Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Growth standardization studies on *Monoraphidium contortum* cultured under pH specific conditions in Bolds Basal medium.

Karthika, S.Menon, Thasnim P.M. and C.C.Harilal
Division of Environmental Science, Department of Botany, University of Calicut.
Corresponding author: karthu9smenon@gmail.com

Abstract
The species *Monoraphidium contortum* is noted to be useful for a wide range of purposes, including biofuel production and as a source of secondary metabolities in pharmaceutical industries. However their growth and multiplication in most of the culture media, including Bolds Basal medium is slow. Hydrogen ion concentration (pH) is reported to be an important factor determining the growth and multiplication of micro algal members, both in natural as well as culture conditions. In this light, the present work is attempted with the objective of finding out the optimum pH favouring the maximum growth of *Monoraphidium contortum* in Bolds Basal medium. For fulfilling this objective, the growth performances of the microalgae under different conditions of pH(pH 3 to 12, with a gradation of 0.5) were monitored for a period of 7 days. During experimentation, characteristics of the culture media like pH, temperature, conductivity, resistivity and micro algal growth responses like cell size, cell count, turbidity and biomass were monitored. Analyses were conducted in triplicates with control at each time. The pH of BB medium favouring maximum growth of *Monoraphidium contortum* was noticed to be 9.

Key words: *Monoraphidium contortum*, Bolds Basal medium, pH.

Introduction

Microalgae, one of the oldest life forms are considered as the most likely resource to replace the conventional resources for its short span of life cycle, rapid replication, higher photosynthetic efficiency, high oil content etc. Considering these properties, several research and development efforts are undertaken worldwide to explore their potentialities in sectors like food, nutrition and medicine, energy and environment etc. Currently they are mainly attempted on a large scale for biofuel production and for extraction of high value biological derivatives.

In all these sectors, production of biomass in sufficient quantities seems to be a major factor in selecting them for meeting required targets. Many attempts have been carried out to develop protocols for mass multiplication of micro algal members, including open ponds and closed photobioreactors, modification of culture medium composition, alteration of culture conditions etc. However as the growth preferences of microalgal members vary in accordance with their species characteristics, standardization of species specific culture conditions is a prerequisite.

Several researchers observed that the physicochemical factors like pH, salinity light, temperature, carbon and nutrients such as nitrate, phosphate and trace metals(Becker, 1994; Grobbelaar, 2000; Mata *et al*., 2010)have significant impact on the growth and photosynthetic activity of microalgae. Through optimization of various environmental factors, the biomass production of desired microalgal species can be maximized and the required targets can be achieved. Optimization of culture conditions for the enhanced growth and metabolism of microalgae have already been attempted by Guedes (2011), Celekli et al., (2009) and Beherens (1996).However, optimal ranges of parameters tends to be species specific and hence need to formulate specific conditions for individual species (Menon *et al*.,2017).

Hydrogen ion concentration (pH) is an important factor for the optimal growth of microalgae and has significant impact on algal metabolism. Previous research works on microalgal mass multiplication and growth perspective reported that pH is an important factor that affects the growth of microalgal members (Pandey and Tiwari, 2010; Khalil *et al*., 2010; Guedes *et al*., 2011; Gong *et al*., 2014). Furthermore, Moheimani (2005)stated that by subjecting the microalgal cultures to temporary changes in environmental factors such as temperature, pH and light, the yield can be increased and the unwanted contaminants can be avoided.

The potentialities of *Monoraphidium contortum* as a source of biofuel has already been reported by Reyes *et al*. (2012) and Bogen *et al*. (2013). Ferriera (2017) noticed the antioxidant properties of *Monoraphidium contortum*. However their mass multiplication process was a major hindrance in their utilization for specific purposes. In this light, an investigation has been carried out with an objective to study the growth responses of *Monoraphidium contortum* in Bolds Basal medium at varying levels of pH.

