

# Yield and biochemical composition of a marine cyanobacterium (*Nodularia* sp.) in outdoor culture

Benjamin Pushparaj, Elio Pelosi, Pietro Carlozzi, Giuseppe Torzillo

Centro di Studio dei Microrganismi Autotrofi, CNR, P. le delle Cascine, I-27-50144 Firenze, Italy

**ABSTRACT:** The results are reported of an investigation into biomass output of N<sub>2</sub>-fixing marine cyanobacterium *Nodularia* sp. grown outdoors in open ponds (OPs) and in tubular photobioreactors (TPRs). The productivity in TPR was 13.2 g (dw) m<sup>-2</sup> d<sup>-1</sup>, while in OP it was 11.0 g (dw) m<sup>-2</sup> d<sup>-1</sup>. The higher productivity obtained in TPR compared to OP was probably due to a better temperature control in the system. Both true protein content and amino acid composition were very similar to that reported for *Spirulina*. The photoinhibitory effects of high light intensity on the photosynthetic activity of *Nodularia* was considerably reduced by using a black net for culture shading, which improved photosynthetic activity of the cyanobacterium.

**KEY WORDS:** Marine cyanobacterium · *Nodularia* sp. · Outdoor culture · Photobioreactors

## INTRODUCTION

Photosynthetic microorganisms are highly efficient in primary production given their simple structural organization and their capacity to efficiently utilize solar energy and available nutrients. In this group, the marine N<sub>2</sub>-fixing cyanobacteria appear to be particularly attractive for biomass production as they are able to fix atmospheric nitrogen using sunlight. This opens important prospects for the exploitation of coastal areas that are unsuitable for conventional aquaculture. Recently, the interest in the mass culture of these microorganisms has been focused mainly on their use as a source of food protein for aquaculture organisms (Mitsui et al. 1981) and for the production of fine chemicals (Benemann & Weisemann 1984). However, it was observed that cyanobacteria in an outdoor culture were very sensitive to high light intensity, often resulting in low productivity. Vonshak & Richmond (1988) demonstrated that shading the culture could reduce the adverse effect of high irradiation. Further mass culture of these phototrophs in seawater presented many technical limitations such as an adequate pH maintenance in the culture medium to avoid precipitation of phosphate and other nutrients. These drawbacks have been successfully overcome in further studies conducted in recent years (e.g. Materassi et al. 1984,

Tredici et al. 1986). In this paper the possibility of growing *Nodularia* sp. outdoors in open ponds and in tubular photobioreactors was examined to compare the yield obtained in both systems. The effect of shading on O<sub>2</sub> evolution and biomass output is also reported.

## MATERIALS AND METHODS

The *Nodularia* strain (S.E. 1) isolated from S. Eufemia gulf seawater (southern Italy), was grown both in the laboratory and outdoors in an artificial seawater medium (ASW) containing (g l<sup>-1</sup>): 33.0 sea salts (Tropic marin neu), 0.03 K<sub>2</sub>HPO<sub>4</sub>, 0.005 Fe (as Fe-EDTA) and Arnon solution A5 (1 ml l<sup>-1</sup>). The pH of the sea water medium was maintained at 7.5 by the addition of pure CO<sub>2</sub> in the culture through a pH stat system (Claind s.r.l., Milan, Italy). The laboratory cultures were maintained at an optimum temperature (28°C) and illuminated under an incidental light intensity of 50 μE m<sup>-2</sup> s<sup>-1</sup>.

**Outdoor culture.** Growth experiments with *Nodularia* sp. were performed in a semi-continuous regimen from June to August in 2 culture systems: open raceway ponds (OPs) and closed tubular photobioreactors (TPRs). In OP (4 m<sup>2</sup> surface area), the culture level was

Table 1. Analysis of variance for yield in 2 culture systems in different months

| Factors        | Sum of squares | df  | Mean square | F-ratio | Sig. level |
|----------------|----------------|-----|-------------|---------|------------|
| Main effects   |                |     |             |         |            |
| Culture system | 157.80         | 1   | 157.80      | 86.31   | 0.0000     |
| Months         | 169.50         | 2   | 84.75       | 46.36   | 0.0000     |
| Residual       | 210.26         | 115 | 1.83        |         |            |
| Total          | 533.87         | 118 |             |         |            |

