X-ray diffraction: An approach to study interaction between cyanobacteria and dairy effluent

Kanika Sharma*,†, N. Lakshmi‡, K. Venugopalan‡, Pooja Mehta*, Ashish Maheshwari* and Shalu Bapna*

*Department of Botany, M.L. Sukhadia University, Udaipur 313 001, India
‡Department of Physics, M.L. Sukhadia University, Udaipur 313 001, India

Dairy effluent and cyanobacteria cultured in effluent for 3, 5, 7, 9 and 11 days were studied by X-ray diffraction to understand the interaction between them. Physico-chemical properties, total protein and carbohydrate content of effluent before and after culture of cyanobacteria, change in biomass, total proteins, carbohydrates, chlorophyll *a* and β-carotene contents of cyanobacteria were analysed. X-ray spectra show an increase in the crystalline nature of the biomass and decrease in crystalline nature of effluent. Some of the metabolites were also identified. Results suggest that organic and inorganic substances present in effluent are absorbed and metabolized by cyanobacteria.

MILK processing consumes large amount of water and generates 6–10 litres of effluent/l of milk processed[1]. Effluent volume is approximately four times the volume of milk processed[2]. The effluent is organic, slightly alkaline, but becomes acidic due to decomposition of lactose into lactic acid followed by precipitation of casein into a highly odoriferous black sludge[3,4]. When discharged into water body, especially a stagnant one, the effluent brings about rapid depletion of the dissolved oxygen, adversely affecting its ecosystem[5–8].

Cyanobacteria are the most dominant and directly affected populations in any water body. In the present study, a new approach was adopted by using X-ray diffraction technique in addition to traditional methods to study *in vitro* interaction between cyanobacteria and dairy effluent. X-ray diffraction has been used to study the crystalline structure of various organic and inorganic molecules[9,10]. Our studies have shown that it is a quick and non-destructive method and that it clearly indicates the change in biochemical profile of both effluent and cyanobacteria.

Effluent samples were collected from a local milk processing plant. Mixed cyanobacteria (500 mg) were cultured in 250 ml of effluent for 3, 5, 7, 9 and 11 days. Physico-chemical properties of dairy effluent were analysed before and after the growth of cyanobacteria by standard methods[11]. Change in fresh weight was estimated. Chlorophyll *a*, β-carotenes, total proteins were estimated by the methods suggested by Mackinney[12], Kaushik[13], Bredford[14] and carbohydrates by Anthrone method[15]. Three sets of three replicates each and respective controls were maintained.

X-ray diffraction patterns of effluent and cyanobacteria, before and after culture were obtained using a Rigaku Miniflex tabletop diffractometer. CuKα X-ray radiation was used. Spectra of each sample were collected at room temperature for a range of angles of 2θ over 5° to 60°. All spectra were recorded with a step size of 0.1° and a scan speed of 2° per minute. Raw recorded data were then corrected for background and for elimination of the Kα-2 peaks. Effluent solution (500 ml) was evaporated and the residual powdered material was mounted in a hollow aluminum holder and used as a sample. Cyanobacterial samples were prepared by spreading one-gram fresh weight of biomass on a glass slide, in approximately 1.5 cm diameter area. Air-dried samples along with the slide were used.

Figure 1 *a–c* shows the physico-chemical properties of dairy effluent before and after growth of cyanobacteria. Maximum decrease of organic matter was seen between 0 and 3 days. Alkaline nature of effluent showed gradual increase as a result of breakdown of bicarbonates into hydroxyl and carbonate ions (Table 1). Manoharan and Subramanian[16] have also reported rise in pH value up to day 10 of growth in paper mill waste. No significant change in chloride content was observed, although initial decrease followed by increase has been reported by other workers[17]. Phosphates, sulphates, proteins, carbohydrates, etc. showed a gradual decline (Table 1). Reduction of sulphate in effluent and its uptake by cyanobacteria has been reported[18,19]. Tam and Wong[20] have reported over 90% removal in total phosphorus within 10 days of algal cultivation.