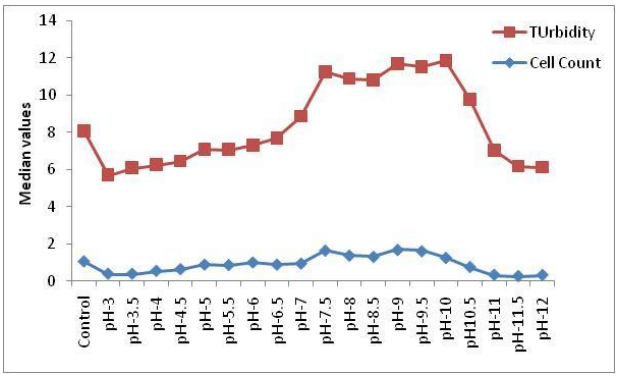
Materials and Methods

Micro algal samples were collected using plankton net from various lentic and lotic fresh water environments of Malappuram district. Pure cultures of *Monoraphidium contortum*, after series of sub culturing in Bolds Basal medium (Bischoff and Bold, 1963)were deposited in Calicut University FreshWater Microalgal Culture Collection (CUFWMACC), maintained in the Division of Environmental Science, Department of Botany, University of Calicut.

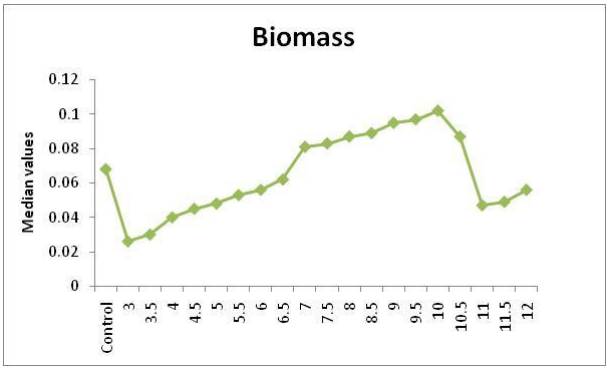
For fulfilling the objectives, treatment sets were maintained with 50 ml each of BB medium taken in 100 ml conical flasks. The pH of culture medium contained in each conical flask was adjusted to a specific pH, ranging from 3.0 to12.0, with a gradation of 0.5 using NaOH (0.5 N) and HCl (0.05 N). To each conical flask, after adjusting to the required pH, 5.0 ml of pure culture of *Monoraphidium contortum* was added. Two control sets were maintained, of which one was worked on the first day of the treatment and the other one was kept for further observation. All the sets were kept at illumination during day time. The pH, temperature, conductivity and resistivity associated with the culture medium and the growth parameters associated with the micro algal species like cell count, cell size, turbidity and biomass were monitored, periodically. Every day after observation, the altered pH was readjusted. Monitoring of the treatment sets were carried out for a period of seven days. The entire experimentation was repeated thrice and the average values were reported.

Results and discussion

Responses of *Monoraphidium contortum* to varying ranges of pH are given in table1A-C. Changes in cell count, turbidity and biomass associated with *Monoraphidium contortum* under different ranges of pH are represented in Figures 1a- b. During the treatment period, in higher alkaline pH ranges, especially above pH 9 the formation of precipitates was noticed. Sirisansaneeyakul *et al*. (2011) also reported that there will be precipitate formation in the culture if pH exceeds a value of 9. Therefore for confirmation of appropriate pH range at which maximum growth occurred, the median values of major growth parameters above pH 9 was neglected.



1a- Cell count and Turbidity



1b Biomass

Figs 1a and 1b. Changes in Cell count, Turbidity and biomass associated with *Monoraphidium contortum* response to varying ranges of pH.

Table 1 A. Comparison in cell count difference noticed in cultures of *Monoraphidium contortum* .

