A review of the role of *Nocardia*-like filaments in activated sludge foaming

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Abstract

The common problem of biological foaming associated with high concentrations of filamentous *Nocardia*-like actinomycetes in activated sludge plants is reviewed. These bacteria appear to be hydrophobic in nature, concentrating at the air/water interface. They are able to metabolise lipids and hydrocarbons which are not readily available to many other bacteria. Foaming control techniques include reduction of MLSS and aeration rate, selection against *Nocardia*-like filaments by increased F/M and decreased solids retention time, and elimination of concentrated filaments in recycle streams from solids handling facilities.

Introduction

In activated sludge systems treating domestic waste water, organic matter removal in the aeration tank usually does not present a problem. Common and serious process problems occur during separation of mixed liquor suspended solids (MLSS) from treated waste water within the final settling tanks. One major such problem is sludge bulking, predominantly caused by excessive growth of filamentous bacteria (Pipes, 1978a, Sezgin *et al.*, 1978; Palm *et al.*, 1980; Strom and Jenkins, 1984; Jenkins *et al.*, 1985), which results in slow settling and poor thickening of the sludge. A second common problem is associated with sludge foaming in both the aeration and final settling tanks (Fig. 1).

Unlike the detergent-type white foam commonly observed in treatment plants in the 1950's (Wells and Garrett, 1971), almost all present day foaming problems are associated with a biological foam that is highly viscous and stable. In most cases, high concentrations of filamentous actinomycetes usually assigned to the genus *Nocardia* (Lechevalier, 1975; Pipes, 1978b, Dhaliwal, 1979; Nelson, 1979; Fleissner and Foes, 1980; Nelson and Puntenney, 1983; Sezgin and Karr, 1984; Awong *et al.*, 1985; Ward, 1986; Richard, 1986; Lemmer, 1986; Pretorius and Laubscher, 1987), or filamentous *Microthrix parvicella* (Jenkins *et al.*, 1985; Daigger *et al.*, 1985; Richard, 1986; Pretorius and Laubscher, 1987) are found in the foam, although other filamentous bacteria may be abundant (Hart, 1985). Although systematically collected data are lacking, the solids concentration and lipid content in the foam are high, e.g. 4 to 6,5% and 16 to 23% (based on mass per volume), respectively, on samples from two plants (unpublished data).

In addition to the operational problems in removing sludge foams and possible increases in effluent solids (Dhaliwal, 1979), accumulation in aeration tanks also presents a sludge inventory control problem. For example, in a typical 4,5 m deep aeration tank with a MLSS concentration of 3 000 mg/ℓ, the solids in the sludge foam would account for approximately 22% of the total biological mass, if a conservative foam solids concentration of 5% and 7,5 cm foam thickness is assumed. Normally, solids in the foam are not included in calculating the total biomass in the system. On the other hand, some types of substrate are available for bacterial utilisation within the foam. This is supported by a recent study that activity levels of actinomycete foams are comparable to those of activated sludge (Awong *et al.*, 1985). Consequently, one cannot neglect the high quantity of biomass present in the sludge foam in terms of food to microorganisms ratio (F/M). Likewise, the solids retention time of the foam may be much greater than that of the MLSS. Foams may also cause unsightly and sometimes odor-producing accumulations of solids on exposed surfaces (Dhaliwal, 1979).

Another potential problem sometimes mentioned is a possible health concern from aerosols formed by air bubbles bursting at the foam surface. The most common microorganism in foam, *Nocardia amarae,* is non-pathogenic, but *N. asteroides* and *N. caviae,* which may sometimes be pathogenic, are also found occasionally (Lechevalier, 1975). However, none of the 11 strains isolated from sludge that Lechevalier (1975) had tested was found to be pathogenic.

Occurrence of sludge foaming has been reported to be associated with several, sometimes conflicting, factors, including longer solids retention time (Pipes, 1978b, Fleissner and Foes, 1980), low (Graham, 1985; Ward, 1986; Richard, 1986) or high temperature (Pipes, 1978b, Richard, 1986), pure oxygen aeration (Lechevalier, 1975; Nelson and Puntenney, 1983), and low F/M ratios or high MLSS (Wells and Garrett, 1971). Some of these relationships have been disputed (Dhaliwal, 1979). It appears that there are no generally accepted conditions for the development of sludge foaming problems.

