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Research Article

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Co-Culture of diatoms and microalgae for improvement in aquaculture ecosystem with development of resources for green energy production- A sustainable model for self-reliable economics and environment

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ABSTRACT

Background study: During the phases of Pandemic at times of COVID-19, many people have lost their livelihood. During this period, many people opt for aquaculture in their native places and became self-reliable as well as created employment opportunity for other native and unemployed youths. Scientific farming using various technologies can increase the productivity, engage with environmental friendly terms, lower dependency on chemical inputs and more important produce sources of green energy substrates. **Prospect:** Development and co-culture of diatoms grown along with the aquaculture help in efficient re-cycling of the nutrients thereby maintaining natural nutrient cycle for growing population of aquaculture fishes, increase DO level, decrease the level of toxic contaminants, act as a natural predator for different parasites and pests, promotes food chain cycle as they can be primary sources of feed for larva and many more. This will also help in reduction in dependency on chemical or synthetic entities leading to lesser generation of pollutant and increasing the healthiness of the aquaculture thereby maintaining natural ecosystem. This also helps restoration of the down town ecosystem stabilizing both commercial and natural ecosystem providing sustainable and suitable agriculture practice. Culture of algae biomass in artificial/natural ponds can efficiently act as CO2 Bio absorber along with their potential to be converted into Biofuels, Food additives, Pharmaceutical and cosmetics products. Better understanding, training, utilization can help in increased economic potential of farmers and associates in these agriculture models.

Keywords: diatoms, microalgae, sustainable agriculture practice, environment

INTRODUCTION

Chaetocerous sp. can be used as aquaculture feed, feed additives, natural colouring agents, antioxidants etc. (Pulz and Gross 2004). They are the natural food for the larval stages of many commercial aquaculture organisms such fish, shrimps, crustaceans and molluscs (Arnaud 2000; Spolaore et al. 2005; Saavedra and Voltolina 2006). They can be used for the enrichment of zooplankton that are the natural feed for the aquaculture organisms (Coutteau 1996; Khatoon et al. 2009; Khatoon et al. 2013; Banerjee et al. 2011). High nutrition such as protein content, carbohydrates content, fatty acid content, vitamins & mineral content, and antioxidants are present in these di-atoms (Natrah et al. 2007; Goh et al. 2010). Therefore, simple and large scale production of microalgae in aquaculture is an important aspect to support larviculture (Lai et al. 2012).

The major challenge is the continuous production of high quality microalgae that require specific environmental conditions such as high salt concentration and high pH. So for sustainable production of microalgae environmental factors plays a pivotal role. (Veron et al. 1996; Cirik and Goksan 2008), Optimization of growth conditions such as medium composition and culture attributes and conditions are very important for the economic viability. (Lai et al. 2012).

Although there are many di-atom species but species important for the commercial aquaculture are limited and the species of diatoms that are commonly used in aquaculture include Chaetoceros, Isochrysis, Chlorella, Skeletonema, Nitzschia, Thalassiosira, and Dunaliella (Pulz & Gross 2004). Amongst all the diatoms, Chaetoceros are used widely as natural feed system to commercial aqua culture (Becker 2004) because of their high nutritional value, high rate of production (Vega et al. 2010), especially for the rearing and maintenance of shrimp larvae, bivalve mollusc larvae and postlarvae, prawn larvae and brine shrimps (Coutteau 1996) that are extensively used in commercial aquaculture. Chaetoceros plays a vital role in shrimp aquaculture lies on the fact that all nutritional requirements of penaeid larvae are met by the Chaetocerous (Rodriguez et al. 2000).

However, experimentation in regards to the optimization of growth conditions and nutrient profile with respect to optimizing of growth conditions and nutrient composition of growth medium Martinus and Caetano 2010).

MATERIALS AND METHODOLOGY

Procurement of Diatoms: The marine diatom Chaetoceros sp. was procured from the Central Marine Fisheries Research Institute, Visakhapatnam, India.

Stock culture: The stock cultures was maintained in F/2 medium (pH 6.00) (Guillard 1975). This is one of the widely used growth media that is enriched with seawater for growth of marine algae, especially diatoms. In order to prepare, 950 mL of filtered natural seawater was taken and the following components (Table 1, Table 2, Table 3) were added. The final volume was made with 1 litre with filtered natural seawater and was sterilized by autoclave at 121°C, 15 psi for 15 minutes.

