Relationships between bacteria and cyanobacteria in the Marrakech waste stabilisation ponds

K. Oufdou*, N. Mezrioui*, B. Oudra**, M. Barakate*, M. Loudiki** and A. Ait Alla***

* Microbiology Laboratory, Cadi Ayyad University, Faculty of Sciences-Semlalia, Department of Biology, P.O. Box S15, Marrakech 40 000, Morocco.

** Algology Laboratory, Cadi Ayyad University, Faculty of Sciences-Semlalia, Department of Biology, P.O. Box S15, Marrakech 40 000, Morocco.

* Microbiology Laboratory, Ibnou Zohr University, Faculty of Sciences, Department of Biology, P.O. Box S28, Agadir, Morocco.

Abstract In waste stabilisation pond systems the interactions between algae and bacteria have an important ecological effect and appeared to play a key role in the self-purification process. The aim of the present study is to evaluate the interactions between two axenic cyanobacteria: *Synechocystis* sp. and *Pseudanabaena* sp. with heterotrophic bacteria and some pathogenic bacteria: *E. coli, Salmonella* sp. and non-O1 *V. cholerae*. The results obtained showed that *Synechocystis* sp. (planktonic cyanobacterium) and *Pseudanabaena* sp. (benthic cyanobacterium) stimulated the growth and the survival of heterotrophic bacteria and non-O1 *V. cholerae* and reduced the survival of *E. coli* and *Salmonella*. Blooms of these cyanobacteria during hot periods which are a particularity in stabilisation ponds of Marrakech, could explain the dynamics of bacteria studied in this sewage treatment process. The presence of heterotrophic bacteria, showing relative high densities during hot periods, could be considered as an important biotic factor which led to the cyanobacteria blooms in the Marrakech ponds which function under an arid Mediterranean climate.
Keywords Heterotrophic bacteria, *Pseudanabaena*, pathogenic bacteria, stabilisation ponds, *Synechocystis*, wastewater.

Introduction

Among the biological treatment systems for wastewater, the stabilisation pond method is recommended in developing countries where sewage water is increasing, being re-used for irrigation particularly in arid and sub-arid areas. It can simultaneously solve the environmental and sanitary problems and it may also be economically efficient if treated wastewater is re-used for agricultural purposes such as occurs in Marrakech (Morocco) (Oron *et al.*, 1985; Hassani *et al.*, 1992). In wastewater stabilisation ponds, algae and bacteria are two major biological components. The interactions between these two populations appeared to have an important ecological effect. Beneficial (Lange, 1973; Cole, 1982) or antagonistic interactions (Dor and Svi, 1980; Forlani *et al.*, 1989) which occur between these two populations, may have a key role in the biological waste purification efficiency (Oswald, 1988). In wastewater lagoons of Marrakech, high densities of heterotrophic bacteria and non-O1 *V. cholerae* and low densities of faecal coliforms and *Salmonella* sp. were noted during hot periods (Oufdou, 1994; Mezrioui and Echab, 1995; Mezrioui *et al.*, 1995). This period coincided with net blooms of two axenic cyanobacteria: *Synechocystis* sp. (planktonic cyanobacterium) and *Pseudanabaena* sp. (benthic cyanobacterium). The occurrence of cyanobacterial blooms is an important particularity of this sewage treatment. There is little information on relationships between cyanobacteria and bacteria particularly in stabilisation ponds. The purpose of the present study was therefore to evaluate the interactions between *Synechocystis* sp. and *Pseudanabaena* sp. with heterotrophic bacteria and the effects of these blue-green algae on the survival and behaviour of pathogenic bacteria

(*Escherichia coli, Salmonella* sp. and non-O1 *Vibrio cholerae*) in the wastewaters of the Marrakech stabilisation ponds.

Material and methods

The stabilisation pond system

The stabilisation ponds are situated at the sewage spreading field of Marrakech (31°36' N, 08° 02'W, Morocco) and receive only part of the city sewage. There are two oval ponds linked in series, each is 2500 m^2 in area. The first pond is anaerobic (depth of water 2.3 m) and the second pond is facultative aerobic (depth: 1.5 m). The raw sewage flow to the system is maintained at 5.4 l/s. The total hydraulic retention time was set at about 18 days with 10.5 days in the first pond and 7.5 days in the second pond.

