Comparative Studies on Growth and Remediation of Waste Water by Two Cyanobacterial Biofertilizers

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Summary

Nitrogen fixing cyanobacteria are ecologically significant inputs in improving the plant productivity in tropical countries like India. Large scale cultivation of these organisms using inorganic media is relatively expensive. In the present study utilization of kitchen waste water emerged from a pilgrim centre as a source of nutrients and its remediation was compared using two blue green algal cultures viz. *Anabeana variabilis* and *Nostoc muscorum*. A complete randomized design was created for the experiment that was performed on BG-11, 100% and 75% KW (Kitchen Water) media. The physicochemical properties of waste water were analyzed before and after cultivation. It was found that the *N. muscorum* was more effective in removal of phosphorous and nitrogen contaminants from waste water to meet the standards of safe discharge besides producing more biomass compared to *A. variabilis*.

Key words

N. muscorum, *A. variabilis*, kitchen waste water, biomass, remediation

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Introduction

With the growing realization that chemical based agriculture is unsuitable and is slowly leading to ecological imbalance, the latter part of the last century witnessed the emergence of the concept of "organic agriculture" advocating minimum use of chemical fertilizers and increasing dependence on biological inputs like compost, farmyard manure, green manure and biofertilizers. Such inputs are sustainable, effective, cheap, easily manageable, fuel energy independent, ecofriendly and improve the nutrient status and health of the soil. Among the array of biofertilizers developed nitrogen fixing blue green algae constitutes the most important inputs for nutrients management in plants. Culturing of blue green algae using inorganic media is relatively expensive because of the need to provide full complement of nutrients. Low cost alternative such as waste water should be evaluated as more cost effective method of cultivating these organisms.

Tirumala, the worlds, richest pilgrim centre is located 3200 feet above the sea level in Chittoor district of Andhra Pradesh in India. The process of cooking on large scale on the hill region generates enormous amounts of waste water, which is a potential source of nutrients for cultivation of useful microorganisms (Ponsano et al., 2003). Remediation of waste water by nonsulphur bacteria (Izu et al., 2001), *Thiobacillus* Sp (Kantachote and Innuwat, 2004) and *Spirulina* sp (Canizares, 1993; Chuntapa et al., 2003) is well documented. The potentiality of *N. muscorum* and *A. variabilis* as biofertilizers has been reported by Subba Rao (2002). The enhanced growth of agricultural crops by the application of *Anabaena variabilis* is reported by Rodrigues (2005). The scope and potential use of *Nostoc muscorum* as nitrogen fixer in soils with low nitrogen status is reported by Sarada (2007) and Radha Khale (1999).

In the present study an attempt has been made to explore the possibility of cultivation of N_2 fixing blue green algal fertilizers and to evolve the potential of *A. variabilis* and *N. muscorum* to use waste water as a growth medium.

Materials and methods

Fifty liter of waste water emerged from food and dish washing was collected from kitchen outlet on Tirumala hill. This water was placed in 50 l cement tank and allowed to ferment for two weeks for microorganisms to breakdown the solid organic wastes. The liquid portion in each tank was then filtered through a 50 microplankton net filter. The filtrate was taken for determination of temperature, pH, dissolved oxygen (DO), BOD (azide modification), COD (closed reflux), NH_3-N (nesserlerization), total nitrogen (TN) by the macrokjeldahl method and total phosphorous (TP) by persulfate digestion/ stannous chloride (APHA 1998). This filtrate or 100% kitchen waste water was diluted to 75% with tap water to make an additional starting solution. Greater dilutions were found to produce significantly lower growth rates, so only 100% and 75% solutions were used as nutrient media in the present study.

