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

Optimization of Panchagavya Concentrations for Mass Culture of Microalgae

***Sivadasan, K.K[1]., Anjana, M[1]., Girishkumar, E[1]., C.P.Ravindran[1] and K.M. Gopinathan[2]**

[1] Post Graduate Dept. of Plant Science, [2] Dept. of Zoology, Mahatma Gandhi Govt. Arts College, Mahe, U.T. of Puducherry, New Mahe Post -673 311)
*Corresponding author: Dr. K. K. Sivadasan, Associate Professor, P.G. Dept. of Plant Science, MGGAC, Mahe 673 311, Email: sivasmahe@gmail.com,

Abstract

Micro algae are known for their significant contributions in the primary production of earth and thereby reducing CO_2 and climate change. The potentialities of microalgae as a resource for biofuel, nutraceutical, pharmaceutical, cosmetics, bioremediation and aquaculture feed attract the mass cultivation of microalgae. Present study focused on the identification of potentiality of modified Panchagavya as a low cost culture medium and the optimization of its concentrations for mass production of microalgae as feed stock for biodiesal and aquaculture. When *Dunaliella salina*, *Chlorella vulgaris*, *Tetraselmis gracilis* and *Synechocystis salina* were cultivated in various concentrations of cow dung, Panchagavya, Walne's Medium and normal sea water or fresh water the growth rates showed that Panchagavya is a natural nutrient source for mass cultivation of microalgae. Fresh water algae *Chlorella vulgaris* showed highest growth in 0.3% concentration (v/v) of panchagavya on 27th day while marine *Dunaliella salina* and *Tetraselmis gracilis* achieved maximum growth in 0.15% and 0.3% concentrations on 22nd and 19th day respectively. Marine blue green algae, *Synechocystis salina* attained maximum growth in 0.15% concentration on 27th day. Optimum concentration of Panchagavya found to be varying with species. The growth enhancing property of Panchagavya as a medium for fresh water, marine and prokaryotic algae is effective. It can be recommended as low cost medium for mass cultivation of microalgae for biodiesal and aquaculture feed production. Higher concentration of Panchagavya can also be used as algicide against red rust disease caused by green algae, *Cephaleuros*.
Key words: panchagavya, microalgal culture, biodiesal, aquaculture

Introduction

Microalgae are the diverse group of primary producers grow in various aquatic habitats contributing 50% of total primary production of earth. In addition to their role as primary producers presently they are utilizing as source of nutraceuticals, pharmaceuticals, cosmetics, bioremediation agents and feed stock for biodiesel and aquaculture. The viability of the first generation biofuels production is however questionable because of the conflict with food security (Vishwanth Patil et al., 2008). Biodiesal from microalgae seems to be the only renewable biofuel that has the potential to completely displace petroleum derived transport fuels without adversely affecting supply of food and other crop products (Chisti, 2007) but an assessment of the economics of production is necessary to establish competitiveness with petroleum derived fuels. One of such major challenges is found to be the cheaper production of biomass. Various attempts were made for the mass cultivation of microalge in various culture media including fermented swine waste water (Kim et al. 2007), compost extract (de Oliveira and Crispim, 2013) and cow dung ash supplemented media (Shweta and Samuel, 2013). As no work has been done so far on the potentiality of Panchagavya as cheaper culture media for various microalgae, the present study focuses on potentiality of Panchagavya as a culture medium for the mass culture of freshwater and marine prokaryotic and eukaryotic microalgae as source of feed stock for biodiesal and as aquaculture feed.

Panchagavya, five products originated from cow is traditionally used in various religious rituals including last rites for purification and house warming ceremonies and is also used as traditional medicine (Balasubrahmanyan et al., 2009). Utilisation in organic farming due to the fertilizer values were attracted various modifications and one of such widely using modification is the modification by Natarajan, 2001. Studies on various crop varieties showed Panchagavya concentrations are most effective as growth promoters (Bindumathi Mohan, 2008), antifungal agents (Baby Joseph and Sankarganesh, 2011) and pesticides (Barati, 2005).

