

**USE OF VERMICAST IN THE PRODUCTION OF *Chlorella sorokiniana***

by

**MARICAR VILLANUEVA CARBONEL**

An Undergraduate Thesis presented to the faculty of the College of Fisheries in partial fulfillment of the requirements for the degree of

**BACHELOR OF SCIENCE IN FISHERIES**

**Department of Aquatic Resources, Ecology and Management**  
**COLLEGE OF FISHERIES**  
**CENTRAL LUZON STATE UNIVERSITY**  
Science City of Muñoz, Nueva Ecija  
Philippines

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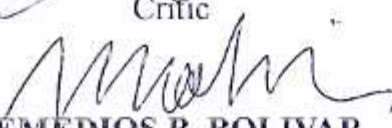
  
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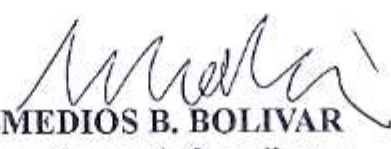
  
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# USE OF VERMICAST IN THE PRODUCTION OF *Chlorella sorokiniana*

## ABSTRACT

*Chlorella* is a potential food source because it is high in protein and other essential nutrients. It is also abundant in calories and vitamins. The objective of the study was to evaluate the *Chlorella* production using vermicast as organic fertilizer. Specifically, this aim to determine the ideal dosage of vermicast in the production of *Chlorella* under the laboratory condition.

The study had six treatments, which were replicated thrice, namely: T1- 1 liter of stock solution and 10 ml inoculant of *C. sorokiniana*, T2- 2g of vermicast, T3- 4g of vermicast, T4- 6g of vermicast, T5- 8g of vermicast, and T6- 10g of vermicast, 1 liter of stock solution and 10 mL inoculant of *C. sorokiniana*. The study was conducted in wet laboratory using 18 wine bottles.

Analysis revealed that T1 (control) was significantly different ( $P < 0.05$ ) among all treatment and this treatment obtained the lowest density count. However, T2 were significantly different ( $P < 0.05$ ) among all treatment except in T3 and this treatment with 2g of vermicast had a highest total density count.

This study was limited on the production of *Chlorella* using vermicast under laboratory condition. It is recommended to use lower dosage of vermicast and evaluate the distance of light source to the culture vessel.

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## LITERATURE CITED

- Agwa, O.K., S.N. Ibe and G.O. Abu. 2012. Economically effective potential of *Chlorella* sp. for biomass and lipid production. *Journal of Microbiology and Biotechnology Research*, 2(1): 35-45.
- Agwa, O.K., S.N. Ibe and G.O. Abu. 2013. Heterotrophic cultivation of *Chlorella* sp. using different waste extracts. *International Journal of Biochemistry and Biotechnology*, 2(3): 289-297.
- Agwa, O.K. and G.O. Abu. 2014. Utilization of poultry waste for the cultivation of *Chlorella* sp. for biomass and lipid production. *International Journal of Current Microbiology and Applied Science*, 3(8): 1036-1047.
- Aira, M., F. Monroy, J. Dominguez and S. Mato. 2002. How earthworm density affects microbial biomass and activity in pig manure. *Eur. J. Soil Biol.*, 38: 7-10.
- Arisha, H.M. and A. Bardisi. 1999. Effect of mineral fertilizers and organic fertilizers on growth, yield and quality of potato under sandy soil conditions. *J. Agric. Res.*, 26: 391-405.
- Brennan, L. and P. Owende. 2008. Biofuels from microalgae - a review of technologies for production, processing and extraction of biofuels and co-products. *Renewable and Sustainable Energy Review*, 14: 557-577.
- Brown, M.R., S.W. Jeffrey, J.K. Volkman and G. Dunstan. 1997. Nutritional properties of microalgae for mariculture. *Aquaculture*, 151: 315-331.
- Cantrell, K.B., T. Ducey, K.S. Ro and P.G. Hunt. 2008. Livestock waste-to-bioenergy generation opportunities. *Bioresource Technology*, 99: 7941-7953.
- Chen, G-Q. and F. Chen. 2006. Growing phototrophic cells without light. *Biotechnology Letters*, 28(9): 607-616.
- Cooper S. 1979. A unifying model for the G1 period in prokaryotes and eukaryotes. *Nature* 280, 17-19.
- Cooper S. 1984. The continuum model as a unified description of the division cycle of eukaryotes and prokaryotes. In: Nurse P, Streiblová E, editors, *The microbial cell cycle*. Boca Raton, Florida: CRC Press, pp 7-18.
- Edwards, C.A. and I. Burrows. 1988. The potential of earthworm composts as plant growth media. In: C.A. Edwards and E.F. Neuhauser (Eds). *Earthworms in Waste and Environmental Management*. 211-219.

