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# A COMPARATIVE STUDY ON VITAMIN B12 AND CO-CULTURE SYSTEM PROMOTES THE GROWTH OF MICROALGAE *NEPHROSELMIS ASTIGMATICA*

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## ABSTRACT

*Microalgae frequently grow in marine environment with the help of symbiotic microbes. Here our study explains in detail with the reciprocity between the microalgae Nephroselmis astigmatica and bacteria Halomaonas meridian in habitat with high saline environment. In this study Co- culture system reveals the possibility to know the bacterial metabolites and their growth promotion in algal cells. However algal cells grown in vitamin solution at various concentrations of 5µg, 7.5µg, 10µg reveals the requirement of vitamins for their growth. According to that the co – culture experiment at different incubation period of 12hr, 24hr, 48hr, and 96hr explains the effect of bacterial growth promoting compounds from late log phase. Hence results of our study show 7.5µg of cyanocobalamine and 48hr of Halomonas meridian improves the algal growth beneficially.*

## KEY WORDS

*co-culture, Nephroselmis astigmatica,cyanocobalamine, symbiotic microbes, Halomonas meridian.*

## INTRODUCTION

Microalgae and their valuable metabolites are the potential source to be used as to control pollution, food and feed additives, cosmetics and medicine production, etc. [16,23,25]. Furthermore, microalgae also considered as most appropriate feedstock of biofuel production. there are several studies have been carried out to improve their culture efficiency as well as to increasing the valuable substances from microalgae such as polyunsaturated fatty acid and neutral lipid [1,5,12,14,24,8]. However, in an aquatic environment Microalgae inhabit along with various flora and fauna. Besides, bacteria play a crucial role in their association by nutrient cycling and energy flowing. There by it enhance the microalgal growth either positively or negatively [11, 13, 19, 21, 22]. According to that, bacteria promote algal growth by reducing dissolved oxygen concentration and consuming excreted organic materials from algae [20]. Instead of that bacteria secreting vitamins such as biotin, cobalt amine and thiamine [9]. through that microalgae reimburse bacteria with oxygen and extracellular compounds this mutualistic association reveals microalgal growth enhanced by specific bacteria [11, 13]. Although light and chemical energy gives extraordinary growth on microalgae [6, 7, 17, 18, 27]. However, the study on co-culture system explores the reciprocity between algae and bacteria which were documented in microalgae/cyanobacteria in wastewater treatment [25]. The mechanism behinds growth promoting effects in bacteria was limited. Predominantly vitamins [9,15] and siderophores are the two components efficiently promotes growth in algae.

Many studies has been reported on *Halomonas sp*., improves the algal growth beneficially. *Halomonas sp*. a halophilic and oligotrophic bacterium, improves the algae *Dunaliella bardawil* under fe- deficient conditions. The symbiotic relationship of bacteria and algae

enhances the availability of fe to the algae by releasing the bacterial siderophores [14]. Not all of the mechanisms behind the growth promoting effect of bacteria are known. Vitamins [9,15] and siderophores are two components produced by the bacteria that promote algal growth. Siderophores are iron chelating molecule facilitates iron uptake in both algal and bacteria [3] while Vitamin B12 act as a cofactor to synthesis methionine [9] . Hence the present study discusses the symbiotic relationship between *Nephroselmis astigmata* a halophile and *Halomonas meridian* improves the growth rate in short period. Moreover, the healthy balanced population of bacterial communities in algal environment protects the unwanted microbial contaminants. In this study we aim to focus the algal growth rate during vitamin uptake as well as co- culture system of different time course.

## 2. MATERIALS AND METHODS

**Sample collection and identification**

The sample collections were carried out using horizontal towing of phytoplankton net (Bolting silk cloth,20µm) at Vellar estuary at high saline environment during early morning. The collected samples were fixed in formalin with 4% buffered solution for qualitative (light microscope Olympus CX21i) and quantitative (haemocytometer) analysis. On the other hand, water samples were transferred to filtered and sterilized seawater (30 Psu) with Guillard's F/2 medium and brought it to the laboratory immediately. samples were examined using the standard manuals [2, 4, 26].

**In vitro culture of microalgae and bacteria**

The isolated microalgal species of *Nephroselmis astigmatica* using aseptic techniques which was maintained Guillard's F/2 medium under optimum conditions of 25±2̊ C,30±2 psu and 4000±500 Lux light intensity with 12:12 light and dark. At the same time associated bacteria from the same environment *Halomonas meridian* was retrieved from pure culture techniques and maintained in 20% glycerol stock at -80̊ C.

**Quantification of Algae and bacteria**

Cell density of both algae and bacteria were determined using manual cell counting (0.1 mm deep neubauer chamber) and measuring Optical Density using spectrophotometer (Spectronic 20, Genesys, Thermos, USA) at 540nm, 650nm, 695nm of *Nephroselmis astigmata*, *Halomonas meridian* during the log and stationary phases.

