

# Invitro Assessment of the Growth and Biomass Level of *Chlorella* in Textile and Tannery Wastewater

P. S. Subashini<sup>1</sup>, Dr. P. Rajiv<sup>2</sup>

<sup>1</sup>Research Scholar, <sup>2</sup>Assistant Professor

Department of Biotechnology, Karpagam Academy of Higher Education,  
Coimbatore, Tamil Nadu, India

## ABSTRACT

*Chlorella* has the capacity to sustain in both fresh water and wastewater. The present study deals with a comparison of the growth and yield of *Chlorella* in fresh water and textile and tannery wastewaters. The wastewaters used in the study were collected from textile and tannery dyeing units. The wastewater was diluted with tap water in different dilutions (60%, 70% and 80%). Higher dilution yields better biomass parallel to tap water. The result of present study confirms that *Chlorella* can sustain in almost all wastewaters but gives the maximum yield in textile wastewater.

**KEYWORDS:** *Chlorella*; textile wastewater; tannery wastewater; fresh weight (FW); dry weight (DW)

## INTRODUCTION

Nowadays, industrial effluents are the major sources of pollution of water bodies worldwide due to human needs and also decreased farm activities (1). Tanneries and textile industries are the most lucrative in India. About 25% of tannery workers are suffering from skin disease, gastric ulcer, respiratory illness, anaemia, dysentery, hypertension and lethargy (2). The colloidal substance along with the color and oily scum prevents the penetration of sunlight necessary for photosynthesis (3). There are number of findings about *Chlorella* applications as bio-fertilizer in plant growth (4) and feed stock for bio-diesel preparation (5). A number of findings have proved the sustainability of *Chlorella* on UV exposure, water stress, low light and heat for about a month (6). The present study confirms the higher sustainability and growth rate of *Chlorella* in textile and tannery wastewaters.

## Methodology

### A. Collection of Industrial Wastewater and Algae

The textile and tannery wastewaters were collected from different sites. The tannery wastewater was collected from a tannery outlet located in Erode District, Tamil Nadu and textile wastewater from a dyeing unit outlet located in Tirupur District, Tamil Nadu. The samples were stored at 4°C in the dark for further deactivation. *Chlorella vulgaris* was collected from Bharathidasan University, Department of Marine Science, Trichy. The collected culture had been stored at 20 to 25 °C on BBM medium to maintain pure culture.

### B. Experimental Set-up

The *Chlorella* was inoculated separately in textile and tannery wastewaters. The different dilutions of the wastewater with tap water containing initial algal count of  $5 \times 10^3$  cells/ml were 60% (T<sub>3</sub>), 70% (T<sub>2</sub>) and 80% (T<sub>1</sub>). This initial count of alga was based on the growth in normal tap water and served as the control (7). The cultivated algal cells were maintained at  $20 \pm 2^\circ\text{C}$  in photoperiod for 12 hr in light and 12 hr in the dark. The experiments were processed for 28 days (8).

### C. Biomass Estimation of *Chlorella*

Tannery and textile wastewaters with algal cells were centrifuged at 10,000 rpm for 20 mins. The supernatant was discarded and algal pellet was weighed to determine the fresh weight and then dried at 80 °C for 2 mins. These dried algal cells were again weighed to determine the dry weight of *Chlorella*. This gave the total mass of viable and dead cells of *Chlorella* (9). Table-1 shows the fresh weight (FW) and dry weight (DW) of *Chlorella* on tap water and diluted textile and tannery wastewaters.

### D. Cell Count and Chlorophyll Estimation

Algal cells were counted using haemocytometer. The haemocytometer consisted of counting grids with 4 squares which were  $1\text{mm}^3$  on the sides. The samples were mixed thoroughly and 2–3  $\mu\text{l}$  of samples were loaded in a chamber. The units used to calculate algal biomass was cells/ml (10). 1ml of algal cells was taken and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were separated from pellets and were mixed with 4.5ml of 95% ethanol. This solution was analysed for chlorophyll a and b content using spectrophotometer at 663 and 645 nm respectively (11).