Proteins and carbohydrates together are responsible for total suspended solids (TSS) and turbid nature of the effluent. Turbidity of effluent showed continuous decrease and by day 11 the effluent became almost clear. With the decrease in TSS, the total dissolved solids (TDS) increase, i.e. organic substances are being broken down into simpler inorganic forms for absorption by growing cyanobacteria. This leads to increase in conductivity from 0 to 5 days and steady behaviour thereafter. Decrease in biological oxygen demand (BOD), chemical oxygen demand (COD) and dissolved organic matter (DOM) further confirms that proteins and carbohydrates are being broken down before absorption. Considerable reduction in all these parameters by micro algal systems has been reported[16,17,21,22].

Ca and Mg contribute to the hardness of effluent. A general decrease in their concentrations with respect to zero day was observed. A slight increase was observed after day 9, although the concentration on day 11 was still less than the zero day value. Probably these ions were absorbed by the biomass and later released back into the effluent. The biomass X-ray spectra also showed increase in intensity of Mg from 3 to 7 days (Figure 2). There are reports of reduction in Mg from sewage and Ca in ossein effluent-treated by microorganisms[17,23].

†For correspondence. (e-mail: kanikasharma@yahoo.com)

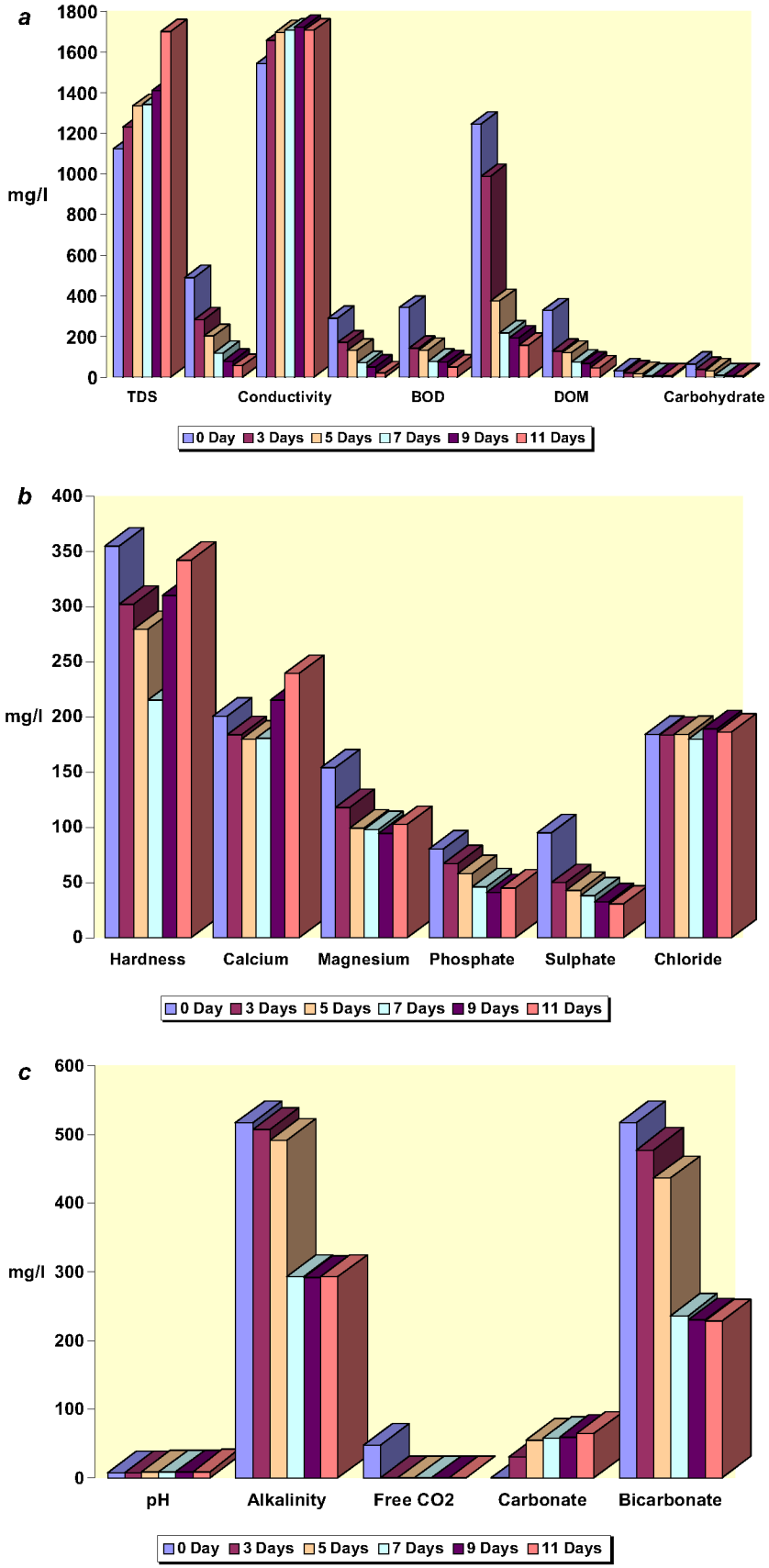

