



Biomass study of *Anabaena sphaerica* cultivated in electroplating industrial effluent

Dr. Yasodha. T ^{1*}, Sugapriya A ², Reethika S ², Christy Hepsiba A ², Abi M ², Ishwarya.M ²

¹ Professor, Dept of Biotechnology Madha Engineering College Tamilnadu ,India

² II year B.Tech Dept of Biotechnology Madha Engineering College Tamilnadu ,India

*Corresponding author E-mail: btmbty@gmail.com

Abstract

The growth of microalga *Anabaena sphaerica* in electroplating industrial effluent with reference to the vegetative cells , heterocysts and dry biomass weight brings about oxygenation and mineralization in addition to the increase in biomass which serve as a multipurpose raw material to the industries. From lab scale experiments it is demonstrated that the present investigation could be converted to pilot study for large scale production of biofuel and other commodity chemicals. Cultivation of microalgal biomass as a potential resource / raw material for various industries to produce commodity chemicals could enhance the economy and curtail the environmental hazards.

Keywords: *Microalgae; Anabaena Sphaerica; Electroplating Effluent.*

1. Introduction

The growing global population and the need of large amounts of energy effected a least care about environment. The direct and indirect anthropological activities had a negative impact on the environment and human health leads to the depletion of resources at faster rate and increase of pollutants at high levels. Hence curtailing the load of pollutants in the environment and remediating the polluted sources are the need of hour. Bioremediation by various microbes is the part of green technology and circular economy (Ryckeboosch, et al., 2014; Tao, et al .2017 ; Srinuanpan et al.2020; Peter et al.2021).

Several wastewaters from domestic, agricultural, and various industries consist of enormous nutrients . Any waste water can be used as an inexpensive alternative resource for the raw nutrients to cultivate microalgae.

(Metcalf and Eddy, 1997; Li et al 2011; Yuan et al 2013; Sforza et al 2015).

Electroplating industries are characterized based on its infrastructure, production and quality of raw materials used. Source of water in each unit varies. Few industries use distilled water as source for chrome plating and zinc plating industries use tap water or ground water based on their availability. Many small scale units of EP industries release not only waste water but also the toxic load. Proper treatment has to be provided for the safe discharge of electroplating effluent (Wood and Haselkorn, 1980; Wolk et al. 1994; Zhu and Lee 1997; Yasodha et al 2009; Kumazaki et al., 2013; Khanzada, 2020).

Blue green, filamentous, intercalary heterocystous , branched trichomes with oxygenic photosynthetic thallus. *Anabaena* are heterocyst-forming, photoautotrophic cyanobacteria that perform oxygenic photosynthesis. *Anabaena* grow in long filaments of vegetative cells. The envelopes of these BGA are similar to those of gram negative bacteria. Lipopolysaccharide of these BGA envelop is helpful in ionic exchange properties/intracellular accumulation/adsorption onto cell surface. Yasodha, 2009. In *Anabaena* the cells are ovoid or barrel-shaped, often giving the filaments (trichomes) the appearance. *Anabaena* possesses heterocysts and can also develop akinetes (thick walled resting cells that can survive in sediments for many years). Biosorption is possible due to the unique and complex structure of the microalgal cell wall (Laamanen and Kuosa, 2005; Corrales-Guerrero et al., 2014 and Tao et al. 2017).

With this background the physico chemical characteristics of the electroplating industrial effluent were analysed for its constituents before and after the microalgal treatment. *Anabaena sphaerica* had been selected to find out its growth response in electroplating industrial effluent with reference to vegetative , heterocyst cells and dry biomass weight.

2. Materials and methods

Physico chemical characteristics of electroplating effluent such as pH, dissolved oxygen, total dissolved solids, total suspended solids , COD, BOD, sulphate, chloride, nickel, chromium, copper, zinc and ferrous were analysed by the methods of APHA, 2012.

