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# Filamentous Growth in Activated Sludge

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## ABSTRACT

Bulking and foaming in activated sludge have been associated with filamentous overgrowth. Filamentous *Nocardia amarae* and nonfilamentous *Pseudomonas auruginosa* were cultured using fatty acids (C2-C24) as the sole carbon. *N. amarae* could utilize all acids tested for growth, whereas *P. auruginosa* hardly grew on acids with 12 or more carbons. Maximum specific growth rate and saturation constant of *N. amarae* on C24, at 0.048 $h^{-1}$ and 1.520 g COD/L, respectively, were much lower than that of *P. auruginosa*, showing that *N. amarae* had a relatively stronger affinity for long-chain fatty acids. *N. amarae* was competitive in activated sludge processes that receive sewage containing a high proportion of long-chain fatty acids, oils, and fats.

**Index Entries:** Activated sludge; bulking; fatty acid; filamentous growth; foaming; *Nocardia*.

## INTRODUCTION

The activated sludge process is among the most widely used biological processes for treating domestic sewage and industrial effluents. The process converts organic matters into flocculent settleable biomass that can be subsequently removed by sedimentation. Since its development in 1914, the activated sludge process has been reported to encounter operational problems of bulking and foaming *(1,2)*. Bulking sludge has poor settling characteristics and compactability, whereas foaming over aeration basins and secondary clarifiers results in safety hazards, deteriorated effluents, and odors. Overgrowth of filamentous microorganisms or microorganisms that can grow into a filamentous form under adverse conditions is commonly accepted as the main cause of bulking and foaming, although the mechanisms are not fully understood *(3)*. Recent surveys of filamentous microorganisms that affect the quality of activated sludge from the US, Japan, Hong Kong, Singapore, Australia, and South Africa revealed more than 20 different morphological types, including fungi and bacteria, such as *Nocardia* spp., *Rhodococcus* spp., and *Microthrix* spp. *(4–10)*.

The relative abundance of filamentous bacteria among nonfilamentous floc-forming bacteria in activated sludge may be related to their relative growth

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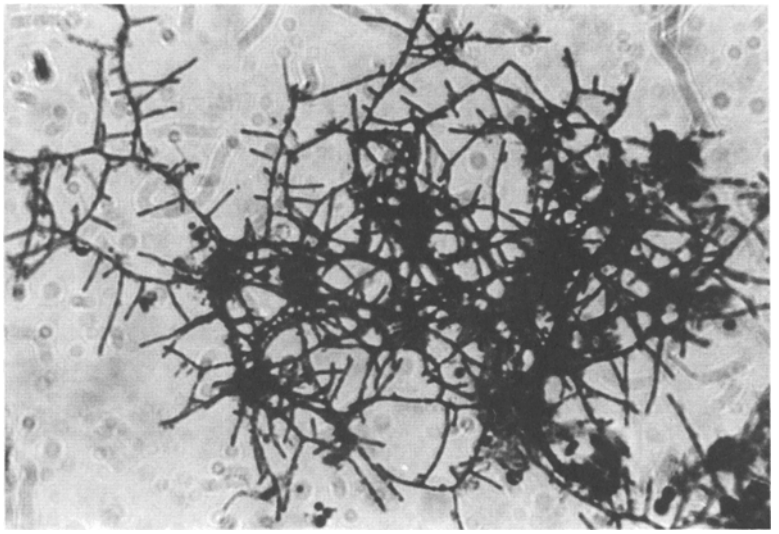

Fig. 1. *Nocardia* spp. Isolated from foaming sludge in Hong Kong (magnification = 400×).

rates, which are, in turn, affected by the characteristics of incoming waste water. Balfours *(11)* reported that increased grease concentrations in the incoming settled sewage coincided with filamentous overgrowth. Other substrates, such as nonionic surfactants, ethoxylates, and fatty acids, either directly promoted filamentous growth or have been observed to enhance foam production and foam stability significantly *(2,12,13)*. On the other hand, environmental factors, plant design, and operating conditions, such as fluctuations of incoming waste water characteristics, aeration rate, and food-to-microorganism (F/M) ratio, also play an important role in governing the relative abundance of filamentous bacteria *(14)*. Control of filamentous microorganisms has been attempted through addition of nutrients or filament-suppressing chemicals, such as hydrogen peroxide or chlorine, use of selectors *(15–17)*, or alteration of dissolved oxygen concentration or F/M ratio *(18)*. However, better understanding of the filamentous growth characteristics is needed for more effective control.