- Forsburg, S.L. and P. Nurse. 1991. Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Annual Review of Cell Biology*, 7: 227-256.
- Gajalakshmi, S. and A. Abbasi. 2003. Earthworms and vermicomposting. *Indian Journal of Biotechnology*, 3: 486-494.
- Garg, S.K. and A. Bhatnagar. 1999. Effect of different doses of organic fertilizer (cowdung) on pond productivity and fish biomass in still water ponds. *Journal of Applied Ichthyol.*, 15: 10-18.
- Hormanseder, E., T. Tischer and TU Mayer. 2013. Modulation of cell cycle control during oocyte-to-embryo transitions. *EMBO Journal*, 32: 2191-2203.
- Iyiola, A.O. and A.P. Ojo-Awo. 2015. Organic manures and phytoplankton production. *Journal of Agriculture and Ecology Research International*, 3(4): 141-146.
- Jansirani, D., S. Nivethitha and M.V.P. Singh. 2012. Production and utilization of vermicast using organic wastes and its impact on *Trigonella foenum* and *Phaseolus aureus*. *International Journal of Research in Biological Sciences*, 2(4): 187-189.
- Kaur, V.I. and M.D. Ansal. 2010. Efficacy of vermicompost as fish pond manure—effect on water quality and growth of *Cyprinus carpio* (Linn.). *Bioresource Technology*, 101: 6215-6218.
- Lazcano, C., M.B. Gomez and J. Dominguez. 2008. Comparison of the effectiveness of composting and vermicomposting for the biological stabilization of cattle manure. *Chemosphere*, 72: 1013-1019.
- Mata, T.M., A.A Martins, and N.S Ceatano. 2010. Microalgae for biodiesel production and other applications: A review. *Renewable Sustainable Energy Review*, 14: 217-232.
- Miao, X.L. and Q. Wu. 2006. Biodiesel production from heterotrophic microalgal oil. *Bioresource Technology*, 97: 841-846.
- Muhammad, A., J. Muhammad., T. Rashid., A. Muhammad, A. Zafar, S. Ali and N. Muhammad. 2011. Replacement of expensive pure nutritive media with low cost commercial fertilizers for mass culture of freshwater algae, *Chlorella vulgaris*. *International Journal of Agriculture & Biology*, 13(4): 484-490.
- Muller-Feuga A. 2004. The role of micro-algae in aquaculture: situation and trends. *J. App. Phycol.*, 12: 527-534.

- Politud, E.R.R. 2016. Growth and yield performance of radish (*Raphanus sativus* L.) 'cv' 'SNOW WHITE' in response to varying levels of vermicast applications. *International Journal of Scientific and Research Publications*, 6(5): 2250-3153.
- Qiao, H., G. Wang and X. Zhang. 2009. Isolation and characterization of *Chlorellasorokaniana*GXNN01 (Chlorophyta) with the properties of heterotrophic and microaerobic growth. *J. Phycol.*, 45: 1153–62.
- Resdi, R., R.S. Jessy and M.H. Ibrahim. 2013. Vermicast from cattle/goat manure as fish pond fertilizer and effect on water quality. *Journal of Environmental Science, Computer Science and Engineering & Technology*, 2(2): 234-240.
- Scott, M.A. 1988. The use of worm-digested animal waste as a supplement to peat in loam less composts for hardy nursery stock. In: C.A. Edwards and E.F. Neuhauser, (Eds.). *Earthworms in Environmental and Waste Management*. (Pp. 231-229). SPB Acad. Publ. The Netherlands.
- Selden, P., M. Duponte, B. Sipes and K. Dinges. 2005. Small-scale vermicomposting. Retrieved on March 29, 2016 from <https://www.ctahr.hawaii.edu/oc/freepubs/pdf/HG-45.pdf>.
- Shi, X.M., X.W. Zhang and F. Chen. 2000. Heterotrophic production of biomass and lutein by *Chlorella protothecoides* on various nitrogen sources. *Enzyme Microbiol. Technol.*, 27: 312–318.
- Singh, J. and S. Gu. 2010. Commercialization potential of microalgae for biofuel production. *Renewable and Sustainable Energy Reviews*, 14: 2596-2610.
- Sinha, R.K., S. Agarwal, K. Chauhan and D. Valani. 2010. The wonders of earthworms & its vermicompost in farm production: Charles Darwin's 'friends of farmers', with potential to replace destructive chemical fertilizers from agriculture. *Agricultural Sciences*, 1(2): 76-94.
- Stahl, M.S. 2009. The role of natural productivity and applied feeds in the growth of *Macrobrachium rosenbergii*. *Proc. World Maricult. Soc.*, 10: 92-109.
- Stotttrup, J. and L. McEvoy. 2003. *Live feeds in marine aquaculture*. Wiley Blackwell. UK. 336 p.
- Subler, S., C. Edwards and J. Metzger. 1998. Comparing vermicomposts and composts. *Biocycle* 39: 63-69.
- Taiganides, E.P. 1978. Principles and techniques of animal waste management and utilization. *FAO Soils Bulls.*, 36: 341-362.

2

Arner, M.F. 1979. Nutrition of some micro-algae with special reference to vitamin requirements and utilization of nitrogen and carbon sources. *J. Marine Biotechnol.*, 159: 535-552.

Amadoss, V.A. 2005. Utilisation of garden waste for the production of vermicompost. *Journal of Ecotoxicology and Environmental Monitoring*, 15: 207-211.

Li, W., M. Gao, Z. Qi, M. Qiu, J. Peng and R. Shao. 2014. Effect of dietary *Chlorella* on the growth performance and physiological parameters of gibel carp *Carassius auratus gibelio*. *Turkish Journal of Fisheries and Aquatic Science* 14: 53-57.

Amaguchi, K. 1996. Recent advance in micro-algal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *Journal of Applied Phycology*, 8(6): 487-490.

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