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Research Article
Utilization of Vermiwash to Promote Growth Rate and Biomass in Fresh Water Microalgae

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Abstract

Background and Objective: Inorganic fertilizers are used to cultivate microalgae causing adverse environmental effects and increase the cost of microalgal cultivation. The objective of this study was to find a cheap, nutrient rich alternate to cultivate freshwater microalgae. **Materials and Methods:** Vermiwash at a concentrations of 25, 50, 75 and 100% were used to cultivate microalgae isolated from fresh water habitats. Specific growth rate and biomass concentrations were determined to evaluate the effect of vermiwash medium in comparison with Bold's basal medium. **Results:** A total of 13 microalgae that belonged to the family Cyanophyceae and Chlorophyceae were isolated. Growth rate and biomass of microalgae were increased with higher concentrations when the vermiwash was used at a concentration of 25, 50 and 75%. At the same time, both the parameters were declined in undiluted vermiwash indicating the algal growth inhibition under high levels of nutrients. **Conclusion:** Utilization of vermiwash is feasible to cultivate fresh water microalgae thereby fulfilling the nutrient requirements and reducing the use of inorganic fertilizers.

Key words: Vermiwash, microalgae, biomass, growth rate, biofuel, inorganic fertilizers, Bold's basal medium

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Microalgae are good source of carotenoids[1,2], long-chain polyunsaturated essential fatty acids[3], bioactive compounds[4,5] and biofuels[6,7]. Microalgae cultivation requires large amount of fertilizers causing high cost during production of value added products. One example is the production cost of biofuel from microalgae is too high to compete with fossil fuel prices. Further, the use of fertilizers for microalgae cultivation has negative impact on the environment[8,9]. To achieve economically feasible products from microalgae, it is necessary to choose a cheap source such as waste water and agro-industrial wastes. Alternatively, leachate derived from vermicompost can be used to cultivate microalgae.

Vermiwash, a leachate from vermicomposting beds is a rich source of nutrients, enzymes, plant growth hormones and vitamins[10]. Both macro and micronutrients are present in the vermiwash as a result of composting of organic matter by earthworms. The nutrients present in vermiwash are easily available to plants[11]. Since, vermiwash contains high concentrations of nutrients especially ammonia, urea, nitrates and phosphates[12], it can be a good source to promote the growth of microalgae. While many studies have shown that microalgae can be cultivated in various wastewaters, the cultivation of microalgae in nutrient rich vermiwash has received less attention. In this study, the viability of vermiwash was evaluated as a substrate under different dilutions to grow fresh water microalgae. The positive effect of vermiwash on microalgae was investigated in terms of promoting growth rate and biomass.

MATERIALS AND METHODS

This study was carried out at Department of Biotechnology, Indian Academy Degree College-Autonomous, Bangalore between August 2018 and February 2019.

Production of vermicompost and vermiwash: Plastic containers with a hole at the base were used to produce vermicompost. The base layer was filled with gravel and above it with sand. Pre-decomposed agro-industrial wastes were used as substrate and cow manure and farm yard manure was mixed in the ratio of 1:1 by weight. *Eisenia foetida* procured from University of Agricultural Sciences, Bangalore was introduced to the beds and the experiment was conducted in a greenhouse. Vermiwash was collected after 45 days for a period of 15 days. Available forms of nitrogen, phosphorus and other measures of vermiwash were performed using standard methods[13].

Microalgae: Microalgae used in this study were isolated from fresh water lakes in and around Bangalore. Water samples that appeared to contain algal growth were inoculated into a 100 mL of autoclaved Bold's basal medium for isolation and incubated under continuous illumination with white fluorescent light for 10 days. Serial dilutions from the isolation broth were done in Bold's basal agar medium and the plates were incubated at 25°C under continuous illumination for 2 weeks. Microscopic observations were performed for identification of the isolates[14].

Cultivation conditions: The physicochemical characteristics of vermiwash used in the study are shown in Table 1. A total of 13 microalgae that belonged to the family Cyanophyceae and Chlorophyceae were isolated from the fresh water habitats (Table 2).