Culture of *A. variabilis* and *N. muscorum* were obtained from Biotechnology Research Centre, Tirupati, A.P., India. They were cultured separately in BG-11 broth in a 2 l bottle and allowed to grow for 10 days until the optical density at 560 nm reached to one ($OD_{560} = 1$). The BG-11 medium composed of g/l: $NaNO_3$ - 1.5; K_2HPO_4 - 0.04; $MgSO_4$. $7H_2O$ - 0.075; $CaCl_2$. $2H_2O$ - 0.036; citicacid - 0.06; ferric ammonium citrate - 0.06; EDTA Na salt - 0.001; Na_2CO_3 - 0.02; and trace metal mix A_5-10 ml (composition of trace metal mix: g/l – H_3BO_3 - 2.86; $MnCl_2$. $4H_2O$ - 1.81; $ZnSO_4$. $7H_2O$ - 0.222; $NaMoO_4$. $5H_2O$ - 0.39; $CuSO_4$. 5 H_2O - 0.079; Co $(NO_3)_2$. $6H_2O$ - 0.04). The pH of the medium is adjusted to 7.0 using 1 N NaOH. *A. variabilis* and *N. muscorum* from BG-11 medium were subclutured in fermented 100% KW and allowed to grow for two weeks until the OD at 560 nm is one ($OD_{560} = 1$) and used as inoculum.

A complete randomized design (CRD) was carried out using three treatments in triplicate. The treatments were 100% KW, 75% KW and BG-11 medium inoculated with 5 ml of either *A. variabilis* or *N. muscorum*. The initial OD of each tank was 0.30 (OD 560 nm). Initial physicochemical properties of waste water were measured before inoculating. The experimental (100% KW and 75% KW) and control (BG-11 medium) flasks were cultured for 15 days with continuous aeration. Samples from each flask were collected every five days from each tank and analyzed for their physicochemical properties viz. temperature, pH, DO, biological oxygen demand (BOD), chemical oxygen demand (COD), total phosphorous (TP), ammonia nitrogen (NH_3 –N), organic nitrogen, total kjeldhal nitrogen (TKN), total oxidized nitrogen (TON) and total nitrogen (TN). Biomass concentration of *A. variabilis* and *N. muscorum* was determined by filtration using a 120 μm planton net. The weight was determined using monopan electronic balance. The values obtained were subjected to paired sample t-test to compare significant (p-values) values in different treatments using SPSS 11.5 ver.

Results and discussion

After completing the 15 day experimental period there was a significant change in the physical and chemical properties of the kitchen waste water. All media showed a slightly change in the pH during the period of growth of two algal biofertilizers.

The optimum level of DO for algal culture is 5 mg/l (Dolly et al., 2007). Cultivation of *A. variabilis* and *N. muscorum* resulted in the increased DO in 100% KW due to aeration. The initial level of DO in the BG-11 medium was high, since it was prepared using tap water containing considerable DO. As 75% KW contained 25% tap water, resulted in slightly elevated DO level compared to 100% KW (Fig 1a).

The chemical composition of BG-11 medium resulted in low BOD since it contained no significant amount of biodegradable organic compounds such as protein, carbohydrate or fat. A significant reduction ($p < 0.000$) in BOD is observed in both 100% KW and 75% KW by *A. variabilis* and *N. muscorum*. Growth of *N. muscorum in* 100% KW resulted in maximum decrease in BOD (71.6%) (Fig 1b).

Similarly the COD values decreased significantly ($p < 0.000$) in 100% KW and 75% KW compared to BG-11 medium. *N. muscorum* was found more effective in reducing the COD content (95.4%) compared to *A. variabilis* (93.3%) (Fig 1c).

Promya et al. (2006) reported 97% decrease in COD by cultivation of blue green algae in 30% effluent from pig manure biogas digester.