Table 2. Analysis of variance for growth yield ( $Y_g$ ) of shaded and unshaded cultures at different hours of the day

| Factors                      | Sum of squares | df  | Mean square | F-ratio | Sig. level |
|------------------------------|----------------|-----|-------------|---------|------------|
| Main effects                 |                |     |             |         |            |
| Hours                        | 2797.92        | 6   | 466.320     | 16.153  | 0.0000     |
| Shaded and unshaded cultures | 6599.32        | 1   | 6599.324    | 228.598 | 0.0000     |
| Residual                     | 3810.67        | 132 | 28.868      |         |            |
| Total                        | 13207.91       | 139 |             |         |            |

maintained at 8 cm depth, with an areal density of 72 g (dw)  $m^{-2}$  and stirred by a paddle wheel. In TPR, whose characteristics have been described in detail elsewhere by Bocci et al. (1987), culture volume in the reactor was 53 l with an areal density of 64 g (dw)  $m^{-2}$ . The pH values in both systems were kept constant at around 7.5 by automatic addition of  $CO_2$ . The temperature of the culture in TPR was maintained at 28°C during the day, while in OP no temperature regulation was adopted and as a result it varied from 18 to 32°C during the experimental period.

Growth yield ( $Y_g$ ) was calculated from the ratio of biomass produced to the amount of light energy received by the cells per unit of surface area.

Measurements of dissolved oxygen concentration in open pond and tubular reactor were taken using a polarographic Clark type electrode.

#### Photosynthetic activity at different light intensities.

Photosynthetic activity was measured from the rate of specific  $O_2$  evolution of *Nodularia* cells grown outdoors in TPR under 2 different light conditions. One of the photobioreactors was exposed to full sunlight while the other was covered at 11:00 h with a black net which cut off 30% of the solar radiation. The cells collected from the photobioreactors were diluted to a final concentration of 0.6 to 0.7  $\mu g$  chl  $ml^{-1}$  with fresh artificial seawater medium. The specific  $O_2$  evolution of the cells was measured at 30°C in the laboratory at a photosaturating light intensity (700  $\mu E m^{-2} s^{-1}$ ) using a biological oxygen monitor (YSI model 5300) with a Clark type electrode.

**Analytical methods.** Biomass was determined in triplicate by filtering 25 ml samples of cultures through a membrane (8  $\mu m$ ) washed with distilled water and dried at 105°C to constant weight. The productivity was calculated from the dry weight increase over 24 h. The elemental composition (C, H, N, O) of lyophilized biomass was determined by means of an elemental analyser (Model 1106, Carlo Erba Strumentazione, Milan). The crude protein, calculated as  $N \times 6.25$ , included nucleic acids and other non-proteic compounds such as chlorophyll *a* (Herbert et al. 1971). Carbohydrate content was determined by the phenol-sulphuric acid method using D(+)-glucose as standard (Dubois et al. 1956). True protein was calculated from amino acid concentration, determined with HPLC (Mod. Enzyme assay). Total solar radiation was measured with a Micros solarimeter, using a Kipp and Zonen CH 5/6 pyranometric sensor.

**Statistics.** The data on the productivity of *Nodularia* sp. grown in the 2 systems (open pond and tubular photobioreactor) were subjected to a multifactor analysis of variance (ANOVA), where the culture systems and months were the main factors (Table 1).  $Y_g$  of this cyanobacterium grown in TPR under shaded and unshaded conditions was compared by means of a similar statistical analysis, where time of sampling, shaded and unshaded culture were the 2 main factors (Table 2). In both cases, the sum of squares related to the interaction was pooled with the experimental error. No data conversion was applied because the factor variances were homogeneous according to Bartlett's test. Means comparisons were performed with Tukey's test with  $p = 0.05$ .

