Effect of different pH, temperatures and Fe\(^{2+}\) concentration on *Sphaerotilus natans* growth and clarity performance index

M. F. Al-Shahwani, M. R. Al-Ghazali, and E. A. Al-Rawi

Biological Research Center, P.O. Box 2371, Baghdad, Iraq

**Summary.** An investigation was made of the effect of various environmental conditions on *Sphaerotilus natans*, an organism which causes sludge bulking, during the application of Fe\(^{2+}\) as inhibitor for bacterial growth. The ratio between non-settleable bacterial aggregates (turbidity) and bacterial plate count was regarded as representing the Clarity Performance Index (CPI).

The results showed that ideal clarity was obtained at pH 6.5 and 30°C when 15 mg l\(^{-1}\) of Fe\(^{2+}\) applied. Under conditions, the percentage of inhibition of *S. natans*, the highest percentage of Fe\(^{2+}\) removal, and the CPI, compared to a control, were 87.50%, 29.58 and 0.962 respectively.

**Introduction**

Filamentous organisms have long been associated with the bulking of activated sludge (Lackey and Wattie 1940; Farguhor and Boyle 1971). Since the operational success of this type of treatment is so dependent on the sludge having good settling characteristics, the presence of these organisms has often caused concern to operational managers. This long-standing association of filamentous organisms with the poor settlement of sludge appear to have arisen through repeated examination of microbial flora during periods when settleability problems were experienced.

Several methods have been used to solve the problem. However, a number of disadvantages and difficulties were encountered when applying these methods in the field. A few years ago a method for controlling sludge bulking through the use of iron salt was introduced by Pfeffer (1967) and its efficacy substantiated by additional research of Carter and Mckinney (1973). The results of the laboratory studies indicated that adding adequate amounts of iron (e.g. 10 mg l\(^{-1}\) as Fe\(^{2+}\) in the form of ferrous sulphate, based on the influent volume) into activated sludge system not only reduced sludge bulking but also increased efficiency of the system. The role of iron in the activated sludge was presumed to be that of inhibiting the growth of filamentous organisms and encouraging the growth of normal organisms.

In pure culture studies, iron salts have been found to inhibit the growth of *Sphaerotilus* (Waitz and Lackey 1959), but this is still a subject of controversy (Wolfe 1964). However Chang et al. (1979) illustrated that there was a close relationship between growth inhibition and the amount of iron absorbed by the organism, which in turn is a function of the type of iron complex used.

One of the important factors that affect iron sorption and subsequently *Sphaerotilus* growth, is the environmental factor. There are reports, however, on the effect of pH (Mulder and Veen 1963; Rouf and Stokes 1964; Stokes 1954) and temperature (Dondero 1961; Rouf and Stokes 1964; Stokes 1954).

This paper describes the effect of different pH and temperatures on growth and iron sorption by *Sphaerotilus* sp. when an "optimal" dose of Fe\(^{2+}\) is applied. The non-settleable bacterial aggregates (turbidity) and bacterial number were used as a criteria for the Clarity Performance Index (CPI).

**Materials and methods**

*Growth media.* The synthetic sewage (S-medium) described by Chang et al. (1980) was employed in this study. The media is
comprised of (concentrations in milligram per liter): Na₂HPO₄, 50; NaCl, 15; KCl, 70; MgSO₄, 5.0; Peptone, 100 (BDH Biochemicals); dextrose, 500; and distilled water.

Solid media. Purification and maintenance of isolates were performed on a solid medium (Chang et al. 1979) with the following compositions (concentration in milligram per liter): Dextrose, 1000; peptone, 1000; MgSO₄·7H₂O, 200; CaCl₂, 50; FeCl₃·6H₂O, 10; and agar, 12.5 g l⁻¹ in tap water.

Bacterial culture. A strain of *Sphaerotilus natans* was isolated from a storage tank receiving the final effluent of a sugar factory in Musil (Iraq).

Incubation of test cultures. Test cultures were incubated in an orbital shaker (Heraeus-RCA-TK), previously adjusted at 150 rpm.