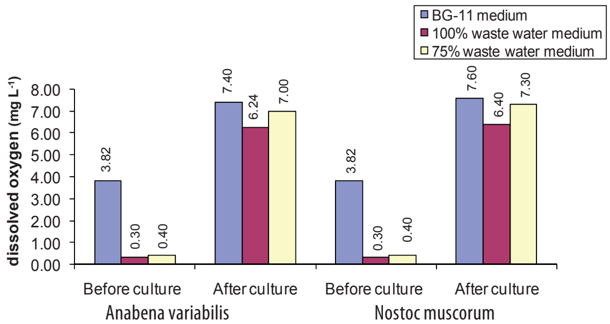

Figure 1a. Changes in dissolved oxygen

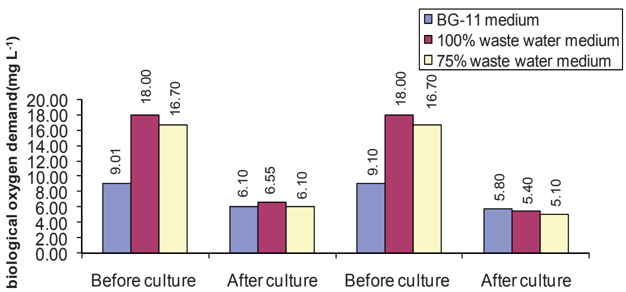

Figure 1b. Changes in biological oxygen demand

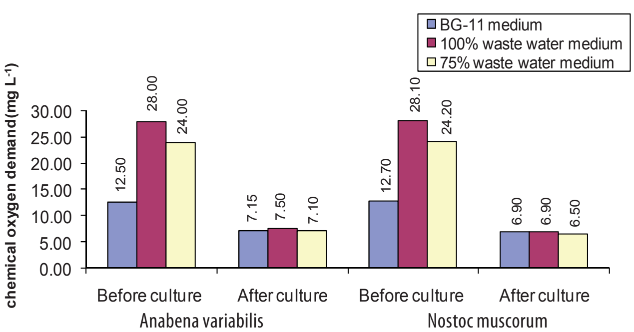

Figure 1c. Changes in chemical oxygen demand

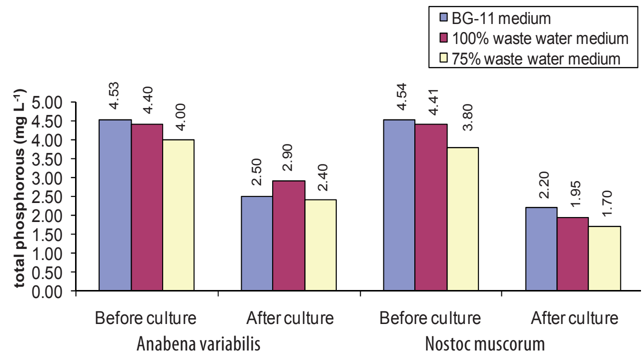

Figure 1d. Changes in total phosphorous

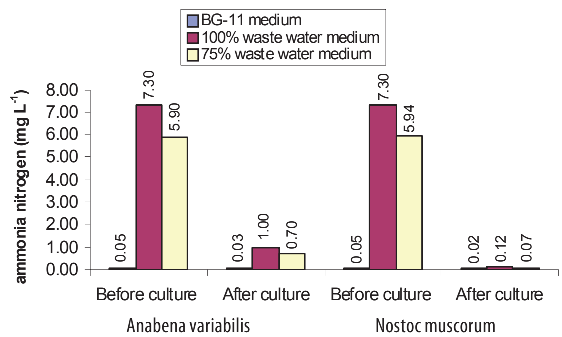

Figure 1e. Changes in ammonia nitrogen

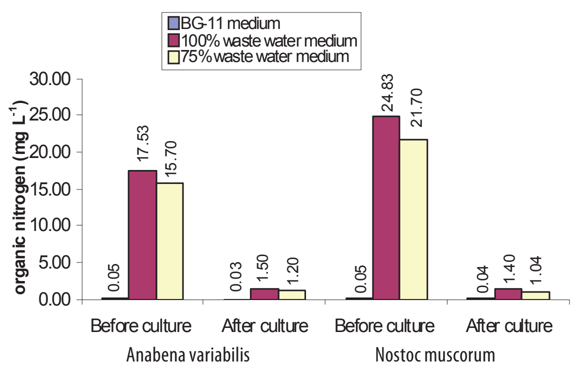

Figure 1f. Changes in organic nitrogen

Improvement in water quality is often assessed by the ability of a process to reduce nutrient matter especially nitrogen and phosphorous. Cultivation of cyanobacteria in water with high nutrient matter can dramatically reduce nitrogen and phosphorous (Bitton, 1994). In the present study cultivating *N. muscorum* in waste water significantly reduced nitrogen and phosphorous levels to ensure safe discharge besides production of blue green algal biomass as a source of biofertilizer. *A. variabilis* was found less effective than *N. muscorum* in removal of nitrogen and phosphorous contaminants from 100% KW and 75% KW.

The TP levels in all three media (BG-11, 100% KW, 75% KW) were initially high (3.8 to 4.53 mg/l) and decreased by 55%, 88% with *N. muscorum* and 34.1% with *A. variabilis* in 100% KW (Fig 1d).

Initial concentration of NH_3-N in 100% KW and 75%KW was 7.30 and 5.9 mg/l respectively (Fig 1e).

Algae prefer to use NH_3-N than NO_3-N and the optimal concentration range of nitrogen for algal growth is 1.3 to 6.5 mg/l (Seema and Trivedi, 2007). After 15 days of cultivation the NH_3–N levels in 100% KW dropped by 98.6% and 87% by *N. muscorum* and *A. variabilis* respectively. TKN is the sum of organic nitrogen and ammonia nitrogen (Stumm and Morgan, 1996). Since BG-11 contained either ammonia or organic nitrogen, the initial and final levels were very low. Kitchen waste water contained a significant amount of both organic and ammonia nitrogen. The TKN values ranged between 24.83 and 21.6 mg/l in 100% KW and 75% KW respectively (Fig 1f, 1g).