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	0.56	0.68	0.56	1.06	1.25	1.31	1.69	1.06
pH-3	0.38	0.43	0.38	0.38	0.31	0.19	0.25	0.38
pH-3.5	0.38	0.38	0.31	0.25	0.38	0.38	0.31	0.38
pH-4	0.44	0.47	0.54	0.5	0.56	0.63	0.69	0.54
pH-4.5	0.63	0.43	0.5	0.56	0.75	1.19	1.07	0.63
pH-5	0.56	0.38	0.44	0.94	0.88	1.38	1.19	0.88
pH-5.5	0.38	0.53	0.75	0.86	0.94	1.5	1.5	0.86
pH-6	0.44	0.64	0.81	1	1	1.63	1.88	1
pH-6.5	0.5	0.71	0.75	0.88	1.25	1.64	2.13	0.88
pH-7	0.56	0.68	0.88	0.94	1.56	1.94	2.25	0.94
pH-7.5	0.63	0.73	0.88	1.65	1.81	2	2.56	1.65
pH-8	0.56	0.83	1	1.38	1.75	2.13	2.81	1.38
pH-8.5	0.5	0.63	1.06	1.31	2.13	2.25	2.81	1.31
pH-9	0.5	0.64	0.94	1.69	2.13	2.44	2.88	1.69
pH-9.5	0.44	0.51	0.81	1.62	2.25	2.5	2.88	1.62
pH-10	0.63	0.78	0.94	1.25	2.5	2.81	3.25	1.25
pH10.5	0.31	0.68	0.75	0.75	1.25	1.63	1.75	0.75
pH-11	0.31	0.32	0.38	0.46	0.44	0.31	0.25	0.32
pH-11.5	0.19	0.18	0.38	0.25	0.25	0.25	0.19	0.25
pH-12	0.31	0.34	0.37	0.36	0.13	0.25	0.13	0.31

Table 1B. Comparison in turbidity difference noticed in cultures of *Monoraphidium contortum* .

Experimental set	First day	Second day	Third day	Fourth day	Fifth day	Sixth day	Seventh day	Median
Control	5.3	6.1	6.4	7	10.1	10.9	10.4	7
pH-3	3.9	4.2	5.8	4.4	5.3	6.2	5.8	5.3
pH-3.5	4.5	4.4	5.7	5	5.7	6.5	6	5.7
pH-4	4.6	4.6	5.7	5.4	6	7.4	6.5	5.7
pH-4.5	4.8	5	5.8	5.5	6.6	8.3	7.3	5.8
pH-5	4.7	4.9	5.9	6.2	7.6	9	7.4	6.2
pH-5.5	4.3	5.1	6.2	6	7.5	9.9	7.3	6.2
pH-6	4	5.2	6.2	6.3	7.7	9.8	8	6.3
pH-6.5	4.3	6.3	6.6	6.8	8.3	10.6	9.1	6.8
pH-7	5	7.1	7.9	7.4	11	12	12.8	7.9
pH-7.5	5.8	6.9	7.5	9.6	11.3	12.6	18.1	9.6
pH-8	6.2	6.6	7.7	9.5	11	13.1	15.9	9.5
pH-8.5	6.3	6.4	8.1	9.5	11.9	14.2	19	9.5
pH-9	6.6	6.9	8.5	10	12	16.2	18.6	10
pH-9.5	6	6.7	8	9.9	12	16.5	14	9.9
pH-10	7	6.7	8.9	10.6	12.1	17	15.7	10.6
pH10.5	6.9	6.7	8.5	9.8	9	11	9.3	9
pH-11	7.1	6	6.7	7.7	5.9	6.5	6.7	6.7
pH-11.5	6.7	5.9	6	6.8	5.7	5.4	5.3	5.9
pH-12	6.9	5.8	6.4	7	5.6	5.2	5.5	5.8

Table 1C. Comparison in biomass difference noticed in cultures of *Monoraphidium contortum* .

Experimental Set	Median	Experimental Set	Median
Control	0.068	**pH-7.5**	0.083
pH-3	0.026	**pH-8**	0.087
pH-3.5	0.03	**pH-8.5**	0.089
pH-4	0.04	**pH-9**	0.095
pH-4.5	0.045	**pH-9.5**	0.097
pH-5	0.048	**pH-10**	0.102
pH-5.5	0.053	**pH10.5**	0.087
pH-6	0.056	**pH-11**	0.047
pH-6.5	0.062	**pH-11.5**	0.049
pH-7	0.081	**pH-12**	0.44

Upon comparison of the pH values associated with the treatment sets, it was noticed that all pH ranges exhibited a tendency to move towards the neutral range from the pre adjusted range. Here in the acidic range pH increases and in the basic range pH decreases towards the neutral range, which is an indication of the adaptabilities of micro algae to survive in modified environments by altering the pH to suitable ranges. Verduin (1964) stated that the microalgae can increase the pH of the culture medium via photosynthesis and similar changes in the pH values of culture medium were also noticed by Dubinsky and Rotem (1974). Al-Shatri (2014) reported that the variations on the pH of the culture medium were due to the metabolites secretions of microalgae in response to the nutrients present in the culture medium. In the control set the pH value ranged from 6.7 on the first day to 6.96 on the seventh day.

