UJMR, Volume 3 Number 1 June, 2018 *ISSN: 2616 - 0668*

Received: 1st Jan, 2018 **Accepted: 6th Feb, 2018**

Bioremediation of Waste Water Using *Chlorella vulgaris* Isolated from River Ginzo Effluents in Katsina State Nigeria

Badamasi, M.,[1]Abdulkarim, B.[1] and Indabawa, I.I.[2]
1- Department of Biology Umaru Musa Yar'adua University Katsina
2- Department of Plants science Bayero University Kano
Email: mudassir.badamasi@umyu.edu.ng

Abstract

The Bioremediation application of *microalgae* on wastewater effluent was conducted using single cultured specie (*Chlorella vulgaris*).Samples were collected from River Ginzo municipal effluent in Katsina metropolis and Wastewater was inoculated with *Chlorella vulgaris* as a single culture. treatments were periodically analysed (every 5th day) for a total period of 20 days for physico-chemical parameters such as pH, TDS, DO, Phosphate, Nitrate, Ammonium, BOD, Potassium and heavy metals using standard APHA method. The results indicated that, the parameter analyzed were statistically significants ($p<0.05$) were; Nitrate, phosphorus, ammonium, BOD, TDS, potassium, copper, cobalt, zinc, lead, iron and chromium recorded(75%, 91%, 87%, 30%, 52%, 80%, 39%, 42%, 21%, 13%, 20% and 19%) reduction composition respectively.Therefore, *Chlorella vulgaris* could be used for wastewater bioremediation as an environmental friendly alternative.

Keywords: Bioremediation, *Chlorella vulgaris* and *physicochemical parameters*.

INTRODUCTION

Algae are oxygen-evolving photosynthetic microorganisms that commonly grow in different aquatic environments, such as fresh and marine water and also wastewater sourcesfrom surface run-offs industrial and municipal wastewaters (Oswald *et al.,* 1967). Some algae species also can grow on rocks, soils and plants due to adequate amounts of Carbon (organic or inorganic carbon), Nitrogen (ammonium, nitrate, urea, yeast extract, etc.), and Phosphate (Phosphorus) andessential trace elements (Zhou *et al.,* 2012). Wastewaters are unique in their chemical profile and physical characteristics as compared to fresh and marine water(Zhou *et al.,* 2012).

The releases of industrial and residential wastewater possess serious environmental challenges to the receiving water bodies (De-Bashan *et al.,* 2010). Godos*et al.,* (2009) reported that, the major effect of releasing wastewater rich in organic compounds and inorganic chemicals such as phosphates and nitrates is mainly eutrophication. This is a global problem that could be remediated using microalgae whereby the wastewater is used as microalgae nutrient for growth (Olguın*et al.,* 2003). The advantage is that while the microalgae will be removing excess nutrients in the wastewater, there will be concomitant accumulation of biomass for downstream processing (Olguın*et al.,* 2003) The use of a various range of microalgae such as *Chlorella, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas*and*Spirulin*a for the treatment of domestic wastewater has been reported and efficacy of this method is promising. (Chinnasamy*et al.,* 2010)

The industrial effluents were discharged into open drains which finally join the rivers as receiving. Rural communities may use such effluent for domestic activities. The studied communities were observed to have used such type of effluents for washing cloth, vehicles and irrigation purposes. Most people in Katsina metropolis rely solemnly on vegetables cultivated from such area which may may be contaminated with chemical pollutants.

The aim of this research isto, isolate and the indigenous micro-algal specie that has potentials for bioremediation of chemical contaminants in waste wastewater.

MATERIAL AND METHODS

Study Area

Waste water was collected from River Ginzo municipal effluent in Katsina metropolis.The sampling point in river ginzois located along the GPS of 12°, 59', 197" N and 007° 36', 875" E.

Collection of waste Samples

The domestic wastewater samples in this study were collected from River Ginzo Katsina respectively. Wastewater samples were collected using 2-litre dark brown bottle based on the techniques described by Indabawa, (2012)

Media Preparation

Allen's Blue-Green Medium was prepared;the media was prepared using one liter of distilled water and the pH was maintained at neutral also the mediaautoclaved at121°C for 20 min.