Isolation and identification. A 50-ml sample of effluent from the sugar factory was incubated at 30°C for 2–3 days. The developed bacterial masses were microscopically examined for sheathed cells and filaments. Each bacterial mass was streaked on the surface of solid media and incubated at 30°C for 48 h. Typical colonies were picked-up for further purification and microscopic examination. Colonies suspected of being *S. natans* were tested and identified according to Bergey’s manual. Isolates were also used to inoculate 100-ml aliquots of a sterile effluent sample and S-medium to observe the formation of cell masses over 48 h at 30°C. This was followed by a further microscopic examination of the developed bacterial masses.

Experimental procedure. The degree of inhibition exerted by an iron compound (FeSO₄) on the growth of *S. natans* was expressed in terms of the total count of the organisms and the turbidity of the non-settleable particles of the tested samples compared with that obtained from the control. The medium for control cultures had the same ingredients as the test cultures except for Fe²⁺. The test cultures and the controls were inoculated with 0.5 ml (20 x 10⁸ cell/ml) of *S. natans* broth culture (Nutrient broth No. 2).

Three sets of flasks of the test cultures together with their control sets were prepared as follows: A set of Fe²⁺ concentrations (mg l⁻¹) of 10, 15, 20, 25, 30 and 50, and pH 6 were incubated at 30°C for 24–48 h. The second set of an Fe²⁺ concentration of 15 mg l⁻¹ consisted of a range of test cultures of pH 5, 5.5, 6, 6.5, 7, 7.5 and 8, was incubated at 30°C. A third set of an Fe²⁺ concentration of 15 mg l⁻¹ and pH 6.5 were incubated at 22°C, 30°C and 36°C for 2–3 days. All the test cultures were made in triplicate.

Bacterial plate count of *S. natans* was carried out by spreading on solid medium plates. Non-settleable bacterial aggregates (turbidity) was performed according to the method described by American Public Health Association (1975) with modification where samples were left for 30 min to allow the settleable particles to precipitate. Hach-2100A turbidimeter was used for measurement of turbidity.

Analytical technique. The amount of iron sorbed by the organisms was determined by measuring the redox in the soluble iron concentration (15 mg l⁻¹) in the test cultures. Three incubation temperatures (22°C, 30°C and 36°C) were used for the incubation of three different test cultures at pH 6.0, 6.5 and 7.0. These values were obtained by comparing the soluble iron concentrations of iron-inhibited cultures with that of blanks (medium with the same ingredients of the test cultures excluded from the inoculum).

The soluble iron was determined by the atomic absorption technique (Pye Unicam-SP9 Spectrophotometer).

Calculations. The percentage of heavy metal removal was calculated according to the following equation:

\[ \frac{B - T}{B} \times 100 \]

where: \( B = \) Concentration of Fe²⁺ in spent medium of the blank samples
\( T = \) Concentration of Fe²⁺ in spent medium of the test samples

Clarity Performance Index (CPI) was calculated as

\[ \frac{\text{Turbidity of the control sample} - \text{Turbidity of the test sample}}{\text{Turbidity of the Control sample}} \times 100 \]

\[ \frac{\text{Growth of the control samples} - \text{Growth of the test samples}}{\text{Growth of the control samples}} \times 100 \]

Results and discussion

The precise effect that filaments have on the settlement of activated sludge has not been determined in practice, although theories have been proposed. These are usually related to the amount of filamentous biomass in the sludge, often quantified as the filament length, and referred to as the “settlement index.” For example, Newton (1980) regarded filament numbers, estimated from photomicrographs, as the settlement index when a pure culture of *S. natans* was used, but his results indicated that no apparent correlation could be obtained between filament number and sludge volume index (SVI). In the present study we considered non-settleable bacterial aggregates (turbidity) and bacterial plate count as a criterion for clarity performance and expressed as Clarity Performance Index (CPI). However, the results of the present work indicate that consideration of such a parameter could give a reliable index that represents the “clarity performance” as the condition when SVI cannot be measured e.g. at bulky condition (Pipe 1979).

Figure 1 illustrates the turbidity of the medium and the bacterial plate count of *S. natans* at different concentrations of Fe²⁺ and at medium pH 6.5 and growth temperature 30°C. It indicates the inverse relation between Fe²⁺ concentration and bacterial plate count (growth). The highest concentration of Fe²⁺ that caused 100% inhibi-