CHAPTER 6

CULTIVATION OF *Nostochopsis* sp.2 MORPHOTYPE CM4

6.1. Introduction

A few studies on *Nostochopsis* spp. cultivation had been reported. In 1978, Tiwari (1978) cultivated these cyanobacteria and found that they formed infrequent intercalary heterocytes in the main trichome, while the short lateral branches (1-4 cells) characteristically produced long lateral branches, during the later stages of growth in ammonium medium. Mungmai (2006) reported that it showed maximal growth in half concentration of BG-11 medium. It also produced higher amount of mucilage substance than that cultivated in the liquid medium. Pandey and Pandey (2008a) studied the enhanced production of *N. lobata* in a full factorial design with supplemental zinc, glutamine, and zinc + glutamine in batch culture. Production of biomass, pigments and antioxidant capacity were higher under immobilized cell cultures in comparison to free cell cultures. Maximum biomass, 2,390 mg.dry wt^{-1}, delta-aminolevulinic acid (δ-ALA) 2.715 μg. mg dry $wt^{-1}.h^{-1}$ were recorded when zinc and glutamine were supplemented together in the growth medium at pH 7.8. In the same year, Pandey and Pandey (2008b) showed potential improvement of immobilized cell cultures of *N. lobata* when a mixture of phosphorus and ferric were supplemented. Algal biomass, pigments, nutritional value and antioxidant capacity increased. Phosphorus appeared to be a better supplement than ferric for the

production of biomass, chlorophyll and carotenoids. However, the factors for growth were not only nutrients but also pH, light, temperature and cell condition. Since, *Nostochopsis* growth attached to the substrate. The type of substrate may be another factor for its growth. In this study, cultivation with various substrates and advantages of substrates were investigated.

6.2 Materials and Methods

6.2.1 Isolation

Samples were collected from Queen Sirikit Botanical Garden, Chiang Mai (CM4). They were isolated by washing with 5% ethanol and repeated with deionize water. The filaments were isolated by micropipette technique. They were then placed on nitrogen-free BG-11 plus 0.75% agar (Rippka *et al.*, 1979) and incubated at 24 ºC under continuous illumination by cool-white fluorescent lamp at light intensity of 20.52 µmole. $m^{-2}.s^{-1}$.

6.2.2 Cultivation using the substrates

Nostochopsis sp.2 morphotype CM4 was cultivated with natural substrates such as granite, limestone and artificial substrates *e.g.* concrete, baled clay, in batch culture. Initial inoculum of 7.5 mg dry mass was cultivated in 150 mL of BG-11 nitrogen free medium and incubated at 25 °C under cool-white fluorescent lamp at 20.52 µmole. $m^{-2}.s^{-1}$ and 15:9 h in light: dark cycle (Pandey and Pandey, 2008a). Biomass was harvested and analyzed for growth every ten days in triplicate. The growth of *Nostochopsis* sp.2 morphotype CM4 was measured in terms of dry weight

and during this period, the specific growth rate (μ) was determined (Oris, 2003) and calculated by the following formula.

$$\text{Specific growth rate } (\mu) = \frac{\ln (X_1/X_0)}{T_1-T_0}$$

X_1= cell dry weight (mg) at anytime

X_0= cell dry weight (mg) at time zero

T_1= time period of cultivation any time

T_0= time period of cultivation time zero

In= the base of the natural system of logarithms

6.2.3 Study on advantage of substrate

Nostochopsis sp.2 morphotype CM4 was cultivated in batch culture. Initial inoculum of 50 mg dry wt^{-1} (Pandey and Pandey, 2008a) was in 150 mL of BG-11 nitrogen free medium as a control treatment. Three treatments were comparatively investigated *i.e* cultivation with BG-11 nitrogen free medium without $NaHCO_3$ (without carbon source), BG-11 nitrogen free medium plus 40 $mg.l^{-1}$ $NaHCO_3$ (with carbon source) (Concentration of $NaHCO_3$ was from the titration of HCO_3^{-1} cultivated with limestone) and BG-11 nitrogen free medium plus limestone. The growth of *Nostochopsis* sp.2 morphotype CM4 was measured as described previously in 6.2.2.