The preliminary test involving plate count method indicated high bacterial populations in the vermiwash hence it was autoclaved before use. Dilutions of vermiwash were prepared in distilled water and the autoclaved vermiwash medium was used to cultivate the fresh water microalgal isolates for a period of 7 days. Bold's basal medium was served as control to determine the influence of vermiwash on

Table 1: Physico-chemical properties of the vermiwash

Parameters	Values
pH	7.24
Electrical conductivity (dS m^{-1})	1.04
Organic carbon (%)	8.21
Available nitrogen (mg L^{-1})	427.50
Available phosphorus (mg L^{-1})	548.20
Available potassium (mg L^{-1})	938.25
Total organic carbon (%)	1.06

Table 2: Isolated microalgae species in this study

Microalgae	Family	Class
Botryococcus	Botryococcaceae	Chlorophyceae
Chlamydomonas	Chlamydomonadaceae	Chlorophyceae
Chlorella	Chlorellaceae	Chlorophyceae
Chlorococcopsis	Chlorococcaceae	Chlorophyceae
Chlorococcum	Chlorococcaceae	Cyanophyceae
Chroococcus	Chroococcaceae	Cyanophyceae
Closterium	Desmidiaceae	Chlorophyceae
Nostoc	Nostacaceae	Cyanophyceae
Oscillatoria	Oscillatoriaceae	Cyanophyceae
Pandorina	Volvocaceae	Chlorophyceae
Phormidium	Phormidiaceae	Cyanophyceae
Scenedesmus	Scenedesmaceae	Chlorophyceae
Spirogyra	Zygnemataceae	Chlorophyceae

the growth rate and biomass of microalgae. The vermiwash at a concentration of 25, 50, 75 and 100% was used in the batch experiments. For dilutions, autoclaved water was used. Before beginning the experiments, pH of the diluted vermiwash was adjusted to neutral pH. The isolated microalgae were inoculated into experimental flasks containing 100 mL of medium and incubated under 12:12 light/dark cycle for 7 days.

Growth rate and biomass: The specific growth rate of the microalgae was calculated using the equation:

$$\mu = \ln \frac{(N_2/N_1)}{(t_2\text{-}t_1)}$$

where, μ is the specific growth rate and N_1 and N_2 are the biomass at initial day (t_1) and final day (t_2), respectively.

Algal growth was monitored by measuring the optical density at 680 nm with a spectrophotometer. Dry weight of microalgae was measured by harvesting the cells by centrifugation and washing with deionized water. The pellet was dried at 105°C for dry weight measurement. All the experiments were carried out in triplicate and data are expressed as mean±SD.

RESULTS

Influence of vermiwash on microalgal growth rate: Growth rate of microalgae was increased with higher concentrations of vermiwash. However at 100% vermiwash concentration (VWM100), most of the microalgal growth rate entered decline phase (Fig. 1). Highest growth rate of 0.76 μ/day was recorded in *Oscillatoria* grown in Bold's basal medium whereas it was 0.71 μ/day in 75% vermiwash medium. The well known biofuel species such as *Chlorella* and *Scenedesmus* have exhibited almost similar growth rates in control and 75% vermiwash medium (VWM75). In most cases, vermiwash medium at a concentration of 75% resulted in similar growth rate as that of Bold's basal medium (control). Vermiwash medium at a concentration of 25 and 100% (VWM25 and VWM100) had promoted the growth of microalgae however it was comparatively lower than VWM75 and Bold's basal medium (BBM).

Influence of vermiwash on microalgal biomass: In most microalgae, maximum biomass was obtained in Bold's basal medium (control) and the second highest was found with VWM75 indicated the optimum concentration. For *Chlorococcum*, *Pandorina* and *Phormidium*, 50% concentration of vermiwash (VWM50) had resulted in highest biomass concentration. In general, the biomass was increased with higher concentrations of vermiwash. Among the isolates, *Scenedesmus* has produced 1.97 g/dry weight L^{-1} (g dw L^{-1}) and 1.8 g dw L^{-1} of biomass in BBM and VWM75, respectively (Fig. 2).

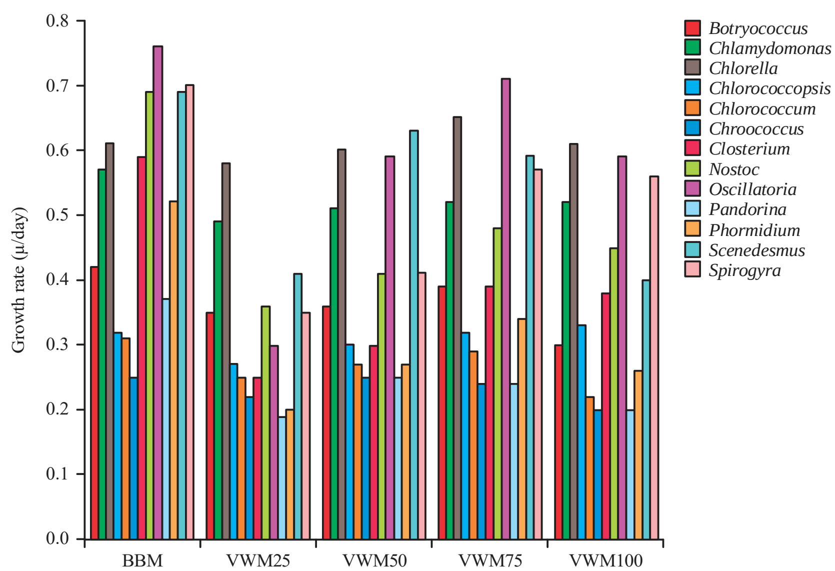

Fig. 1: Comparison of specific growth rate of microalgae cultivated in Bold's Basal Medium (BBM) and Vermiwash medium (VWM)

