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Utilization of Inorganic Nitrogen Compounds by *Sphaerotilus natans* Growing in a Continuous-Flow Apparatus[1]

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Sphaerotilus natans was grown, attached, in a continuous-flow apparatus with inorganic nitrogen compounds (NH_4Cl, $NaNO_2$, or $NaNO_3$) as the only sources of nitrogen. The growth rate with NH_4^+-containing medium did not differ from that with media containing glutamate or Casitone as the nitrogen source.

The ability of the filamentous, sheathed bacterium, *Sphaerotilus natans*, to utilize inorganic nitrogen compounds has been the subject of several contradictory reports. A survey of the earlier work led Harrison and Heukelekian (5) to conclude that "there is general agreement that the luxurious growths similar to those found under stream conditions cannot be reproduced in the laboratory with inorganic sources of nitrogen," although some workers did report limited utilization of inorganic nitrogen or utilization under certain conditions. More recently, Mulder and van Veen (7) reported that *S. natans* could utilize inorganic nitrogen compounds, but not as well as it utilized organic nitrogen compounds. This was suggested by A. D. Adamse (Ph.D. Thesis, Wagenigen, The Netherlands) as one of the reasons why *S. natans* does not dominate in activated sludge treating dairy wastes, the dominant organisms in the sludge, *Arthrobacter* species, utilizing inorganic nitrogen compounds as well as they did organic compounds.

Our investigations on the growth of *Sphaerotilus* in continuous-flow cultures suggested that *S. natans* was, in fact, able to utilize readily inorganic nitrogen compounds. Because of the ecological implications (most of the nitrogen in the natural habitat might be expected to occur in inorganic form), we investigated the problem in greater detail.

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MATERIALS AND METHODS

The continuous-flow apparatus was designed by N. C. Dondero (*unpublished data*). It consists of 12 chemostats. It is important to emphasize that we are not dealing with a continuous culture in the usual sense of the term. *S. natans* grows attached to the sides of the culture vessel, and there is very little wash-out of cells. Moreover, values recorded in this report do not represent "steady states."

Medium was continuously fed into the culture vessels (chemostats) at a flow rate of 180 ml/hr. The culture vessels were fitted with overflow tubes which maintained volume in culture vessels at 150 ml. Contents of the culture vessels were stirred with magnetic stirring bars and aerated by passing air through the medium through glass gas diffusers (coarse) at a flow rate of 400 ml/min. Culture vessels were placed in a water bath maintained at 20 C. A diagrammatic representation of a culture vessel is given in Fig. 1, while Fig. 2 shows the complete arrangement. The experimental details of the continuous culture runs were as follows. Culture vessels, each containing 150 ml of medium, were inoculated with 10 ml of a 48-hr culture of *S. natans* growing in 5% CGY broth [5 ml of CGY broth (8) diluted to 100 ml with water] on a rotary shaker (180 rev/min) at 20 C. After incubation for 24 hr, flow of medium through the chemostats was started. After the requisite amount of medium was used, adhering growth was harvested with the aid of a rubber policeman. Either all the harvested cells were used for dry weight determination or suitable samples were used. When samples were used, the cell crop, after suitable dilution with water, was homogenized in an Omni Mixer (Ivan Sorvall, Inc., Norwalk, Conn.) at 800 rev/min for 1 min. In all cases, cells were washed twice with water (by centrifugation at about 8,000 $\times$ *g*) before dry weight (100 C, 24 hr) or nitrogen determinations. Cellular nitrogen was

determined as described by Okrend and Dondero (8) with the method of McKenzie and Wallace (6).

NH_4^+ in culture media was estimated by the method of Ecker and Lockhart (3).

The strains of *S. natans* (except strain ffd which was isolated just before conducting this study from a natural slime infestation) were revived from lyophilized stocks (2). Subsequent maintenance was done in CGY broth (8) at 20 C by semimonthly transfers.