6.2.4 Data evaluation

The computer statistical packages, SPSS for Windows version 14.0 and Microsoft Excel were used for statistical analysis of variance (ANOVA), correlation and cluster analysis.

6.3 Results and Discussion

The highest growth of *Nostochopsis* sp.2 morphotype CM4 was observed in the cultivation using limestone as substrate. The biomass was approximately 5-6 folds higher than that of control (free cell) and other substrates ($p<0.05$) (Figure 6.1). This cyanobacterium could not survive when concrete was used as substrate. Since concrete is composed of CaO, SiO_2, Al_2O_3 (Sinney *et al*., 2003), when it was soaked in the medium or water, $Ca(OH)_2$ was released and the medium became strongly alkaline (pH 9-11) and was not suitable (pH 7.8) for *Nostochopsis* growth (Pandey and Pandey, 2008a). *Nostochopsis* sp.2 morphotype CM4 is a filamentous branched cyanobacterium which grows luxuriantly attached to the rock surface in streams (Pandey and Pandey, 2008a; Thaimdao *et al*., 2011). This affected the high biomass in the cultivation using limestone as substrate. The specific growth rate of *Nostochopsis* sp.2 morphotype CM4 cultivated with limestone and baked clay increased until 30 days of cultivation and decreased afterwards. On the contrary, the specific growth rate of the cultivation using granite as substrate decreased only after 20 days (Figure 6.2). The growth of this cyanobacterium stopped after the 60^{th} day (specific growth rate ~0), therefore the cyanobacterial biomass should be harvested within those periods.

However, it was still not clear whether limestone was a suitable substrate because it was a matrix for attachment or it provided $CaCO_3$ into the medium which *Nostochopsis* could utilize as a carbon source. To clarify this, cultivation with limestone or supply of $NaHCO_3$ as a carbon source was done.

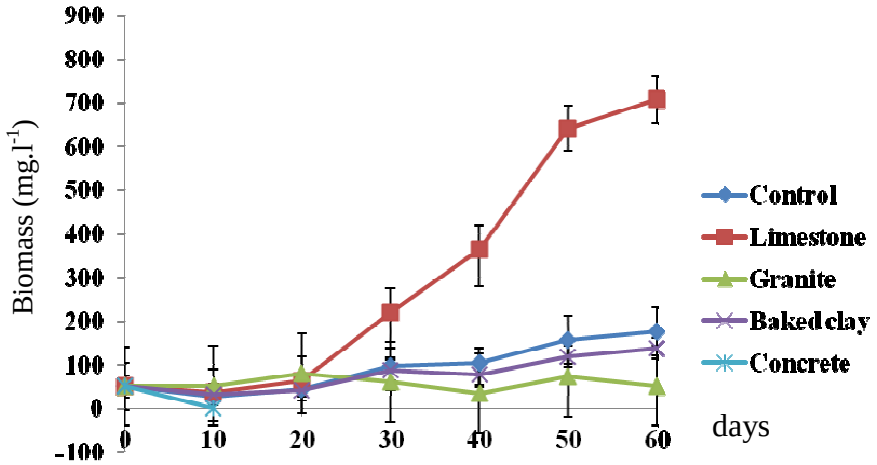

Figure 6.1 Biomass of *Nostochopsis* sp.2 morphotype CM4 cultivated in BG-11 nitrogen free medium with varied substrates