Cyanobacterial and bacterial strains

Cyanobacterial strains studied were unicellular planktonic picocyanobacterium: *Synechocystis* sp. and pluricellular filamentous benthic cyanobacterium: *Pseudanabaena* sp. These strains were isolated from the second pond of Marrakech lagoons. They were cultivated and purified on BG$_{13}$ agar (Ferris and Hirsch, 1991). The *Synechocystis* inoculum (8 ×10^5 cells/ml) was comprised of some colonies of this algae growing on BG$_{13}$ sugar and resuspended in sterile wastewater of the first stabilisation pond, filtered on Whatman GF/C (Glass fiber filter) and autoclaved at 120°C for 15 minutes. The *Pseudanabaena* inoculum was constituted of a few ml of this algal culture growing on sterile wastewater medium having final chlorophyll a content of 3.157 µg/l. Pathogenic bacterial strains tested were *E. coli*, non-L1 *V. cholerae* and *Salmonella* sp. They were isolated from the stabilisation pond's inflow and identified according to the method described by Oufdou *et al.* (1988). All studied bacteria were grown on trypticase-soja agar (Institute Pasteur Production; IPP) for 37°C for 18 hours. The monobacterial inoculum (approximately 10^6 colonies forming units / ml; cfu / ml) was constituted of some colonies of these cultures which were suspended in sterile wastewater. The heterotrophic bacterial strains were isolated from stabilisation pond's inflow. Their inoculum (3.5×10^4 cfu / ml) was made up of 40 different strains, previously grown by prick on nutrient agar (IPP) and resuspended in 10 ml of sterile wastewater. According to Bianchi and Bianchi (1982), samples of 20 or 30 colonies were statistically significant to have a good idea of the qualitative composition of bacteria in the medium.

Interactions between cyanobacteria with heterotrophic bacteria and pathogenic bacteria

The reaction medium used was the wastewater of the first pond of Marrakech lagoons, filtered (glass fiber filter, Whatman GF / C), autoclaved at 120°C for 15 minutes and adjusted at pH 7.3 with sterile solutions of NaOH (1N) or HCl (1N). The wastewater was distributed at the rate of 700 ml in each Erlenmeyer flask. We compared the growth of heterotrophic bacteria in wastewater with and without algae, and in the supernatant of *Synechocystis* or *Pseudanabaena*. At the same time, the growth of each cyanobacterial strain was compared in wastewater with or without heterotrophic bacteria, and in the supernatant of these bacteria. The supernatants were prepared as follows: bacteria and algae (*Synechocystis* or *Pseudanabaena*) were separately grown in sterile wastewater and samples were collected at the exponential phase of growth, filtered through a filter Millipore (0.45 µm pore) and adjusted at pH 7.3. All culture flasks were incubated at 27 ± 1°C in a 14:10 light / dark regime provided by 40 W white fluorescent tubes under an intensity of 60 µE / m^2 / s. The culture flasks were agitated on a table shaker set at 100 rev./min. The effect of *Synechocystis* sp. and *Pseudanabaena* sp. on the survival of pathogenic bacteria, under sunlight conditions, was done in 700 ml of sterile wastewater which was distributed in each

PVC microcosm (surface $= 142^2 \times$ height $= 6.7$ cm). The microcosms were inoculated separately with each pathogenic bacteria (*E. coli, Salmonella* or non-O1 *V. cholerae*) and/or with *Synechocystis* or *Pseudanabaena*. Some microcosms were exposed to sunlight, whereas other microcosms were protected by aluminium foil (control microcosms).

Bacterial counts and physico-chemical parameters

The temporal evolution of heterotrophic bacteria was enumerated, by indirect count of cfu, on nutrient agar (IPP) incubated at 25°C for 5 to 7 days. Enumeration of pathogenic bacteria was also done by indirect count of cfu, at sunset, on selective media: TTC-Tergitol 7 agar (IPP) incubated at 44.5°C for 24 hours (*E. coli*), *Salmonella-Shigella* agar (Difco) incubated at 37°C for 24 to 48 hours (*Salmonella* sp.) and TCBS agar (Difco) incubated at 37°C for 24 hours (non-O1 *V. cholerae*). Throughout this experimental study, pH was measured by using ORION Research, model 601 A pH meter. The temporal evolution of *Synechocystis* sp. was done by hematocytometer cells (Malassez cells, depth 0.2 mm) (Sournia, 1978), whereas the growth of *Pseudanabaena* sp. was evaluated by the dosage of chlorophyll a according to the method of Marker *et al.* (1980). The organic carbon (O.C.) was estimated from chemical oxygen demand (C.O.D.). According to Akiyama (1973), Somiya and Fujii (1984), the ratio C.O.D./O.C. was estimated to 3 for bacteria, algae, organic matter released by algae and for wastewaters. The *Synechocystis* sp. and heterotrophic bacteria growth was also estimated by the rate expressed as follows: $\mu = \text{Ln} (Nt / No) / (t\text{-to})$ in which No was the initial number of cells (at to) and Nt was the cells number at the instant t (Guilllard, 1973).