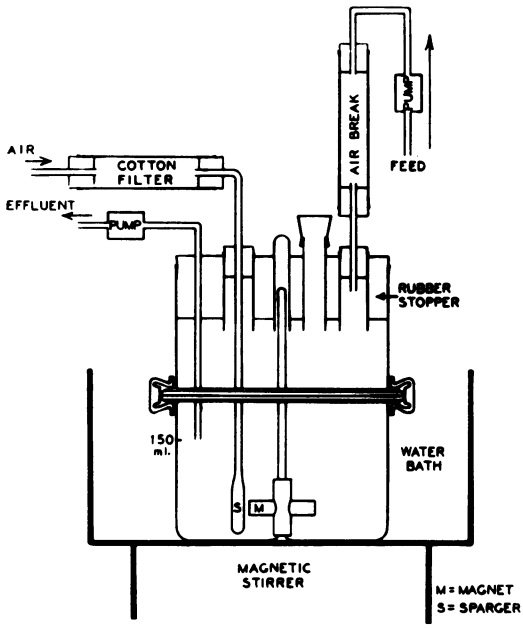

FIG. 1. *Diagrammatic representation (not to scale) of a culture vessel of the continuous-flow apparatus. Culture vessels were fabricated by Bellco Glass Co., Vineland, N.J. Model 12 AP Dial-A-Pump (Durrum Instrument Corp., Palo Alto, Calif.) pumps were used. The water bath was made of Plexiglas, and temperature was maintained at 20 C by pumping water from a constant temperature bath. Bell-Stir Multi-Stir (Bellco Glass Co.) magnetic stirrers were used.*

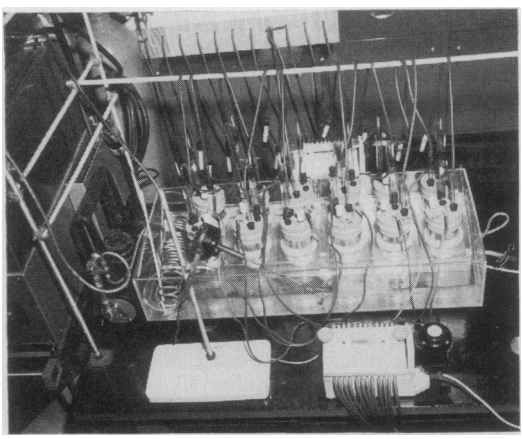

FIG. 2. *Continuous-flow apparatus.*

RESULTS

The results show that *S. natans*, strain 47, is able to utilize all three forms of inorganic nitrogen tested (Table 1), viz., NH_4^+, NO_2^- and NO_3^-. The highest concentration of NH_4Cl tested did not seem to have any toxic effect on growth of *S. natans*, contrary to the reports of some earlier investigators (5) who used batch cultures. Other experiments (not described in this paper) had shown a decrease in the amount of NH_4^+ in the effluent collected during a 30-min period before harvesting cells as compared with the concentration of NH_4^+ in feed (NH_4Cl in feed, 20 mg/liter; NH_4Cl in effluent, 15 mg/liter). Limited growth with nitrite and nitrate is probably a reflection of the toxicity of nitrite. However, this point has not been investigated. The other strains of *S. natans*

TABLE 1. *Utilization of inorganic nitrogen by Sphaerotilus natans strain 47*

Nitrogen source added to medium[a]	Concn	Dry wt of attached growth[b]
	mg/liter	*mg*
NH_4Cl	5	46
NH_4Cl	10	47
NH_4Cl	25	47
NH_4Cl	50	58
$NaNO_2$	5	22
$NaNO_3$	10	37

[a] Dextrose, 50 mg/liter; vitamin B_{12}, 5 μg/liter; micronutrient solution (1), 0.001 ml/liter; $Na_2MoO_4 \cdot 2H_2O$, 0.005 mg/liter; $MgSO_4$, 0.01 mM; $CaCl_2$, 0.1 mM; PO_4 buffer (K^+, *p*H 7.2), 01 mM; $FeCl_3$, 0.01 mM; in distilled water. A total of 9 liters of medium was fed through each culture vessel.

[b] Average of duplicate culture vessels.