Figure 1 *a–c.* Physico-chemical properties of dairy effluent after culture of cyanobacteria at different time intervals. TSS, Total suspended solids; TDS, Total dissolved solids; BOD, Biological oxygen demand; COD, Chemical oxygen demand; DOM, Dissolved organic matter.

Table 1. Physico-chemical properties of untreated and residual effluent

Physico-chemical properties	Time period of culture of biomass (No. of days)					
	0	3	5	7	9	11
pH	6.81 ± 0.16	7.36 ± 0.08	8.14 ± 0.06	8.85 ± 0.14	8.94 ± 0.08	9.06 ± 0.06
Conductivity	1542.33 ± 30.56	1655.33 ± 16.04	1695.66 ± 8.02	1705.33 ± 18.58	1722 ± 29.86	1705.33 ± 16.28
Chloride	184.19 ± 11.86	183.47 ± 11.24	184.14 ± 11.86	179.98 ± 9.97	189.16 ± 8.07	186.31 ± 5.48
Total alkalinity	567.33 ± 17.00	529.33 ± 18.58	491.33 ± 9.23	293.33 ± 9.45	291.33 ± 6.42	292.66 ± 7.21
$CO_3 + OH^-$	46.66 ± 4.16	52.00 ± 7.2	54.66 ± 7.02	57.33 ± 7.02	59.33 ± 7.02	64.66 ± 8.00
HCO_3	517.33 ± 15.53	477.33 ± 13.01	436.66 ± 4.16	236.00 ± 4.00	230.00 ± 2.00	228.00 ± 2.00
Mg	154 ± 16.37	118 ± 13.85	99.3 ± 13.01	98.0 ± 2.0	94.66 ± 5.03	102.66 ± 11.01
Ca	200.66 ± 19.0	184.00 ± 17.77	180.00 ± 16.0	180.66 ± 17.0	215.33 ± 5.03	239.33 ± 3.05

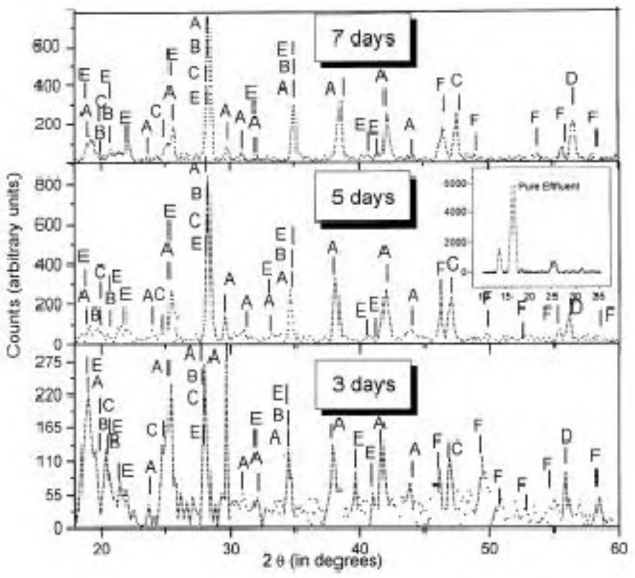

Figure 2. X-ray spectra of dried cyanobacteria samples cultured after days 3, 5 and 7 assigning peak positions to different substances: A, Glutaconic acid; B, Poly analnyl bis (Glycl); C, p-hydroxybenzoic acid; D, Sulphur oxide graphite; E, Magnesium phenol sulphonate; F, Unassigned peaks.

