophrya sp.	rare
Metazoa		
nemathelminthes		
rotifera	*Cephalodella* sp.	frequent
	Rotaria rotatoria	frequent
	Philodina sp.	widespread
	Proales sp.	widespread
nematodes		frequent
gastrotrichs		widespread
tardigrades		common
plathelminthes		
turbellaria		rare
annelids	*Aelosoma hemprichi*	rare
oligochaetes	*Nais* sp.	rare

Summarized are results of a 2-year survey (1995/1996) of 85 wastewater treatment plants (Lind, personal communication).

Whereas members of this group were underestimated by cultivation techniques, members of the gamma-subclass of Proteobacteria, most notably the genera *Acinetobacter* [24] and *Aeromonas* [17], were overestimated. Manz et al. [28] used the group-specific probes to demonstrate differences in the community structure between municipal and industrial activated sludge plants. The probes used in these and other studies were still based mainly on sequences of cultured bacteria.

Other scientists have used direct rRNA sequence retrieval and subsequent comparative data analysis of 16S rDNA clone libraries to analyze the microbial diversity present in activated sludge [29,30]. Whereas Schuppler et al. [30] focused on clones from the group of Gram-positive Bacteria with a high DNA G+C content, Bond et al. [29] partially sequenced 100 statistically selected clones each of two laboratory-scale sequencing batch reactors with strong differences in their phosphate-removing capabilities. Most clones were affiliated with the beta-subclass of Proteobacteria and only few *Acinetobacter* clones were found. Both these results were in agreement with the earlier results of oligonucleotide probing [16,24]. Bond et al. [29] tried to use the frequencies of the clones for a comparison of the community structures of the two reactors. The clone library from the reactor with the higher phosphate removal contained more clones related to the genus *Rhodocyclus* within the beta1-subgroup of Proteobacteria. However, it has been pointed out before that for several reasons [27] clone frequencies should not be regarded as a valid means to estimate abundances of certain populations. Clearly, the combination of the two different rRNA-based techniques, direct rRNA sequence analysis followed by hybridization with probes based on the retrieved sequences, would be the best. However, this is also a very laborious and time-consuming way to characterize the community structure of activated sludge at high resolution. Two such studies were performed recently for the first stage of a municipal wastewater treatment facility receiving a high load of organic waste. In the first, simultaneous in situ visualization of up to seven distinct beta-proteobacterial genotypes corroborated that the high diversity found in the respective clone library was indeed present in the complex activated sludge community [31]. The second study resulted in interesting and surprising findings such as members of the genus *Arcobacter* (formerly thermophilic campylobacters), which are epsilon-subclass Proteobacteria, to form quite abundant populations (4% of total cell counts) in activated sludge [32].

One important advantage of all rRNA-based hybridization techniques is that they are automatically linked to modern bacterial systematics. A good example is the work of Rossello-Mora and colleagues [33] on a famous wastewater bacterium, *Zoogloea ramigera*. Most strains once designated as *Z. ramigera* have nothing in common with the neotype strain, *Z. ramigera* ATCC 19544. By probing various activated sludge samples with rRNA-targeted oligonucleotide probes specific for the type strain and two misclassified *Z. ramigera* strains it was demonstrated that only populations related to the neotype strain make up a significant fraction above 1% and, in the more highly loaded stages sometimes even constitute more than 10% of the total community. The neotype-specific probe was also the one that visualized the typical zoogloeal finger-like structures (Fig. 2). Today, many other bacterial groups important for wastewater treatment can be tracked by in situ hybridization. For example, probe sets are available for nitrifying bacteria [34–36] and for filamentous bacteria causing bulking and foaming [37–39].

The monitoring of specific populations in wastewater treatment plants by fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes can be compromised by high background fluorescence, low cellular rRNA content, or limited probe permeability of target cells. These problems and approaches to solve them have been discussed in detail before [27].

Attempts have been made to combine fluorescent oligonucleotide probing with advanced microscopic techniques such as confocal laser scanning microscopy for analyzing the spatial distribution of specifically labeled target cells within activated sludge flocs and trickling filter biofilms [40]. Using this approach the 3-D arrangements of Gram-negative filamentous bacteria [37], ammonia-oxidizing bacteria [34] and *Paracoccus* sp. [41] were studied. Schramm and colleagues [42] have combined in situ localization of *Nitrosomonas* spp. and *Nitrobacter* spp. with in situ measurements of concentrations of O_2, NO_2^-/NO_3^-, and N_2O using microsensors. They found a good

match between structure and function, the distribution of the nitrifying bacteria and the zone of nitrification. Such studies are intended to increase our understanding of the various wastewater treatment processes. They are, however, too complex to become routine methods in the monitoring of activated sludge plants in the near future.