As previously stated, *Nocardia*-like actinomycetes or *M. parvicella* are usually associated with sludge foaming. This paper, however, restricts the review to the role of the *Nocardia*-like organisms in sludge foaming. Other less closely related actinomycetes (Lechevalier, 1975) or other filaments (Hart, 1985) have been found in foam, but seldom in the absence of the *Nocardia*-like organisms. On the other hand, many of the recognised species of *Nocardia* have never been reported as present in activated sludge. It is also possible for the *Nocardia* foaming organisms to be present in the absence of foam (Pipes, 1978b; Strom and Jenkins, 1984).

Biological foam containing *Nocardia* as a predominant organism was first reported in a 1969 article called Milwaukee Mystery (Anonymous, 1969). Research on the microbiology of foam samples was initiated after R. Lewis of USEPA noted that foam samples contained a profusion of actinomycete hyphae (Lechevalier, 1975). Lechevalier (1975) demonstrated that sludge foam was associated with several species which he assigned to the genus *Nocardia,* and that *Nocardia* may indeed cause foam production. The most predominant *Nocardia* was a new species,

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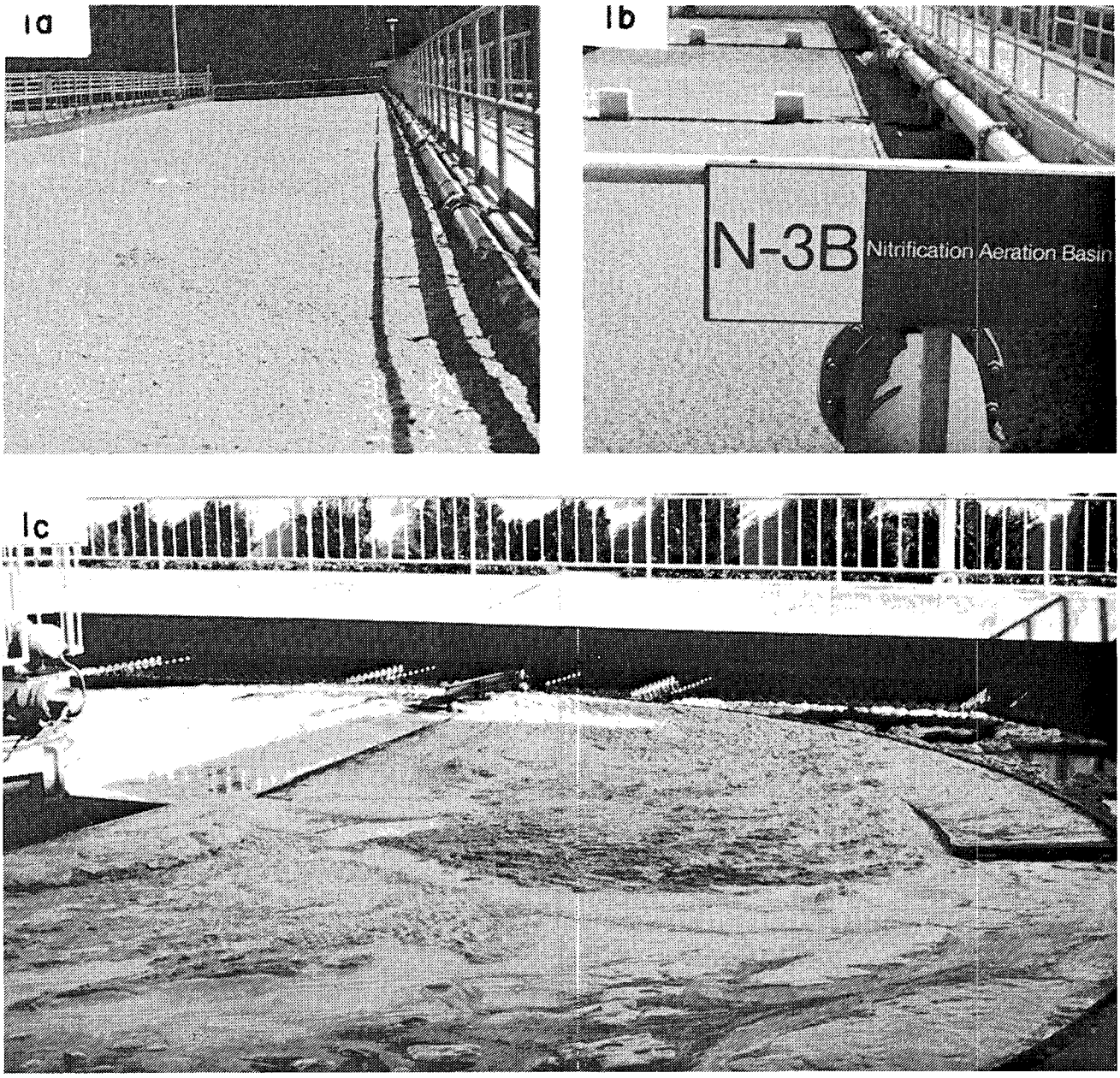