Results and discussion

In order to have axenic algar cultures, earlier studies have recommended the use of antibiotics such as streptomycin, penicillin G, gentamycin, chloramphenicol (Droop, 1967) or a chemical method using potassium tellurite (Rosowski and Hoshaw, 1970). The use of these antibiotics, singly or in combination, or of potassium tellurite, however they kill or inhibit the growth of tenaciously bacteria contaminants, affected the growth and the survival of the studied blue-green algae especially of the unicellular cyanobacterium; *Synechocystis* sp. These cyanobacterial strains were rendered axenic after several treatments. At first, a new treatment to reduce significantly the contaminants associated bacteria, was the supplying of the citric acid from the BG_{13} medium. The citric acid is the sole organic source of carbon in this medium (Ferris and Hirsch, 1991) that will be preferably consumed by bacteria. The initial number of bacteria in close association to *Synechocystis* and *Pseudanabaena* after agitation of algar cultures for 30 min, was respectively of 38×10^{10} and 41×10^{12} cfu / ml. After one use of the BG_{13} sugar without citric acid, the number of bacteria decreased by approximately 2.6 logarithmic units to 87×10^7 cfu /ml for *Synechocystis* sp. and 92×10^9 cfu / ml for *Pseudanabaena* sp. This treatment was repeated many times. In addition, colonies of *Synechocystis* sp. and filaments of *Pseudanabaena* were aseptically transferred and grown in few ml of liquid BG_{13} medium without citric acid in continuous illumination and agitation under the conditions of growth previously described. At the stationary growth phase, 0.1 ml of the samples, checked for 30 min., or its appropriate dilutions were plated on BG_{13} agar without citric acid. Algar cells were resuspended and washed in sterile distilled water at least three times, centrifuged at 5000 rev. / min and aseptically resuspended in liquid BG_{13} medium. These treatments were repeated until we had axenic cultures of *Synechocystis* and *Pseudanabaena*. The axenicity of these strains was always confirmed. A few ml of liquid BG_{13} medium containing cyanobacteria and algal suspension in distilled water of colonies on BG_{13} agar, were added with nutrient broth and incubated at 30°C for 5 to 7 days. No tube or flask showed turbidity. Furthermore, 0.1 ml of the samples or of their dilutions spreading on nutrient agar also confirmed the absence of bacteria. These strains were conserved as

173

axenic by successive pricking on BG_{13} medium agar under aseptic conditions. It is interesting to note that the studied blue-green algae did not possess heterocysts on which numerous bacteria are tenaciously encountered (Paerl, 1988).

The growth of heterotrophic bacteria isolated from Marrakech stabilisation ponds was affected by *Synechocystis* sp. and *Pseudanabaena* sp. As shown in figure 1, bacterial growth improvement was obtained during the exponential algal growth phase (figure 2). The bacterial exponential growth rate was significantly higher ($p < 0.05$) in the presence of *Synechocystis* sp. ($4.02\ d^{-1}$) than that noted in its absence ($2.88\ d^{-1}$). At the same time, the growth of heterotrophic bacteria was promoted by *Pseudanabaena* sp. ($3.43\ d^{-1}$). This stimulation of bacterial growth was weaker than that provided by *Synechocystis* sp. ($4.02\ d^{-1}$). The increasing bacterial growth could be attributed to the assimilation of organic matter produced by cyanobacteria. In axenic culture of *Synechocystis* sp., we have noted at the fifth day of the experiment, 200 mg/l of dissolved organic carbon, whereas in the mixed *Synechocystis*-bacterial culture, the quantity of dissolved organic carbon was weaker (114.4 mg/l) (figure 3). Previous studies have shown that phytoplankton products can be used by bacteria and account for 30 to 90% of bacterial carbon uptake (Bell *et al.*, 1983; Feuillade *et al.*, 1988). Although it is obvious that other sources of bacterial organic carbon exist in aquatic systems, phytoplankton appeared to be the major source in most cases. Cole *et al.* (1982) noted that a variety of interactions between algae and bacteria occur in aquatic systems, but one of the major processes is the production by algae of organic carbon materials suitable for bacterial assimilation. This result was also confirmed by the significant increasing of heterotrophic bacterial growth in the supernatants of *Synechocystis* sp. ($\mu = 4.25\ d^{-1}$) and *Pseudanabaena* sp. ($\mu = 3.72\ d^{-1}$) (figure 1). Our experiment, in concert with correlative and experimental studies of others, revealed that materials from cyanobacteria are important substrates for bacterial growth and stimulation of the degradation of organic matter in wastewater stabilisation ponds. Blooms of *Synechocystis* sp. and *Pseudanabaena* sp. in Marrakech lagoons can explain the relatively high densities of heterotrophic bacteria in this aquatic ecosystem.