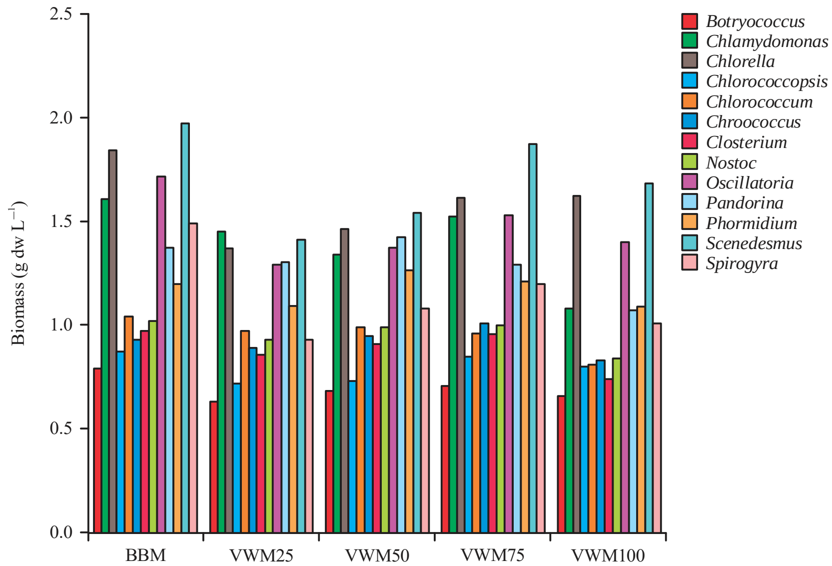

Fig. 2: Comparison of microalgal biomass (g dw L^{-1}) cultivated in Bold's Basal Medium (BBM) and Vermiwash medium (VWM)

DISCUSSION

The findings of the study revealed the positive growth promoting effects of vermiwash in terms of growth rate and biomass in freshwater microalgae. Comparison of growth promotion with synthetic medium (Bold's basal medium) indicated that vermiwash could be used as an alternate for inorganic fertilizers while cultivating microalgae. Since vermicompost contains high concentrations of nitrates and phosphates, it is an excellent way to promote the growth of microalgae. Utilizing vermicompost and vermiwash to improve crop productivity was reported elsewhere[15-20] but the use of vermiwash to cultivate microalgae is demonstrated for the first time in this work. Results of this study indicated that the role of vermiwash as growth media for microalgae can be no less than commercial synthetic media.

Higher growth yield of *Chlorella pyrenoidosa* and *Scenedesmus quadricauda* on refuse compost extract was reported earlier[21]. In their study, dry weight of 0.387 and 0.992 mg mL^{-1} was produced by *C. pyrenoidosa* and *S. quadricauda*, respectively. In another study[22], *Selenastrum* was cultivated in composting plant leachate where highest fatty acid content was obtained in 10% biowaste press leachate. Similarly, biowaste leachate was used to cultivate *Euglene gracilis*, *Selenastrum* sp. and *Chlorella sorokiniana* for growth and lipid production[23]. In a study by Tan *et al.*[24], chicken, goat and plant compost water was used to cultivate *Chlorella vulgaris* and the highest specific growth rate of 0.1628 μ/day was obtained in chicken compost medium.

High concentrations of nitrogen in the form of ammonia or ammonium can inhibit growth of algae[25]. In this study, the drop in both growth rate and biomass of microalgae at higher concentrations of vermiwash is likely caused by an algal growth inhibition under high levels of nutrients.

CONCLUSION

The result in this paper indicated that it is feasible to utilize vermiwash to cultivate fresh water microalgae for value added products such as nutraceuticals, biomass and lipid. Vermiwash at a concentration of 75% was found optimum for higher growth rate and biomass concentration in microalgae.

SIGNIFICANCE STATEMENT

This study explored the use of vermiwash as alternate to inorganic medium for cultivating freshwater microalgae. Both the growth rate and biomass were promoted which was in accordance to the results obtained in inorganic medium. The scope of future research involves cultivation of economically important microalgae using vermiwash medium to fulfill the nutrient requirements and reduce the use of inorganic fertilizers.

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