Figure 1
Activated sludge foaming

Figure 1a
Aeration tank

Figure 1b
Nitrification basin

Figure 1c
Final clarifier

which was named *N. amarae* (Lechevalier and Lechevalier, 1974). Furthermore, the concentration of nocardomycolic acid, a specific constituent of *Nocardia* cell walls, was directly related to the extent of foam observed in four waste-water treatment plants (Lechevalier *et al.*, 1976; 1977). Subsequent observation by various investigators all more or less confirmed the predominance of *Nocardia* in foam samples. The *Nocardia* enrichment factor in foam, defined as the ratio of the number of bacteria per mℓ in the surface layer to that in the subsurface water, is tenfold or more (Richard, 1986).

The taxonomy of *Nocardia* is still somewhat uncertain. *N. rhodochrous,* for example, has been called *Mycobacterium rhodochrous* (Lechevalier, 1975) or *Rhodococcus rhodochrous* (Goodfellow and Alderson, 1977). Dr. Ruth E. Gordon, American Type Culture Collection, has previously indicated her belief that some of these strains are intermediates between several closely related genera (Gordon, 1980). The eventual assignment of all these related strains from activated sludge foam to one or more genera is not yet resolved.

In this paper the role of these *Nocardia*-like bacteria in relation to sludge foam is reviewed and analysed. Because of the apparent relationship of these organisms to *Nocardia*, characteristics

of this genus are also examined. Control methods and research needs are identified.

Nocardia morphology and characteristics

Members of the genus *Nocardia* are generally filamentous heterotrophic aerobic gram positive organisms that exhibit true branching (Fig. 2), as compared to the false branching of *Sphaerotilus natans*. Sudan B Black staining indicated the presence of poly-≈-hydroxybutyrate in a laboratory-grown *N. amarae* strain isolated from activated sludge. In activated sludge the individual cells in the filaments ("crosswalls") usually are not visible. Older filaments may break up into smaller fragments (arthrospores).

Nocardia utilise and decompose a wide variety of hydrocarbons (Raymond and Jamison, 1971). *Nocardia* metabolise alkanes with relatively long chains more efficiently than those shorter chains (Tarnok, 1976). The lipid content of a *Nocardia* isolate was as high as 70 % of the total cell weight when n-hexadecane or n-octadecane was the carbon and energy source (Raymond and Davis, 1960). Raymond and Jamison (1971) reported a lipid content between 9 to 24 %. Some *Nocardia* can degrade Venezuelan crude oil, heavy fuel oil, and hexadecane at temperatures as low as 5 °C (Mulkins-Phillips and Stewart, 1974), and grow on paraffin wax (Tarnok, 1976). Furthermore, the chain length of fatty acids incorporated into the glyceride and wax compounds of nocardial cells reflects the length of the starting substrates of alkane substances (Davis, 1964). In general, the lipid composition is influenced quantitatively and qualitatively by the growth conditions, carbon source, and the species studied (Ioneda and Silva, 1978). Surface active substances (e.g. Tween 40 or Tween 60) may also provide a fatty acid component as a precursor for biosynthesis of surface cell wall lipids (Schömer and Wagner 1980).