## RESULTS AND DISCUSSION

### Biomass yield in the 2 systems

Fig. 1 illustrates the comparative biomass yield obtained in the outdoor experiments conducted with marine cyanobacterium *Nodularia* sp. in OP and TPR from June to August. The average productivity [11.0 g (dw)  $m^{-2}$  in OP and 13.2 g (dw)  $m^{-2}$  in TPR], during the experimental period, was significantly higher (+ 20%,  $p = 0.01$ ) in the photobioreactor than that in the open pond. The higher productivity observed in TPR was possibly due to the better temperature conditions maintained during both night and day. Indeed, the

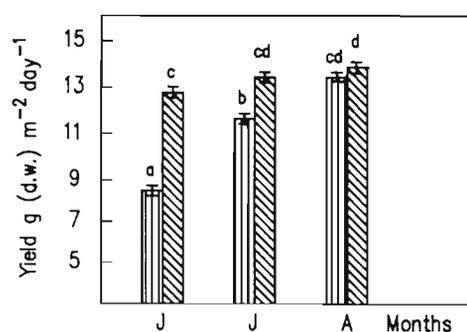


Fig. 1. Comparison of the mean yield of *Nodularia* sp. grown in ponds (vertical hatched columns) and photobioreactors (oblique hatched columns). Average of 20 replicates  $\pm$  confidence intervals for  $p = 0.05$

temperature maintained in the closed system possibly allowed a better utilization of light energy during the day compared to open pond, where the low morning temperature, far below 28°C, probably prevented a full exploitation of morning radiation for a few hours, thus delaying the resumption of an active photosynthesis. A similar observation was made by Balloni et al. (1981) in outdoor mass culture of a green algae, by Vonshak & Richmond (1988) and Torzillo et al. (1991) with *Spirulina*.

Dissolved oxygen concentration in the OP varied from 7 ppm in the morning to 25 ppm at the maximum light intensity period (13:00 h).

### Chemical composition

In Tables 3 & 4, the biochemical, elemental and amino acid composition of *Nodularia* sp. are shown. The biochemical composition of *Nodularia* sp. biomass grown in the 2 systems at full sunlight showed no significant difference. Furthermore, the cultures grown in TPR under shaded and unshaded conditions did not evidence any significant difference in the biomass

Table 3. Protein and carbohydrate content of *Nodularia* sp. grown in open pond (OP) and tubular photobioreactor (TPR). Data, expressed as percentage of dry weight, are the average of 6 analyses, each  $\pm$  standard deviation

|                    | OP              | TPR             |
|--------------------|-----------------|-----------------|
| Crude protein      | 61.5 $\pm$ 0.39 | 63.9 $\pm$ 0.15 |
| True protein       | 49.3 $\pm$ 0.13 | 50.9 $\pm$ 0.20 |
| Carbohydrate       | 19.0 $\pm$ 0.23 | 20.6 $\pm$ 0.18 |
| Ash                | 8.3 $\pm$ 0.19  | 5.3 $\pm$ 0.08  |
| Heat of combustion | 5.5 $\pm$ 0.058 | 5.3 $\pm$ 0.05  |

Table 4. Amino acid composition of protein from *Nodularia* sp. and *Spirulina platensis* (data from Paoletti et al. 1980) grown in TPR at full sunlight expressed as mg aa per 100 mg protein. Data are the average of 3 replicate samples  $\pm$  standard deviation

