



EFFECT OF DIFFERENT MEDIA COMPOSITION ON GROWTH, BIOMASS AND PHOTOSYNTHETIC PIGMENT OF *AMPHORA* SP. (BACILLARIOPHYCEAE) – A MICROCOSM APPROACH

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ABSTRACT

Microalgae have enormous potential because of their rapid growth and tolerance to varied environmental conditions. *Amphora* sp., an epipelagic benthic pennate diatom, holds promise as a nutraceutical source and may be useful for aquaculture. The purpose of this study was to investigate the effect of the use of different technical culture medium (F/2, F/2+Nualgi, TMRL, TMRL+Nualgi) on the growth rate, biomass and pigment concentration of diatom culture *Amphora* sp. Nualgi, a commercially available micronutrient ready-mix with eight elements adsorbed as nanoparticles on a modified silica sol, was found to drastically boost growth in *Amphora* sp. *Amphora* sp. was isolated from seawater through the dilution technique. The strain was inoculated into seawater enriched with different media under 16:8 light/dark photoperiod with $50\mu\text{molm}^{-2}\text{sec}^{-1}$. Optical density was recorded by using UV-Visible spectrophotometer at 680 nm. Besides, dry weight and chlorophyll-‘a’ and ‘b’ concentration of strain were determined. The highest growth rate (OD) (2.16 ± 0.11), dry weight ($0.91\pm0.07\text{gL}^{-1}$), chlorophyll-‘a’ ($27.59\pm0.4\mu\text{gL}^{-1}$) and chlorophyll-‘b’ ($16.32\pm0.4\mu\text{gL}^{-1}$) production were observed in the TMRL+Nualgi medium followed by F/2 + Nualgi than the other media. Supplementation of the nutrient medium with Nualgi, a commercial formulation of micronutrients on a silica base was found to promote growth of *Amphora* sp.

Keywords: Nualgi, Diatom, Laboratory culture, Aquaculture and growth rate.

1. INTRODUCTION

Diatoms are unicellular eukaryotic algae that entered the fossil record 150 million years ago [1]. They are found in fresh water, brackish water and marine ecosystem and also in benthic sediment regions. It's have a unique structural cell wall which contains the hydrated silica wall made up of glass. They are widely used for the climate and environmental studies although their use is strongly hampered by the fact that silica cell walls are prone to dissolution [2]. Among the microalgae, diatoms are found to be predominant in ocean ecosystem which involves in CO_2 sequestration to mitigate the fossil fuel gases present in atmosphere. The microalgae diatoms are potentially candidate to absorb unlimited nitrogen and carbon from natural resources and dynamically focused on the biofuel industry as an alternative energy production based on easy for biomass cultivation in large scalable. Particularly, the diatoms have a high content of oil, fatty acids, steroids and other primary and secondary metabolites.

The highly branched metabolites such as long chain fatty acids and lipids are mainly useful for pharmaceutical applications and biodiesel production [3].

In the development of algal products, one of the major targets is to be selecting suitable culture nutrient medium [4, 5]. The choice of culture media mainly depends on several factors that include its chemical composition [6]. It is known that microalgae respond with physiological alterations to the environmental conditions where they grow [7]. This behavior can be viewed as a biotechnological attribute that can be manipulated in order to control the algae biochemical composition and growth focusing on specific compounds and higher productivity. Therefore, the selection of cheap and promising media to improve microalgae production yield is of great interest [8]. Harrison and Berges [9] proposed that three main experimental categories, culture maintenance, algal biomass yield and physiological/growth experiments are a good way to search for ideal production conditions. Growth rates give a general index of algal health status and physiological condition in the cultures, since it reflects algal metabolism, as a response to all of its cellular cycles. From this rationale, it is clear that batch cultures can offer adequate growth conditions for microalgae only

during a short period of time, after which cell metabolism begins to collapse and photosynthesis is reduced [10]. A high production of microalgal biomass is one of the critical requirements of successful finfish and shellfish hatchery aquaculture [11, 12]. In this study, *Amphora* sp. a new isolate from seawater was evaluated for its capacity to grow in different media composition. The goal of this study was to evaluate the growth rate, pigment concentration and biomass production of the newly isolated benthic diatom *Amphora* sp. by micronutrient-mix enrichment medium under controlled condition.