The implication of the degradation of some hydrocarbons, and oil and grease by *Nocardia* is that little substrate limitation for *Nocardia* in activated sludge aeration tanks exists, since grease constitutes the most abundant single component in domestic waste water (Heukelekian, 1943). Consequently, *Nocardia* species may be ecologically important in degrading the lipid/hydrocarbon constituents of waste water.

It has been reported that biosurfactants are produced from a *Nocardia* species grown on hydrocarbons (Margaritis *et al.*, 1979; MacDonald *et al.*, 1981). The surface-active materials produced stimulate the emulsification of lipid/hydrocarbon substrates. Cairns *et al.* (1982) reported that *N. amarae* induced coalescence of emulsions. The de-emulsifying activity by *N. amarae* varied with the type of growth medium, culture age and post-harvest treatment. Furthermore, the de-emulsifying properties are due to the bacterial cell surface which perhaps is related to its hydrophobic characteristics.

Nocardia/foaming relationship

In general, microorganisms that utilise long-chain hydrocarbons as carbon/energy sources tend to have a hydrophobic surface (Hatch, 1983). *Nocardia* cells are highly hydrophobic. When air is injected through a reactor containing a pure culture of *Nocardia*, cells tend to float on the air/water interface within a short period (unpublished data). The attachment of *Nocardia* to air bubbles that are subsequently carried to the air/water interface is a further indication of the high degree of hydrophobicity. Dahlbäck *et al.* (1981) reported that there is a positive correlation between the degree of enrichment of bacteria at the surface along the Swedish west coast and their hydrophobicity. Thus, the high degree of hydrophobicity of *Nocardia*-like cells may play a dominant role in their accumulation in the interfacial area.

In activated sludge, MLSS-containing *Nocardia*-like filaments are also carried to the water surface during aeration. Once the *Nocardia*-like cells enter the air/water interfacial area, they tend to stay there. In many plants, the MLSS flows through a subsurface outlet to the final clarifiers. Under these conditions, the concentrated *Nocardia*-like bacteria in the foam can accumulate on the surface of the aeration tank. Coupled with the loss of foam film water through evaporation, this gradually increases the solids concentration of the foam. Finally, a stable and rigid foam is produced, resulting in a separation of two phases — the MLSS phase (liquid) and the foam phase (semi-solid). The stability of *Nocardia*-like foam is also due to other physical,