Materials and Methods

Present study conducted in the laboratory with room temperature 30±with light of 2300lux by fluorescent tube kept on the panel. Freshwater *Chlorella vulgaris*, marine *Dunaliella salina* and *Tetraselmis gracilis* and marine prokaryotic blue green *Synechocystis salina* culture maintained in Walne's medium (Walne, 1970) were used for the experiment. Algal cultures were collected from Department of Marine Botany, Cochin University of Science and Technology.

Modified Panchagavya (Natarajan, 2001) prepared by using fresh cow dung (5kg), cow urine (3L), cow milk (2L), cow ghee (500gm), cow curd (2L), sugar cane juice (3L or 500gm jagiri in 3L water), tender coconut water (3L), ripe banana fruits (12) and toddy (2L or 100gm yeast and 100gm jagiri in 2L warm water) , then different concentrations were made through dilutions. In the agriculture field recommended Panchagavya concentrations for various crops ranging from 2% to 5% and hence for microalgal culture similar concentrations and further low concentrations were used. For the fresh water culture autoclaved tap water was taken and for the marine forms seawater was autoclaved before adding medium. Sterilised

250ml Erlenmeyer's flasks were filled with 200ml of freshwater or seawater and filtered modified Panchagavya added to them to get the concentrations of 0.5 1.0, 2.0,3.0,4.0 and 5.0 for the first set up of experiments followed by the concentrations (v/v) of 0.05, 0.1, 0.15, 0.2, 0.3 and 0.4 %. Fresh cow dung (5kg) dissolved in 20L sterilised water to get similar contribution of cow dung as in the Panchagavya and then various concentrations were prepared from 0.05 to 0.5% (v/v). Algal cultures grown in Walne's medium, sterilized tap water or seawater were used for growth comparison. Growth rates were evaluated via periodic counting of cells using haemocytometer.

Results and discussions

Growth characteristics of freshwater green *Chlorella vulgaris*, marine green *Tetraselmis gracilis*, *Dunaliella salina* and marine prokaryotic blue green *Synechocystis salina* were studied to analyse the effective concentration of Panchagavya as culture medium.

Chlorella vulgaris

Growth of *Chlorella vulgaris* in Panchagavya showed highest cell numbers on 27th day with 61.2X10^6 cells/ml in 0.3% concentration (fig.1). This standing crop is about 50 times higher to inoculum, nearly 7 times higher than that of maximum growth in freshwater and 2.7 times higher to maximum growth in Walne's medium. Interestingly at 0.4% concentration of Panchagavya maximum growth observed on 15th day with 47.2X10^6 cells/ml (40 times to inoculum) that means when two culture is maintaining in 0.4% concentration one after other the harvest on 30th day the standing crop will be 94.4 X10^6 cells / ml, which is about 1.5 times higher to maximum growth in 0.3% on 27th day. Maximum growth rate of 3 observed for the culture in 0.4% concentration on 15th day also support that 0.4% concentration is the optimum concentration for large biomass production in short period.

When cow dung was used as nutrient source maximum standing crops in all concentration (fig.2) were found to be less than that of Walne's medium except in 0.05% concentration.

Fig. 1. Growth of *Chlorella vulgaris* (cells/ml) in Panchagavya medium at different concentration to that of standard Walne's medium and fresh water