2. MATERIALS AND METHODS

2.1. Collection and Isolation of marine microalgae

The microalgae samples were collected from Hare Island Tuticorin (Lat. 9° 14' N; Long. 79° 13'E) Tamil Nadu coast, southern India. The microalgae samples were collected by using phytoplankton net (No. 10, bolting cloth, 48µm) during early morning. Collected samples were filtered through zooplankton mesh (158 µm) to segregate zooplankton exist in the collected sample and microalgae filtrate was transferred to filtered seawater and brought to the laboratory. At laboratory micro algal species were isolated using agar plating and serial dilution technique and identified using standard keys [13].

2.2. Growth in different culture media

Selected diatom species were grown on various synthetic media in order to check which of the medium was able to support the best growth of diatom. In order to find out the best culture medium, isolated algal species were cultured in four different seawater enriched media composition having pH of 8.2 to 8.7 such as F/2 medium [14], F/2 with Nualgi enriched medium, TMRL medium [15] and TMRL with Nualgi enriched medium. Before experimental setup all the media prepared and sterilized in autoclave at 15 psi, 121°C for 20mins. All the media pH was adjusted by using 1N NaOH/1N HCl prior to autoclave. Autoclaved media were carefully transferred in sterilized plastic tubs, covered with muslin clothes and placed under continuous illumination of 3000 lux light intensity at 25±2°C and selected algal species were cultured for 15 days in order to obtain sufficient biomass.

2.3. Effect of Nualgi – a micronutrient mix

Nano silica based micronutrient mixture called “Nualgi” which has nano silica as its major constituent along with iron and 9 other trace metals. The silica becomes both the carrier for other nutrients and the nutrient by itself.

It is in a water dispersible particulate form. Nualgi because of its nano size is able to pervade very small spaces in the subsurface and remain suspended in water, allowing the particles to travel farther than larger, macro-sized particles there by increasing the bioavailability of the nutrients for easy absorption by micro algae and achieve wider distribution. A patented (PCT/IN05/00195 US patent application no. 0070275856) micronutrient ready-mix is available in the market with the trade name Nualgi at ~6.25 US\$ kg⁻¹. In water, Nualgi causes diatom algae to bloom, though any pond, lake, estuary or coastal water has many species of organisms, only diatoms require silica and they consume Nualgi rapidly and bloom. The optimal concentration of Nualgi was determined by setting up cultures in media with concentration of 0.5g L⁻¹.

2.4. Experimental setup

The initial axenically culture of the microalgal strain was obtained in 250 ml Erlenmeyer flasks containing 150 ml of seawater enriched with four different media composition. A 2-weeks old culture, at vegetative cell growth phase, was used as inoculum for all experiments. The pH of nutrient media was adjusted as 8.2 to 8.6. The implementation of 12:12 light/dark photoperiod (50 µmol photons m⁻² s⁻¹) was applied on cultures and they were cultivated under at 22-25°C room temperature. Growth parameters of microalgae were evaluated at each growth phase for the period of 15 days.

2.5. Growth rate and Biomass analysis

Optical density of microalgae cultures were measured at 5 days interval of time by checking culture turbidity at 680 nm [16] with the help of spectrophotometer (Shimadzu UV/VIS). Vortexing of diatom cultures was done to get homogenous culture to prevent the settling and erroneous result while reading the OD.

Cells were concentrated by centrifugation, washed with de-ionized water and dried at 60°C using hot air oven to determine dry weight (expressed as g/l). Cultures were harvested and dry biomass was estimated at 5 days of intervals.

2.6. Measurement of photosynthetic activity (Mackinney, 1941)

Chlorophyll was extracted by taking 0.5 gram of algae to which 5 ml 80% acetone was added. It was kept undisturbed overnight and the optical density of the chlorophyll was measured next day with a UV/VIS spectrophotometer at 663 nm. The amount of

chlorophyll extracted was calculated according to the equations of Mackinney [17].

