BIOMASS PRODUCTION AND TREATMENT OF KITCHEN WASTE WATER WITH *NOSTOC MUSCORUM*-A POTENTIAL BIOFERTILIZER

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Kitchen waste water emerging from Tirumala hills, a world famous pilgrim centre was used as a source of nutrients for cultivation of *Nostoc muscorum*. A complete randomized design (CRD) was created for the experiment which was performed on BG-11 medium, 100% kitchen waste water (100% KW) and 75% kitchen waste water (75% KW). The physicochemical properties of kitchen waste water and the yield of biomass were determined from the cultures harvested every three days for a period of 15 days. After cultivation the kitchen waste water met the standards of safe discharge in India. The organisms could able to remove maximum of 98% of contaminants during its growth in waste water. The highest production of biomass was achieved in BG-11 medium followed by 100% KW and 75% KW.

Key words: Kitchen waste water, nutritional value, physicochemical properties, *Nostoc muscorum*, biomass.

1. INTRODUCTION

Treatment of waste water to remove unwanted nutrients using microorganisms has been widely applied throughout the world. Being the base for all food chains and primary production, aquatic phytoplankton play a key and indispensable role in cycling of elements and pollutants in any ecosystem. Cyanobacteria have a great deal of potential as a source of biofertilizer and biofuel¹⁾. Recently, there has been increasing interest about using cyanobacteria as pollution control agents since they possess many advantages over the microorganisms isolated from soil. Some of the important advantages of using blue green alga such as *Nostoc muscorum* include relative safety of the microorganism, the efficient removal of the nitrogen and phosphorous nutrients, rapid growth rate and the ability to utilize the resulting organism as a biofertilizer for fixing atmospheric nitrogen. Tirumala hill region is located

3200 ft above the sea level in Chittoor District of Andhra Pradesh, India. It is visited by an average of one hundred thousand devotees per day. The process of cooking for such a large number of people in one place generates enormous amount of waste water from kitchen. Kitchen waste water has received relatively little attention although it can make a significant contribution to the polluted stream especially from dining facilities in large institutions and organisations. It is rich in nutrients like nitrogen, phosphorous, fats, proteins, carbohydrates etc²⁾. Culturing of *Nostoc muscorum* as a source of biofertilizer using inorganic culture media is relatively expensive because of the need to provide a full complement of nutrients. Low cost alternative such as waste water evaluated as a more cost effective method of producing biofertilizer. In addition cultivation of *Nostoc muscorum* improves water quality by reducing the waste water nutrient levels. Cultivation and treatment of sago starch factory waste water by *Spirulina*³⁾, pig waste water

by *Scenedesmus obliquus*⁴⁾, fish farm effluent by *Phormidium bohneri*⁵⁾ high strength organic waste water by *Rhodobacter sphaeroides* and *Chlorella sorokiniana*⁶⁾, poultry slaughter house waste water by *Rhodopseudomonas gelatinosus*⁷⁾ is well documented. The present study is aimed to compare the biomass production of *Nostoc muscorum* at 100% and 75% dilutions of kitchen waste water and to know the percent of nutrients removed in both the dilutions after harvesting the biomass.

2. MATERIALS AND METHODS

Twenty five liters of kitchen waste water emerging on Tirumala hills from food and dish washing was collected. This waste water was placed in 100 L cement tanks and allowed to ferment for 2 weeks at 32°C±1°C under aerobic conditions with periodic stirring for microorganisms to breakdown the solid organic wastes. The liquid portion was filtered through 80 micron net filter after complete breakdown of solid particles. The filtrate was taken for determination of temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), ammonia nitrogen (NH₃-N), nitrate nitrogen (NO₃-N), nitrite nitrogen (NO₂-N), total kjeldhal nitrogen (TKN) as per the methods described in "Standard methods for the examination of water and wastewater American Public Health Association"⁸⁾. Total phosphorous (TP) content was determined by persulfate digestion method⁹⁾. The solid phase was used as a source of nutrients for growth of tropical medicinal plants (data yet to be published). This filtrate or 100% solution (100% KW) was diluted to 75% with tap water to compare the growth of *N.muscorum* at lower dilutions of kitchen waste water. Greater dilutions were found to produce significantly lower growth rates, so only 100% and 75% solutions were used in this study.

Cultures of *Nostoc muscorum* were obtained from Biotechnology Research Center, Tirupati, A.P, India. The cyanobacteria are mostly associated with bacteria that live symbiotic relations, since bacterial contamination in the miscellaneous sheath of cyanobacteria can affect the results leading to artifacts. During the present study, the selected species was provided as axenic culture. However, before using the organism for growth and bioremediation its axenicity was checked using agar phototactic response method¹⁰⁾. The organism was cultured in standard BG-11 broth as outlined by¹¹⁾ in a 2 liter bottle and allowed to grow for 10 days until the optical density at 560 nm reached 1 (OD_{560nm} = 1).