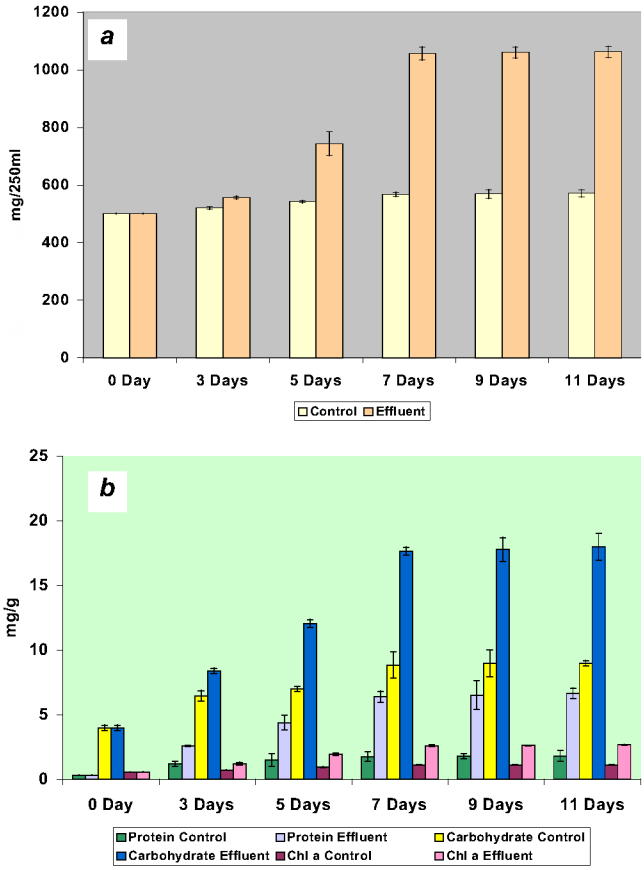

Figure 3. *a*, Change in biomass of cyanobacteria after different days of culture in effluent. *b*, Biochemical composition of cyanobacteria after different days of culture in effluent.

Figures 3 and 4 show the change in biomass, chl *a*, β-carotene, total protein and carbohydrate contents of cultured cyanobacteria. Significant increase in all these parameters was observed till day 7 followed by gradual decrease or no change at day 9 and day 11, suggesting that effluent enhances the growth of cyanobacteria and after day 9, growth decreases due to nutrient depletion. Tam and Wong[20] have reported 6 and 9 days as optimum culture time for maximum biomass cultivation.

Appearance of sharp peaks in an X-ray spectrum indicates the crystalline nature of the product. Meusel *et al.*[24] have established the crystalline nature of plant waxes by X-ray diffraction. Figure 5 shows the X-ray spectra of dried samples of cyanobacteria cultured in effluent along with control, i.e. cyanobacteria grown in distilled water. It can be seen that there is considerable enhancement of certain peaks compared to respective controls in the biomass spectra. Some new peaks, which were not seen in the control spectra, were also observed. Figure 6 shows the spectra of dried samples of residual effluent at days 0, 3, 5, 7, 9 and 11. A general decrease was observed in all peaks of days 3, 5, 7 samples. But an increase in most peaks for effluent samples at day 9 and day 11 was seen. For angles (2θ) between 18.8° and 21°, it can be seen that the day 3 sample shows appearance of new peaks with intensity more than the ones treated for days 5 and 7 (Figure 5).

On comparison with the standard ICDD[25] cards, it was observed that these peaks mainly correspond to the organic polymer poly alanyl bis (Glycl) $(C_7H_{11}N_3O_3)_n$

and few of the more intense peaks of *p*-hydroxybenzoic acid $(C_7H_4O_2)_n$ (Figure 2). Although these peaks are also present in the other two samples, they do not show as much intensity as in the day 3 sample. This shows that maximum absorption of these compounds takes place within three days of treatment with effluent. Other prominent peaks observed correspond to organic compounds magnesium phenol sulphonate ($C_{12}H_{10}MgO_8S_2$) and glutaconic acid ($C_5H_6O_4$) (Figure 2).

