

Extracellular Products of Photosynthesis in a Tropical Environment

HELGA do R GOMES and A PANT

National Institute of Oceanography, Dona Paula, Goa 403 004

Extracellular products in the Arabian Sea averaged $0.42 \text{ g/cm}^2/\text{day}$ and represented 35% of the total carbon fixed by phytoplankton. No seasonal changes are observed during the two seasons i.e. premonsoon (Jan-Feb) and onset of southwest monsoon (April-May). Maximum extracellular production occurred at the surface in contrast to the sub-surface maxima in particulate production. Percentage extracellular release was higher (35%) in the nutrient limited waters of the Arabian Sea as compared to only 7.6% in the estuarine waters of Dona Paula, Goa, where nutrients are seldom limiting.

A partially modified radiochromatographic method was used to identify some of the photosynthetic products released. Glycollic acid, a product of photorespiration and one of the more commonly occurring products of excretion did not appear to be a major excretory compound. It constituted only 2-32% of the total radioactivity and was present in only 34% of the samples. Citrate was tentatively identified as another product of extracellular release. Unidentified acids comprised 2-78% of the total radioactive material recovered from the phytoplankton exudate.

Key Words: Extracellular productivity, Primary productivity, Photosynthate, Glycollic acid

Introduction

Exudation of recently fixed photosynthate as a source of dissolved organic matter in the environment has been the subject of considerable study. Substantial excretion rates with values varying from 23% in a tropical estuary (Shailaja & Pant 1986) to 34% in temperate waters (Al-Hasan & Coughlan 1976) have been reported. Although experimental evidence has shown that cells in the exponential phase excrete negligible amounts of extracellular products (Myklesstad 1977) there is ample evidence that high light intensity and low nutrient levels (Verity 1981, Jensen 1984) bring about release of organics into the medium by single-celled algae. The excretory compounds consist of mono- and di-saccharides (Shailaja & Pant 1986), amino acids (Hammer & Eberlain 1981) and organic acids such as glycollic acid (Watt 1966, Al-Hasan & Coughlan 1976, Shailaja & Pant 1986).

The biological advantage of release of extracellular products to the photosynthesizing cells themselves is obscure, except that at high light intensities it appears to act as an overflow mechanism, thus avoiding the

mechanical effects of accumulation of excess byproducts. Extracellular products of photosynthesis released under stress may serve as sources of reduced carbon energy for heterotrophic bacteria (Jensen 1983, review by Fogg 1983).

In the present study primary and extracellular productivity estimates were made in the Arabian Sea and compared to similar data in a high-nutrient estuary, Dona Paula Bay. An attempt was made to characterize the excretory compounds using a radiochromatographic technique.

Materials and Methods

Samples were collected during cruises: 91 (April-May 1981), 103 (Jan-Feb 1982) and 118 (April-May 1983) on *R V Gaveshani* from depths corresponding to 100%, 30% and 1% light intensities using 51 PVC Niskin Samplers. The area covered by these cruises was between 10°N & 21°N lat. and 72°E & 58°E long. in the Arabian Sea.

Three litres of the sample were used to determine cell numbers, species, composition, chlorophyll *a*, adenosine triphosphate and particulate oxidisable carbon as biomass estimates, according to methods reported in Strickland and Parsons (1972).

After prefiltration (200 μ m nybolt net) to remove zooplankton, 1 L samples were incubated with 40 μ Ci NaH¹⁴CO₃ (BARC, Trombay), generally from 0800 to 1200 hours, under simulated *in situ* conditions. Temperature was kept constant by flowing sea water at 27°C during the period of incubation. Samples were covered with neutral density filters to approximate 100%, 30% and 1% light intensities representing actual depths of sampling. After incubation, samples were filtered through 0.45 μ m Millipore[®] membrane filters. Activity of the filter paper as particulate production (Strickland & Parsons 1972) and of the filtrate as extracellular production (Watt 1966) was measured using a Packard Prias Liquid Scintillation System by the External Standard Ratio Method.

Glycollic acid was extracted from the filtrate (figure 1) estimated colorimetrically (Calkins 1943) and further identified chromatographically. Pre-coated silica gel plates (J T Baker, USA) were used instead of cellulose plates (Coughlan & Al-Hasan 1976). Plates were developed (propanol-ammonia, 70-30) upto the 15 cm mark, dried and divided into strips of 1 cm \times 5 cm. Strips corresponding to the sample and standard labelled glycollate co-chromatographed with the sample were scraped and the activity on the silica gel was estimated as dpm.