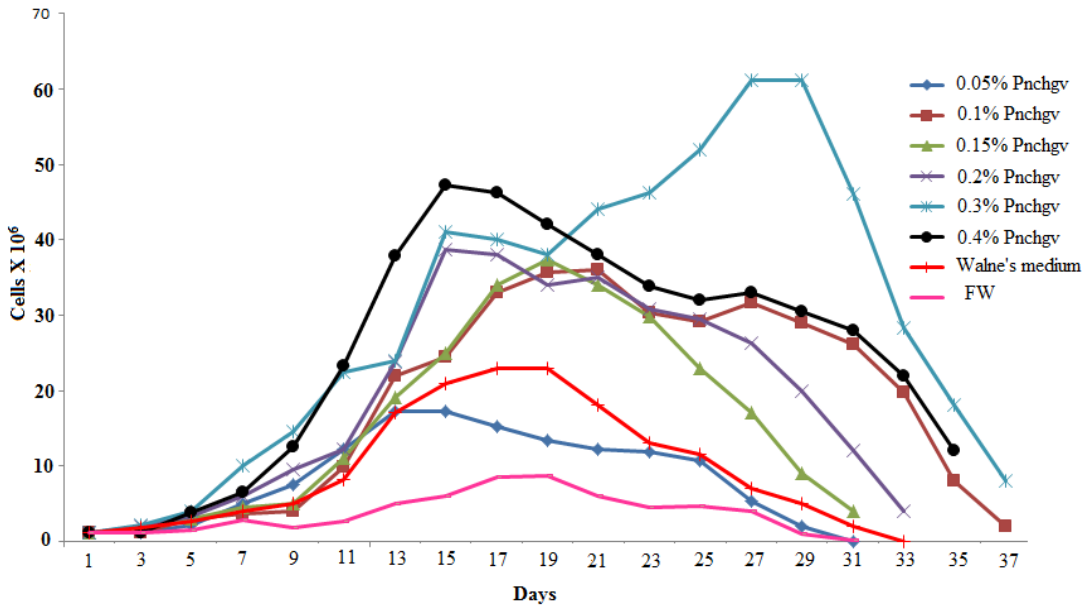
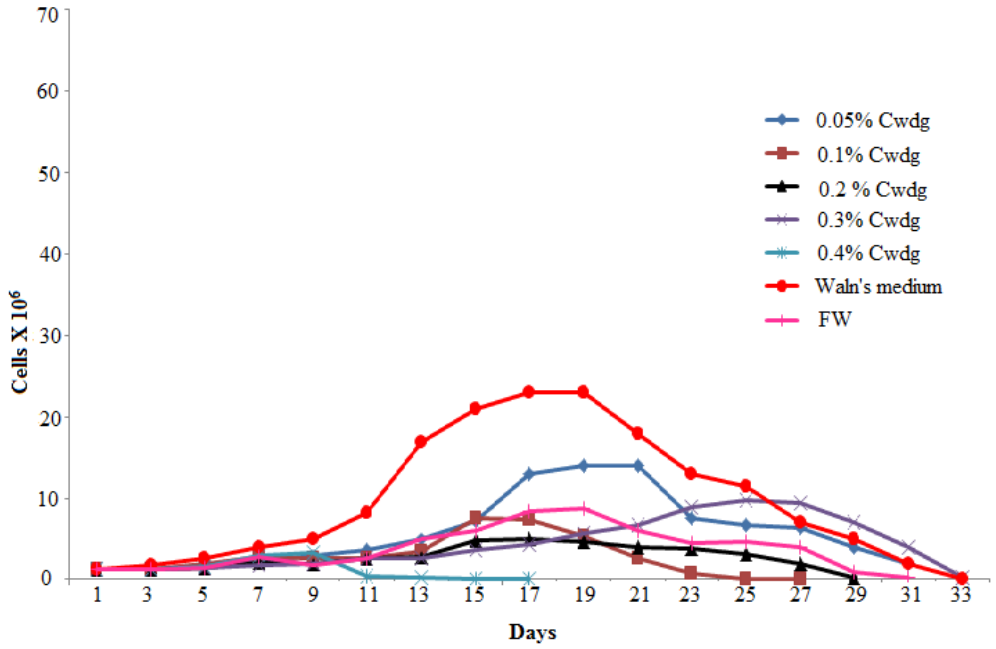


Fig. 2. Growth of *Chlorella vulgaris* (cells/ml) in Cow dung medium at different concentration to that of standard Walne's medium and fresh water



Tetraselmis gracilis

A maximum standing crop of 49X10^6 cells/ml was observed on 19th day in 0.2% concentration of Panchagavya. This concentration is 4.9 times higher than that of culture in seawater without nutrient supply (fig. 3) and is 70 times higher to inoculum). Other Panchagavya concentrations such as 0.15%, 0.3% and 0.4% also showed higher growth rate than Walne's medium supporting the growth promoting character. When cow dung concentrations of cow dung solutions were used growth patterns in 0.4% were somewhat similar to that of Walne's medium (fig.4). This result proves that cow dung solution does not have growth promoting properties as Panchagavya.