At the stationary algal phase, the growth of heterotrophic bacteria in the mixed culture decreased as compared to the control microcosm containing only bacteria (figure 1). The decrease of bacterial population may be attributed, but not totally, to the alkaline pH caused by the algal growth. During this period, pH of the mixed algal-bacterial cultures was of 9 units whereas, it was only 8 units in control microcosm. It can also be explained by antibacterial substances produced by the studied blue-green algae. In a previous paper (Oufdou *et al.*, 1988), we reported that *Synechocystis* sp. released, at the stationary growth phase, bioactive compounds which have antibacterial activity. The competition to nutrients in the batch cultures may not be excluded. This last phenomenon was the possible explanation for the reduced bacterial growth at the stationary phase of algar growth, since the bacterial densities were significantly higher in supernatants of these cyanobacteria as compared in the microcosm containing only bacteria (Figure 1).

As for the growth of cyanobacterial strains, it was stimulated by heterotrophic bacteria. The exponential growth rate of *Synechocystis* sp. was significantly higher ($p < 0.05$) in the mixed culture ($0.72\ d^{-1}$) than that noted in the axenic culture ($0.54\ d^{-1}$) (Figure 2a). Similar results were obtained for the growth of *Pseudanabaena* sp. evaluated by the dosage of chlorophyll a (Figure 2b). This result is in contrast to the finding of Dor and Svi (1980) who have observed that sewage heterotrophic bacteria reduced the growth of green algae; *Chlorella* and *Scendesmus*. Nevertheless, our result was in agreement with the works of Lange (1973) and Wang and Priscu (1994), who have demonstrated that cultures of various cyanobacteria were enhanced in the presence of bacteria. Several factors may explain the stimulation of algal growth by the autochthonous bacteria. There are some reports supporting the beneficial effect of bacterially-produced CO_2 vitamins and growth factors on algae
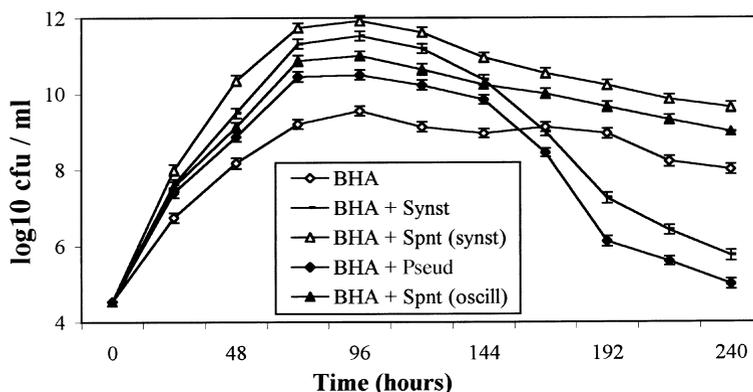
Figure 1 Temporal development of heterotrophic bacteria (BHA) (confidence intervals, 95% : n = 3) in the absence and the presence of *Synechocystis* sp. (Synst) or *Pseudanabaena* sp. (Oscill) and in their culture supernatant (Spnt)
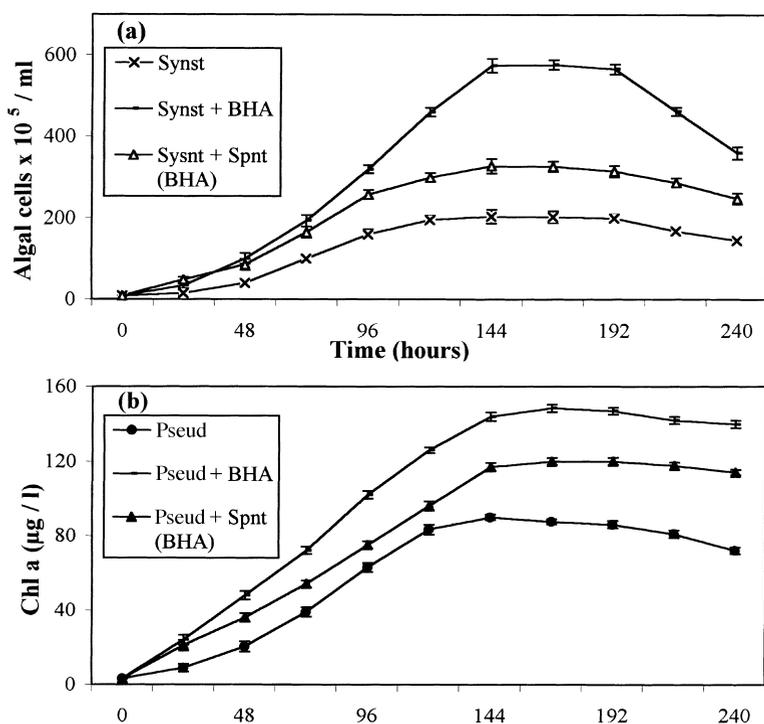