## Results and Discussion

There was a gradual increase of cell count in both textile ( $1272 \times 10^3$  cells/ml) and tannery ( $1188 \times 10^3$  cells/ml) wastewaters (Fig. 1). This was because of low turbidity which transmitted light and the requirement of minimum nutrients for the growth of algae (12). In case of Ajayan *et al* (13) due to low dilution, there was a decline in growth on 7<sup>th</sup> day. This confirmed that *Chlorella* was able to adapt very well in wastewater only due to higher dilution (80%). There was a parallel increase in chlorophyll count with cell count which confirmed the observations of Cinderlla das *et al* 2016 (14) on tannery wastewater (Fig. 2). Table 1 shows the comparison of biomass yield on viable cells and dead cells of *Chlorella*. On lower dilutions (60%), the biomass yield was reduced but on higher dilution (80%) there was an appreciable increase in biomass yield (Tannery – 2.75 g/L, Textile – 3.21 g/L). This confirms that the biomass of micro-algae mainly depends on carbon source and light (15).

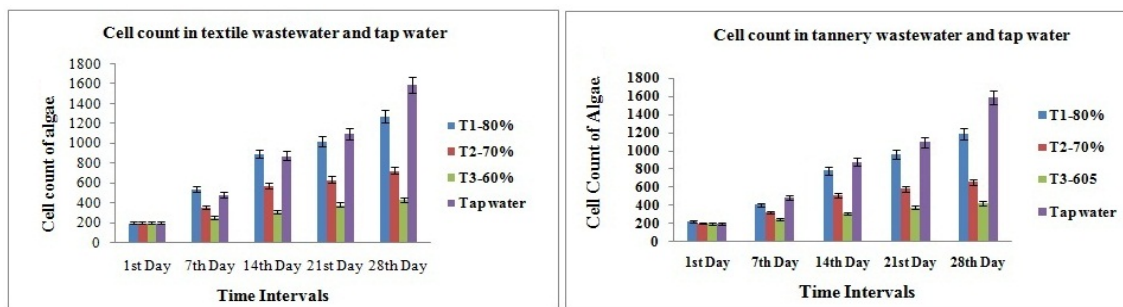


Figure 1; Cell count on textile and tannery wastewater (cell count/ml)

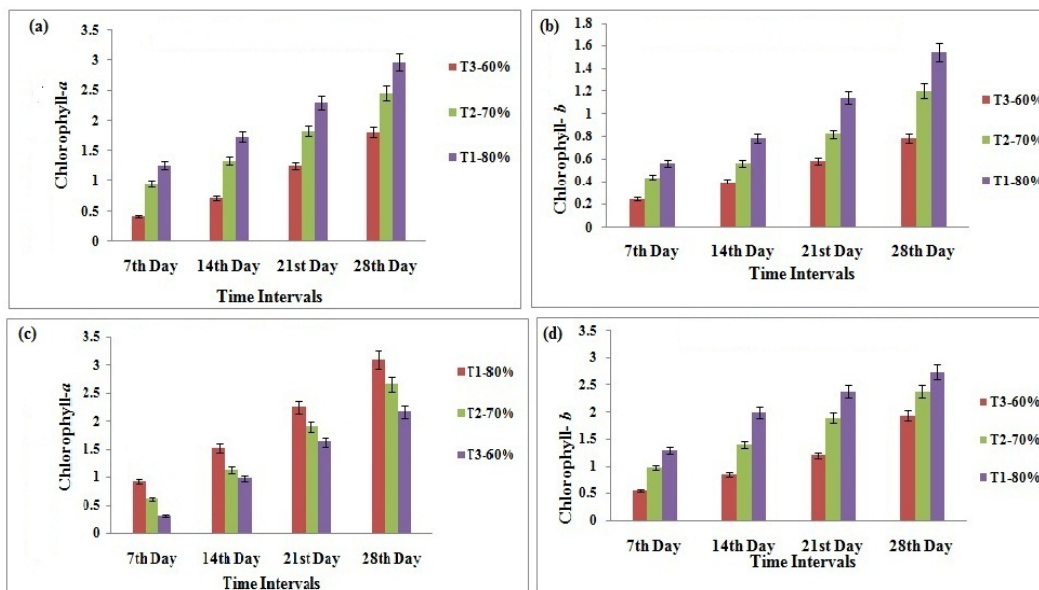


Figure 2; Chlorophyll estimation on textile and tannery wastewater ( $\mu\text{g/ml}$ )

- (a) Chlorophyll *a* on tannery effluent
- (b) Chlorophyll *b* on tannery effluent
- (c) Chlorophyll *a* on textile effluent
- (d) Chlorophyll *b* on textile effluent