Fig.3 Growth of *Tetraselmis gracilis* (cells/ml) in Panchagavya medium at different concentration to that of standard Walne's medium and marine water

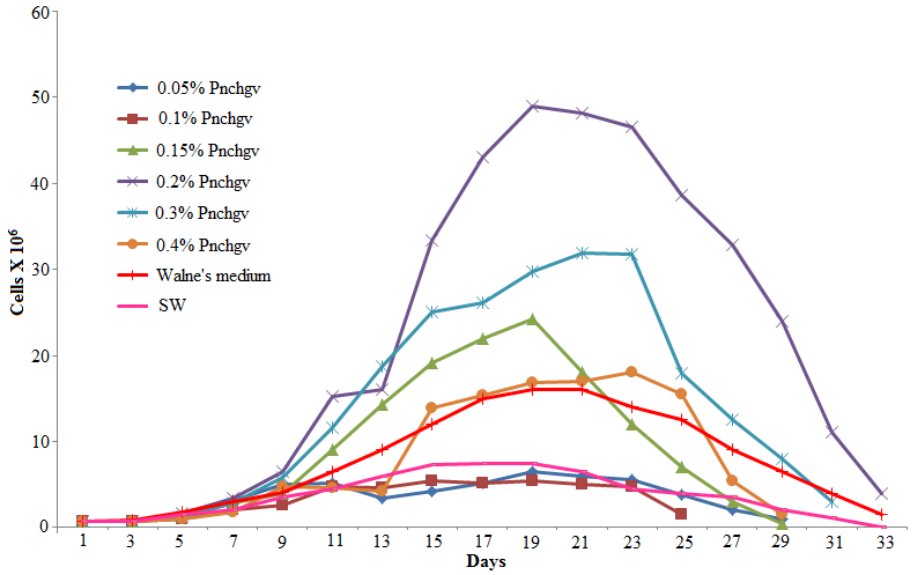
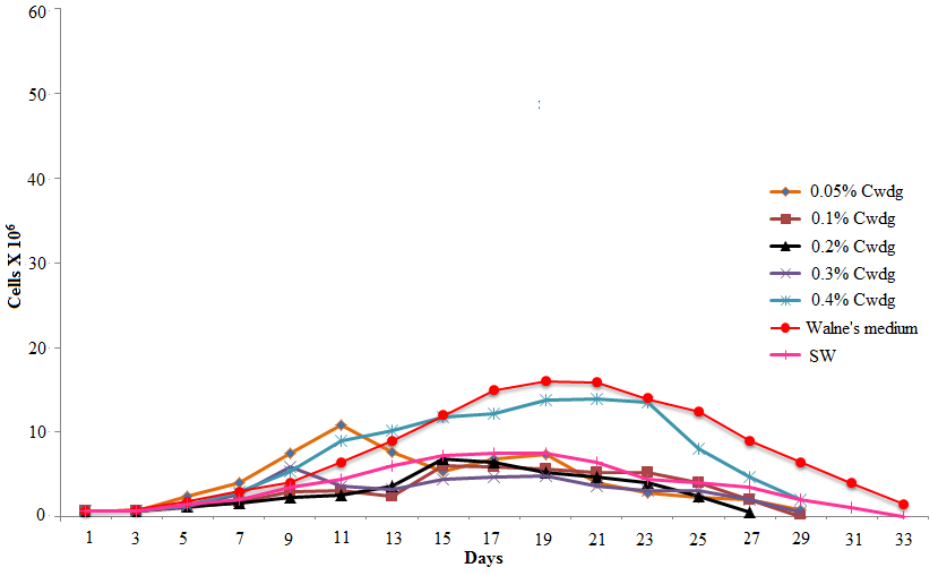


Fig.4 Growth of *Tetraselmis gracilis* in Cow dung medium at different concentration to that of standard Walne's medium and marine water



Dunaliella salina

Growth curve of *Dunaliella salina* reached its peak in 0.15% concentration of Panchagavya on 21st day of inoculation with 52X10^6 cells /ml (fig.5), while maximum standing crop in Walne's medium is 14X10^{6}cells/ml on 7th day. Other concentrations Panchagavya enhanced growth initially and growth rate of culture in Walne's medium is high on third day of experiment with early exponential phase. But in Panchagavya medium with with 0.15% concentration, the standing crop is 4 times higher than that of in Walne's medium and 5.3 times than culture grown in sterile sea water without nutrient supplementation.