Figure 2 Growth of *Synechocystis* sp. (Fig. 2a) and *Pseudanabaena* sp. (Fig. 2b) (confidence intervals, 95% : n = 4) in the absence and the presence of heterotrophic bacteria and in their culture supernatant (Spnt BHA)

(Lange, 1973; Chirac *et al.*, 1985). This symbiotic association might prevent the accumulation of alkalinity and oxygen within the micro-environment of algae (Caldwell, 1977). Escher and Characklis (1982) reported that in various aquatic environments, photosynthetic carbon fixation may be limited by a low $CO_2 : O_2$ ratio in the aqueous phase. These observations were supported by our finding. Indeed, the stimulation of cyanobacterial growth obtained in the supernatant of bacterial cultures was significantly weaker as compared in the mixed cultures containing living bacteria. The role of heterotrophic bacteria might be an active one, involving the possible recycling of algal extracellular products and the production of CO_2 which can benefit the algae. Lange (1973) concluded that the presence of bacteria-assimilable matter is one of the important factors leading to the blue-green algae

175

blooms in aquatic ecosystems. Algal growth in wastewater was determined chiefly by the quality of bacterial flora, while fluctuations in the chemical composition were of minor importance as was reported by the finding of Dor and Svi (1980). Our results, over previous studies of cyanobacterial-bacterial relationships in lakes and marine ecosystems, proved that synergistic effect of heterotrophic bacteria on *Synechocystis* sp. and *Pseudanabaena* sp. growth occur in Marrakech stabilisation ponds and could be considered as the important biotic factor which led to the cyanobacterial blooms in this ecosystem.

The effects of *Synechocystis* sp. and *Pseudanabaena* sp. under sunlight conditions, on the survival of pathogenic bacteria are given in Figure 4. These cyanobacteria have a strong inhibitory effect on *E. coli* and *Salmonella*. Densities of these bacteria were significantly ($p < 0.05$) higher in the presence of *Synechocystis* sp. and *Pseudanabaena* sp. as compared to their absence. *Synechocystis* sp. had a stronger inhibitory effect than that obtained by *Pseudanabaena* sp. (Figure 4). Several studies have been conducted on the production of
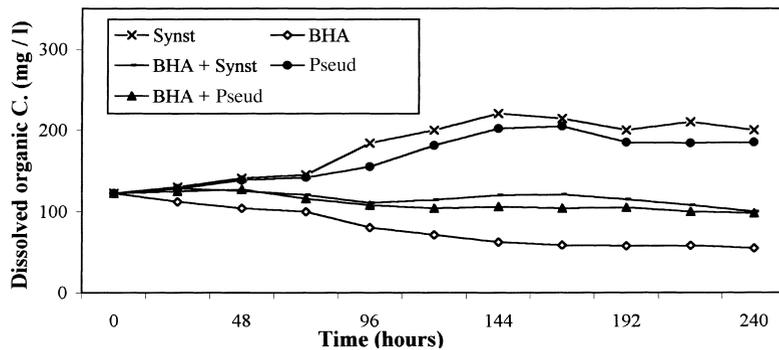