The very high enhancement of the peak at 28.4° in particular was due to the overlap of peaks of these two compounds and Glycl. Although this peak was present in all control samples, it was approximately of the order of 20% of the most intense peak in their respective spectra. The enhancement is therefore about four times in the day 5 and day 7 effluent-treated samples and about five times for the day 3 effluent-treated sample. Peaks corresponding to the compound sulphur oxide graphite (C_4SO_3) are also seen to be present. Few distinct peaks corresponding to peak positions (2θ) 46.1°, 47.5°, 55.7° and 56.7° in the day 5 and day 7-treated samples have not been able to be assigned satisfactorily (Figure 2). Some preliminary work seems to indicate that these unassigned peaks point to the presence of metals like Co, Ni, Ti, Al and Na as salts or complexes. Further supporting studies are required to completely identify and establish their identities.

A comparison between diffractograms of pure effluent (inset in Figure 2) and effluent-treated samples of cyanobacteria (Figure 2) indicates that the effluent has not merely been absorbed into the organism but has been assimilated. This conclusion can be drawn from the fact that the main and very intense peaks of the pure effluent samples in no way correspond to any of the peaks obtained for the effluent-treated samples.

X-ray studies suggest that till day 7 there is absorption and assimilation of nutrients present in the effluent. This can be seen by the appearance of new and well-defined peaks, indicating the crystalline nature of new products, at different time intervals in the diffractogram of biomass with corresponding reduction in the peaks of effluent. Diffractograms of days 9 and 11 show disappearance of several peaks, which indicates that the structural integrity of various metabolites has been destroyed and the biomass is converting into an amorphous substance (Figure 5).

On the basis of results obtained, it can be concluded that cyanobacteria utilize the organic and inorganic matter present in the effluent as nutrients. Zero to eleven-day observations show that initially nutrient absorption is rapid till the 7th day but it gradually slows down thereafter. This trend is reflected in the physico-chemical properties of the effluent. After 7 days, due to nutrient depletion, cyanobacteria start undergoing degeneration leading to release of several substances, ions, etc. back into the

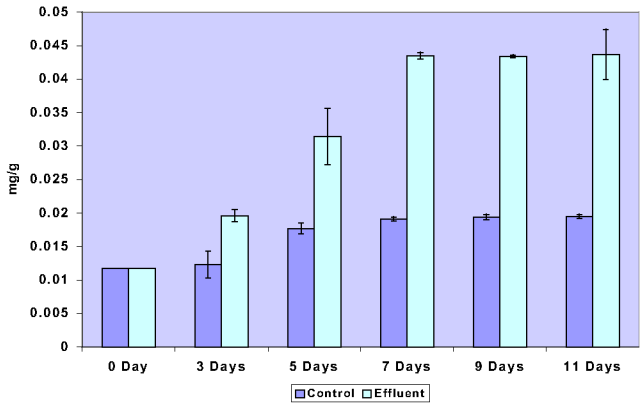

Figure 4. β-carotene content of cyanobacteria after different days of culture in effluent.

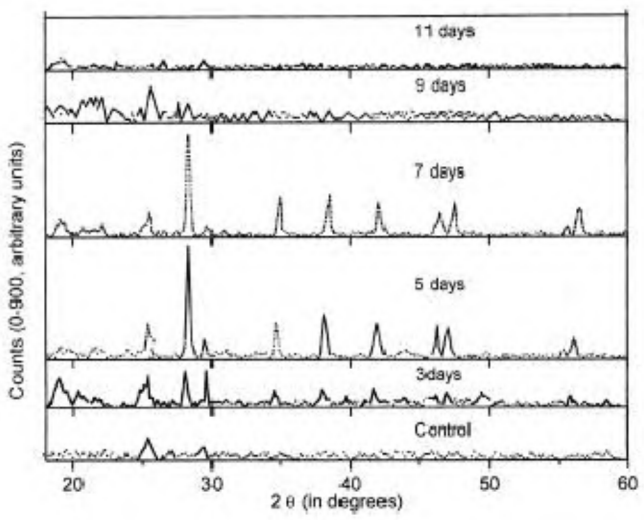

Figure 5. X-ray spectra of dried samples of cyanobacteria cultured in effluent for 0, 3, 5, 7, 9 and 11 days.