In the case of temperature (˚c) of the culture medium in the treatment sets, the lower temperature was observed on the seventh day (27) and higher temperature noticed on the first day (32). Fernandez *et al*. (2013) reported that a range of 25-35˚c is ideal for the growth of freshwater microalgae and also pointed that for a short period, freshwater microalgae can tolerate temperature up to 40˚c. The data pertaining to the temperature ranges associated with the culture conditions were almost within the range to support the growth and proliferation of *Monoraphidium contortum.*

The evaluation of cell count at specific intervals provides information pertaining to the growth status of microalgae under experimentation. While comparing the median values of treatment set, the maximum cell count ($\times 10^4$ cells/ml) was observed in pH 9 and 9.5 on the seventh day (2.88)and minimum value in pH 12 (0.13) on the fifth and seventh day. In control set, the cell count was ranging from 0.56on the first day to 1.69on the seventh day. Throughout the study, it was observed that in the higher alkaline and higher acidic ranges of pH, cell division and growth was low. Significantly reduced growth at elevated pH ranges was also observed by Rai and Rajashekhar (2014). Chen and Durbin in 1994 and Guckert and Cookseyin 1990 also reported the minimal growth of microalgae in higher pH ranges. Moreover Gensemer *et al.* (1993) noticed the reduced algal growth due to altered nutrient uptake in the acidic conditions. Kumar *et al*.(2010) also commented that declining pH may have undesirable effects on the growth and proliferation of microalgae.

A change in the cell size of *Monoraphidium contortum* during the treatment period has been monitored through micrometry. In the case of cell size (µm), the lower cell size was observed in pH 9.5 and higher cell size in acidic pH (3.5). In control set, the cell size was ranging from 72 on the first day to 67.5 on the second and fifth day. Visviki and Santikul (2000) conducted experiments on the pH tolerance of *Chlamydomonas applanata* and observed larger single cells with increased pyrenoidal volume and thicker cell walls in acidic pH than the control cells. The authors stated that the microalgae undergo dormancy in extreme conditions to ensure their survival. However increased cell size cannot be taken as a growth parameter as larger cells appeared in acidic ranges may be due to the survival strategy of microalgae to overcome the extreme conditions.

Assessment of turbidity (NTU) can be taken as the most general method for estimating the microalgal concentration in culture medium (Becker, 1994). Here in the present study, maximum turbidity value was observed in pH 9 (18.6) on the seventh day and minimum turbidity in pH 3 (3.9) on the first day. In control set, turbidity ranged from 5.3 on the first day to 10.9 on the sixth day. The minimum turbidity observed in extreme acidic pH may be due to the low cell count that the acidic pH reduces the growth and multiplication of the microalgae (Hargreaves and Whitton, 1976).

In the cultures of *Monoraphidium contortum*, throughout the treatment period, maximum conductivity (µS) was found in pH 12 (4318) on the seventh day and minimum conductivity in pH 6.5 (739.2)on the first day. In control set, conductivity ranged from 781.8 on the first day to 801.8 on the seventh day. It was also noticed that from the first day onwards, there was a significant increase in conductivity in all ranges of pH, especially in higher acidic and alkaline pH ranges, which may be due to the addition of HCl and NaOH for maintaining specific ranges of pH.

The maximum resistivity(kΩ) of the culture medium was noted in pH 6.5 (1.32)on the first day and minimum resistivity in pH 12 (0.23) on seventh day of the treatment. In control, resistivity ranged from 1.22 on the seventh day to 1.25 on the first day. From the first day onwards, there was a gradual decrease in resistivity in all treatment sets of microalgae retained at varying pHs. The resistivity values of cultures under experimentation were generally noted to be in an agreement of conductivity values observed in treatment sets.