Figure 3 Temporal evolution of dissolved organic carbon in axenic algal cultures, in bacterial cultures and in mixed algal-bacterial cultures
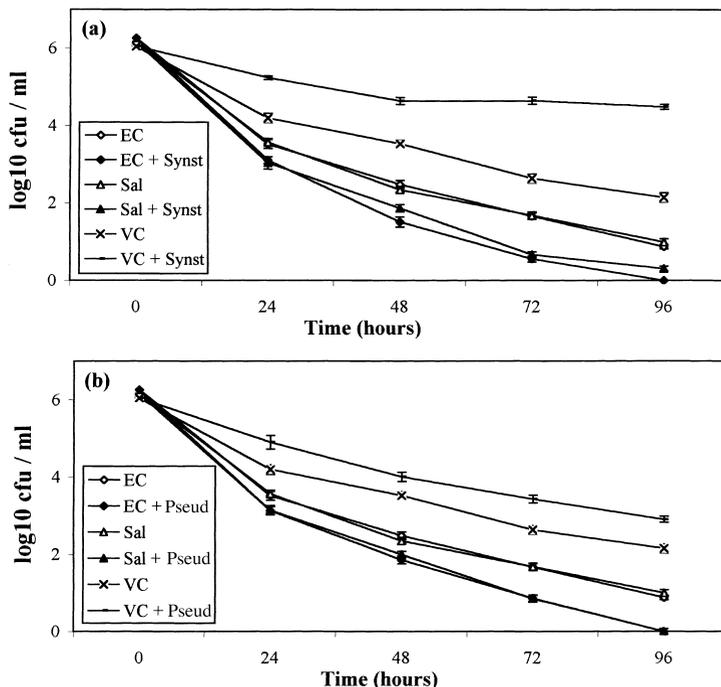

Figure 4 Temporal evolution of *E. coli* (EC), *Salmonella* sp. (Sal) and non-O1 *V. cholerae* (VC) abundances (confidence intervals, 95% : n = 3) in the presence and the absence of *Synechocystis* sp. (Fig. 4a) or *Pseudanabaena* sp. (Fig. 4b) in microcosms exposed to sunlight

antibacterial substances by cyanobacteria (Cano *et al.*, 1990; Caire *et al.*, 1993; Oufdoue *al.*, 1998). As for non-O1 *V. cholerae*, its survival was higher with these algae than that noted in their absence. The stimulation of non-O1 *V. cholerae* survival was more pronounced by *Synechocystis* sp. (figure 4a) than by *Pseudanabaena* sp. (Figure 4b). The studied blue-green algae seemed to lead to specific bacteria flora and to play an important ecological role in bacterial community composition in the Marrakech stabilisation ponds. During hot periods, cyanobacterial blooms coincided with low levels of the human pathogenic bacterium, *Salmonella* sp. (Mezrioui and Echab, 1995) and of the indicators of faecal pollution, faecal coliforms (Mezrioui *et al.*, 1995) and high densities of the pathogenic opportunistic bacteria, non-O1 *V. cholerae (*Mezrioui and Oufdou, 1996). Non-O1 *V. cholerae* was, however, recognised as an autochthonous bacterium in aquatic ecosystems (Singleton *et al.*, 1982; Colwell 1986). Cole (1982) has reported that among bacterial isolates from phytoplankton, 70% belonged to *Vibrio* sp. and *Aeromonas* so. We can presume according to this study and our previous works (Mezrioui *et al.*, 1995; Mezrioui and Oufdou, 1996) that non-O1 *V. cholerae c*old be an autochthonous bacterium and was an important population among heterotrophic bacteria in Marrakech stabilization ponds since their abundances were higher in this treatment system during hot periods. Blooms of cyanobacteria may be considered as a major biotic factor leading to proliferation of non-O1 *V. cholerae* in such aquatic ecosystems.