This article presents in vitro studies and comparisons of the growth kinetics of filamentous and nonfilamentous bacteria of activated sludge. The effects of common fatty acids on filamentous growth and foaming were investigated.

## MATERIALS AND METHOD

### Cell Cultures

The predominant filamentous microorganisms isolated from the bulking and foaming activated sludge in Hong Kong have been identified as *Nocardia* spp. *(5)* (Fig. 1). *Nocardia amarae* (American Type Culture Collection, Rockville, MD, ATCC 27810) and *Pseudomonas auruginosa* (Cell Reserved Culture Collection in Taiwan, CRCC 10261), were used in this study to represent filamentous and nonfilamentous bacteria in activated sludge, respectively. The cultures were maintained on yeast-extract-glucose agar slants at 2–4°C. Liquid broth cultures of *N.*

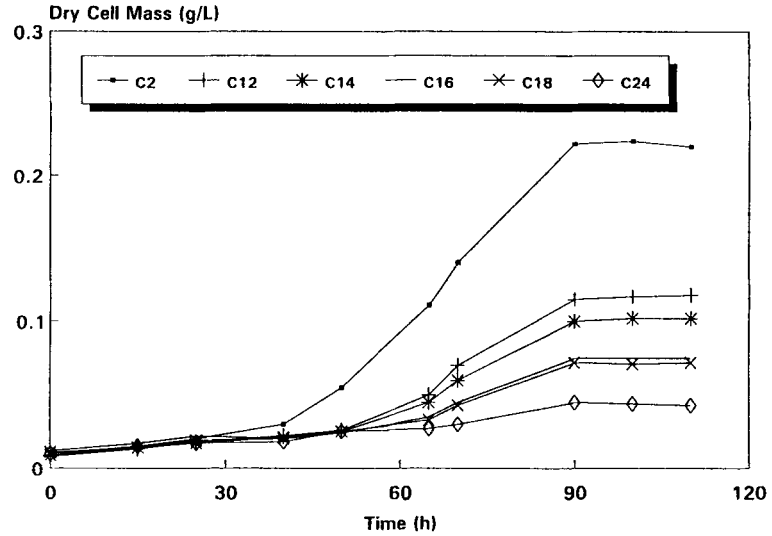


Fig. 2. Growth of *N. amarae* with different fatty acids as sole carbon source.

*amarae* and *P. auruginosa* were derived from the agar slants and maintained at 30°C for 72 and 24 h, respectively, before being used as the inocula. The compositions of yeast-extract-glucose agar slant and liquid broth have been described elsewhere *(5)*

Minimal salt medium (MSM) was prepared with the following formulation: $KH_2PO_4$ 2.796 g/L, $Na_2HPO_4$ 2.834 g/L, nitrilotriacetic acid 0.200 g/L, $MgSO_4$ 0.289 g/L, $CaCl_2 \cdot 2H_2O$ 0.067 g/L $(NH_4)_2Mo_7O_{24} \cdot 4H_2O$ 0.185 mg/L, $FeSO_4 \cdot 7H_2O$ 2.480 mg/L, EDTA 0.250 mg/L, $ZnSO_4 7H_2O$ 1.095 mg/L, $MnSO_4 \cdot 4H_2O$ 0.203 mg/L, $CuSO_4$ 0.020 mg/L, $CoSO_4 \cdot 7H_2O$ 0.024 mg/L, and $Na_2B_4O_7 \cdot 10H_2O$ 0.018 mg/L. The MSM was adjusted to pH 6.8 with NaOH solution and autoclaved. Fatty acid stock solutions were separately prepared with 4 g/L of acetic ($C_2$), lauric ($C_{12}$), myristic ($C_{14}$), palmitic ($C_{16}$), stebaric ($C_{18}$) or lignoceric ($C_{24}$) acids, adjusted to pH 7.0 with NaOH solution to solubilize the acids and filtered through a 0.45-μm membrane filter. Ninety milliliter of MSM, 10 mL of fatty acid stock solution, and 5 mL of inoculum were added to a 500-mL baffled conical flask. The cultures were maintained in an incubator shaker (Forma Scientific Model 4518 Orbital Shaker) at 30°C and 200 rpm for a period necessary to obtain stationary growth phase in each culture.