Enrichment of Culture in the Media

Collected waste water samples were brought to the laboratory and the samples were centrifuged to remove the supernatant sample which was then inoculated into sterile conical flasks containing media. This was incubated for 10 days by providing required environmental condition such as 12:12 light/dark photoperiods and a temperature of 25°C, which allowed further growth and multiplication of algae organisms (*Mohan et al.*, 2009, 2010).

Identification of Microalgae

The micro algal samples were subjected to microscopic observation for physical identification. *C. vulgaris* was vied under digital Compound microscope (SWIFT M10). Standard phycological keys described by Palmer (1980), Edward and David (2010) was used to determined species. Algal cell count was done using haemacytometer as described by Guillard (1978) and Schoen (1988).

Growth and maintenance in Media

For the maintenance of algal cultured, broth was prepared and each identified algal species was inoculated and incubated at 25°C under 12:12 light/dark photoperiods. The culture was maintained both in slants and broth cultures for further 7 days and algal cell count was done very day(*Mohan et al.*, 2009, 2010).

Collection of wastewater (Sampling of water Effluent)

For the treatment of effluent the domestic wastewater samples in this study were collected from sewage municipal wastewater at River Ginzo Katsina.According toKaul and Gautum, (2002).

Physico-chemical characteristics of water samples

The initial physico-chemical parameters of water samples were measured before inoculation of algae and at final stage, the total content in each flask was filtered to remove algae and then used for the analysis of various parameters pH, TDS, phosphate, nitrate, ammonium, potassium, DO(dissolved oxygen), BOD (biological oxygen demand) and heavy metals using standard methods (APHA, 2005).

Remediation Bioassay

Twelve flasks (100ml waste water samples in 250ml conical flasks) were prepared. Each of the twelve flasks was inoculated with 10ml of 1-week cultured individual (*Chlorella vulgaris*) microalgae suspensions. This was further Incubated under the stated condition for a period of 20 days.Samples wereperiodically (every 5th day) analyzed for physico-chemical parameters (APHA, 2005)

Statistical Analysis

The result was analyzed using ANOVA at ($p < 0.05$) significance. Graph pad prism statistical software version (6.04) were used for the analysis

RESULT AND DISCUSSION

Table 1: *C. vulgaris* identified, isolated and cultured for bioremediation in waste water sample from river ginzo Katsina metropolis were *Chlorella vulgaris* shows the high growth and development with $41.33x10^4 \pm 0.58$ at day one (1) and $91.67x10^4 \pm 0.57$ at day seven (7),

NUMBER OF DAYS	*C. vulgaris* (cell/ml)
1	$41.33x10^4 \pm 0.58$
2	$48.66x10^4 \pm 0.58$
3	$60.67x10^4 \pm 0.57$
4	$74.67x10^4 \pm 1.16$
5	$83.33x10^4 \pm 1.56$
6	$88.66x10^4 \pm 2.08$
7	$91.67x10^4 \pm 0.57$

Chlorella vulgaris shows high growth and development with $41.33x10^4$±0.58 cell/mlat day one and$91.67x10^4$±0.57 cell/mlat day seven from table 1 above. The finding of this research is in line with finding of Chan, (2011) who conducted research by cultivating the microalgae in wastewater from a fish farm and established that they can promote the growth of *Chlorella* sp. and they obtained a 90% growth rate during the experimental period. Growth of *Chlorella vulgaris* microalgae depends mainly on nutrients present in the synthetic wastewater (Changfu*et al.*, 2013). The finding of this research also corresponds with the finding ofChinnasamy*et al.*, (2009), that showed growth response of *C. vulgaris* in terms of biomass production in their finding.

The bioremediation of wastewater from of river ginzo Katsina metropolis using single specie (*Chlorella vulgaris)* is shown in Table 2 below. Wherephysico-chemical parameters such as pH, TDS, phosphate, nitrate, ammonium, (Dissolved Oxygen) DO,(Biological Oxygen Demand) BODanalyzed for total period of 20days

Table 2: Bioremediation of waste water using single species (*Chlorella vulgaris*) isolated from River Ginzo Katsina metropolis.