**Assay for algal growth promotion**

**Vitamin assay**

Microalgae *Nephroselmis astigmata* with their growth performance on vitamin uptake was monitored using cyanocobalamine, biotin, nicotinic acid and folic acid at various concentrations (5µg, 7.5µg &10µg). The positive control was made sea water with vitamin solution (cyanocobalamine + thiamin hcl + biotin). on the other hand, seawater with f/2 media components except vitamin solution considered as negative control. Algal growth rate was monitored every day and the final cell density was determined with a haemocytometer as well as spectrophotometer at 650nm.

***Co-culture system***

Co- cultivation of algae in the log phase *Nephroselmis astigmatica* was about 1.9 × $10^5$ cells mL−1 inoculated into100ml of fresh autoclaved seawater at the same time the associated bacteria of *Halomonas meridian* at the stationary phase of 8.2 ×$10^8$ was inoculated into the same 250ml conical flask which was already inoculated with algae. These above-mentioned Co-culture systems was maintained at 25 ± 1 °C under 70 µmol photons m−2 s−1 irradiation of 12 h light and 12 h dark condition. The final cell density was monitored every day using spectrophotometer at 650nm.

**Time course assay on growth of microalgae – bacteria**

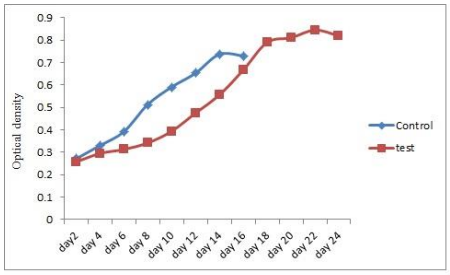
Time course assay for algae – bacteria growth was monitored by using six well assay plate of 4ml algal culture and 1ml bacterial culture at different time intervals (12hr, 24hr, 48 hr,72hr & 96 hr) were inoculated. The experiments were monitored for 2-3 days and their cell densities were measured using Spectrophotometer at 650nm.

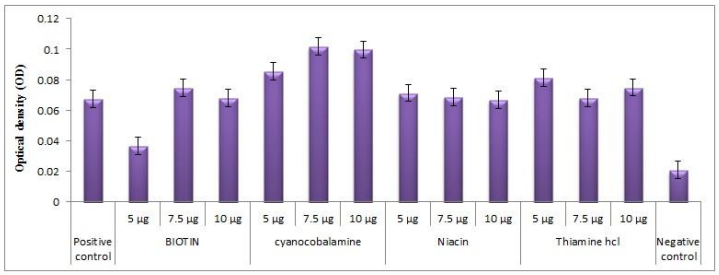
**Results and discussion**

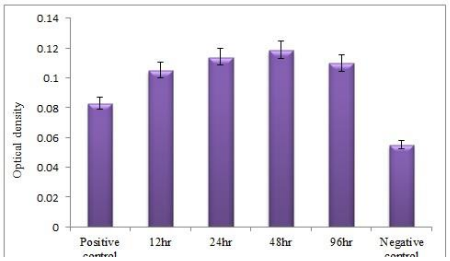
**Effect of growth rate on *Nephroselmis astigmatica* with their associated bacteria as well as vitamin treatment**

Initially we study the growth rate of *Nephroselmis astigmatica* in F/2 medium as well as vitamin solution. **Fig 1** shows the growth of both control and test of *Nephroselmis astigmatica* reveals the efficient growth on test. Whereas *Nephroselmis astigmatica* grown under F/2 guillar medium shows maximum growth in earlier days. Table 1 shows the algal cell numbers in detail. Thus, explores the maximum growth rate was observed in treatment with vitamin solution at 20 th

day. **Fig 2** explains the requirements of vitamins on *Nephroselmis astigmatica* growth at different concentrations. When compared to other vitamins Cyanocobalamine at 7.5µg enhances the growth of *Nephroselmis astigmatica* at short period. Meanwhile the co-culture experiment with the bacterial associates of *Halomonas meridiana* shows greater activity on algal growth promotion. Fig 3 explains the efficient algal growth under different incubation period of associated *Halomonas meridian*. It shows maximum growth at 48 hours incubation period. the late stationary phase of *Halomonas meridian* may release some growth promoting factors such as vitamins or irons improves the algal growth efficiently.




## CONCLUSION

In conclusion the co- culture system explains the rapid algal growth promotion in large scale. Thus, indicates the increasing amount of promising effect on valuable products from algae. *Nephroselmis astigmatica* a halophillic algae and their associated bacteria *Halomonas meridian* shows maximum growth at late log phase at which the bacterial secondary metabolites were released and efficiently promoting the algal growth within 3-4 days. Whereas algae cultured with F/2 guillard medium it takes 10-12 days.in future co-cultivation system would be considered as a promising field to study the valuable compounds from algae in largescale.