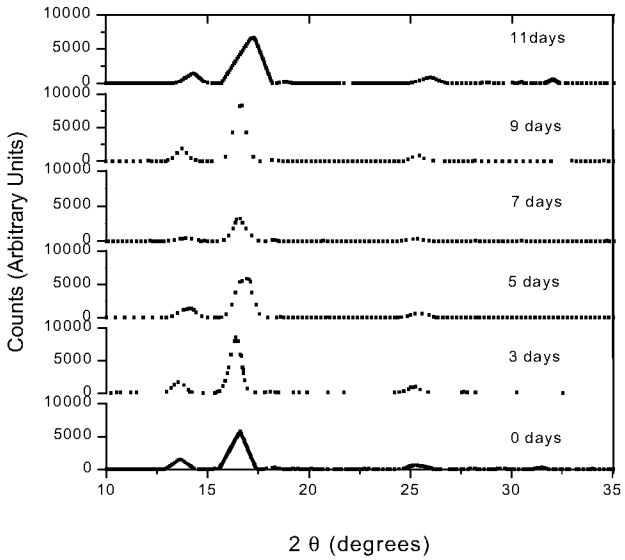

Figure 6. X-ray spectra of dry leftover effluent samples after removal of cyanobacteria at the end of 0, 3, 5, 7, 9 and 11 days culture period.

medium. This could be a possible explanation for increase in calcium, magnesium, phosphate, etc. in the medium, after 7 days. This conclusion is also supported by an increase in the peak intensities of effluent X-ray spectra of days 9 and 11 (Figure 6).

Thus X-ray diffraction study, in addition to corroborating the results obtained by conventional methods, gives more specific information. It is a fast and more efficient method of monitoring the systematics of biomass–effluent interactions.