2.7. Calculation

Chlorophyll 'a' $\mu\text{g/l} = \text{O.D} \times 12.63 \times \text{D.F.}$

(Where O.D is optical density and D.F dilution factor)

Chlorophyll 'b' $\mu\text{g/l} = \text{O.D} \times 19.3 \times \text{D.F.}$

(Where O.D is optical density and D.F dilution factor)

3. RESULTS AND DISCUSSION

3.1. Cell morphology

Cells are solitary, frustules lanceolate to narrowly elliptical with amphoroid symmetry. Central area on dorsal side, narrow fascia is expanding and branching near dorsal margin, on ventral side indistinct. Dorsal margin is convex, ventral margin is straight to slightly gibbous. Raphe branches are straight, running near the ventral margin, proximal raphe endings straight. The length of the *Amphora* sp, is $5.9 \mu\text{m}$ and width is about $2 \mu\text{m}$. Initially, colonies were golden brown in colour, but later turned grey-green due the presence of chloroplasts. The microscopic image of isolates *Amphora* sp was shown in Fig 1.

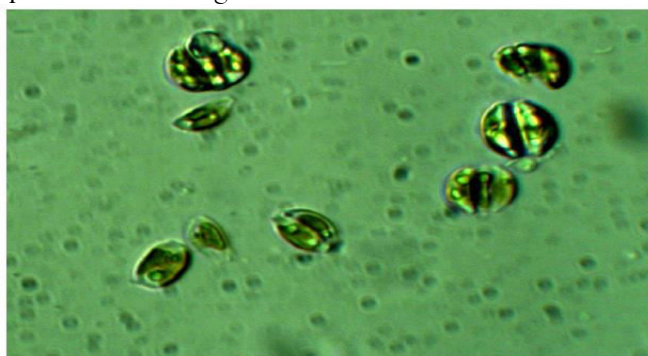


Fig.1: Microscopic image of *Amphora* sp. (40X)

3.2. Effect of different media composition on growth, biomass and photosynthetic pigment of *Amphora* sp.

There are differences among microalgae species in terms of cell growth and different microalgae groups have different physiological requirements in their natural habitats or under culture conditions [18]. Environmental and culture condition parameters such as light, photoperiod, medium and temperature influence the growth of microalgae [19, 20]. The compositions and the amount of nutrients have great effect on reproduction of the microalgae. The lack of those substances may result in physiological and morphological changes in the microalgae. Especially, microalgae need the macronutrient elements (i.e. carbon, nitrogen,

phosphorus), basic ions (i.e. Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cl^- , SO_4^{2-}) and micronutrient metals (i.e. iron, manganese, zinc, cobalt, copper, molybdenum, nickel and cadmium) in their habitats [21]. Phosphorus is the most needed macronutrient by the microalgae after nitrogen. Phosphorus is necessary for many phosphorylation syntheses and the Calvin cycle. Therefore, the lack of phosphorus affects not only the synthesis of chlorophyll but also the growth and metabolism of cells [22]. The KH_2PO_4 and K_2HPO_4 are the sources of phosphate for algal growth in the present study. Nitrogen is a significant substance for synthesis of protein, nucleic acids and chlorophyll molecules [23]. NaNO_3 and EDTA are the nitrogen sources of both media which were used in this study. The magnesium plays a significant role in the growth of microalgae as a co-factor of some key enzymes in the metabolic pathway [24]. A series of measurements were performed in order to estimate growth rate and to calculate the rate of change occurring in biomass concentration. The cell number was determined through optical density (Fig. 2).