Results and Discussion

In tropical oceanic environments where surface light (I_0) intensity is about 600 langley/day, phytoplankton are probably inhibited by light particularly in the first few cm of the water column. One effect of such inhibitory light intensities is the enhanced production of extracellular matter. As seen in table 1, the sub-surface maximum in particulate production was much pronounced and clearly related to the optimal light intensity (60% I_0) at this depth. In contrast, surface extracellular production decreased significantly with depth, becoming vanishingly small at 1% I_0 . The averages noted in table 1 are the result of some 25 samples at each depth in the Arabian Sea, collected at different locations and different times of the year. The high standard deviations probably reflect the activity of different species of phytoplankters at varying stages of their growth phases.

It has been suggested by Kabanova (1968) and Sengupta et al. (1976) that surface productivity in the Arabian Sea is nitrate-N limited. In the instances reported in table 1, nutrient values were uniformly low: PO₄-P ranged from 0.11 to 0.55 μ g-at/l, NO₃-N was even lower from non-detectable to 0.38 μ g-at/l and

Table 1 Particulate and extracellular production* per unit of chl *a* at different depths within the euphotic zone

I_z	Particulate prodn/chl <i>a</i> /hr	Extracellular prodn/chl <i>a</i> /hr
$I_{100\%}$	1.44 \pm 1.00	0.65 \pm 0.64
$I_{60\%}$	2.54 \pm 1.71	—
$I_{30\%}$	1.5 \pm 1.00	0.23 \pm 0.28
$I_{16\%}$	1.22 \pm 1.00	—
$I_{1\%}$	0.37 \pm 0.31	<0.02 (negligible in most cases)

*Means of 25 values

Table 2 Surface particulate and extracellular production in two tropical environments

	Arabian Sea	Dona Paula	
		Pre-monsoon	Monsoon
Particulate prodn/chl- <i>a</i> /hr	1.44	2.0	3.4
Extracellular prodn/chl- <i>a</i> /hr	1.2	0.61	0.14
Extracellular prodn as % of total fixation	45	25	4

Table 3 Radioactivity in glycollate and other fractions of extracellular matter produced by phytoplankton in the open sea

I_z	Arabian Sea			
	Lat. 15° N Long. 72°30' E	15°N 58°20.3' E	18°59.8' N 62°29.0' E	
$I_{100\%}$	Total extracellular dpm	2066	1617	5516
	% Glycollate	2.7	1.7	0.5
	% Citrate	0.72	Nil	Nil
$I_{30\%}$	Total extracellular dpm	1355	70572	2615
	% Glycollate	Nil	Nil	Nil
	% Citrate	1.8	Nil	Nil
$I_{1\%}$	Total extracellular dpm	41	2797	Nil
	% Glycollate	Nil	Nil	Nil
	% Citrate	Nil	0.7	Nil