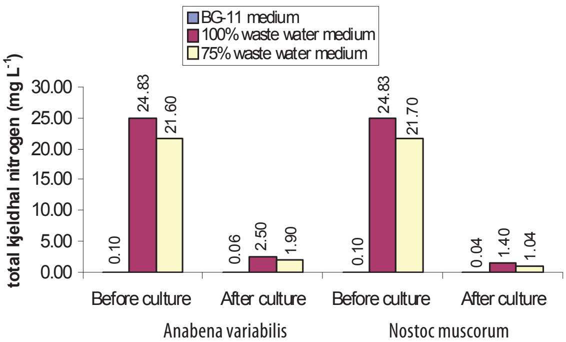

Figure 1g. Changes in total kjeldhal nitrogen

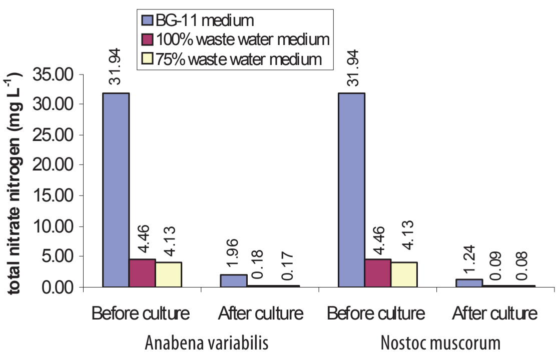

Figure 1h. Changes in total nitrate nitrogen

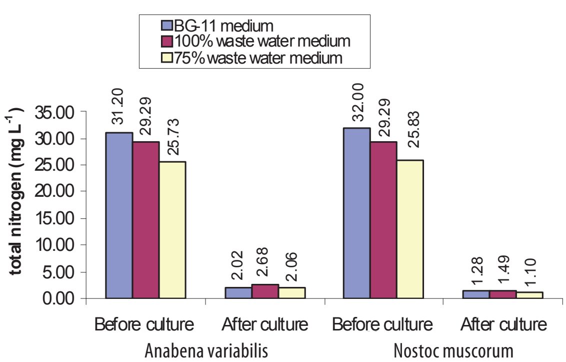

Figure 1i. Changes in total nitrogen

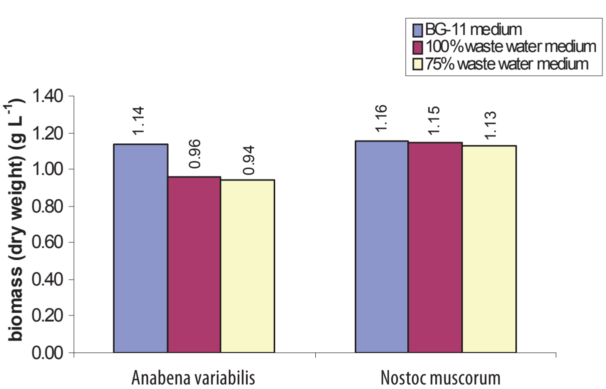

Figure 1j. Changes in biomass

N. muscorum was found more effective in treatment of waste water as it removed 95% of TKN.

NO_3-N was the sole source of nitrogen for BG-11 medium, so the initial level was very high (31.94 mg/l). Cultivation of *A. variabilis* and *N. muscorum* reduced this by 94% and 97% respectively in BG-11 medium. The lower starting level of NO_3-N in 100% KW and 75% KW was 4.46 and 4.13 mg/l that was reduced by 98.3% in 100% KW and 75% KW to meet the standards for safe discharge in India according to standards of ICMR (1996) (Fig 1 h).

TN is the sum of TKN and total oxidized nitrogen (TON). The cultivation of blue green algae as a source of biofertilizer resulted in removal of 82 to 96% of total nitrogen. The highest percentage of TN removal was achieved by cultivating *N. muscorum* (Fig 1i).

After cultivation of algae for 15 days in BG-11, 100% KW and 75% KW it was found that the biomass of *N. muscorum* was high in all the media compared to *A. variabilis* (Fig 1j).

Similarly the waste water arising from the production of sago starch with high carbon and nitrogen ration was used for production of *Spirulina* cultivation. This waste water supported the growth of *Spirulina platensis* with an average specific growth of 0.51 g/day compared with average 0.54 g/day in the inorganic medium (Laliberte et al., 1997). Supplementation of K_2HPO_4 at 2.1 m.mol concentration and urea 6 m.mol concentration enhanced biomass productivity by 14%. The content of protein carbohydrate and lipid of *Spirulina platensis* was 68%, 23% and 11% respectively in both waste water and inorganic cultivated media. Ebtesam (2008) cultivated cyanobacterial biomass in industrial wastewater for remediation process to eliminate highly toxic pollutants.

The 100% KW from Tirumala hills was nearly as effective as BG-11 medium in biomass production. In the present study *N. muscorum* cultivation in wastewater is cost effective in production of N_2 fixing biofertilizer besides remediation of waste water to meet the standards of safe discharge. The biomass of *A. variabilis* and *N. muscorum* obtained from inorganic medium and kitchen wastewater has similar potentiality in promoting the growth of important medicinal plants at nursery level (data to be communicated)

Conclusion

Kitchen wastewater retains high concentration of inorganic phosphorous and nitrogen that may lead to eutrophication of the water bodies besides polluting the underground wastewater. Over the last few decades efforts have been made to cultivate the beneficiary microorganisms as a biological treatment for remediation of waste water. Results of the present study proved that the kitchen wastewater can serve as best nutrient medium for cultivation of *N. muscorum* and *A. variabilis* to be used as biofertilizers. *N. muscorum* is more efficient in removal of contaminants from kitchen wastewater than and *A. variabilis*.

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