Microalga *Anabaena sphaerica* was cultivated in growth chamber (under 12/12 h light/dark cycle by fluorescent illumination of 40 $\mu\text{Em}^{-2}\text{s}^{-1}$) in 250 mL flasks with 150 mL, in BM and EPI effluent incubating at 25 \pm 2° C. (Allen and Arnon , 1955). Triplicates were maintained in each treatment.

Sterilized glass beads were added to the culture flasks .So that microalgal cells sticking on the glass wall and clumping of cells were avoided. Gentle shaking of the cultures was done manually every day to reduce the clumping of cells.



Treatments followed for microalga cultured in BM and EPI effluent

T1-Concentrated Basal Medium

T2,T3 and T4-diluted Basal Medium

T5-Concentrated Electro plating industrial effluent

T6,T7,T8 - diluted Electro plating industrial effluent

Growth Characteristics of *Anabaena* sp in terms of number of vegetative cells and number of heterocysts were observed in both BM and EPI effluent and dry weight of algal biomass were recorded during the experimental period of 20 days. Growth was observed and subsequent readings were taken on 4th, 8th, 12th, 16th and 20th days of growth period. Microscopic observation: From the 100 randomly selected filaments, the total number of cells, number of vegetative cells and heterocysts were counted.

For biomass study, a known amount of culture was harvested on the last day (20th day) of the study and oven dried at 105^oC until constant weight was obtained and was expressed in mg/l.

All the data were analysed statistically using Duncan's test as per the method suggested by Snedecor and Cochran,1991.

3. Results and discussion

Results of physico chemical characteristics of electroplating effluent recorded before and after algal treatment were predicted in Table-1. The heavy load of pollutants from the EPI effluent were absorbed by *Anabaena sphaerica* effected a sustainable way to treat the EPI effluent. The previous investigations based on waste waters and microalgal treatments are in agreement with Dufosse et al. 2005; Desbois et al 2009; Razzak et al. 2017.

Table 1: Physico Chemical Characteristics of Electroplating Effluent BM

S.No	Parameters	Electroplating effluent (before treatment)	Electroplating effluent (after treatment)
1	pH	5.7	7.9
2	dissolved oxygen (ppm)	7.4	8.1
3	Total dissolved solids (mg/ml)	5	3
4	Total suspended solids (mg/ml)	6	5.2
5	Chemical Oxygen Demand (ppm)	38.4	18.4
6	Biological Oxygen Demand (ppm)	150	193
7	Sulphate(mg/ml)	3.1	2.19
8	Chloride (mg/ml)	1.4	0.4
9	Nickel (mg/ml)	1.2	0.2
10	Chromium (mg/ml)	3.7	0.19
11	Copper (mg/ml)	2.81	0.21
12	Zinc (mg/ml)	1.02	0.04
13	Ferrous (mg/ml)	2.76	0.06

Table 2: Composition of Basal Medium

Medium component	Weight (g/l)
Calcium Chloride	0.2
Magnesium Sulfate (anhydrous)	0.098
Potassium Chloride	0.4
Sodium Bicarbonate	2.2
Sodium Chloride	6.8
Sodium Phosphate Monobasic (anhydrous)	0.12
L-Arginine • HCl	0.021
L-Cystine • 2HCl	0.016
L-Histidine (free base)	0.008
L-Isoleucine	0.026
L-Leucine	0.026
L-Lysine • HCl	0.03647
L-Methionine	0.0075
L-Phenylalanine	0.0165
L-Threonine	0.024
L-Tryptophan	0.004
L-Tyrosine • 2Na • 2H ₂ O	0.02595
L-Valine	0.0235
D-Biotin	0.001
Choline Chloride	0.001
Folic Acid	0.001
myo-Inositol	0.002
Niacinamide	0.001
D-Pantothenic Acid (hemicalcium)	0.001
Pyridoxal • HCl	0.001
Riboflavin	0.0001
Thiamine • HCl	0.001
D-Glucose	1
Phenol Red • Na	0.011