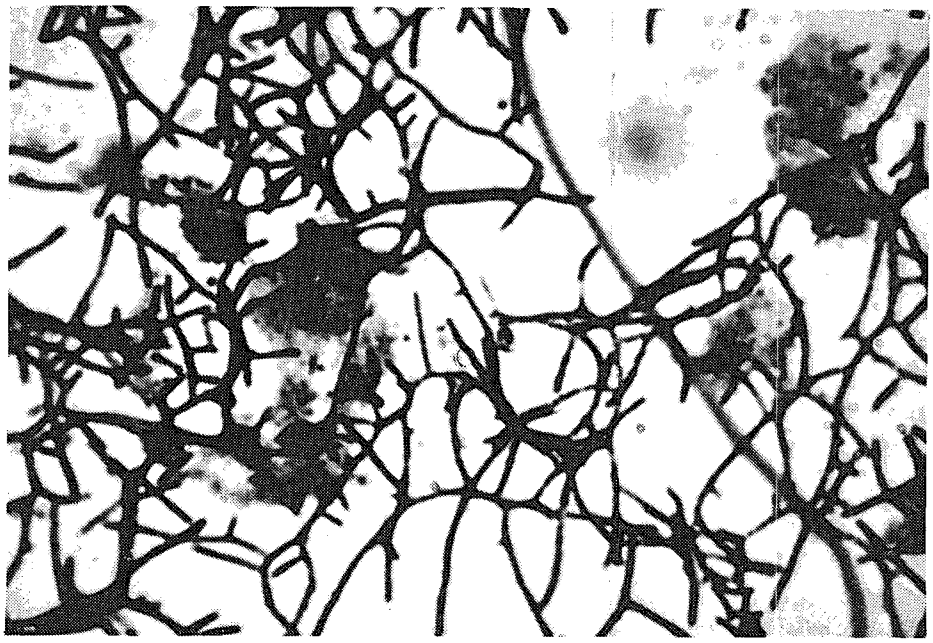

Figure 2
Nocardia *in activated sludge foaming*

chemical and biological factors as discussed later. The foam environment in activated sludge also appears to provide a niche for the growth of *Nocardia*-like organisms.

The phenomenon of bacteria floating on the air/water interface under aeration was first reported by Dognon in 1941. He found the bacillus was easily removed from suspension by foaming, while other bacterial species were not concentrated on foam unless electrolytes were used. Boyles and Lincoln (1958) stated that only cells with hydrophobic surfaces could be collected on foam. The concentration of microorganisms by flotation from culture media and separation of the desirable species from mixed culture suspensions has been studied by several investigators (Gaudin *et al.*, 1962a, 1962b; Rubin *et al.*, 1966; Grieves and Wang, 1966; Rubin, 1968; Viehweg and Schügerl, 1983), with the addition of salts (e.g. NaCl), surface active substances (e.g. a cationic surfactant), or a variety of collectors (e.g. long-chain fatty acids or amines). For example, *A. aerogenes* was removed in a foam using both lauric acid and laurylamine, and its removal efficiency was pH dependent (Rubin, 1968). Wozniak *et al.* (1976) studied the transfer of *E. coli* from the water to the air with different air bubble sizes. The enrichment factor decreased with anincreased bubble droplet size.

A variety of colloidal and dissolved materials tend to concentrate on the surface film (Lemmer, 1986). These surface-active substances produced by microorganisms or initially present in the waste water may serve three functions in foam production and stability. First, they may serve as "collector" agents by enhancing the accumulation of MLSS-containing *Nocardia*-like cells at the water/air interface. The lateral cohesive forces between the hydrocarbon chains of these surfactants and other organic compounds have a strong influence on the microbial interaction (Norkrans, 1980). Secondly, these substances may form viscous films, even when MLSS viscosity is low. Thirdly, these substances are likely to be good carbon and energy sources for *Nocardia* (Lemmer, 1986). Other water insoluble substances (e.g. oil and grease) and other organic matter that is concentrated in the interfacial area also might serve as substrates. In fact, the presence of a large grease and fat component in the waste water has been hypothesised to account for foaming observations (Ligthelm, 1986; Richard, 1986). It was also reported that bacteria at the interface could use lower concentrations of nutrients for growth than bacteria in the bulk media (Kjelleberg *et al.*, 1980; Hermansson and Dahlbäck, 1983).

Other physical parameters, such as the air flow (or mechanical mixing) rate, size of air bubbles, and temperature (through effect on evaporation and growth rate) may all influence the concentration of *Nocardia*-like organisms in foam. Aeration tremendously increases the accumulation of surface-active material. This phenomenon contributes partially to the stability of the foam system. Some plants are able to control foaming by reducing aeration, thus reducing the chance for *Nocardia* to float to the top and form a stable foam.

Foaming problems were severe in a pure oxygen activated sludge plant while there was no foaming problem in a side-by-side air activated sludge system (Nelson, 1979; Nelson and Puntenney, 1983). It should be noted that no evidence has been presented that oxygen plants are generally more prone to foaming. In fact, no such correlation was noted in one extensive survey of filamentous organisms in activated sludge (Strom and Jenkins, 1984). In the particular case cited, among other possible differences, the waste water directed to the two plants received separate primary treatment, perhaps resulting in different oil and grease removals.