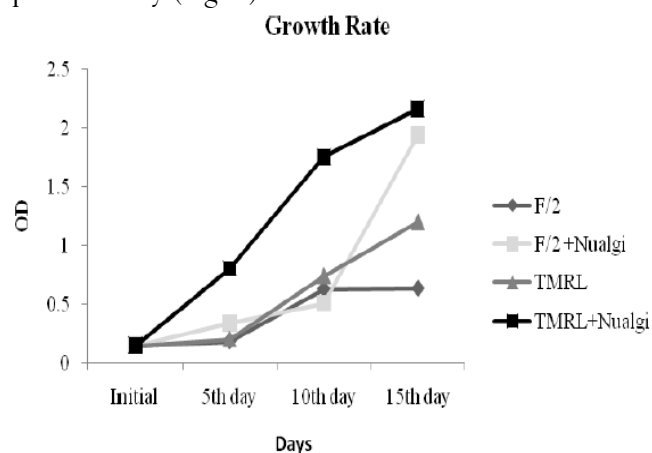


Fig. 2: Effect of different media composition on growth of *Amphora* sp.

The present study mainly dealt with the different media such as F/2, F/2 with Nualgi, TMRL and TMRL with nualgi growth media use for *Amphora* sp. The growth of *Amphora* sp. in different culture media was evaluated by biomass and pigment concentration. In general, TMRL with micronutrient (Nualgi) was found to greatly influence the growth of *Amphora* sp. followed by F/2 + Nualgi than the other compositions. Based on the density concentration measurement, it was observed that the growth of *Amphora* sp was highly favoured by TMRL with nualgi (Fig. 2). The cell density was found to be maximum in (2.16 ± 0.11) TMRL+ nualgi followed by F/2+ Nualgi (1.96 ± 0.02) , TMRL (1.2 ± 0.01) and F/2 (0.635 ± 0.06) at the end of 15th day. The maximum

biomass was observed in seawater enriched with TMRL+ nualgi (0.91 ± 0.07 g/L) than the other growth medium (Fig.3) tested. Suman *et al* [25] studied the culture medium optimization and lipid profiling of *Cylindrotheca* sp., a lipid- and polyunsaturated fatty acid-rich pennate diatom and potential source of eicosapentaenoic acid and reported that substitution of Nualgi for micronutrients and silicate contained medium improved growth *C. fusiformis*. The ready-made micronutrient mix adsorbed on metallate silica available under the trade name “Nualgi” promoted good growth of diatom *Cylindrotheca* species. This could be due to the relatively high quantities of iron in the formulation and the minor elements being in a readily available (nano) form in the product. The product is not expensive and can be used in mass production of these nutraceutically important diatom species.

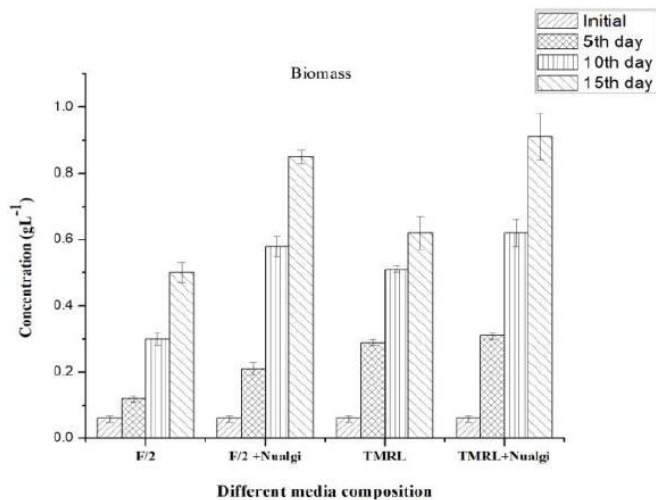


Fig.3: Effect of different media composition on biomass of *Amphora* sp.

Pigments can also be subjected to oxidation by molecular oxygen, which is known to be a potent inhibitor of microbial processes including photosynthesis [26]. The environmental variables can influence growth and pigment production which in turn may change the composition of the biomass by affecting the metabolism. Several kinds of chlorophylls are found in photosynthetic organisms; however, only two forms occur in diatoms. The predominant Chl ‘a’ plays a central role in the photochemical energy conversion of the majority of photosynthesizing organisms, while Chl ‘b’ participates functional activity of higher plants. In the present study, the maximum pigment concentration was observed in the maximum biomass containing growth media (TMRL+Nualgi) with the concentration Chl ‘a’ 27.59 ± 0.4 $\mu\text{g L}^{-1}$ and Chl ‘b’ 16.32 ± 0.4 $\mu\text{g L}^{-1}$ at the end

of the experiment period followed by F/2+nualgi than the F/2 and TMRL media respectively (Fig 4 & 5).