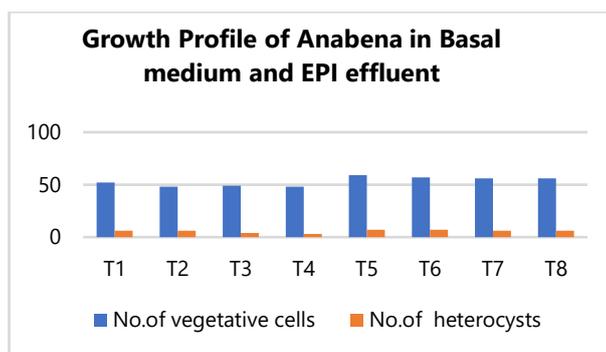


Fig. 1: Dry Biomass of Anabaena SP Cultured from Basal Medium and EPI Effluent.

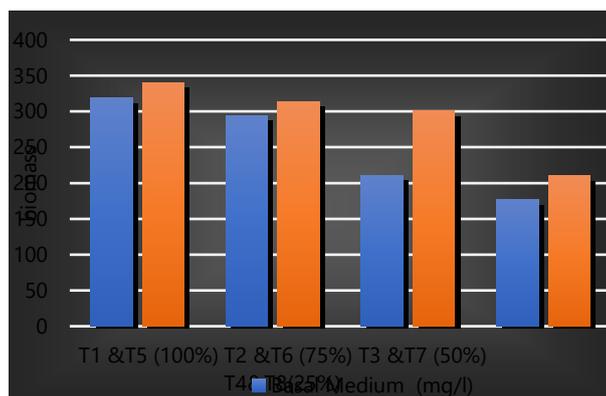


Fig. 2: Dry Biomass of Anabaena SP Cultured from Basal Medium and EPI Effluent.

Results pertaining to the nutrient uptake by the vegetative cells and heterocyst cells was faster and enhanced the efficient growth of wet biomass of experimental microalga. Similar kind of experiments with reference to cell differentiation and heterocyst cells were conducted by Luther 1990 ;Chen et.al,1997; Laamanen and Kuosa ,2005;Bothe et al.,2010.

Similar culture studies were performed by Helena et al.1986 ; Cellular differentiation studies revealed the faster nutrient uptake of microalgae from different nutrient sources are well documented by earlier studies of : Valladares et al.,2007; Kumar et al.,2010; Ehira,2013.

Studies on growth of microalgae in various nutrient composition and metabolic profile were revealed by Cardona et al., 2009; Flores et al., 2010; Herrero et al., 2013; Sforza et al.,2015;Santamaría-Gómez et al., 2018.

Growth performance with reference to the heterocyst cells of different microalgae in various waste waters are well documented by Meeks et al. 2002 and Carey et al. 2012.

The result of present investigation showed an increasing trend in the growth of microalga Anabaena sp biomass grown in all concentrations of electroplating industrial effluent. In 100% basal medium, dry biomass weight was good but a decreasing trend in other dilutions of basal medium. This might be due to the less supply of nutrient impact on the dry weight. Assessments were recorded for the Anabaena sp biomass grown in the electroplating industrial effluent revealed that in all concentrations there was an increasing trend. The results for dry biomass weight production are in agreement with Jais et al.2017; Lima,2020.

Dry biomass of Anabaena sphaerica cultivated from the treatments T5 and T6 showed high performance compare to the dry weight obtained in basal medium in which sufficient nutrients are supplied. Hence it had been concluded that the EPI effluent is effectively enhancing the growth microalga which could be an alternative and sustainable nutrient source.

Dry microalgal biomass yield from waste waters by Zhu and Lee, 1997;Yasodha 2009; Yuan et al.,2013 and Sforza et al.,2015 supported the view of the present study . Brandao et.al, 2023 reviewed about various microalgae yield in terms of biomass production from domestic waste waters revealed the potentiality of nutrient uptake of micaroyalgae.