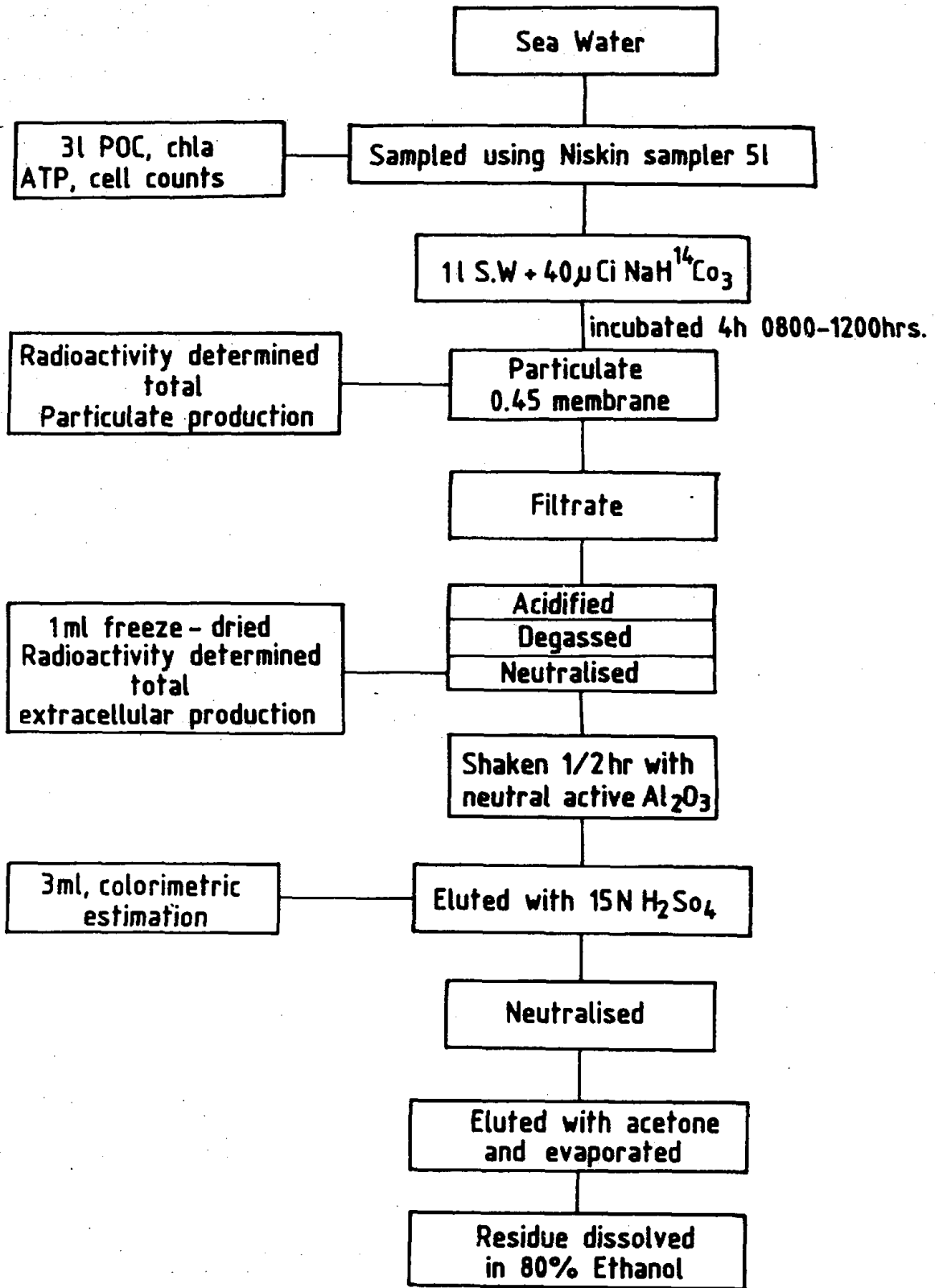


Figure 1 Technique used for extraction of Glycollate (after Al-Hasan & Coughlan 1976)

extracellular fixation averaged 45% (table 2) of surface primary production. In comparison with a nutrient-rich environment like Dona Paula Bay, where $\text{PO}_4\text{-P}$ was up to $2\mu\text{g-at/l}$ and $\text{NO}_3\text{-N}$ up to $1\mu\text{g-at/l}$, the open ocean extracellular fixation values were significantly higher. It appears reasonable to conclude from the above that high light intensity and low nutrient concentrations result in production of extracellular exudates in the open sea.

One of the pathways for the production of extracellular matter that has been studied in some detail involves the photorespiratory release of glycollate produced by the oxidation of ribulose by Ribulose biphosphate carboxylase: oxygenase (Tolbert 1979 review). The glycollate component of extracellular products was determined following the method of Al-Hasan and Coughlan (1976). Of the total dpm at the three representative stations, significant amounts of glycollate were produced only at the surface in the water column (table 3). A second excretory product citrate, probably from the TCA intermediates, was also identified, on the basis of Rf values, on the thin layer chromatograms.

Glycollic acid *per se* formed a fairly small fraction of total extracellular products when fixed-time incubations were used as the basis for assays. Evidence suggests that mono or disaccharides or their polymers may form the major component of extracellular matter produced by photosynthesizing cells in tropical seas.