|                    | <i>Nodularia</i> sp. | <i>Spirulina platensis</i> |
|--------------------|----------------------|----------------------------|
| Aspartic acid      | 10.8 $\pm$ 0.26      | 11.7 $\pm$ 0.16            |
| Histidine          | 1.8 $\pm$ 0.17       | 2.0 $\pm$ 0.06             |
| Arginine           | 6.1 $\pm$ 0.21       | 7.4 $\pm$ 0.10             |
| Serine             | 2.4 $\pm$ 0.32       | 4.9 $\pm$ 0.60             |
| Glutamic acid      | 13.8 $\pm$ 0.29      | 17.5 $\pm$ 0.64            |
| Proline            | 5.1 $\pm$ 0.13       | 4.1 $\pm$ 0.06             |
| Glycine            | 5.8 $\pm$ 0.32       | 6.3 $\pm$ 0.07             |
| Alanine            | 7.0 $\pm$ 0.22       | 9.3 $\pm$ 0.07             |
| Cysteine           | 0.4 $\pm$ 0.14       | 0.3 $\pm$ 0.13             |
| Valine             | 6.7 $\pm$ 0.28       | 8.1 $\pm$ 0.21             |
| Methionine         | 0.8 $\pm$ 0.11       | 2.6 $\pm$ 0.10             |
| - - diaminopimelic | Trace                | 1.3 $\pm$ 0.07             |
| Isoleucine         | 5.9 $\pm$ 0.28       | 6.7 $\pm$ 0.21             |
| Leucine            | 8.6 $\pm$ 0.17       | 10.9 $\pm$ 0.17            |
| Tyrosine           | 3.3 $\pm$ 0.29       | 4.8 $\pm$ 0.16             |
| Phenylalanine      | 5.8 $\pm$ 0.37       | 5.7 $\pm$ 0.00             |
| Lysine             | 4.6 $\pm$ 0.26       | 5.2 $\pm$ 0.20             |
| Threonine          | 4.4 $\pm$ 0.16       | 5.3 $\pm$ 0.07             |
| Tryptophan         | nd                   | 1.5 $\pm$ 0.10             |
| Ammonia            | 3.8 $\pm$ 0.17       | 0.9 $\pm$ 0.06             |

nd: not determined

compositions (results not shown). It was also interesting to note that a high true-protein content (51%) and amino acid pattern were in the range of that reported for *Spirulina* (Paoletti et al. 1980), a cyanobacterium utilized as a source of protein in various feed trials. The phycocyanin and chlorophyll contents of the biomass obtained from OP was significantly higher ( $p = 0.01$ ), by 24% and 15% respectively, than that grown in TPR (1-way ANOVA, data not shown).

### Photosynthetic activity at different light intensities

The photosynthetic activity of cultures grown in TPRs, exposed to 2 different light intensities, is shown in Fig. 2. The rate of oxygen evolution of *Nodularia* cells taken at 2 h intervals from the photobioreactor exposed to full sunlight (850 W m<sup>-2</sup>) showed a drastic decrease at about 13:00 h to 65% of that measured in the morning at the beginning of the experiments. In the late afternoon (18:30 h), the photosynthetic activity was back to 80% of the morning level. In contrast, in the shaded culture the specific rate of O<sub>2</sub> evolution remained at an almost constant high level throughout the day. The corresponding biomass yield obtained in the 2 photobioreactors exposed to different light intensities showed only an 8% increase in the shaded cul-

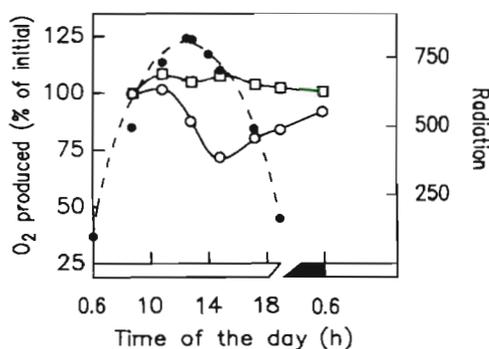


Fig. 2. Effect of shading on oxygen evolution of *Nodularia* sp. grown outdoors in TPR. (○) O<sub>2</sub> without black net; (□) O<sub>2</sub> with black net; (●) solar radiation (W m<sup>-2</sup>). Each point represents the average of 10 replicates; confidence intervals were omitted as the values were very low (0.79% of O<sub>2</sub>)

ture. However, the growth yields ( $Y_g$ ) in TPR covered with a black net was 0.665 g (dw) MJ<sup>-1</sup>, while in the uncovered reactor it was 0.596 g (dw) MJ<sup>-1</sup>, showing a very significant difference ( $p = 0.006$ ) between them. The results indicated that the increase in growth yield (11.6%) and the high efficient photosynthetic activity observed in the shaded culture were probably the consequence of a low photostress experienced by the cultures during high light intensity.

**Acknowledgements.** The authors thank A. Sacchi, D. Mannelli, and M. Anichini for their help and technical assistance and Dr G. Bartolini and P. Pestelli, of the Institute for the Propagation of Woody Plants, CNR, Scandicci (Italy) for statistical analysis.

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