S/N	PARAMETERS	Before Treatment	After Treatment			
			5^{th} day	10^{th} day	15^{th} day	20^{th} day
1	PH	7.44±0.02	7.59±0.01	7.66±0.01	7.50±0.01	7.60±0.01
2	DO(ppm)	1.05±0.01	1.07±0.01	1.10±0.01	1.11±0.10	1.14±0.00
3	TDS(mg/l)	1373±1.00	992±1.00	712±20.00	644±8.30	655±35.15
4	Nitrate(mg/l)	136.66±0.06	96.45±1.88	63.50±0.50	42.01±1.01	33.57±3.50
5	Phosphorus(mg/l)	33.27±0.25	33.27±0.25	11.15±1.56	5.71±0.21	2.9±0.42
6	NH_4(mg/l)	126.07±0.05	84.02±0.04	34.68±0.58	21.37±1.14	16.82±0.89
7	BOD(ppm)	0.39±0.01	0.37±0.01	0.30±0.01	0.28±0.01	0.27±0.00
8	Potassium(mg/l)	24.87±0.07	13.27±0.04	8.64±0.88	5.88±0.42	4.97±0.04

The Table 2 shows there were significant differences between the initial pH and 5^{th}, 10^{th} and 20^{th} days respectively (P<0.01). However, there was no significant differences between initial pH and that of 15^{th} day (P>0.05). The pH was maintained around neutral values throughout the cultured period. The finding of this research was in line with the finding of(Aarti*et al.*, 2008;Makarevicien*eet al.*, 2011;Mostafa*et al.*,2015). whose reported that *Chlorella vulgaris* sustained the maximum growth rate at the range of pH between 6.0 and 9.0.

The concentration of dissolved oxygen (DO) shows significant differences between the initial DO and that of 5^{th}, 10^{th}, 15^{th} and 20^{th} days respectively (P>0.05).The value of DO increases due to photosynthesis process that took place in the culture, which provided energy source. This is the similar finding with the finding of(Oswald *et al.*,2003) who reportedthat, using light as an energy source, microalgae uptake CO_2 from the environment and produce O_2 as a byproduct and this is similar observations with my finding.

The concentration of BOD shows there is no significant differences between initial BOD and that of 5^{th}days (P>0.05). However, there is significant different between the initial and and that of 10^{th}, 15^{th}, 20^{th} respectively (P<0.01). The obtained results revealed that remarkable decrease in BOD concentration. This is the same observation made by Ganapathy*et al.*, (2011) the value of BOD indicates level of toxicity of wastewater and they further reported the reduction in BOD of distillery effluent by 53 % using Algae species.

The concentration of TDS shows that there is significant differences between initial TDS and that of 5^{th}, 10^{th}, 15^{th}, and 20^{th} respectively (P<0.01).The removal of TDS for cultured considered extremely significant.These reduction in TDS might be as the result of utilization of various nutrients by algae (Rao*et al.*, 2011 ; Ahmad *et al.*, 2013) which is in line with our finding.

The concentration of Nitrate,Phosphorus,Ammonium and Potassium showed that, there is significant differences between initial nitrate,Phosphorus,Ammonium and Potassium and that of 5^{th}, 10^{th} ,15^{th} and 20^{th} respectively (P<0.01). However the concentration of Potassium showed that, there is significant differences between initial potassium concentration and 5^{th}, 10^{th} ,15^{th} and 20^{th} respectively (P<0.01). The finding of this research correspond with the finding of Aslan and Kapdan, (2006) who used *C. vulgaris* for nitrogen and phosphorus removal from wastewater with an average removal efficiency of 72% for nitrogen and 28% for phosphorus.

