Investigating the impact of salinity level on growth and lipid accumulation in*Chlorella vulgaris* as a feedstock for biodiesel production

A. Gammanpila[1]*, C. Rupasinghe[1] and S. Subasinghe[2]

[1]*Department of Agriculture Engineering, Faculty of Agriculture, University of Ruhuna*

[2]*Department of Crop Science, Faculty of Agriculture, University of Ruhuna*

Abstract

High biomass and lipid production are the two most important desirable characteristics of a microalgae species in order to consider it as a suitable feedstock for intensive biodiesel production. It is reported that changes in environmental factors such as, light, salinity and nutrient availability can affect these characteristics of microalgae. Therefore, the research aim was to study the effect of salinity on the growth and the lipid content of the microalgae, *Chlorella vulgaris* because of its potential utilization as feedstock for biofuels. *Chlorella vulgaris* was grown in eight different salinity levels; 35, 30, 25, 20, 15 10, 5 and 0 ppt for 21-day growth period in an outdoor culture system. At the end of the growth cycle, cells were harvested by flocculation and dry weight and the percentage lipid content were estimated.

Chlorella vulgaris was able to tolerate all the salinity levels, but showed significantly different growth and lipid accumulation ratesat different salinities. The highest dry biomass weights (0.77, 0.78, 0.83 and 084 g/l) were observed when cells were cultured at lower salinities (15, 10, 5 and 0 ppt respectively). The highest salinity (35 ppt) saw the lowest dry biomass weight (0.24 g/L). *Chlorella vulgaris* also showed decreased lipid contents when the salinity was increased. Percentage lipid content was significantly higher (14.6 %) at 5 and 0 ppt salinity levels, and this was about three times higher than lipid accumulation at the highest salinity (35 ppt). The present study concluded that the lower salinity levels of 5 and 0 ppt are the optimum levels for culturing *Chlorella vulgaris* for biodiesel production, as these salinity levels reported the significantly highest lipid yield of 0.12 g/l.

Keywords: dry weight, lipid yield, *Chlorella vulgaris*, salinity

**Corresponding author: shanigam@yahoo.com*

Introduction

Fuel production from phyto-biomass is increasingly important these days due to problems with global warming, pollutants emission and increases in the cost of petroleum fuels. Further,it is understood that the present petroleum reserves are to be depleted in less than 50 years at the present rate of consumption (Huang*et al.* 2010). Therefore, in recent years biodiesel has received considerable attention as a biodegradable and renewable source of energy.Production of biodiesel from microalgae is a newly emerging field because of their high oil content and rapid biomass production. They are one of the fastest growing photosynthesizing organisms and can complete an entire growing cycle every few days. Further, microalgae are capable of utilizing a wide variety of water sources, such as fresh water, marine water, brackish water and waste water (Huang *et al.* 2010). Therefore, microalgae have strong potential as a biofuel feedstock that will not compete with production of food, fodder and other products derived from crops for food production.

Some species of microalgae already possess high oil concentrations and these can be manipulated to produce more oil. According to previous studies, the lipid content in some microalgae could be increased by changing the nutrient concentration, temperature, CO_2 aeration, salinity and light intensity (Gu*et al.* 2012). Accordingly, the main objective of the present study was to determine the effect of salinity on the cultivation of a robust microalgae species, *Chlorella vulgaris,* in order to determine the best conditions to maximize the lipid yield for biodiesel production.

Materials and Methods

For determination of optimum salinity level, *Chlorella vulgaris* was cultured in eight different salinity levels; 35, 30, 25, 20,15 10, 5 and 0 ppt in an outdoor culture system using Guillard and Ryther's modified F/2 media (Guillard, 1975) as the nutrient media for 21 days growth period. The cultures were aerated with mechanical aerators. All the glassware and media were sterilized prior to inoculation. All the experiments were carried out in triplicates. The cultures were harvested during the stationary phase of the growth cycle by chemical flocculation method using NaOH as the flocculent agent. The cells were washed three times with distilled water and dried in an oven for 6 hours at 105 °C for dry weight estimation. The lipid contents of the dried algal samples were then determined using a Soxlet apparatus. The statistical analysis was done by using Minitab 14 version. One-way Analysis of Variance (one-way ANOVA) was performed at 95 % level of probability in order to test the significance differences of lipid content and growth performances under different treatments. When the test reported $p \leq 0.05$, a Turkey post-hoc test was performed for pair wise comparisons.