### Analytical Methods

Bacterial growth was monitored by quantifying cell mass via optical density with a Milton Roy Spectrophotometer (Spectronic 601) at 520 nm. Substrate utilization was monitored by measuring the residual chemical oxygen demand (COD) *(19)* in the culture medium.

## RESULTS AND DISCUSSION

### Growth on Different Carbon Sources

The growth of *N. amarae* with different fatty acids as the sole carbon source is shown in Fig. 2. In acetic acid culture, the logarithmic growth phase lasted for about 50 h slowly reaching a maximum of 0.22 g dry cell mass/L. All the fatty acids tested

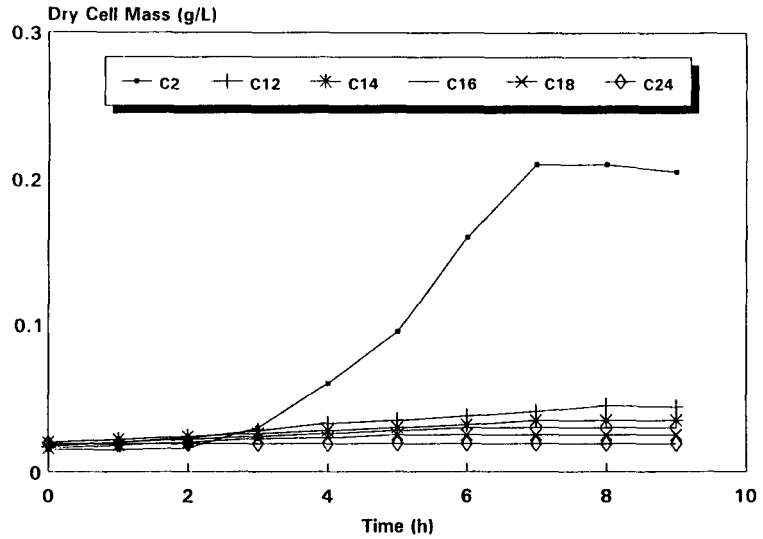


Fig. 3. Growth of *P. auruginosa* with different fatty acids as sole carbon source.

could support growth, although growth was less pronounced with the long-chain fatty acids (12 or more carbons). Even with lignoceric acid, the culture could grow to a maximum of 0.05 g dry cell mass/L. The growth of *P. auruginosa* with different: fatty acids as the sole carbon source is shown in Fig. 3. *P. auruginosa* grew rapidly in acetic acid. The logarithmic phase lasted for about 5 h, and the culture grew to 0.21 g dry cell mass/L. On the other hand, the long-chain fatty acids were hardly utilized for growth. When lignoceric acid was used as the sole carbon source, little growth was detected.

## Kinetic Selection

The growth and substrate utilization data were processed by Lineweaver-Burk and linear-regression techniques to obtain the maximum specific growth rates ($\mu_m$) in $h^{-1}$, saturation constants ($K_s$) in g COD/L, and growth yields ($Y_{x/s}$) in g/g COD for *N. amarae* and *P. auruginosa* with different fatty acids as the sole carbon source (Table 1). As a general trend, the maximum specific growth rates were higher and the saturation constants were smaller with short-chain fatty acids. *N. amarae* had lower maximum specific growth rates and smaller saturation constants than *P. auruginosa*, compared on the basis of the same carbon source.

For instance, maximum specific growth rate and saturation constant of *N. amarae* on stearic acid, at 0.069 $h^{-1}$ and 1.400 g COD/L respectively, were much lower than that of *P. auruginosa*. The specific growth rate, at varying stearic acid concentrations for the two bacteria are compared in Fig. 4. In this kinetic selection, *N. amarae* was a $K_s$-strategist that grew slowly, but had a strong affinity towards and could survive on low concentrations of the fatty acid. *P. auruginosa*, on the contrary, was a $\mu_m$-strategist that grew rapidly, but required high concentrations of fatty acids for growth. The ability of filamentous bacteria to switch between $\mu_m$-strategy when readily biodegradable substrates are available and $K_s$-strategy when only refractory substrates are available was described by Chudoba et al. *(20)*. However, this phenomenon Was not observed in the growth of *N. amarae* with fatty acids as the carbon source. With the readily biodegradable acetic acid, the maximum specific growth