The dot blot hybridization technique for quantification of specific rRNA signature sequences [43] is yet another modern molecular technique that has recently been applied to the monitoring of specific populations in anaerobic wastewater treatment [44]. Here, radioactively labeled, rRNA-targeted oligonucleotides are applied to quantify the relative abundance of a specific rRNA compared to the total rRNA pool quantified by a universal probe. The advantages and disadvantages of this approach are similar to those discussed for the chemotaxonomic approach. Dot blot hybridization allows rapid examination of multiple samples in a short time. However, the cellular rRNA content is not stable and therefore it is difficult to extrapolate from relative fractions to absolute cell numbers. In the case of rRNA the cellular content is linked to growth rate for many bacteria and the relative abundance of a specific rRNA might consequently be a good measurement for the metabolic activity of the respective population. Based on dot-blot hybridization alone, it is, however, impossible to attribute changes to fluctuations in cell number or cellular rRNA content.

6. Other hybridization techniques and nucleic acid based patterns

In contrast to in situ hybridization, dot blot hybridization techniques are already applicable today to the quantification of nucleic acids other than rRNA. Whereas rRNA based techniques allow only indirect conclusions on the function of a population the detection of a functional gene representative of a defined physiological activity (or its mRNA) offers more direct evidence for the presence or abundance of a certain physiological group of microorganisms and its potential activity. Along these lines the group of van der Meer (EAWAG, Switzerland) recently did a thorough study on a continuous culture of *Paracoccus denitrificans*. Induction and repression of denitrification activity were studied in a chemostat during changes from aerobic to anaerobic growth conditions [45]. The denitrification activity was monitored by measuring the formation of denitrification products, individual mRNA levels for the nitrate, nitrite, and nitrous oxide reductases, and the concentration of the nitrite reductase enzyme was assessed with antibodies. On a change from aerobic to anaerobic respiration, mRNA formation was induced and raised 15- to 45-fold. However, this increase was transient and the mRNA levels declined within a few hours to levels only slightly higher than that under aerobic steady-state conditions. Interestingly, the level of nitrite reductase protein increased slowly during the anaerobic period, reaching a stable value about 30 h after the switch. Upon a switch back to aerobic conditions the denitrification stopped immediately, whereas the levels of mRNA returned only slowly to their uninduced levels. The nitrite reductase was not actively degraded and its decline followed the washout curve. These results also clearly show that the screening of functional genes or mRNAs alone is not a straightforward means to determine in situ activities. Combined studies that use a suite of methods to correlate functional and structural parameters of wastewater communities are at this time the most promising approach to gain a more thorough understanding of the important processes in sewage treatment, such as nitrification, denitrification and phosphorus removal.

Last but not least, new techniques should be mentioned here that also have considerable potential for monitoring population dynamics in complex microbial communities. They essentially all work on nucleic acids extracted from the environment and convert – in the ideal case – every organism to one clearly distinguishable band in a pattern. As soon as the population behind a particular pattern is identified (e.g. by sequencing the band once), these techniques allow rapid monitoring of multiple samples. The techniques may target rRNA as well as functional genes. For its sensitivity and speed many of these techniques are based on PCR [46]. They can assess differences in the length of restriction fragments of PCR products (e.g. ARDRA [47]) or even discriminate fragments of identical length but varying sequence by techniques such as DGGE (denaturing gradient gel electrophoresis [48]).

7. Conclusions

In the last years considerable progress in the analysis of the bacterial part of the biocenoses catalyzing wastewater treatment has been achieved with new molecular techniques. However, if we really want to understand the structure and function of modern wastewater treatment plants it is important to realize that these are not any longer the relatively simple high load systems applied to eliminate carbonaceous substrate. Today, in the various wastewater treatment plants microbial communities experience, at certain time intervals, anoxic and/or anaerobic periods. It could be that the more complex modes of operation in the various types of plant have also resulted in a higher diversity of community structure. Furthermore, prolonged biomass retention times allow the development of more slowly growing protozoa and even metazoa. Table 1 gives an overview of the different species that have been identified as regular inhabitants of activated sludge plants in Germany (Lind, personal communication). Even though protozoa and metazoa already serve as indicator organisms we are far from an understanding of their impact on community structure. Intuitively, most microbiologists and engineers dealing with the 'living part of wastewater treatment' still regard the bacterial community structure as mainly resulting from the physico-chemical environment, and neglect the potential impact of grazing. In the field of microbial ecology the discussion on the relative impact of 'bottom-up' (e.g. nutrient availability) vs. 'top-down' (e.g. grazing by protozoa) regulation on the actual community structure has been most fruitful. It is overdue that environmental biotechnologists start to consider the potential effects of microbial food webs. Along these lines, sludge scumming, a nuisance observed in many of the modern nutrient removal plants and frequently linked to high abundance of hydrophobic, Gram-positive filamentous bacteria, might be explained in the future by top-down regulation, resulting from the formation of grazing resistant forms. From lake systems it is well known that protozoan and metazoan grazing can cause shifts in bacterial community structures towards filamentous forms [49]. The integration of higher trophic levels into our models of wastewater treatment might indeed be the key to a better understanding of structure-function correlations. Molecular techniques, such as those described in this MiniReview for studying bacteria, might also be highly useful for the enumeration and identification at least of the smaller protozoa [50]. A thorough description of the microbial community – including the Eucarya in addition to the Bacteria – could help in the future to elucidate adaptation and selection mechanisms in the various trophic levels. This type of basic biological understanding could indeed support the theory and practice of wastewater treatment.

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