4. Conclusion

The growth of microalga Anabaena in electroplating industrial effluent with reference to the vegetative cells and heterocysts brings about oxygenation and mineralization in addition to the increase in biomass which serve as a multipurpose raw material to the industries. The enhancement of dry biomass was ranging from 11.5% to 45% for Anabaena revealed the potentiality of its nutrient uptake from EPI effluent and cleared the pollutants such as the heavy metals . The dry biomass of microalgae Anabaena sphaerica grown in electroplating industrial effluents could be utilized as an economical resource potential for the commodity chemicals.

References

- [1] Allen MB, Arnon DI (1955) Studies on nitrogen-fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. Plant Physiol 30(4):366–372. <https://doi.org/10.1104/pp.30.4.366>.
- [2] APHA.2012, Standard Methods of Water and Wastewater Analysis.22nd Edn.American Public Health Association.Washington DC.USA.
- [3] Brandão, Barbara & Oliveira, Carlos Yure B. & Santos, Elizabeth & Lima de Abreu, Jéssika & Oliveira, Deyvid & Silva, Suzianny & Galvez, Alfredo. (2023). Microalgae-based domestic wastewater treatment: a review of biological aspects, bioremediation potential, and biomass production with biotechnological high-value. Environmental Monitoring and Assessment. 195. 1384. <https://doi.org/10.1007/s10661-023-12031-w>.
- [4] Bothe H, Schmitz O, Yates MG, Newton WE (2010) Nitrogen fixation and hydrogen metabolism in cyanobacteria. Microbiol Mol Biol Rev 74(4):529–551. <https://doi.org/10.1128/MMBR.00033-10>.