TABLE 2. *Utilization of NH_4^+ by Sphaerotilus natans*[a]

Strain	Dry wt of attached growth
	mg
2	45
10	32
32	55
110	41
ffd	56

[a] The medium was as described in Table 1, containing 25 mg of NH_4 Cl per liter. A total of 9 liters of medium was fed through each culture vessel.

tested were all able to utilize NH_4^+ (Table 2); their ability to utilize NO_2^- and NO_3^- was not tested.

The experiment described in Table 3 shows that the amount of growth produced with NH_4^+-containing medium is the same as that produced with medium containing sodium glutamate, but more than that produced with a Casitone-containing medium when the nitrogen compounds were added at identical nitrogen values (about 1.3 mg of N/liter). The Casitone culture was apparently limited by the supply of utilizable nitrogen compounds. This is reflected not only by the decreased growth but also in the lower proportion of cellular nitrogen. Microscopically, the cell chains in all three media were enclosed in sheaths. Interestingly, however, many of the cell chains in the Casitone medium were coiled into spirals, some resembling corkscrews. Attempts to induce formation of such forms in nitrogen-deficient batch cultures have not proved successful.

Attempts to obtain growth curves of *S. natans* 47 in different media were not entirely successful because of the wide scatter of points, especially with the complex medium. However, growth rate in the NH_4^+-containing medium is not much different (if at all) from that in either the glutamate- or Casitone-containing media (Fig. 3). Curves were obtained from three different experiments, and the differences in the lag phase probably are due to variation between experiments which we have not been able to control.

DISCUSSION

There is little doubt that *S. natans* is able to utilize inorganic nitrogen compounds as well as it can utilize organic nitrogen compounds. However, the data fail to reveal the reason why batch cultures give erratic results. They do indicate, however, usefulness of continuous culture methods in studying "aquatic bacteria" which are normally accustomed to dilute media. A similar approach to the study of marine bacteria was recently reported by Hamilton et al. (4).

TABLE 3. *Growth of Sphaerotilus natans strain 47 in media containing NH_4Cl, sodium glutamate, or Casitone as nitrogen source*

Addition to medium[a]	Concn	Attached growth	
		Dry wt	Nitrogen
	mg/liter	*mg*	*mg*
NH_4Cl	5	56	4.6
Sodium glutamate	15.66	56	3.2
Casitone	12.42	27	1.1

[a] Medium was as in Table 1; 9 liters of medium was fed through each culture vessel.

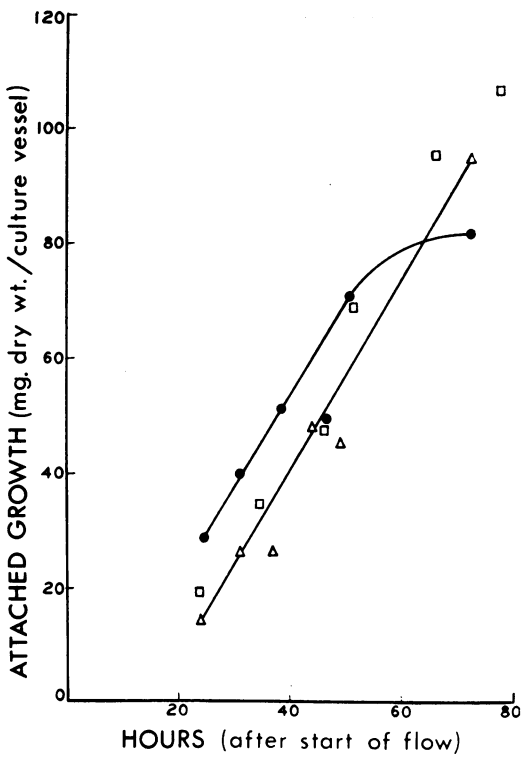

FIG. 3. *Attached growth of Sphaerotilus natans strain 47 in continuous-flow apparatus fed with media containing NH_4Cl (25 mg/liter), ●; sodium glutamate (30 mg/liter), ▲; or Casitone (50 mg/liter) plus yeast autolysate (10 mg/liter), □, as nitrogen sources. Other constituents of the medium are given in Table 1. Dilution rate was 1.2 hr^{-1}. The curve for Casitone-yeast autolysate medium was not drawn because of the scatter in points.*

ACKNOWLEDGMENT

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