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## REFERENCE

1. Adarme-Vega TC, Lim DK, Timmins M, Vernen F, Li Y, Schenk PM (2012). Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microbial Cell Fact.11:96.
2. Al-Kandari, M., Al-Yamani, F.Y., & Al-Rifaie, K. (2009). Marine Phytoplankton Atlas of Kuwait's Waters in Printed and bound by Lucky Printing Press, Kuwait, ISBN 99906-41-24-2, PP. 1-351.
3. Amin, S.A., Parker, M.S., andArm brust,E.V.(2012).Interactions between diatoms and bacteria. *Micro biol. Mol. Biol.Rev.* 76, 667–684.doi: 10.1128/MMBR.00007-12
4. Botes, L (2003). Phytoplankton Identification Catalogue Saldanha Bay, South Africa, April 2001. GloBallast Monograph Series No. 7. IMO London, ISSN 1680-3078.
5. Bougaran G, Bernard O, Sciandra A (2010). Modeling continuous cultures of microalgae colimited by nitrogen and phosphorus. J Theor Biol.265:443–54.
6. Brennan L, Owende P (2010). Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and coproducts. Renew Sustain Energ Rev.14:557–77.
7. Burkholder JM, Glibert PM, Skelton HM (2008). Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. Harmful Algae.8:77–93.
8. Cheng-Wu Z, Zmora O, Kopel R, Richmond A (2001). An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). Aquaculture.195:35–49.

9. Croft MT, Lawrence AD, Raux-Deery E, Warren MJ, Smith AG (2005). Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature.438:90–3.
10. Croft, M.T. et al. (2005) Algae acquire vitamin B12 through a symbiotic relationship with bacteria. Nature 438, 90–93
11. de-Bashan LE, Bashan Y, Moreno M, Lebsky VK, Bustillos JJ (2002). Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*. Can J Microbiol.48:514–21.
12. EL-Sheekh MM, Bedaiwy MY, Osman ME, Ismail MM (2012). Mixotrophic and heterotrophic growth of some microalgae using extract of fungal-treated wheat bran. Int J Recycl Org Waste Agric.1:12.
13. Ethier S, Woisard K, Vaughan D, Wen Z (2011). Continuous culture of the microalgae *Schizochytrium limacinum* on biodiesel-derived crude glycerol for producing docosahexaenoic acid. Bioresour Technol.102:88–93.
14. Ety Keshtacher-Liebson, Yitzhak Hadar and Yona Chen. (1999). Fe nutrition demand and utilization by the green alga Dunaliella bardawil. Plant and Soil 215: 175–182.
15. Gonzalez LE, Bashan Y (2000). Increased growth of the microalga *Chlorella vulgaris* when coimmobilized and cocultured in alginate beads with the plant-growth-promoting bacterium *Azospirillum brasilense*. Appl Environ Microbiol.66:1527–31.
16. Kazamia, E. et al. (2012) Mutualistic interactions between vitamin B12-dependent algae and heterotrophic bacteria exhibit regulation. Environ. Microbiol. 14, 1466–1476
17. Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, *et al* (2010). Enhanced CO2 fixation and biofuel production via microalgae: recent developments and future directions. Trends Biotechnol. 28:371–80.
18. Lee Y-K (2001). Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol.13:307–15.
19. Liang Y, Sarkany N, Cui Y (2009). Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol Lett.31:1043–9.
20. Lorincz Z, Preininger E, Kósa A, Pónyi T, Nyitrai T, Sarkadi L, et al (2010). Artificial tripartite symbiosis involving a green alga (*Chlamydomonas*), a bacterium (*Azotobacter*) and a fungus (*Alternaria*): morphological and physiological characterization. Folia Microbiol (Praha).55:393–400.
21. Mouget J-L, Dakhama A, Lavoie MC, de la Noüe J (1995). Algal growth enhancement by bacteria: is consumption of photosynthetic oxygen involved? FEMS Microbiol Ecol.18:35–43.
22. Ortiz-Marquez JC, Do Nascimento M, Dublan Mde L, Curatti L (2012). Association with an ammonium-excreting bacterium allows diazotrophic culture of oil-rich eukaryotic microalgae. Appl Environ Microbiol.78:2345–52.
23. Rivas MO, Vargas P, Riquelme CE (2010). Interactions of *Botryococcus braunii* cultures with bacterial biofilms. Microb Ecol.60:628–35.
24. Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr Opin Biotechnol.19:430–6.
25. Sharma KK, Schuhmann H, Schenk PM (2012). High lipid induction in microalgae for biodiesel production. Energies.5:1532–53.
26. Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R (2011). Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. Biotechnol Adv. 29:896–907.
27. Verlencar, X.N., Desai, S (2004). Phytoplankton Identification Manual. National Institute of Oceanography. Dona paula, Goa India. Pp33.
28. Yang C, Hua Q, Shimizu K (2000). Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light-autotrophic/dark-heterotrophic conditions. Biochem Eng J.6:87–102.

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