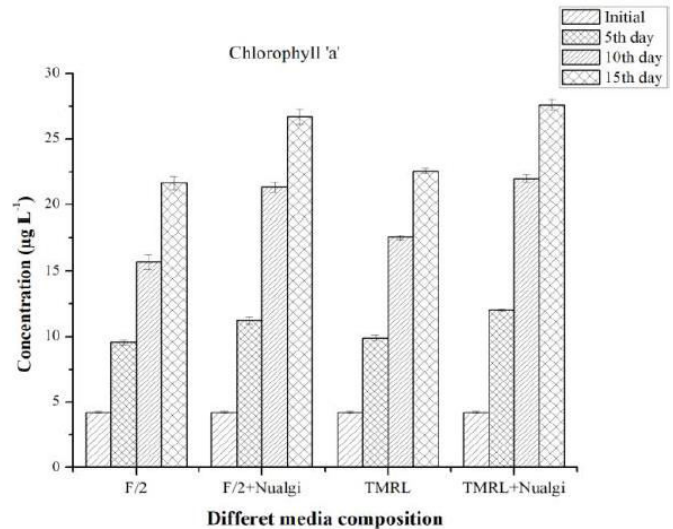


Fig.4: Effect of different media composition on Chlorophyll ‘a’ pigment of *Amphora* sp.

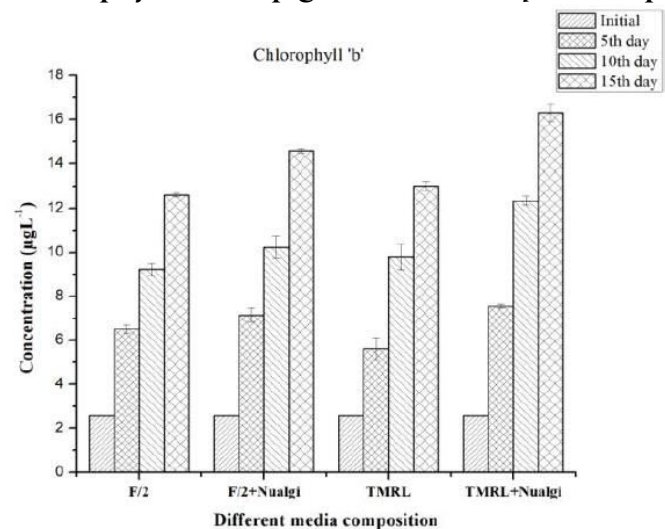


Fig.5: Effect of different media composition on Chlorophyll ‘b’ pigment of *Amphora* sp.

The change in pigment composition was evident from the color of cultures. Iron controls the process of chlorophyll synthesis directly and indirectly and iron stress induces chlorotic cells in diatoms [27]. According to the findings of the present study, the presence of the micronutrients (iron) concentration was an increased in chlorophyll and soluble protein content, as well as increase the growth of diatom in relation to control cultures grown under optimal nutrient conditions. Similar study has been observed by Haimanti and Debasmitta [28].

4. CONCLUSION

Diatoms served to be multifunctional candidate which may possibly be used for various ecological and

biotechnological applications. *Amphora* sp. has many good characteristics, such as rapid growth and multiplication rate; they are easy to culture and harvest. Thus, they can be used in aquaculture and can also be cultured for use in poultry and animal feed to improve the nutritional status of meat and eggs. Among various tested, culture medium TMRL with Nualgi was found to be most appropriate medium for culturing of *Amphora* sp. Furthermore, this study recommends the use of the nualgi –Micronutrient mix to increase diatom biomass in indoor and outdoor culture system and this by-product is increased aquaculture productivity.

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