Shi *et al.,* (2007) performed experiments with *Chlorella* to remove nitrate from municipal wastewater and reduce levels of phosphate, ammonium and nitrate in synthetic secondary wastewater which is also in line with our finding. The specifics depend on the type of wastewater, the type of algae and their growth conditions, and most importantly on the relationship between the amount of biomass applied and the hydraulic loading of the wastewater (Shi et al., 2 007). Freshwater unicellular microalgae, mainly *Chlorella* have been used in wastewater treatment for the removal of nitrogen and phosphorus compounds (Hammouda*et al*., 1995; Gonzalez *et al*., 1997). This is similar observation made by Azab (2002) who stated that the application of algae for wastewater treatment showed variable percentages of decrease in minerals. This above observations are in line with my observations.
The bioremediation of waste water (Heavy metals) from river ginzo Katsina metropolis using single species (*Chlorella vulgaris)* is showed in table 3 below.Whereheavy metals analysed for total period of 20days after inoculations of C.*vulgaris*.

Table 3: Bioremediation of waste water (Heavy metals) using single specie (*Chlorella vulgaris*) isolated from river ginzo Katsina metropolis

S/N	PARAMETERS	Before Treatment	After Treatment			
			5^{th} day	10^{th} day	15^{th} day	20^{th} day
1	Copper (mg/l)	0.46±0.44	0.45±0.01	0.41±0.01	0.31±0.01	0.28±0.01
2	Cobalt (mg/l)	0.77±0.12	0.70±0.01	0.59±0.02	0.51±0.03	0.45±0.02
3	Zinc (mg/l)	10.14±0.16	9.95±0.15	8.76±0.12	7.96±0.18	7.29±0.19
4	Lead (mg/l)	0.70±0.17	0.69±0.01	0.67±0.01	0.62±0.01	0.61±0.01
5	Chromium (mg/l)	0.32±0.11	0.31±0.01	0.29±0.01	0.27±0.01	0.26±0.01

HEAVY METALS

Table 3 above shows that there was no significant differences between initial Copper and cobalt and that of 5^{th},10^{th}, 15^{th} and 20^{th} days respectively (P>0.05). However, there is significant differences between initial Cobalt and that of 10^{th}, 15^{th} and 20^{th} days (P<0.01,P<0.01and) respectively. There was no significant differences between initial Zinc value and Zinc at 5^{th} days respectively (P>0.05). However, there was significant differences between initial Zinc and Zinc at 10^{th}, 15^{th} and 20^{th} days (P<0.001).There is no significant differences between initial Lead and that of 5^{th},10^{th}, 15^{th} and 20^{th} days respectively (P>0.05).There is no significant differences between initial Chromium (Cr) and Chromium (Cr) at 5^{th},10^{th}, 15^{th} and 20^{th} days respectively (P>0.05). The concentration of heavy metals in the wastewater samples inoculated with *C. vulgaris* shows reduction with increase in the number of days, this is in line with observation made by Al- Qunaibit, (2009) who reported that, dried dead *C.vulgaris*was studied in terms of its performance in binding divalent Cu, Cd and Pb ions from their aqueous solutions. Percentage uptake of cadmium ions exhibited general decrease with decrease in dielectric constant values while that of copper, Lead ions shows decrease with increase in donor numbers. (Chan *et al.*, 2014) also reported, microalgae removed up to 81.7% Cu reaching lowest final concentration of 7.8ppb after 10 days. Zn reduced up to 94.1% reaching 0.6ppb after 10 days this is also in line with our finding.

Conclusion

From the result it has shown that *C. vulgaris*was easier to cultivate and developed on media.Also from the results, it is concluded that remediation of diverse waste water contaminants is possible by using *Chlorella vulgaris*. The present result showed that *Chlorella vulgaris* had very good potentials to remediate the toxic level of all physico-chemical parametersanalysed. These finding confirmed that *Chlorella vulgaris* may be considered as efficient algae for the removal of all level of toxic physico-chemical parameters.

Recommendation

From the finding of this research, it is recommended that;

1. Cultivation of micro algae on media or wastewater treatment should be encouraged as it produces an appreciated biomass which can be used for biodiesel, bio fertilizer, biofuel and production or obtaining valuable items.
2. Also government should pay more emphasis on bioremediation of toxic chemicals in waste water and enlightens people on importance of bioremediation.

