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BRIEF COMMUNICATION

The effect of immobilization on carbon flow through *Anabaena variabilis* and *A. azollae*

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Abstract

Immobilization in polyvinyl foam resulted in an increased carbon fixation and release of fixed carbon in *Anabaena variabilis* while in *A. azollae* both processes decreased.

Additional key words: acid insoluble and soluble fractions; amino acids; cyanobacteria; organic acids; polyvinyl foam.

The use of immobilized microbial and plant cells as biocatalysts has become a rapidly advancing area of biotechnology since it offers increased biocatalytic capacity due to increased cell densities, stabilization of enzyme activities and the possibility for continuous operation (Brouers and Hall 1986). Cyanobacteria contribute 3-8 g(N) m^{-2} y^{-1} to flooded paddy soils (Fogg *et al.* 1973), and in India small farmers are encouraged to use cyanobacteria to obtain the benefit of 2-3 g(N) m^{-2} y^{-1} in rice fields (Venkataraman 1977). Foam immobilized cyanobacteria perform better as biofertilizer than inocula with free living cells (Brouers *et al.* 1989, Sophia Rajini 1995). So far, there is no report on the influence of immobilization on the flow of carbon through these nitrogen fixing cyanobacteria although certain other aspects have been studied (Shi *et al.* 1987, Subramanian *et al.* 1992).

The cyanobacteria used for the study were *A. variabilis*, a free-living form, and *A. azollae*, a symbiotic form isolated from the sporocarp of *Azolla mexicana* and maintained in the germplasm collection of our laboratory. They were grown in Bothe's (1968) medium with molecular nitrogen as nitrogen source. The pH of the medium was 7.8. Immobilization of cells was carried out in hydrophillic polyvinyl foams. The experiments were performed in petri dishes as still cultures, with 6 cm^3 of medium occupying half the height of foam pieces. Free-living control cultures were also grown paralell in petri dishes with 6 cm^3 of medium and the same amount

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of inoculum as that of the immobilized cultures. The medium was renewed once in four days by centrifugation in the case of free-living cultures and by decanting in immobilized cultures. The cultures were maintained under 14/10 h (light/dark) with fluorescent tubes (13.7 W m^{-2}) at a temperature of 25 ± 2 °C. After 30 d of growth (ensuring proper immobilization inside foam pieces), 18.5×10^4 Bq of radioactive sodium bicarbonate ($NaH^{14}CO_3$) was added to each petri dish and the cultures were allowed to grow for further 10 d without changing the medium. The amounts of carbon fixed, retained and released in various forms were studied using different fractions of the sample from column chromatography with *Dowex-1* and *Dowex-50* (for details see Subramanian and Shanmugasundaram 1986).

In *A. variabilis*, immobilized cultures fixed much more carbon than the free-living ones while it was reverse in *A. azollae* (Table 1). Though both species of *Anabaena* differed in the pattern of ^{14}C fixation under free-living and immobilized conditions, the increased ^{14}C fixation had always resulted in an increased release of fixed carbon in both the species (Table 1). Free-living and immobilized cultures of *A. variabilis*

Table 1. Extracellular release of ^{14}C [$\times10^6$ s^{-1} kg^{-1}(Chl)] fixed in *Anabaena variabilis* and *A. azollae*. In brackets % values are given: * % of total fixed, ** % of total released.

Organism	Fixed ^{14}C		Released ^{14}C				
	total	retained	total	acid insol.	amino acid	organic acid	neutral
A. variabilis	5800	3350	2433	1783	139	54	462
(free-living)		(58)	(42)*	(73)**	(5.7)**	(2.2)**	(18.9)**
A. variabilis	9067	2633	6450	5283	280	83	817
(immobil.)		(29)	(71)*	(82)**	(4.3)**	(1.2)**	(12.6)**
A. azollae	7233	3200	4000	3667	144	56	109
(free-living)		(45)	(55)*	(92)**	(3.5)**	(1.4)**	(2.7)**
A. azollae	7100	3983	3117	2000	195	63	715
(immobil.)		(56)	(44)*	(69)**	(6.2)**	(2.0)**	(22.9)**

released 42 and 71 %, respectively, of the carbon fixed while free-living and immobilized cultures of *A. azollae* released 55 and 44 %, respectively. The possible reason for such a high release of fixed carbon could be due to overproduction which in turn may be the result of the lack of end-product repression (Hood *et al.* 1969) and lack of metabolic control (Pearce and Carr 1969). Although a net release of 6-40 % of the total carbon fixed has already been reported (Subramanian and Shanmugasundaram 1986), a further increase in release observed in these two cases was probably a differential stress response. The extracellular carbon was essentially in the form of acid insoluble fraction such as mucopolysaccharides, and the increased release of fixed carbon was always in the form of acid insoluble fraction in both species of *Anabaena*. The release of this fraction amounted to 73 and 82 %, respectively, in the free-living and immobilized cultures of *A. variabilis* and 92 and 69 %, respectively, in the free-living and immobilized cultures of *A. azollae* (Table 1) suggesting that immobilization was a stress to *A. variabilis* which is a free-living form and not so to *A. azollae*, a symbiotic form, since increased

mucopolysaccharide production is associated with stress (Reed *et al.* 1984). Immobilization increased the release of amino acids, organic acids and sugars within the acid soluble extracellular fraction in *A. azollae* (Table 1). This probably explains the observation that the application of foam immobilized *A. azollae* was better than of the free-living ones in improving the growth of rice plants (Sophia Rajini 1995). Amino acids probably serve as nitrogen source, organic acids aid in phosphate solubilization, and sugars support the heterotrophic microflora in the soil. This study supports the view that foam immobilization of cyanobacteria may have agricultural applications such as the suspension of foam pieces with immobilized cyanobacteria in irrigation ponds (Brouers *et al.* 1989).

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