Optical density of cyanobacterial cell suspension is directly related to its cell mass or number and the growth

is measured by turbidity (spectrophotometrically at 560 nm). The BG-11 medium composed of g/L (NaNO₃ - 1.5, K₂HPO₄ - 0.04, MgSO₄.7H₂O - 0.075, CaCl₂.2H₂O - 0.036, Citric acid - 0.006, Ferric ammonium citrate - 0.006, EDTA (disodium salt) - 0.001, Na₂CO₃ - 0.02, agar - 10.0), distilled water - 1.0 L, trace metal mix A5 - 1.0 mL; (composition of trace metal mix A5 g/L -H₃BO₃-2.86, MnCl₂.4H₂O-1.81, ZnSO₄.7H₂O - 0.222, NaMoO₄.5H₂O-0.39, CuSO₄.5H₂O-0.079, Co(NO₃)₂. 6H₂O-0.04, distilled water-1.0 L). The pH of the medium was adjusted to 7.0 using 1N NaOH. *N.muscorum* from BG-11 medium was subcultured in fermented 100% kitchen waste water in 1 L flask and allowed to grow for 2 weeks until the OD at 560nm reached 1 (OD_{560nm} > 1). This stock was then used as *N.muscorum* inoculum for the experiments in 25L cement tanks at 30°C under 2500 lux of illumination in a 12 h photoperiod. A complete randomized design (CRD) was carried out using 3 treatments, each performed in triplicates.

Initial physico-chemical properties of the waste water were measured before inoculating with *N.muscorum*. The control tanks consisted of the 25 L of BG-11 medium. The treatments were 100% kitchen waste water and 75% kitchen waste water+25% tap water inoculated with *N.muscorum* culture. To avoid the contamination 10 µg/ml neomycin is supplemented before inoculating *N.muscorum*. The initial OD of each tank was 0.30. The experimental and control flasks were cultured for 15 days with continuous aeration at 32°C±1°C. Samples were collected for every 3 days and analyzed for their physicochemical properties viz. temperature, pH, DO, BOD, COD, NH₃-N, NO₃-N, NO₂-N, TP and TKN. As the values of waste water met the standards of safe discharge on 15th day of experiment the experiment was terminated. The biomass of the *N.muscorum* was separated by filtration using 120 µm plankton net. The weight was determined using a monopan electronic balance. The values obtained were subjected to statistical analysis.

3. RESULTS AND DISCUSSION

After completing the 15 days of experimental period there was a significant change in the physical and chemical properties of the waste water. The percent removal of the nutrients from waste water ranged between 50-98 in different treatments being minimum in BG-11 medium and maximum in 75% kitchen waste water medium. All media showed an increase in pH during the experimental period, consistent with usual behavior of blue green algal cultures. A large increase in DO occurred in both waste water media (100% KW and 75% KW) as a result of aeration.

Aeration is one of the most elemental technique frequently employed in the improvement of physical and chemical characteristics of water. Aeration improves the quality of water by supplying deficient oxygen, rescuing the free carbon dioxide (CO_2) and eliminating much of hydrogen disulphide (H_2S) and other odours from the waste water. Removal of H_2S would facilitate the optimal growth of cyanobacterial which used H_2O instead of H_2S as a source of electrons and hydrogen for fixing CO_2 (www.ucmp.berkeley.edu). The optimum level of DO for algal culture in water is normally more than 5 mg /liter¹²⁾. The final DO in waste water after cultivating the *N.muscorum* was (6.12 to 6.80 mg/liter) (Fig. 1).

Fig. 1 Changes in dissolved oxygen

The initial level in the BG-11 medium was high since it was prepared using tap water containing considerable amount of DO, 75% waste water medium contained 25% tap water resulting in slightly elevated DO level compared to 100% waste water medium. For 100% and 75% waste water, the BOD decreased by 71.9% and 68.6% respectively (Fig. 2).

Fig. 2 Changes in biological oxygen demand

The chemical composition of BG-11 medium resulted in low BOD initially since it contained no significant amount of bio-degradable organic compounds such as protein, carbohydrate or fat. The present study showed a larger decrease in BOD during *N.muscorum* cultivation using kitchen waste water than the earlier reports by Chuntapa et al¹³⁾ with 100% waste water. The COD values decreased dramatically (73.1% and 73.6%) (Fig. 3).