1. Tiwana, N. S., Nature and magnitude for water pollution in Punjab, Proceedings of Seminar on Status of Environmental Pollution, PAU, Ludhiana, 1985.
2. Subrahmanyam, P. V. R. and Mohan Rao, G. J., Treatment of dairy wastes by unmechanised methods. *Indian J. Environ. Health*, 1972, **14**, 236–245.
3. Pandit, B. R., Prasannakumar, P. G. and Kumar, M. R., Effect of dairy effluent on seed germination, seedling growth and pigment of *Pennisetum typhoides.* Barm. and *Sorghum bicolor* L., *Pollut. Res.*, 1996, **15**, 103–106.
4. Datar, M. T. and Kale, S. B., Performance of a dairy effluence treatment plant. *Indian J. Environ. Health*, 1997, **39**, 52–60.
5. Sharma, B. M., Aslam, M. and Gupta, H. O., Impact of industrial effluents on the aquatic system of the Doon valley. *J. Environ. Pollut.*, 2000, **7**, 59–65.
6. Chockalingam, S. and Balaji, A., Effect of dairy effluent on the rate of oxygen consumption and survival of the tadpole larva of *Bufo bufo*. *J. Environ. Biol.*, 1991, **12**, 377–379.
7. Amudha, P., Nagendran, R. and Mahalingam, S., Studies on the effects of dairy effluent on the behaviour of *Cyprinus carpio* (Cyprinidae). *J. Environ. Biol.*, 1997, **18**, 415–418.
8. Rai, R. K., Verma, S. and Goyle, M., Growth and biochemical profile of two cyanobacteria effected by carpet industry effluent. *Pollut. Res.*, 1999, **18**, 71–74.
9. Russell, P. J., The structure of genetic material. In *Genetics,* Addison Wesley, New York, 1998, pp. 303–304.
10. Davis, B. D., Dulbecco, R., Eisen, H. N. and Ginsberg, H. S., Antibody–antigen reactions. In *Microbiology*, Harper International Editions, Philadelphia, 1980, 3rd edn, pp. 292.
11. APHA, Standard methods for the examination of water and wastewater, American Public Health Association, AWWA and WPCF, Washington DC, 1985.
12. Mackinney, G., Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, 1941, **140**, 315–322.
13. Kaushik, B. D., *Laboratory Methods for Blue Green Algae,* Associated Publishing Company, New Delhi, 1987, 1st edn, pp. 65–69.
14. Bredford, M. M., Rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein dye binding. *Ann. Biochem.*, 1976, **72**, 248–254.
15. Tandon, P., Further studies on the process of gall induction on *Zizyphus* and the factors involved. Ph. D. thesis, Jodhpur University, Jodhpur, India, 1976.
16. Manoharan, C. and Subramanian, G., Interaction between papermill effluent and the cyanobacterium *Oscillatoria pseudogerminata* var. *unigranulata. Pollut. Res.*, 1992, **11**, 73–84.
17. Manoharan, C. and Subramanian, G., Feasibility studies on using cyanobacteria in ossein effluent treatment. *Indian J. Environ. Health*, 1993, **35**, 88–96.
18. Dash, A. K. and Mishra, P. C., Role of blue green alga *Westiellopsis prolifica* in reducing pollution load from paper mill wastewater. *Indian J. Environ. Protect.*, 1999, **19**, 1–5.
19. Mittal, S. and Sengar, R. M. S., Toxic effect of sulphate and its uptake in algae. *Natl. Acad. Sci. Lett.*, 1989, **12**, 17–19.
20. Tam, N. F. Y. and Wong, Y. S., The comparison of growth and nutrient removal efficiency of *Chlorella pyrenoidosa* in settled and activated sewages. *Environ. Pollut.*, 1990, **65**, 93–108.
21. Manoharan, C. and Subramanian, G., Sewage–cyanobacterial interaction – a case study. *Indian J. Environ. Protect.*, 1992, **12**, 251–258.
22. Fallowfield, H. D. and Barret, M. K., The treatment of wastes by algal culture. *Agric. Wastes*, 1985, **12**, 111–136.
23. Uma, L. and Subramanian, G., Effective use of cyanobacteria in effluent treatment. Proceedings of the National Symposium on Cyanobacterial Nitrogen Fixation, IARI, New Delhi, 1990, pp. 437–444.
24. Meusel, I., Barthlott, W., Kutzke, H. and Barbier, B., Crystallographic studies of plant waxes. *Powder Diffr.*, 2000, **15**, 123–129.
25. William, F. M. (ed.), Powder diffraction file, organic and organometallic phases search manual, ICDD 1998, Card numbers a: 37–1943; b: 36–1744; c: 27–1665; d: 38–1212; e: 1–567.

ACKNOWLEDGEMENTS. This work has been partly supported by UGC DRS COSIST programme to the Department of Physics, M. L. Sukhadia University Udaipur, and also by the DST (Rajasthan).

Received 20 January 2003; revised accepted 18 September 2003

Structural characteristics of marine atmospheric boundary layer and its associated dynamics over the Central Arabian Sea during INDOEX, IFP-99 campaign

D. Bala Subrahamanyam* and Radhika Ramachandran†

Space Physics Laboratory, Vikram Sarabhai Space Centre, Thiruvananthapuram 695 022, India
*Present address: Indian National Centre for Ocean Information Services, Department of Ocean Development, Hyderabad 500 033, India

The Indian component of the Intensive Field Phase of Indian Ocean Experiment conducted onboard Oceanic Research Vessel *Sagar Kanya* during its SK-141 cruise provided valuable meteorological data over the data-sparse region of tropical Indian Ocean and Central Arabian Sea (CAS). The upper air meteorological data obtained from balloon-borne GLASS sonde launches during the campaign are analysed for studying the thermodynamic structure of the Marine Atmospheric Boundary Layer over the CAS. Analysis of profiles of the thermodynamic variables revealed a double mixed layer structure over the region of CAS. We have made an attempt here to provide a possible explanation of the double mixed layer.

THE Indian Ocean Experiment (INDOEX), a major international and multi-disciplinary field experiment, had several objectives focused towards the studies of aerosols, radiation and transport of pollutants over the western tropical Indian Ocean region[1,2]. The Indian component of the

†For correspondence. (e-mail: radhika@md3.vsnl.net.in)