TABLE 1 Biomass estimation of *Chlorella* on textile and tannery wastewater

Fresh weight of <i>Chlorella</i> before and after treatment (g/L)						Dry weight of <i>Chlorella</i> before and after treatment (g/L)				
	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
Tap water	0.85	1.99	3.38	4.25	5.63	0.28	1.05	2.29	3.18	4.23
<b>Textile wastewater</b>						<b>Textile wastewater</b>				
Dilutions	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
T1-80%	0.25	0.86	1.33	2.58	3.21	0.10	0.53	0.98	1.61	2.45
T2-70%	0.25	0.61	1.12	1.76	2.86	0.10	0.31	0.72	1.22	1.78
T3-60%	0.25	0.42	0.77	1.28	2.06	0.10	0.18	0.57	0.81	1.20
<b>Tannery wastewater</b>						<b>Tannery wastewater</b>				
Dilutions	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day	1 <sup>st</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	28 <sup>th</sup> day
T1-80%	0.25	0.79	1.09	1.95	2.75	0.10	0.46	0.86	1.22	1.98
T2-70%	0.25	0.51	0.93	1.36	1.91	0.10	0.33	0.63	0.87	1.36
T3-60%	0.25	0.33	0.66	0.97	1.30	0.10	0.17	0.35	0.59	0.97

### Acknowledgement

We thank Department of Marine Science, Bharathidasan University for providing us the algal culture *Chlorella vulgaris*. Due to this help, we were able to start bio-remediation research and completed successfully.

### References

- [1] V. Diagomanolin, M. Farhang, M. Ghazi-khansari, and N. Jafarzadeh, "Heavy metals (Ni, Cr, Cu) in the Karoon waterway river, Iran", *Toxicol. Lett.*, vol. 151, pp. 63-67, 2004.
- [2] M.K. Mohanta, A.K. Saha, and M.A. Hasan, "Prevalence and determination of occupational diseases of leather tannery workers", *Univ. j. zool. Rajshahi. Univ.*, vol. 31, pp. 79-82, 2012.
- [3] Rita Kant, "Textile dyeing industry an environmental hazard", *Nat. Sci.*, vol. 4, pp. 22-26, 2012.
- [4] N.H. Brahmabhatt and S.K. Hareesh, "Effect of algae on seedling growth of "Queen of Forages", *IJERGS*, vol. 3, pp. 827-833, 2015.
- [5] Y. Chisti, "Biodiesel from microalgae", *Biotech. Adv.*, vol. 25, pp. 294-306, 2007.
- [6] S.C. Agrawal and V. Singh. "Viability of dried cells, and survivability and reproduction under water stress, low light, heat, and UV exposure in *Chlorella vulgaris*", *ISR. J. Plant. Sci. J.*, vol. 49, pp. 27-32, 2001.
- [7] Y. Sanjay and G.P. Satsangi, "Allelopathic effect of algal leachates on seed germination and seedling growth of Paddy", *J. Algal. Biomass. Util.*, vol. 5, pp. 80-84, 2014.
- [8] K.V. Ajayan and M. Selvaraju, "Phycoremediation of tannery wastewater using microalgae *Scenedesmus* species", *Int. J. Phytoremediation*, vol. 17, pp. 907-916, 2015.
- [9] Y. Li, X. Fei and X. Deng, "Novel molecular insights into nitrogen starvation induce triacylglycerols accumulation revealed by differential gene expression analysis in green algae *Micractinium pusillum*", *Biomass. Bioener.* Vol. 42, pp. 199-211, 2012.
- [10] Lenore S Clesceri, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, 20<sup>th</sup> ed., Washington DC: USA, 1998.
- [11] S. Nayek, I.H. Choudhury, N. Jaishee and S. Roy, "Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents", *Res. J. Chem. Sci.*, vol. 4, pp. 63-69, 2014.
- [12] E.E. Nour Eldeen, M.A. Zayed, K.A. Rabie and M.E. Moattassem, "The seasonal variation of the Nile water quality", *J. Environ. Sci. Heal A*, vol. 30, pp. 1957-1972, 1995.
- [13] K.V. Ajayan, M. Selvaraju, P. Unnikannan and P. Sruthi, "Phycoremediation of tannery wastewater using microalgae *Scenedesmus* species", *Int. J. Phytoremediation*, vol. 17, pp. 907-916, 2015.
- [14] D. Cindrella, K. Naseera, Anirudh Ram, Ram Murti Meena and R. Nagappa, *Bioremediation of tannery wastewater by a salt-tolerant strain of Chlorella vulgaris*, *J. Appl. Phycol.*, 2016.
- [15] A. Richmond, *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, 2004.