Conclusions

The treatment by supplying the citric acid from the BG_{13} agar medium to create axenic cultures of *Synechocystis* sp. and *Pseudanabaena* sp., reduced significantly the number of bacteria associated to these blue-green algae. The citric acid which is the sole organic source of carbon in this medium appeared to be preferably consumed by contaminant bacteria. Few studies have been carried out on interactions between axenic cultures of *Synechocystis* sp. and *Pseudanabaena* sp. with heterotrophic bacteria and even fewer on their effects on pathogenic bacteria survival in wastewaters of stabilisation ponds. Materials from cyanobacteria appeared to be important substrates for heterotrophic bacterial growth and stimulation of the degradation of organic matter in Marrakech lagoons. Blooms of *Synechocystis* sp. and *Pseudanabaena* sp. can explain the relative high densities of heterotrophic bacteria in this aquatic ecosystem. Synergistic and active effect of heterotrophic bacteria on *Synechocystis* sp. and *Pseudanabaena* sp. growth was noted. These autochthonous bacteria could be considered as the important biotic factor which led to the cyanobacterial blooms. The blue-green algae efflorescences seemed to lead to specific bacterial flora and to play an important ecological role in reducing bacterial survival of some pathogenic bacteria such as faecal coliforms and *Salmonella* sp. during their passage in stabilisation ponds. As for non-O1 *V. cholerae*, it appears that this bacterium is an autochthonous population. Blooms of cyanobacteria may be considered as a major biotic factor leading to high densities of non-O1 *V. cholerae i*n Marrakech lagoons.

Acknowledgements

References

Akiyama T. (1973). Index of water pollution and their application. *Wat. Purif. Liquid Waste Treat.*, **14**, 361-368.

Bell R., Ahlgren G. M. and Ahlgren I. (1983). Estimating bacterioplankton production by measuring [³H] thymidine incorporation in a eutrophic Swedish lake. *Appl. Environm. Microbiol.*, **45**, 1709-1721.

Bianchi M. A. G. and Bianchi A. J. M. (1982). Statistical sampling of bacterial strains and its use in bacterial diversity measurement. *Microb. Ecol.*, **8**, 61-69.

Caire, G. Z. De, Cano M. S. De, Mulé M. C. Z. De and Haplerin D. R. De (1993). Screening of cyanobacterial bioactive compounds against human pathogens. *Int. J. Experim. Botany (Phyton)*, **54**(1), 59-65.

Caldwell D. E. (1977). The planktonic microflora of lakes. In: *Critical reviews in Microbiology*. A. Laskin and H. Le Chevalier (eds.), vol. 5, CRS Press Inc., Cleveland, Ontario, pp. 305-370.

Cano M. S. De, Mulé M. C. Z. De, Caire G. Z. De and Halperin D. R. De (1990). Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyanobacterium *Nostoc muscorum*. *J. Appl. Phycol.*, **2**, 79-81.

Chirac C., Casadevall E., Largeau C. and Metzger P. (1985). Bacterial influence upon growth and hydrocarbon production of the green alga *Botrycoccus braunii*. *J. Phycol.*, **21**, 380-387.

Cole J. J. (1982). Interactions between bacteria and algae in aquatic ecosystems. *Ann. Rev. Ecol. Syst.*, **13**, 291-314.

Cole J. J., Likens G. E. and Strayer D. L. (1982). Photosynthetically produced dissolved organic carbon. An improvement carbon source for planktonic bacteria. *Limnol. Oceangr.*, **27**, 1080-1090.

Colwell, R. R. (1986). *Vibrio cholerae* and related Vibrios in the aquatic environment - an ecological paradigm. Proc. IV SIME, 426-434.

Dor I. and Svi. B. (1980). Effect of heterotrophic bacteria on the green algae growing in wastewater. In: *Algal biomass*. G. Shelef and C. J. Soeder (eds.), Elsevier/North Holland, Biomedical Press, pp. 425-429.

Droop, M. R. (1967). A procedure for routine purification of algal cultures with antibiotics. *Brit. Phycol. Bull.* **3**, 295-297.

Escher A. and Characklis W. G. (1982). Algal-bacterial interactions within aggregates. *Biotechnol. Bioengng.*, **24**, 2283-2290.

Ferris J. M. and Hirsch C. F. (1991). Method for isolation and purification of Cyanobacteria. *Appl. Environ. Microbiol.*, **57**, 1448-1452.

Feuillade M., Dufour Ph. and Feuillade J. (1988). Organic carbon release by phytoplankton and bacterial reassimiliation. *Schweiz. Z. Hydrol.,* **50**, 115-135.