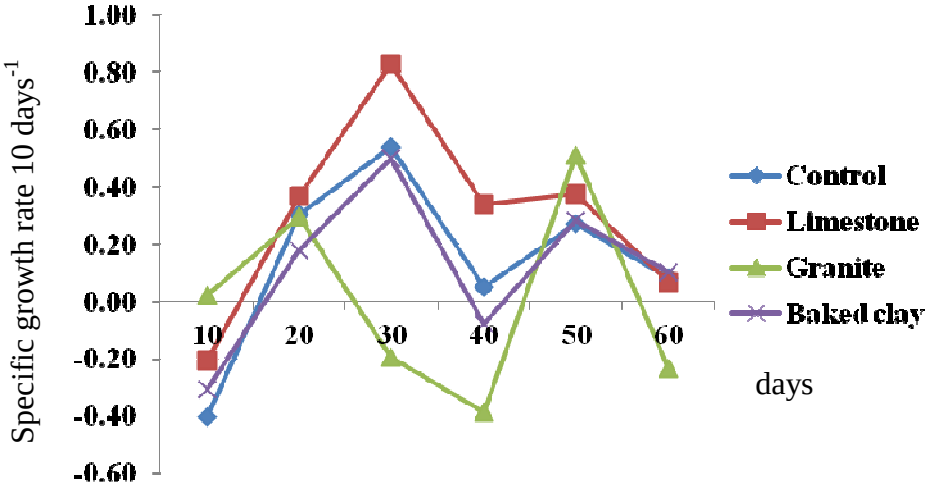

Figure 6.2 Specific growth rate of *Nostochopsis* sp.2 morphotype CM4 cultivated in BG-11 nitrogen free medium with varied substrates

The results showed that, the growth of *Nostochopsis* sp.2 morphotype CM4 cultivated in control medium with or without $NaHCO_3$ and limestone as a carbon

source was not significantly different (p>0.05) at 0-50 days of cultivation, but the biomass from cultivation with limestone was highest after 60 days of cultivation (p<0.05). The biomass was 1,061±0.01 mg dry wt.l^{-1} (Figure 6.3).

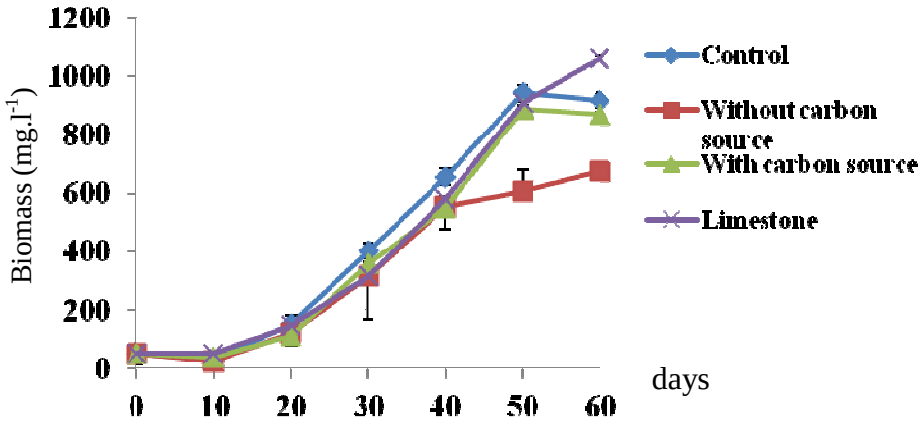

Figure 6.3 Biomass of *Nostochopsis* sp.2 morphotype CM4 cultivated in BG-11 nitrogen free medium with and without carbon sources.

Pandey and Pandey, (2008a) studied the cultivation of *N. lobata* with immobilizated cell and showed that biomass was higher than that cultivated with free cell as 2,390 mg dry wt.l^{-1}. Consequently, the immobilized cell cultivated likewise cultivation by using substrate. In addition, ball forming cyanobacteria such as *Nostoc commune* need solid support for their growth that can be provided by 1.5 -1.8% (w/v) agar in the complete growth medium (García-Pichel, 2006). Mungmai (2006) reported that *Nostochopsis* sp. showed maximal growth in a half concentration of BG-11 medium and cultivation in semi-solid media produced higher amount of mucilage substance than those cultivated in the liquid media. Moreover, *Nostochopsis* sp.2 morphotype CM4 attached to the flask surface when it was cultivated in the medium without substrate. Thus, the substrate may be required for *Nostochopsis* sp.2 morphotype CM4 attachment in the cultivation.