During experimentation maximum biomass (gm) was obtained in pH 9(0.095)and minimum in pH 3(0.026). In control set, biomass obtained was 0.068. In higher alkaline ranges, the death of microalgae and precipitate formation were observed and as a result, biomass values above pH 9 were discarded. From third day of the treatment, the culture medium containing *Monoraphidium contortum* retained in higher alkaline pH turned to white colour due to the formation of precipitates and the growth was also reduced, which was evidenced by minimal cell count. The precipitation of calcium salts was also noticed by Becker (1994) and Sirisansaneeyakul*et al.* (2011) when the pH value exceeds above 9. Andersen (2005) stated that the decreased growth of microalgae in higher alkaline ranges was due to osmotically stressful microenvironment and high levels of precipitate formations, which may results in nutrient limitations. Taraldsvik and Myklestad (2000) stated that the reduction in growth at pH greater than 9.0 may due to the decrease in the rate of significant biochemical reactions, as well due to changes occurred in cell membrane properties.
For confirmation of the appropriate pH level in which maximum growth occurred, the median values of the seven days triplicate data were worked out and compared. Upon analyzing the median values of the major growth indicators like cell count, turbidity and biomass, the augmented growth of the algae was noticed in pH 9.

Conclusion

The attributes like higher photosynthetic efficiency, increased productivity, minimal nutrient requirements, carbon dioxide sequestration efficiency, ideal source of biofuels, generation of valuable byproducts etc. makes etc. makes microalgae a promising tool for researchers. One among the major constraints in their utilization is the lower cell density in culture media that the growth preferences of microalgae vary from strain to strain. Several reports suggested that the successful alterations on the culture conditions can results in maximized growth and reduction of contamination due to unwanted organisms.

In the present study, an attempt has been carried out to evaluate the optimum pH conditions favoring maximum growth and multiplication of *Monoraphidium contortum* in bolds basal medium. For experimentation, the culture conditions were maintained in laboratory using BB medium kept at pH ranging from 3 to 12, with an interval of 0.5. Monitoring of the treatment sets were carried out for a period of 1 week. For confirmation of the appropriate pH level at which maximum growth of *Monoraphidium contortum* occurred, the median values of the seven days triplicate data were worked out and compared. Upon analyzing the median values of the major growth parameters like cell count, turbidity and biomass, it can be concluded that for ensuring better biomass production and maximum growth of *Monoraphidium contortum* in BB medium ,a pH of 9 can be maintained.

Acknowledgement
The authors are grateful to Geraldo José Peixoto Ramos from State University of Feira de Santana, Brazil for the identification of micro algae *Monoraphidium contortum.*

References

Al-Shatri, A.H., Ali, E., Al- Shorgani, N.K.N. and Kail M.S. 2014.Growth of *Scenedesmus dimorphus* in different algal media and pH profile due to secreted metabolites. *Afr. J.Biotechnol*.**13**:1714-1720.

Andersen, R.A. 2005. Algal Culturing techniques, 155pp.Academic Press**.**

Becker, E.W .1994. Microalgae biotechnology and microbiology, pp 56- 280.Cambridge Univ. Press.

Behrens, P.W.1996. Microalgae as a source of fatty acids.*J. Food Lipids*.3: 259–272.

Bischoff, H.W. and Bold, H.C., 1963.Some soil algae from enchanted rock and related algal species, Phycological Studies. 4: 1-95. University of Texas Publications 6318.

Bogen, C., Klassen, V., Wichmann, J., Russa, M.L., Doebbe, A., Grundmann, M., Uronen, P., Kruse, O. and Mussgnug, J.H.2013.Identification of *Monoraphidium contortum* as a promising species for liquid biofuel production. *Bioresour Technol*.**133**:622-626.

Celekli, A., Yavuzatmaca, M. 2009. Predictive modeling of biomass production by *Spirulina platensis* as function of nitrate and NaCl concentrations. *Bioresour Technol*.**100**:1847– 1851.

Chen, C. Y. and Durbin, E. G.1994.Effects of pH on the growth and carbon uptake of marine phytoplankton. *Mar. Ecol. Prog. Ser.***109**: 83-94.