Forlani, G., Tosti, E. and Volterra L. (1989). Growth interactions of phytoplanktonic algae and enteric bacteria. *Rev. Int. Oceangr. Med.*, **93-94**, 15-30.

Guillard R. R. L. (1973). Division rates. In: *Handbook of physiological methods - culture methods and growth measurements*. J. R. Stein (ed.), Cambridge University Press, pp. 289-311.

Hassani L., Imziln B. and Gauthier M. J. (1992). Antibiotic-resistant *Escherichia coli* from wastewater before and after treatment in stabilization ponds in the arid region of Marrakesh, Morocco. *Lett. Appl. Microbiol.*, **15**, 228-231.

Lange W. (1973). Bacteria-assimiliable organic compounds, phosphate, and enhanced growth of bacteria-associated blue-green algae. *J. Phycol.*, **9**, 507-509.

Marker, A. F. H., Crowther, C. A. and Gunn R. J. M. (1980). Methanol and acetone as solvents for estimating chlorophyll a and phaeopigments by spectrophotometry. *Arch. Hydrobiol./Beih. Ergenb. Limnol.*, **14**, 52-69.

Mezrioui N. and Echab K. (1995). Drug resistance in *Salmonella* strains isolated from domestic wastewater before and after treatment in stabilization ponds in an arid region (Marrakesh, Morocco). *World J. Microbiol. Biotechnol.*, **11**, 287-290.

Mezvioui N. and Ofdou Kh. (1996). Abundance and antibiotic resistance of non-O1 *Vibrio cholerae* strains in domestic wastewater before and after treatment in stabilization ponds in an arid region (Marrakesh, Morocco). *FEMS Microbiol. Ecol.*, **21**, 277ª284.

Mezrioui N., Oufdou Kh. and Baleux B. (1995). Dynamics of non-O1 *Vibrio cholerae* and faecal coliforms in experimental stabilization ponds in the arid region of Marrakesh, Morocco, and the effect of pH, temperature and sunlight on their experimental survival. *Can. J. Microbiol.*, **41**, 489-498.

Oron G., Wildschut L. R. and Porath D. (1985). Wastewater recycling by duckweed for protein production and effluent renovation. *Wat. Sci. Technol.*, **17**(4–5), 803-818.

Oswald W. J. (1988) Microalgae and wastewater treatment. In: *Microalgal biotechnology*. M. A. Borowitzka and L. J. Borowitzka (eds.), Cambridge University Press, Cambridge, New York, pp. 357-392.

Oufdou Kh. (1994) *Etude de la dynamique et de la survie de V. cholerae, d' E. coli et de P. aeruginosa au cours d'un traitement des eaux usées par lagunage naturel sous climate aride à Marrakech*. Doctoral Thesis, Department of Biology, Faculty of Sciences-Semlalia, Marrakech, 132 p.

Oufdou Kh., Mezrioui N., Oudra B., Barakate M. and Loudiki M. (1998). Effect of extracellular and endocellular products from cyanobacterium, *Synechocystis* sp., on the growth of some sanitation system bacteria. *Archiv. Hydrobiol. / Algological Studies,* **125**(90), 139-148.

Paerl, H. W. (1988). Growth and reproductive strategies of freshwater blue-green algae (Cyanobacteria). In: *Growth and reproductive strategies of freshwater phytoplankton*. C. D. Sandgren (ed.), Cambridge University Press, pp. 261-315.

Rosowski J. R. and Hoshaw R. W. (1970). Cultivation of a filamentous alga in quantity on agar plates. *J. Phycol.*, **6**, 220-222.

Singleton F. L., Atwell R. W., Jangi M. S. and Colwell R. R. (1982). Influence of salinity and organic nutrient concentration on survival and growth of *Vibrio cholerae* in aquatic microcosms. *Appl. Environ. Microbiol.*, **43**, 1080-1085.

Somiya I. and Fujii S. (1984). Material balances of organics and nutrients in an oxidation pond. *Wat. Res.*, **18**(3), 325-333.

Sournia A. (1978). Phytoplanktonic Manual. Paris. UNESCO (ed.), 337 p.

Wang L. and Priscu J. C. (1994). Stimulation of aquatic bacterial activity by Cyanobacteria. *Hydrobiologia*, **277**, 145-158.