The studies in the open sea used no antibiotics to suppress bacterial activity. Azam and Ammerman (1984) have suggested that microzones of bacterial activity might be formed around live phytoplankton in

Table 4 Radioactivity in glycollate and other fractions of extracellular matter produced by phytoplankton in a coastal environment

	Dona Paula $I_2 = 100\%$	
	Pre-monsoon (10th April 84)	Monsoon (20th June 84)
Total extracellular dpm	29840	15440
% Glycollate	24	1.6
% Amino Acids	18	58
% Mono & disaccharides	48	23

nature and that the consumption of extracellular matter might be as rapid as its production. Although in nutrient-rich Dona Paula Bay the work of Shailaja and Pant (1986), using antibiotics, has shown that the nature of extracellular products changes significantly with season (table 4), bacterial-uptake of exudates remains more or less constant throughout the year. Some of our data (Gomes, unpublished) indicate that up to 40% of CO_2 assimilated photosynthetically may be transferred from phytoplankton to bacteria by extracellular products. Thus, what is a stress reaction for phytoplankton may be of considerable benefit to the ecosystem as a whole.

Acknowledgements

This work was partly funded by the Department of Science and Technology, Project 12 (13)/78-SERC. Thanks are due to the Director, National Institute of Oceanography for encouragement.

References

- Azam F and Ammerman J W 1984 Mechanism of organic matter utilisation by marine bacterioplankton; in *Lecture Notes on Coastal and Estuarine Studies: Proc. Symp. Marine Phytoplankton and Productivity*. 8 eds O Holm-Hansen, L Bolis and R Giles (Berlin: Springer-Verlag) 45-54
- Calkins V P 1943 Micro-determination of glycollic and oxalic acids; *Indian Engg. Chem. Analyt.* 15 762-763
- Coughlan S J and Al-Hasan R H 1976 A method for the determination of glycollic acid in the extracellular products of cultured and natural phytoplankton populations; *J. exp. mar. Biol. Ecol.* 25 141-149
- Fogg G E 1983 The ecological significance of extracellular products of phytoplankton photosynthesis; *Bot. Mar.* 26 3-14
- Hammer K D and Eberlain K 1981 Parallel experiments with *Thalassiosira rotula* in outdoor plastic tanks: Development of dissolved free amino acids during an algae bloom; *Mar. Chem.* 10 533-544
- Jensen A 1984 Excretion of organic carbon as function of nutrient stress in *Lecture Notes on Coastal and Estuarine Studies: Proc. Symp. Marine Phytoplankton and Productivity* 8 61-73 eds. O Holm-Hansen, L Bolis and R Gilles (Berlin: Springer-Verlag)
- Jensen L M 1983 Phytoplankton release of extracellular organic carbon, molecular weight composition and bacterial assimilation; *Mar. Ecol. Prog. Ser.* 11 39-48
- Kabanova J G 1986 Primary production of the northern part of the Indian Ocean; *Oceanology* 8 214-225
- Myklestad S 1977 Production of carbohydrates by Marine

- Planktonic Diatoms. II. Influence of the N/P ratio in the Growth Medium on the Assimilation Ratio, Growth rate and Production of Cellular and Extracellular Carbohydrates by *Chaetoceros affinis* var. *Willei* (Gran.) Hustedt and *Skeletonema costatum* (Grev.) Cleve; *J. exp. mar. Biol. Ecol.* **29** 161-179
- Sengupta R, Sankaranarayanan V N, De Souza S N and Fondekari S P 1976 Chemical Oceanography of the Arabian Sea: Part III—Studies on Nutrient Fraction & Stoichiometric Relationships in the Northern & the Eastern Basins; *Indian J. Mar. Sci.* **5** 58-71
- Shailaja M S and Pant A 1986 Relationship between particulate and extracellular carbon compounds of phytoplankton photosynthesis in a tropical estuary; *Mar. Ecol. Prog. Ser.* **32** 161-167
- Strickland J D H and Parsons T R 1972 *A Practical Handbook of Seawater Analysis*; *Bull. Fish. Res. Bd Can.* **122** pp. 311
- Tolbert N E 1979 Glycollate metabolism in higher plants and algae; in *Photosynthesis. II. Photosynthetic Carbon Metabolism and Related Processes*, pp 338-352 eds M Gibbs and E Latzka (Berlin: Springer-Verlag)
- Verity P G 1981 Effects of temperature, irradiance and daylength on the marine diatom *Leptocylindrus danicus* Cleve. II. Excretion; *J. exp. mar. Biol. Ecol.* **55** 159-169
- Watt W D 1966 Release of dissolved organic matter from the cells of phytoplankton populations; *Proc. R. Soc.* **B164** 521-551