Dubinsky, Z. and Rotem, J. 1974. Relations between algal populations and pH of their media.*Oecologia*.**16**: 53-60

Ferreira, C.R.S., Gama, F.S., Faustino, S.M.M. and Almeida, S.S.M.S.2017.Study of the Secondary Metabolites, Cytotoxic and Antioxidant Activity of the Methanolic *Monoraphidium contortum* Crude Extract. *J.Chem.Pharm.Res*. **9**:327-331.

Gensemer, R.W., Smith, R.E.H. and Duthie, H.C.1993. Comparative effects of pH and aluminum on silica limited growth and nutrient uptake in *Asterionella ralfsii* var. Americana (*Bacillariophyceae*) *J. Phycol.**29***: 36–44.

Gong, Q.,Feng, Y.,Kang, L., Luo,M. and Yang, J. 2014.Effects of light and pH on cell density of *Chlorella vulgaris.Energyprocedia.**61**:* 2012-2015
.
Grobbelaar, J.U.2000. Algal biotechnology: real opportunities for Africa. *S. Afr. J. Bot.* **70**: 140-144.

Guckert, J.B. and Cooksey, K.E. 1990. Triglyceride accumulation and fatty acid profile changes in *Chlorella* (*Chlorophyta*) during high pH induced cell cycle inhibition. *J. Phycol.***26**:72–79

Guedes, A. C., Amaro, H. M., Pereira, R. D. and Malcata, F. X.2011.Effects of temperature and pH on growth and Antioxidant Content of the microalga *Scenedesmus obliquus. Biotechnol. Prog*.**27**:1218–1224.
Hargreaves, J.W. and Whitton, B.A.1976. Effect of pH on tolerance of *Hormidium rivulare* to zinc and copper. *Ecologia*. 26: 235-243.

Khalil, Z I., Asker, M M. S., El-Sayed, S. and Kobbia, I.A. 2010.Effect of pH on growth and biochemical responses of *Dunaliella bardawil* and *Chlorella ellipsoidea*. *World J.Microbiol.Biotechnol*.**26**:1225–1231.

Kumar, A., Ergas, S., Yuan, X, Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X. and van Langenhove, H. 2010. Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends Biotech.***28**: 371- 380

Mata, J.A.,Martins, T.M. and Caetano, N.S. 2010.Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technol.***74:**3-16

Menon,K.S., Sini,K.P. and Harilal, C.C. 2017.Standardization of pH favouring augmented growth and biomass production of fresh water micro algal species of *Coccomyxa,* in Bolds Basal medium. *Indian J.Sci.Res*. **13** : 116-121.

Moheimani, N.R. 2005. The culture of Coccolithophorid Algae for carbon dioxide bioremediation. PhD thesis, Murdoch University.

Pandey, J. P., Pathak N. and Tiwari, A.2010. Standardization of pH and Light Intensity for the Biomass Production of *Spirulina platensis J. Algal Biomass Utln*. **1:** 93 – 102.

Rai S.V. and Rajashekhar, M.2014.Effect of pH, salinity and temperature on the growth of six species of marine phytoplankton.*J. Algal Biomass Utln.***5**: 55-59

Reyes,Y., Chenard,G., Aranda,D.G., Mesquita,C., Fortes,M., Joao,R. and Bacellar,L. 2012 Biodiesel production by hydroesterification of microalgal biomass using heterogeneous catalyst. *Nat. Sci***. 4**:778–783.

Sirisansaneeyakul, S., Singhasuwan, S., Choorit, W., Phoopat, N., Garcia, J. and Chisti, Y. 2011 Photoautotrophic production of lipids by some *Chlorella* strains. *Mar Biotechnol.***13**:928–941.

Taraldsvik, M. and Myklestad,S. 2000.The effect of pH on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom *Skeletonema costatum. Eur*. *J*. *Phycol*.**35**: 189-194

Visviki, I. and Santikul, D. 2000.The pH tolerance of *Chlamydomonas applanata* (Volvocales, Chlorophyta). *Arch Environ ContamToxicol*.**38**: 147–151

Verduin, J.1964. Principles of primary productivity: Photosynthesis under completely natural conditions. In: Algae and man pp. 221–238. New York: Plenum.