Synechocystis salina

Maximum growth (50X10^6 cells/ml) observed on 27th day of incubation in 0.15% concentration of Panchagavya. This high standing crop is about 4.9 times higher than that of maximum growth in Walne's medium on 19th day and 7.6 times higher than in sterilized seawater without the supplement of nutrients (fig.6). Lower concentrations of Panchagavya also found to be enhancing growth while higher concentrations are showing an adverse effect. Study was also conducted in still higher concentrations of Panchagavya media and it was found to be lethal showing algicidal effect. Initial growth rate never showed much variation in all concentrations and variations started only after 6th day of inoculation. Compare to other species studied *Synechocystis salina* had a prolonged stationary phase for about 5days..

Fig.5 Growth of *Dunaliella salina* (cells/ml) in Panchagavya medium at different concentration to that of standard Walne's medium and marine water

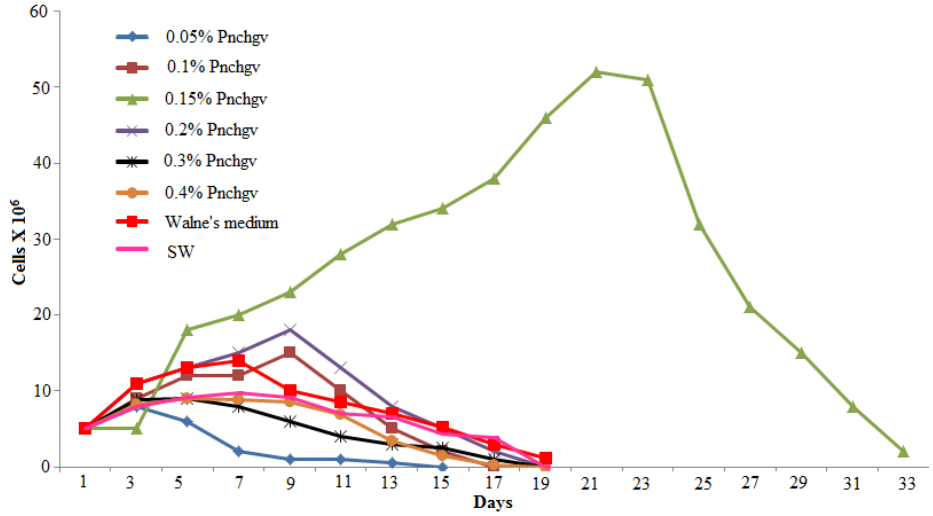
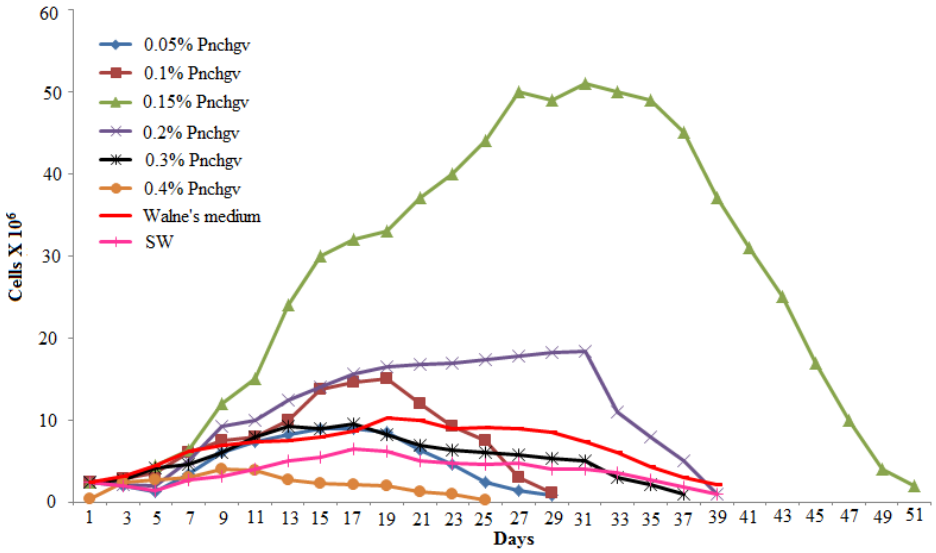