Other physical/chemical factors, in addition to the solids concentration, also may affect the stability of sludge foam. Electrostatic reactions are involved in the interaction of negatively charged bacteria with charges on organics that are also concentrated at the interface. Since pH affects the charge on these molecules, it could influence this interaction. *Nocardia*-like organisms, as living colloids, capable of growth and metabolism, may enhance the attraction and adhesion of biomass to interfaces. Other factors, such as viscosity and interfacial tension, also affect foam stability. Organisms surviving and reproducing at the air/water interface are exposed to higher amounts of UV radiation and wider fluctuations in temperature.

Nocardia-like organisms have been found to be a predominant species in MLSS samples from activated sludge plants (Strom and Jenkins, 1984; Jenkins *et al.*, 1985; Richard, 1986), including a number of plants where sludge foaming was not a problem. *Nocardia* are commonly found in soil but rarely in water, except in sediments or at interfaces (Cross *et al.*, 1976; Al-Diwany and Cross, 1978).

The following summary of the foaming problem is offered on the basis of the preceding discussion, while recognising that other unidentified factors may be involved. Non-aqueous surface phases provide loci for *Nocardia*-like bacteria, and substrates concentrated in such environments provide nutrients for the growth of these organisms. *Nocardia*-like cells in the foam may have less competition and a much longer retention time than organisms in the MLSS. Thus, they may still increase in numbers even with a relatively lower maximum growth rate. Additionally, some substrates (e.g. emulsified oil, grease and hydrocarbons) probably serve as excellent carbon and energy sources for *Nocardia*-like organisms.

Control of Nocardial foam

No universal method of *Nocardia*-like foam control can be offered at this time. One approach is to try to limit the growth of the organisms, another is to try to minimise its foaming action. The prevention of sludge foam depends on the prevention of high concentrations of *Nocardia*-like filaments at the air/water interface. Once these bacteria concentrate on the surface, they may continue to grow there. Future research might develop various physical/chemical agents to reduce the chances of *Nocardia*-like organisms being concentrated in foam. For example, any compound that reduces the hydrophobicity of *Nocardia* would reduce the efficiency of its flotation (Gochin and Solari, 1983). The same principles apply to sludge foam and parallel the use of antifoam agents in microbial fermentations. For example, several drops of cooking oil immediately dispersed the *Nocardia* foam in a pure culture (Unpublished data). No long-term control is expected, however, as the oil would be quickly metabolised. Silicone-based antifoams had essentially no effect. Lechevalier (1975) reported that a polyglycol antifoaming agent reduced foaming in his laboratory cultures. However, using this principle is not practical in solving activated sludge foaming problems, and successful field application has not been reported.

A reduction in MLSS level will alleviate foaming problems because less MLSS (with entrapped *Nocardia*-like organisms) will float to the surface, and less foam will be formed. Similarly, a reduced air flow or mechanical mixing rate can reduce the foam potential, since air entrainment plays a major part in the formation of scum (Hart, 1985). Care must be taken not to induce low dissolved oxygen bulking when employing this approach. This temporarily relieves the foaming problem, but does not remedy the cause. Once a stable foam is present, the usual method of

controlling detergent-type foam with water spray is not successful. It is best to control the foam before it reaches a stable condition.

Lowering the solids retention time (SRT), or increasing F/M, is probably the most successful method used to reduce foam problems. The lower SRT favors more rapidly growing bacteria, thus washing out *Nocardia* species present in aeration tanks. It also leads to a lower MLSS (and perhaps less accumulation of metabolic surface-active by-products), thus reducing the symptom of foaming. Sezgin and Karr (1984) recently reported a successful full-scale application of this approach. This method will be more effective if the SRT of the foam is also lowered. Furthermore, the lowering of the SRT is not practical in the nitrification process where higher growth rate would also wash out nitrifiers. Where a sufficiently low SRT (high F/M) is not practical, use of a "selector" tank may be appropriate. This approach has proved successful in controlling other filamentous bacteria (Lee *et al.*, 1982; Daigger *et al.*, 1985).