REFERENCES

Aarti. N., Sumathi, P. and Subrahmanian, V. (2008). Phycoremediation to improve algal water quality. *IndHydrobiol* 11:173–184

Ahmad, F., Khan, A.U. and Yasar, A. (2013). Comparative phycoremediation of sewage water by various species of algae. *Proc Pak AcadSci* 50:131–139

Akan, J.C., Moses, E.A. and Hati, S.S. (2006). Some physical and chemical pollutants in holborn textile industrial effluents from Kano Metropolis, Kano State, Nigeria. J. Life Environ. Sci., 8: 457-460.

Aksu, Z. and Kustal, T. A., (1991). Bioseparation process for removing lead ions from wastewater by using *Chlorella vulgaris*. *J. Chem. Technol. Biotechnol.*, 52, 109-118.

Al-Qunaibit M. H. (2009). Divalent Cu, Cd, and PbBiosorption in Mixed Solvents. *Bioinorganic Chemistry and Applications.*

APHA. (2005). Standard methods for the Examination of Water and Wastewater. *(American Public Health Association)*

Aslan, S. and Kapdan, I.K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, 28(1), 64–70.

Azab, M.S. (2008). Waste-waste treatment technology and environ-mental management using sawdust bio-mixture. J *TaibahUnivSci* 1:12–23

Azarpira, H., Behdarvand, P., Dhumal, K. and Pondhe, G. (2014). Comparative studies on phycoremediation of sewage water by using blue green algae. *Int J Biosci* 4:58–64

Chan, A., Hamidreza, S. and Ed, M. (2014). Heavy Metal Removal (Copper and Zinc) in secondary effluents from waste water treatment plants by Microalgae.ACS*Sustainable Chemistry and Engineering*, Vol. 2: issue 2 pp 130-137.

Chan, H. (2011). Recycling of Nutrients from Trash Fish Wastewater for Microalgae Production as Health and Pharmaceutical Products and Renewable Energy. *WebmedCentral Microbiology*, 2 (7):WMC002027.

Changfu, W., Xiaoqing, Y., Hong, L. and Jun, Y. (2013). Nitrogen and phosphorus removal from municipal wastewater by the green alga Chlorella sp. *Journal of Environmental Biology*, 34, 421-425.

Chinnasamy, S., Bhatnagar, A., Hunt R.W and Das, K.C. (2010). Microalgae cultivation in awastewater dominated by carpet mill effluents for biofuel applications.*Bio resource Technol* 101:3097–105.

Edward, G. B. and David, C. S. (2010): A Key to the More Frequently Occurring Freshwater Algae.Freshwater Algae: Identification and Use as Bioindicators.C_ 2010 John Wiley & Sons, Ltd.

Edward, W., Wilde., John. R.andBenemann. (1993). Bioremoval of heavy metals by the use of microalgae. *Biotechnology Advances*.Volume 11, Issue 4, Pages 781–812.

Fontes, A., Vargas, M., Moreno, J., Guerrero, M., &Losada, M. (1987). Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture . *Biomass*, 33–43.

Gonzalez, C., Marciniak, J., Villaverde, S., Garcia-Encina, P.A. and Munoz, R.(2008). Microalgae based processes for the biodegradation ofpretreated piggery wastewaters. *ApplMicrobiolBiotechnol*80:891–898

González, L., Cañizares R. and Baena S. (1997). Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorellavulgaris* and *Scenedesmusdimorphus*. *Bioresour Technology*, 60, 259–262.

Guillard, R.R.L. (1978): Counting Slides Phytoplankton Manual. UNESCO. P. 182 From http://www.marine.csiro.au/microalgae/methods/haemacytometer%20counting.html retrieved on 4/7/2014.

Gutzeit, G., Lorch, D., Weber, A., Engels, M., &Neis, U. (2005). Bioflocculentalgalbacterial biomass improves low-cost wastewater treatment. *Water Science & Technology*, 9-18.

Hammouda, O., Gabe,r A. and Abdel-Raouf, N. (1995). Microalgae and wastewater treatment. *Ecotoxicol. Environ. Safety.*, 31:205–210.