Table 1
Growth Characteristics of *N. amarae* and *P. auruginosa*

| Sole carbon | *N. amarae* | | | *P. auruginosa* | | |
|---|---|---|---|---|---|---|
| | Specific growth rate, $\mu_m$, $h^{-1}$ | Saturation constant $K_s$, g/g COD | Growth yield $Y_{x/s}$, $h^{-1}$ | Specific growth rate, $Y_{X/s}$, $h^{-1}$ | Saturation constant, g COD/L | Growth yield, g/g COD |
| Acetic Acid | 0.095 | 0.320 | 0.487 | 3.557 | 2.004 | 0.665 |
| Lauric Acid | 0.073 | 1.097 | 0.426 | 1.003 | 7.435 | 0.105 |
| Myristic Acid | 0.071 | 1.156 | 0.428 | 0.997 | 8.724 | 0.097 |
| Palmitic Acid | 0.070 | 1.308 | 0.433 | 0.724 | 10.468 | 0.070 |
| Stearic Acid | 0.069 | 1.400 | 0.413 | 0.347 | 11.155 | 0.041 |
| Lignoceric Acid | 0.048 | 1.520 | 0.453 | 0.123 | 12.625 | 0.009 |

rate and saturation constant of *N. amarae*, at. 0.095 $h^{-1}$ and 0.302 g COD/L respectively, were still much lower than that of *P. auruginosa*. Growth yields of *N. amarae* remained fairly constant, ranging between 0.413 and 0.487 g/g COD, with all fatty acids tested. Growth yield of *P. auruginosa* with acetic acid was high, at 0.665 g/g COD, but the values were significantly lower, <0.105 g/g COD, when long-chain fatty acids were used as the sole carbon source.

These results indicated that *N. amarae* was more competitive than *P. auruginosa*, both being microorganisms commonly found in activated sludge, in a process that receives sewage containing high proportion of long-chain fatty acids. Long-chain fatty acids, with 12–24 carbons, are commonly produced from the microbial hydrolysis of oils and fats, which often account for more than 30% by weight of total organic content in municipal sewage; the percentage can be much higher in food-processing waste waters *(21,22)*. Therefore, overgrowth of *N. amarae* may also occur with sewage containing a high proportion of oils and fats. Nonfilamentous floc-forming bacteria, including the genera of *Psuedomonas*, and presumably *Zoogloea*, *Achromobacter*, *Alcaligenes*, and *Flavobacterium*, cannot or hardly utilize long-chain fatty acids for growth and proliferation. The observation that *N. amarae* is a $K_s$-strategist and basis of kinetic selection was also consistent with the literature that F/M ratio, treated-effluent recycle, and biodegradability of the organic constituents in the influent had an effect on the relative abundance of *N. amarae* in activated sludge processes.

## CONCLUSION

All the fatty acids tested could be utilized for growth by *N. amarae*. Long-chain fatty acids, namely lignoceric acid, could support the growth of *N. amarae*, but not *P. auruginosa*. *N. amarae* had lower maximum specific growth rates and smaller saturation constants than *P. auruginosa*. These results indicated that *N. amarae* was more competitive than *P. auruginosa*, both being microorganisms commonly found in

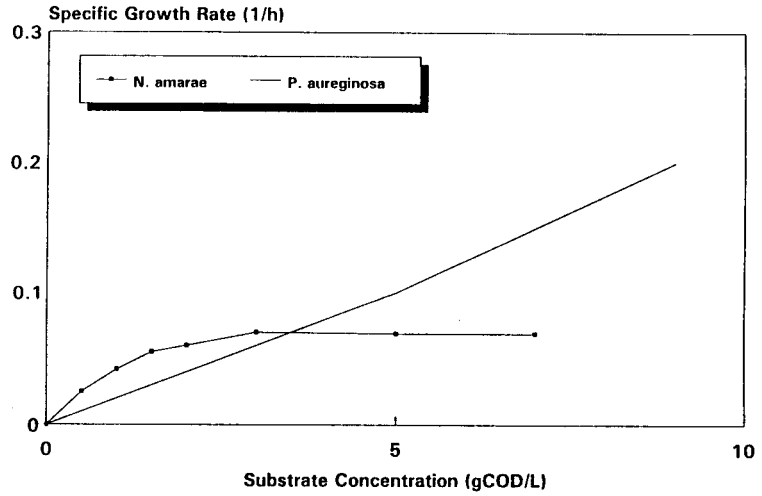


Fig. 4. Specific growth rates at varying substrate concentrations.

activated sludge, in a process that receives sewage containing a high proportion of long-chain fatty acids, oils, and fats

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