The control of *Nocardia* foam problems by nocardiotoxic substances from anaerobic digester supernatant has been suggested (Lechevalier, 1975; Lechevalier *et al.*, 1977), and the method has been widely and frequently repeated (e.g. Genetelli and Genetelli, 1983). There were two major bases for the original assertion. The first was an apparent correlation between the presence of *Nocardia* foaming problems and the absence of anaerobic digesters at the plants initially examined. However, this initial observation was based on 6 plants, and it is now known that foaming also occurs in plants with anaerobic digesters. In fact, at two plants with activated sludge foaming problems, we also observed *Nocardia* in the supernatant from the digesters (which were also foaming). While *Nocardia* cannot grow in an anaerobic environment, it seems unlikely that any specific nocardiotoxic agents were active in these systems.

The second basis for this premise was a series of laboratory experiments (Lechevalier, 1975). Dilutions of digester supernatant were added to pure cultures of *N. amarae* in a favorable growth medium. Subsequent growth of the *Nocardia* was found to be considerably reduced even at a supernatant dilution of 10^{-6}. While specific nocardiotoxic agents could produce such an effect, it is far more likely that the cause was competition with the other microorganisms introduced along with the supernatant. This explanation is strongly supported by the fact that filtration or autoclaving of the supernatant eliminated all or most of the apparent inhibition, respectively. The remainder probably resulted from the observed decrease in pH to 5,7. Under field conditions, the increase in F/M resulting from the return of supernatant might likewise select against *Nocardia*-like organisms. In fact, the return of anaerobic digester supernatant to control undesirable species, such as filaments associated with bulking, was reported as early as the 1940's (Kraus, 1945; 1946). Thus, despite the apparent early evidence for the hypothesis of specific nocardiotoxic agents being present in anaerobic digester supernatant (Lechevalier, 1975), such claims now appear to be unfounded.

Individual operator initiative and ingenuity can play an important role in foam control. Care should be taken to avoid recycle of side-streams from solids handling facilities containing concentrated *Nocardia*-like organisms. Such recycles may include skimmings, anaerobic digester supernatant, aerobic digester decant, or gravity thickener overflow, among others. If recycle is unavoidable, heavy doses of chlorine or hydrogen peroxide may at least minimise the return of viable organisms. Wherever possible, foam should be removed and directed out of the plant to disposal, rather than to units where it may cause further problems.

In a recent study, Pretorius and Laubscher (1987) successfully removed excessive scum by a process of flotation. Although the continous removal of scum slightly reduces the SRT, there are no adverse effects on COD removal and nitrification.

Controlled chlorination of return activated sludge (RAS), as is practiced for sludge bulking (Jenkins *et al.*, 1982), may also be applicable for *Nocardia*-like foaming control. However, since the *Nocardia*-like filaments tend to extend less from the flocs than other types of filamentous bacteria, this approach may be less promising for foam control. Also, once a stable foam has formed, much of the *Nocardia* will not enter the RAS stream and hence will not be chlorinated. Direct chlorination of the foam on the aeration and/or settling tank surface may produce some beneficial effect, although no test of this approach is reported yet in the literature.

Conclusion

A general description of the role of *Nocardia*-like organisms in activated sludge foaming has been presented. The presence of these bacteria at the air/water interface apparently is due mainly to their hydrophobic characteristics. Growth in the interfacial area probably also occurs, through assimilation of substances which concentrate at the interface. The continuing accumulation of the *Nocardia*-like organisms, associated MLSS, and other substances eventually leads to formation of a stable foam layer several centimetres thick. The high concentration of solids in the foam, along with other chemical factors, leads to its stability.

It must be emphasised that the foam environment is distinct from the MLSS in terms of bacterial distribution, substrate availability and environmental considerations. The SRT may be low for the MLSS, yet still be very high in the foam environment. Once a stable foam is observed, it is difficult to control it by simply lowering the SRT of the MLSS.

Further research is needed to confirm or refute some hypotheses outlined in this paper. The research might also focus on identification of the factors which select for *Nocardia*-like organisms in the activated sludge environment, as well as the main mechanisms involved in foam production. Hopefully, such efforts will soon lead to reliable control and/or prevention of activated sludge foaming problems.

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