Indabawa, I.I. (2012): Bioinstrumentation Lecture Notebook for Postgraduate Students, Bayero University Kano.

Karlander, E. P. and Krauss, R. W. (1996).Responses of heterotrophic cultures of *Chlorellavulgaris* Beyerink to darkness and light. II. Action

spectrum and mechanism of the light requirement for heterotrophic growth. *J. Plant Physiol.,* 41, 7-14.

Kaul, S. N. & A. Gautum. *Water and Wastewater Analysis*. Daya Publishing House. Dehli, India.

M.B.Pescod. (1992). Wastewater treatment and use in agriculture - *FAO* irrigation and drainage paper 47, Rome,

Makarevi, V., Andrulevi, V., Skorupskai, V. and Kasperovi, J. (2011). Cultivation of Microalgae Chlorella sp. and Scenedesmus sp. as a Potentional Biofuel Feedstock. *Environmental Research, Engineering and Management*, 3(57), 21-27.

Mohan, N., Rao, P.H., Kumar, R.R. and Sivasubramanian, V. 2010. Mass cultivation of *Chroococcusturgidus*and *Oscillatoria*sp. and effective harvesting of biomass by low-cost methods..*NaturePrecedings,* <http://dx.doi.org/10.1038/npre. 2010.4331.1

Mohan, N., Rao, P.H., Kumar, R.R., Sivasankaran, S. and Sivasubramanian, V. 2009. Studies on mass cultivation of Chlorella vulgaris and effective harvesting of bio-mass by low-cost methods. *J Algal Biomass Utln*, 1(1), 29-39.

Mostafa, M., Abla A., Hamdy R., Hani S. (2015). Bioremediation of different types of polluted water using microalgae *AccademiaNazionaledeiLincei* DOI 10.1007/s12210-015-0495-1

Nanda, S., Sarangi, P.K and Abraham, J. (2010). Cynobacterial remediation of industrial effluents II. *Paper mill effluents*. N Y Sci J. 3:37–41

Olguın, E.J. (2003). Phycoremediation: key issues for cost-effective nutrient removalprocesses. *BiotechnolAdv* 22:81–91.

Oswald, W. (1988). Microalgae and wastewater treatment . In W. Oswald, *Microalgal biotechnology* (pp. 305–328). Cambridge: Cambridge University press.

Oswald, W. J. (2003). My sixty years in applied algology. *Journal of Applied phycolog*, 99-106.

Oswald, W.J., Gotaas, H.B., Golueke, C.G. and Kellen, W.R. (1957). Algae in waste treatment. Sewage Ind Wastes 29: 437-455.

Palmer, C.M. (1980): Algae and Water Pollution.Castle House Publication, *England*.123 P.

Rao, H.P. Ranjith, Kumar., R.Raghavan., B.G. Subramanian, V.V. and Sivasubramanian, V. (2011). Application of phycoremediation technology in the treatment of wastewater from a leather- processing chemical manufacturing facility. *Water SA* 37:7–14

Schoen, S. (1988): Cell Counting Experimental Phycology- a Laboratory Manual From http://www.marine.csiro.au/microalgae/metho ds/haemacytometer%20counting.html retrieved on 4/7/2014

Shi J., Podola B., Melkonian M., (2007). Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *Journal of Applied Phycology*, 19, 417–423.

Travieso, L., Cañizares, R. O., Borja, R., Benítez, F., Domínguez, A. R., Dupeyrón, R. and Valiente, V. (1999). Heavy metal removal by microalgae. *Bull. Environ. Contam. Toxicol*, 62, 144-151.

Volesky B., (1990), Biosorption of heavy metals, Boca Raton, Flo.: Press CRC, Florida. pp 3-6.

WHO and UNICEF Joint Monitoring Programme. (2000). Globalwater supply and sanitation assessment report. *New York:UNICEF and Geneva*: WHO

Zhou, W., Li. Y., Min, M., Hu, B., Zhang, H., Ma, X., Li, L., Cheng, Y., Chen, P., and Ruan, P. (2012). *Appl. Energy*, 98, 433-40