Fig.6 Growth of *Synechocystis salina* (cells/ml) in Panchagavya medium at different concentration to that of standard Walne's medium and marine water



The recommended concentrations of modified Panchagavya in the agriculture field vary from 2 to 3% for various crop varieties (Natarajan, 20010. Therefore, initially 0.5 to 4 percentage solutions were prepared and used as medium for algal culture. But no algal growth was observed in these concentrations and it was algicidal. Hence this concentration can be used against red rust diseases caused by green algae *Cephaleuros* sp. in tea and gua. Potentiality of Panchagavya as insecticide (Barati, 2005) and antifungal agent (Baby Joseph and Sankarganesh, 2011) were already proved but no report is available regarding its effect against algal red rust disease. As higher concentrations are lethal to microalgae, the present studies growth studies were carried out lower concentrations from 0.05 to 0.4%. Results showed that lower concentration of Panchagavya medium are the best natural organic media giving higher standing crop than Walne's medium or any other media tried. Optimum concentrations are found to be species specific similar to growth of microalgae in compost extract medium as observed by de Oliveira and Crareispim (2013). The maximum growth of Dunaliella *salina* was reported only 1.3 times higher in compost extract medium than of Conway medium but in Panchagavya concentration it is 4 times higher than Walne's Medium (fig. 6) indicating Panchagavya is the best low cost medium. Though cow dung is one of the ingredients of Panchagavya, it alone as a medium has much effect on the growth of studied microalgae.

Acknowledgement

Authors are thankful to Dr. Jayachandran, Principal, Mahatma Gandhi Govt. Arts College, Mahe, U.T of Puducherry for his encouragement. Authors are also express sincere gratitude to Dr. A.V. Saramma and Dr. K.J. Joseph (Prof. Rtd.), Department of Marine Botany, School of Marine Science, Cochin University of Science and Technology, Kochi for their support by providing algal cultures.

Reference

Balasubrahmanyan, A.V, T.D Nirmaladevi and M. Merlin Franco, 2009 *Use of Animal Products in Traditional Agriculture*, (Centre for Indian Knowledge systems, Chennai, pp146

Bharati, S.M 2005 *Role of Organics and indigenous components against Spodoptera litura (Fab.) in groundnut and Soybean*, pp66 MSc (Agriculture) Thesis, University of Agriculture Sciences, Dharwad, India.

Bindumathi Mohan, 2008 Evaluation of organic growth promoters on yield of dryland vegetable crops in India. *Journal of Organic Systems*, 3 (1): 23-36.

Chisti Yusuf, 2007 Biodiesel from microalgae beats bioethanol *Trends in Biotechnology* 26 (3): 126-131

de Oliveira Flavia Martins Franco and Crispim Maria Cristina Basillo, 2013 Compost extract as a nutrient source for algal cultures *J. Aquac Res Development* 4 (5):

Kim M K, J W Park, C.S Park, S.J. Kim, K.H Jeune, M.U Chang and J. Acreman, 2007 Enhanced production of Scenedesmus spp. (green microalgae) using a new medium containing fermented swine waste water. *Bioresource Technology* (Science Direct- Elsevier) 98: 2220-2228.

Natarajan, K, 2001 *Panchagavya, a versatile potentiator for living beings* Google web

Shweta Jain and Samuel G Singh, 2013 Low cost medium formulation using cow dung ash for the cultivation of Cyanobacterium: *Spirulina* (*Arthrospira*) *platensis*, *J. Food Agric* 25(9): 682-691

Vishwanath Patil, Khanh-Quang Tran and Hans Ragnar Giselrod 2008 Towards sustainable production of biofuels from microalgae Review, *Int. J. Mol. Sci*. 9: 1188-1195.

Walne PR 1970 Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera ***Ostrea, Crassostrea, Mercenaria*** and ***Mytilis***. *Fish Invest*. 26: 1-62