Fig. 3 Changes in chemical oxygen demand

The present findings on COD are in consistence with the earlier findings of Promya and Traichaiyaporn¹⁴⁾. As the waste water was collected directly from the kitchen outlet it exhibited low BOD and COD values compared to the values of stagnated waste water. To reduce the concentrations of phosphorous and nitrogen contaminants, the water was further diluted.

The total 'P' levels in all three media (BG-11, 100% and 75% waste water medium) were initially high 4.7 - 5.5 mg/L and decreased nearly by 50.1, 51.1% and 51.0 respectively (Fig. 4).

Fig. 4 Changes in total phosphorus

After cultivation of *N.muscorum* the 100% KW and 75% KW met the standards of safe discharge for BOD, COD, TP (< 20 mg/L; <120mg/L; <2.4 mg/L respectively). There were significant levels of $\text{NH}_3\text{-N}$ initially in 100% and 75% KW (Fig. 5). Algae prefer to use $\text{NH}_3\text{-N}$ than $\text{NO}_3\text{-N}$ and the optimal concentration range of nitrogen for algal growth is 1.3-6.5 mg/L¹⁵⁾. After 15 days of cultivation the $\text{NH}_3\text{-N}$ levels dropped dramatically by 94.4% and 92.8% for 100% KW and 75% KW respectively to those with in the allowable discharge limit for $\text{NH}_3\text{-N}$ (<1.4 mg/L) as per Indian Council of Medical Research. These are similar to those in other studies where 54% reduction was achieved¹⁶⁾.

Fig. 5 Changes in ammonia nitrogen

The initial level of $\text{NO}_3\text{-N}$ in BG-11 was high as it was the sole source of nitrogen. The 100% KW and 75% KW contained 4.10 and 3.72 mg/L respectively (Fig. 6) which reduced by 98% in both by cultivation of *N.muscorum* to meet the standards of safe discharge (<0.7 mg/L).

Fig. 6 Changes in nitrate nitrogen

In the present study $\text{NO}_2\text{-N}$ in all media was in the limits of 0.003 to 0.01 mg/L hence it was not considered as identifiable source of nutrient. Total kjeldhal nitrogen is the sum of organic nitrogen and ammonia nitrogen. Kitchen waste water contained significant amount of both organic and ammonia nitrogen so the initial total kjeldhal nitrogen was high (21.5 and 20.0 mg/L for 100% and 75% waste water medium respectively) (Fig. 7). Culturing of *N.muscorum* in kitchen waste water resulted in 95% decrease in TKN for both 100% and 75% KW and the resulting water was improved to within the discharge limit of <50 mg/L.

Fig. 7 Changes in total kjeldhal nitrogen

In the present study the tap water is used as a source for dilution of 100% waste water to 75%. The

amount of P and N present in the tap water is the reason for not obtaining three fourths of the values in 75% waste water after dilution.

The 100% waste water medium was nearly as effective as BG-11 medium in biomass production (0.83 and 0.85 g/L respectively) (Fig. 8).

Fig. 8 Production of biomass

The biomass production in the present study is more compared to the earlier reports of 0.50 g/L¹⁷. The waste water treatment can be achieved by biological, physical and chemical processes. A physical process usually treats suspended rather than dissolved pollutants. Chemical treatment is often used for removal of nitrogen and phosphorous pollutants by precipitation. Compared to engineering based technological approaches, the phytoremediation technique currently being employed effectively. Phytoremediation ensures quality with low nutrient content in treated waste water. In contrast to physical and chemical process phytoremediation produces smaller sludge and occurs at ambient temperature. It is of high sustainability, require low input in energy and manpower, and offer possibilities of carbon sequestration in biomass as well as the recycling of materials and matter¹⁸.

In the present study an attempt had been made to compare the biomass production of *N.muscorum* at 100% and 75% dilutions of kitchen waste water and to know the percent of nutrients removed in two different dilutions after harvesting the biomass. Improvement in water quality is assessed by the ability of a process to reduce nutrient matter especially nitrogen and phosphorous. In the present study culturing of *N.muscorum* in waste water with high nutrient matter dramatically reduced nitrogen and phosphorous levels to ensure the safe discharge besides the production of useful product such as nitrogen fixing blue green algae as a source of biofertilizer.

4. CONCLUSION

Results of the present study proved that waste water obtained from pilgrim centre can form a

best nutritional media for large scale cultivation of nitrogen fixing cyanobacteria with low cost. The technique can be exploited for commercial production of biofertilisers which inturn is useful in treatment of domestic waste water. The biotechnological importance and advantage of using *N.muscorum* for waste water treatment was promising in removal of nitrogen and phosphorous nutrients from the waste water to the extent to meet the safe discharge limits in India.

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