- [5] Burnat M, Herrero A, Flores E (2014) Compartmentalized cyanophycin metabolism in the diazotrophic filaments of a heterocyst-forming cyanobacterium. *Proc Natl Acad Sci U S A* 111(10):3823–3828. <https://doi.org/10.1073/pnas.1318564111>.
- [6] Cardona, T.; Battchikova, N.; Zhang, P.P.; Stensjo, K.; Aro, E.M.; Lindblad, P.; Magnuson, A. Acta (2009) Electron transfer protein complexes in the thylakoid membranes of heterocysts from the cyanobacterium *Nostoc punctiforme*. *Biochim. Biophys.*, 1787, 252–263. <https://doi.org/10.1016/j.bbabbio.2009.01.015>.
- [7] Carey C.C., Ibelings B.W., Hoffmann E.P., Hamilton D.P., Brookes J.D. (2012) Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Res.*; 46:1394–1407. <https://doi.org/10.1016/j.watres.2011.12.016>.
- [8] Corrales-Guerrero, L., V. Mariscal, D.J. Nurnberg, J. Elhai, C.W. Mullineaux, E. Flores, A. Herrero, Subcellular localization and clues for the function of the HetN factor influencing heterocyst distribution in *Anabaena* sp strain PCC 7120, *J.Bacteriol.* 196 (2014) 3452–3460. <https://doi.org/10.1128/JB.01922-14>.
- [9] Desbois, A. P., Mearns-spragg, A., and Smith, V. J. (2009). A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including multi-resistant *Staphylococcus aureus* (MRSA). *Mar. Biotechnol.* 11, 45–52. <https://doi.org/10.1007/s10126-008-9118-5>.
- [10] Dufosse, L., Galaup, P., Yaron, A., Arad, S. M., Blanc, P., Murthy, K. N. C (2005). Microorganisms and microalgae as sources of pigments for food use: Asciantific oddity or an industrial reality? *Trends Food Sci. Technol.* 16 (9), 389–406. <https://doi.org/10.1016/j.tifs.2005.02.006>.
- [11] Ehira, S.(2013).Transcriptional regulation of heterocyst differentiation in *Anabaena* sp.strain PCC 7120, *Russ. J. Plant Physiol.* 60. <https://doi.org/10.1134/S1021443713040043>.
- [12] Flores, E.; Herrero, A. (2010) Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat. Rev. Microbiol.*, 8, 39–50. <https://doi.org/10.1038/nrmicro2242>.
- [13] Helena A. Cmiech, Gordon F. Leedale and Colin S. Reynolds (1986) Morphological and ultrastructural variability of planktonic cyanophyceae in relation to seasonal productivity., *British Phycological Journal*, 21:1, 81–92, <https://doi.org/10.1080/00071618600650081>.
- [14] Herrero A.; Picossi, S.; Flores, E.2013. Gene expression during heterocyst differentiation. In *Advances in Botanical Research, Genomics of Cyanobacteria*; Chauvat, F., Cassier-Chauvat, C., Eds.; Academic Press: Cambridge, MA, USA, ; pp. 281–329. <https://doi.org/10.1016/B978-0-12-394313-2.00008-1>.
- [15] Jais, N. M., Mohamed, R. M. S. R., Al-Gheethi, A. A., and Hashim, M. K. (2017).
- [16] The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. *Clean Technol. Environ. Policy* 19 (1), 37–52. <https://doi.org/10.1007/s10098-016-1235-7>.
- [17] Khanzada, Z.T. Phosphorus removal from landfill leachate by microalgae. *Biotechnol. Rep.* 2020, 25, e00419. <https://doi.org/10.1016/j.btre.2020.e00419>.
- [18] Kumar K, Mella-Herrera RA, Golden JW (2010) Cyanobacterial heterocysts. *Cold Spring Harb Perspect Biol* 2(4): ARTN a000315. <https://doi.org/10.1101/cshperspect.a000315>.
- [19] Kumazaki, S.; Akari, M.; Hasegawa, M. Transformation of Thylakoid Membranes during Differentiation from Vegetative Cell into Heterocyst Visualized by Microscopic Spectral Imaging. *Plant Physiol.* 2013, 161, 1321–1333. <https://doi.org/10.1104/pp.112.206680>.
- [20] Laamanen M and Kuosa H (2005). Annual variability of biomass and heterocysts of the *Nostoc*. *Boreal Environ. Res.* ;10:19–30 Li, Y.; Chen, Y.-F.; Chen, P.; Min, M.; Zhou, W.; Martinez, B.; Zhu, J.; Ruan, R. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresour. Technol.* 2011, 102, 5138–5144. <https://doi.org/10.1016/j.biortech.2011.01.091>.
- [21] Lima, S., Villanova, V., Grisafi, F., Caputo, G., Brucato, A., and Scargiali, F. (2020). Autochthonous microalgae grown in municipal wastewaters as a tool for effectively removing nitrogen and phosphorous. *J. Water Process Eng.* 38, 101647. <https://doi.org/10.1016/j.jwpe.2020.101647>.
- [22] Luther, M. Degradation of different substituted aromatic compounds as nutrient sources by the green alga *Scenedesmus obliquus*. In *Proceedings of the Dechema Biotechnol Conference 4*, Weinheim, NY, USA, 28–30 May 1990; pp. 613–615.