Results and discussion

Chlorella vulgaris was able to tolerate all salinity levels, but showed significantly different growth and lipid accumulation rates at different salinities. The effects of salinity on dry weight and lipid content of *Chlorella vulgaris* are shown in Fig. 1 and 2. Results revealed that, dry biomass weight in the cultures with different salinity levels were significantly different ($p \leq 0.05$).The significantly highest dry biomass weight of 0.77, 0.78, 0.83 and 0.84 g/l was observed when cells cultured at lower salinities of 15, 10, 5 and 0 ppt respectively. The highest salinity (35 ppt) gave the significantly lowest dry biomass weight of 0.24 g/l. Rao*et al.* (2007) showed reduced growth in some microalgal species at higher salinities due to decreases in photosynthetic rate. Some previous studies also reported that many freshwater species are able to withstand higher salinity ranges (Ruangsomboon, 2014).

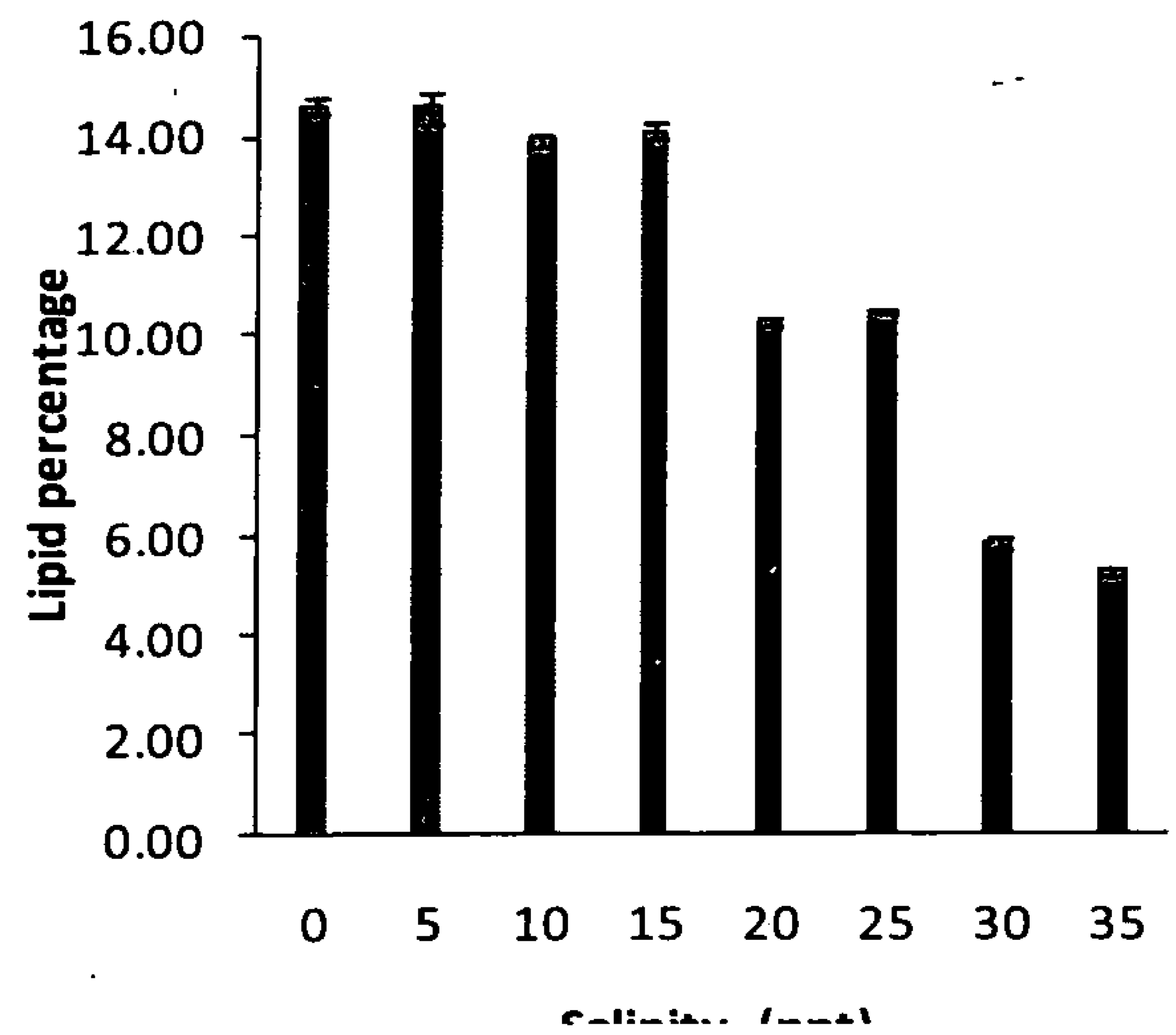

Fig. 1: Dry weight of *Chlorella vulgaris* under different salinity levels

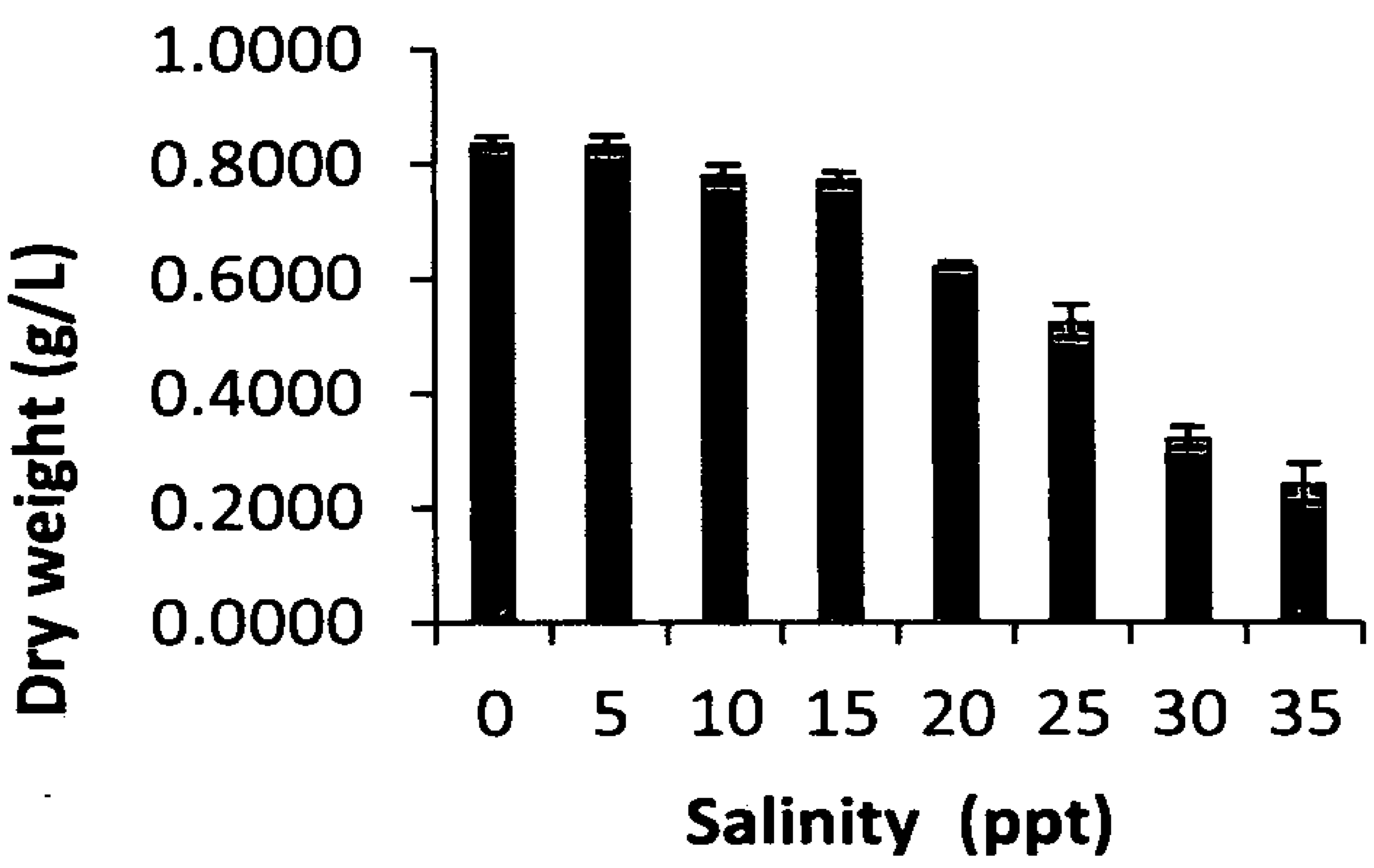

Fig.2: Lipid percentage of *Chlorella vulgaris* under different salinity levels

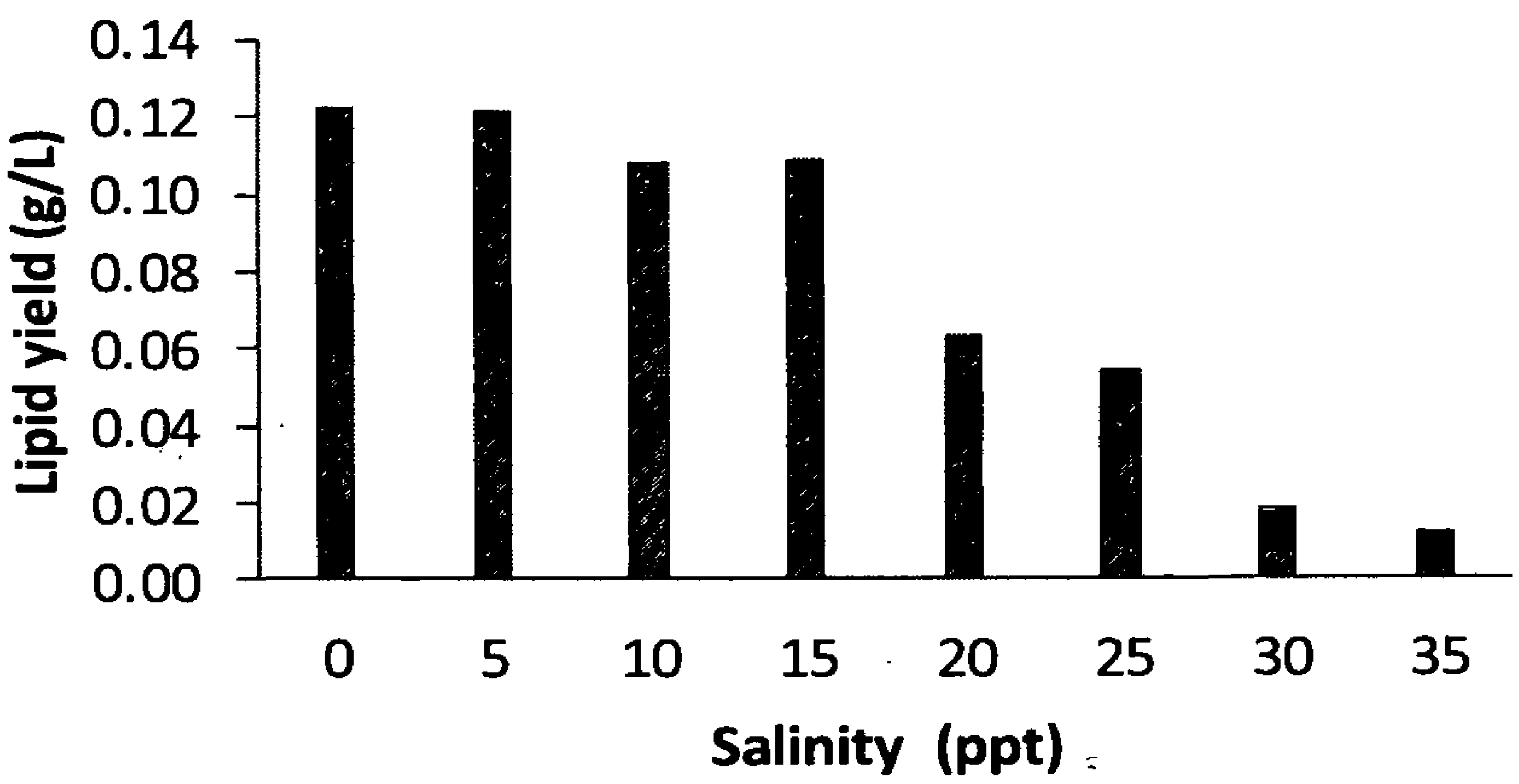

Fig.3: Lipid yield of *Chlorella*

There were also significant differences in the percentage lipid content in different treatments of salinity levels (Fig. 1).*Chlorella vulgaris* showed remarkably decreasing lipid content when increasing the salinity. Percentage lipid content was significantly higher with 14.6 % lipid accumulation at 5 and 0 ppt salinity levels, and these were about three times as high as lipid accumulation at the highest salinity (35 ppt). Finally, the overall lipid yield (dry weight x percentage lipid content) was significantly higher in 5 and 0 ppt salinity levels with 0.12 g/ l lipid yield (Fig. 3).

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