Table 1: f/2 Stock Solution Chemical Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75 g/L dH_2O	1 mL	8.82 $x10^{-4}$M
$NaH_2PO_4.H_2O$	5 g/L dH_2O	1 mL	3.62 x 10^{-5} M
Na_2CO_3	30 g/L dH_2O	1 mL	1.06 x 10^{-4} M
Trace Metal Solution	See Table 2	1 mL	---
Vitamin Solution	See Table 3	0.5 mL	---

Table 2: Trace Metal Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
$FeCl_3.6(H_2O)$	---	3.15 g	1.17 $x10^{-5}$M
$Na_2(EDTA)_2(H_2O)$	---	4.36 g	1.17 $x10^{-5}$M
$CuSO_4,.5(H_2O)$	9.8 g/L dH_2O	1 mL	3.93 $x10^{-8}$ M
$Na_2MoO_4.2(H_2O)$	6.3 g/L dH_2O	1 mL	2.60 $x10^{-8}$ M
$ZnSO_4.7(H2O)$	22.0 g/L dH_2O	1 mL	7.65 $x10^{-8}$ M
$CoCl_2.6(H_2O)$	10.0 g/L dH_2O	1 mL	4.20 $x10^{-8}$ M
$MnCl_2.4(H_2O)$	180.0 g/L dH_2O	1 mL	9.10 $x10^{-7}$ M

Table 3: Vitamin Composition

Components	Stock Solution	Quantity	Molar Concentration in Final Medium
Thiamine HCl (Vitamin B1)	---	200 mg	2.96 $x10^{-7}$ M
Biotin (Vitamin H)	0.1 g/L dH_2O	10 mL	2.05 $x10^{-9}$ M
Cyanocobalamin (Vitamin B12)	1 g/L dH_2O	1 mL	3.69 $x10^{-10}$ M

Biomass estimation: The biomass was calculated as factor of dry weight from the standard calibration curve with Abs_{750nm} vs. dry biomass. For establishment of standard calibration curve, 10mL of algal culture was taken and the absorbance at 750nm (Abs750nm) that ranges from 0.1 to 0.5. Then they were filtered through pre-combusted (100°C, 4 hours) and preweighed glass-microfibre filters (pore size-1.2µm) and rinsed with ammonium formate. The filters were then dried at 100°C for 4 hours and cooled in a dessicator till a constant weight was obtained. The dried biomass so obtained was recorded by difference in weight of dried filter paper (after and before filtration) by filtered volume (Banerjee et al. 2011).

To determine test dry biomass, 2ml of 10 days old culture was taken and A_{750nm} was calculated by plotting the absorbance in the slope equation to get unknown dry biomass.

Nutritional assessment: The proximate composition for nutrition information were analysed in terms of total carbohydrate (%age dry weight), total protein (%age dry weight) and total lipid content (%age dry weight). Culture of 10-day old were taken for analysis. The total protein was determined by Lowry's method (Lowry et al. 1951), total carbohydrate by phenol sulphuric acid method (Dubois et al. 1956) and total lipid by Bligh and Dyer method (Bligh and Dyer 1959).

Optimization of medium composition and culture conditions: The experimental design was based on the optimization of different attributes. Process attributes- physicochemical parameters taken for study were Temperature (20°C-30°C), pH (6-9), salinity index (15gm L^{-1}- 40 gm L^{-1}) and agitation speed (100 rpm- 200 rpm). Nutritional attributes such as nitrate (7.5 gm L^{-1}-150 gm L^{-1}), phosphate (0.5 gm L^{-1}-10.0 gm L^{-1}) and silicates (3.0 gm L^{-1}- 60.0 gm L^{-1}).

RESULTS

Biomass estimation: From the standard curve Abs_{750nm} vs. dry biomass; the unknown dry mass can be calculated from the regression curve equation (Equation 1)

$$Dry\ mass\ (mg\ ml-1)(x) = \frac{Abs\ 750nm\ (y) + 0.0378}{0.0009}$$

(R^2 = 0.995; p<0.001) .....Equation 1

The biomass production of 10 days old culture of *C. muelleri* under the non-optimized conditions in F/2 medium found to be 0.42±0.01 mg mL^{-1}.

Nutritional assessment: The experimental values of nutritional attributes of *C. muelleri* for Total protein content (13.02 ± 1.13%), Total lipid content (19.58 ± 1.2%) and Total carbohydrate content (0.97 ± 0.03%) were predicted by standard methodology.

Optimization of process attributes: The different nutrient attributes were optimized for parametres such as nitrate (0.19 mg L^{-1}), phosphate (7.5 mg L^{-1}), silicate (30 mg L^{-1}) along with physico-chemical attibutes such as temperature (31°C), pH (6.5), salinity index (35 mg L^{-1}) and agitation speed (150 r.p.m)._1,

CONCLUSION

Chaetoceros (grown along with the aquaculture help in efficient re cycling of the nutrients and thereby maintaining natural nutrient cycle for growing population of aquaculture fishes, increase DO level, decrease the level of toxic contaminants, act as a natural predator for different parasites and pests, promotes food chain cycle as they can be primary sources of feed for larva and many more. This will also help in reduction in dependency on chemical or synthetic entities leading to lesser generation of pollutant and increasing the healthiness of the aquaculture thereby maintaining natural ecosystem This in turn also helps restoration of the down town ecosystem stabilizing both commercial and natural ecosystem. More important, these models can be also being helpful for farmers those who can produce and supply to these large aquaculture firms results in rural livelihood self-sustained model of employment and economy This indeed will also reduce dependency of those anthropological entities on environment.

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