- [23] Masukawa H, Sakurai H, Hausinger RP, Inoue K (2014) Sustained photobiological hydrogen production in the presence of N₂ by nitrogenase mutants of the heterocyst-forming cyanobacterium *Anabaena*. *Int J Hydrogen Energy* 39(34):19444–19451. <https://doi.org/10.1016/j.ijhydene.2014.09.090>.
- [24] Meeks JC, Campbell EL, Summers ML, Wong FC (2002) Cellular differentiation in the cyanobacterium *Nostoc punctiforme*. *Arch Microbiol* 178(6):395–403. <https://doi.org/10.1007/s00203-002-0476-5>.
- [25] Metcalf and Eddy, 1997. *Waste water Engineering - Treatment, Disposals and Reuse*. Tata McGraw Hill, New Delhi.
- [26] Peter, A. P., Khoo, K. S., Chew, K. W., Ling, T. C., Ho, S. H., Chang, J. S., et al. (2021). Microalgae for biofuels, wastewater treatment and environmental monitoring. *Environ. Chem. Lett.* 19 (4), 2891–2904. <https://doi.org/10.1007/s10311-021-01219-6>.
- [27] Razzak, S. A., Ali, S. A.M., Hossain, M.M., and deLasa, H. (2017). Biological CO₂ fixation with production of microalgae in wastewater – a review. *Renew. Sustain. Energy Rev.* 76, 379–390. <https://doi.org/10.1016/j.rser.2017.02.038>.
- [28] Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J Gen Microbiol* 111(1): 1-61. <https://doi.org/10.1099/00221287-111-1-1>.
- [29] Ryckebosch, E., Bruneel, C., Termote-Verhalle, R., Goiris, K., Muylaert, K., and Foubert, I. (2014). Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. *Food Chem.* x. 160, 393–400. <https://doi.org/10.1016/j.foodchem.2014.03.087>.
- [30] Srinuanpan, S., Cheirsilp, B., and Kassim, M. A. (2020). “Oleaginous microalgae cultivation for biogas upgrading and phytoremediation of wastewater,” in *Microalgae cultivation for biofuels production*. Editor A. Yousuf (Cambridge, Massachusetts: Elsevier Academic Press), 69–82. <https://doi.org/10.1016/B978-0-12-817536-1.00005-9>.
- [31] Santamaría-Gómez, J.; Mariscal, V.; Luque, I. Mechanisms for Protein Redistribution in Thylakoids of *Anabaena* during Cell Differentiation. *Plant Cell Physiol.* 2018, 59, 1860–1873. <https://doi.org/10.1093/pcp/pcy103>.
- [32] Sforza, E.; Khairallah Al Emar, M.H.; Sharif, A.; Bertucco, A. Exploitation of urban landfill leachate as nutrient source for microalgal biomass production. *Chem. Eng. Trans.* 2015, 43, 373–378.
- [33] Snedecor GW, Cochran WG. *Statistical methods*. 8th edition. IOWA state University Press/Ames IOWA-50010. 1991.
- [34] Tao, R., Kinnunen, V., Praveenkumar, R., Lakaniemi, A. M., and Rintala, J. A. (2017). Comparison of *Scenedesmus acuminatus* and *Chlorella vulgaris* cultivation in liquid digestates from anaerobic digestion of pulp and paper industry and municipal wastewater treatment sludge. *J. Appl. Phycol.* 29 (6), 2845–2856. <https://doi.org/10.1007/s10811-017-1175-6>.
- [35] Valladares, A.; Maldener, I.; Muro-Pastor, A.M.; Flores, E.; Herrero, A. Heterocyst development and diazotrophic metabolism in terminal respiratory oxidase mutants of the cyanobacterium *Anabaena* sp strain PCC 7120. *J. Bacteriol.* 2007, 189, 4425–4430. <https://doi.org/10.1128/JB.00220-07>.
- [36] Wolk CP, Ernest A, Elhai J (1994) Heterocyst metabolism and development. In: Bryant DA (ed) *The molecular biology of cyanobacteria*. Springer (Kluwer Academic Publishers), Dordrecht, pp 769–823. https://doi.org/10.1007/978-94-011-0227-8_27.
- [37] Wood NB, Haselkorn R (1980) Control of phycobiliprotein proteolysis and heterocyst differentiation in *Anabaena*. *J Bacteriol* 141(3):1375–1385. <https://doi.org/10.1128/jb.141.3.1375-1385.1980>.
- [38] Yasodha, T., 2009. Biosorptive potentiality of agarose immobilised biomass of *Anabaena* (AIBA) for Ni (II) recovery. *Adv. Plant Sci.*, 22: 11–13.
- [39] Yuan Z, Wang Z, Takala J, Hiltunen E, Qin L, Xu Z, Qin X, Zhu L. Scale-up potential of cultivating *Chlorella zofingiensis* in piggery wastewater for biodiesel production. *Bioresour Technol.* 2013; 137:318–25. <https://doi.org/10.1016/j.biortech.2013.03.144>.
- [40] Zhu, C.J., Lee, Y.K. Determination of biomass dry weight of marine microalgae. *Journal of Applied Phycology* 9, 189–194 (1997). <https://doi.org/10.1023/A:1007914806640>.