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Microalgae as Primary Producers for Fry Feeding of Some Marine Fishes

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The marine microalgae *Chlorella salina* and *Nannochloropsis salina* were batch-wise grown under different concentrations of fermentation liquor waste ranged from 0.5 to 2.0%. The aim was to identify the best concentration of waste that could enhance the maximum cell mass as compared to basal medium. In addition studying environmental living conditions to stimulate the growth of the tested algae. The maximum growth of *Chlorella salina* and *Nannochloropsis salina* were obtained in 1.0 and 1.75% of fermentation liquor, at temperature 25 and 30°C respectively, with light intensity 1500 Lux and under salinity concentration 30%. In order to get the maximum protein content and the optimum level of essential polyunsaturated fatty acids. The *Chlorella salina* was recorded the highest value of protein; on the other hand, *Nannochloropsis salina* was recorded highest values of polyunsaturated fatty acids (parinaric acid, eicosapentaenoic acid and docosahexaenoic acid).

Key words: Microalgae; Growth; Protein; Polyunsaturated fatty.

Marine microalgae are mainly the floating microscopic plant components of the sea water which from the basic food of almost all the larval organisms, either crustaceans, molluscs or fishes. They are primary producers of any aquatic ecosystem and they belong to various classes of algae[1]. The important components of microalgae are Diatoms, Dinoflagellates, silicoflagellates, coccolithophores, blue green algae and the nannoplankters. Among these the diatoms and phytoflagellates are the significant organisms since, they form the primary link in the food chain of the aquatic system[2].

Green algae do have some beneficial aspects, some are important members of the aquatic food web, particularly in fresh water, and are important oxygenators of such waters. Some such as *Chlorella* has been utilized experimentally as a source of human food and in the future, these algae may become a major food source to man[3]. There has been renewed interest in producing single cell protein (SCP) by mass culturing of the unicellular algae such as *Chlorella* by man and fishes[4,5].

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Recycling waste material not minimize the problem of pollution but also is used in the production of single cell protein, with a view to the increasing universal problem of food shortage. The optimization of culture conditions is importance for the development of any biotreatment process for removing contamination[6].

Marine unicellular algae are used also as primary food source that supplied essential fatty acids as for example, the polyunsaturated fatty acids of the Omega-3 group which have recently been recognized as important component in the diet of cultivated marine organisms[1].

The aim of the present work is therefore to study the principle growth feature of the two microscopic green algae. Also, investigating how valid to use and recycle such wastes for treatment water pollution for producing edible cell protein, for evaluating the nutritional value of some fatty acids synthesized by the two algae.

MATERIALS AND METHODS

Experimental Organisms and Culture Conditions

The unicellular green algae *Chlorella salina* and *Nannochloropsis salina* were obtained from the Institute of Oceanography and Fisheries in Alexandria (ARE).

All algal materials used for experimental studies were axenic. Both of them were grownaxenically in enriched seawater medium as described by Boussiba[7].

Chlorella salina and *Nannochloropsis salina* were cultivated on the industrial waste namely Fermentation Liquor, obtained from the Starch and Yeast Company at Alexandria. Different waste concentrations were prepared by using the natural seawater to get the following concentrations: 0.5, 1, 1.25, 1.5, 1.75 and 2%. These waste concentrations were chosen according to preliminary experiments. Three set of triplicate cultures of two micro-algae were grown in the suitable concentration of fermentation liquor medium. The first set was cultivated under different light intensities:300, 600, 900, 1200, 1500 and 1800 Lux. For the second set, was cultivated under different salinities 10, 20, 30, 35 and 45%. The third set was cultivated under temperatures: 25, 30, 35°C, in order to obtain the optimization growth condition of the two organisms.

The last experiment, the organisms were cultured in big tanks for thirteen days under the optimum growth conditions and suitable fermentation liquor concentration for biochemical analysis.

Growth Evaluation

Cell growth was measured by daily counting cell number under a microscopic using a haemacytometer.

Estimation of some essential polyunsaturated fatty acids

Algal lipid was extracted using n-hexane[8]. The crude lipid extract was esterified according to Radwan[9], and analyzed by a Shimadzu gas liquid chromatography (GLC), equipped with a flame ionization detector with packing column material HP-5. The carrier gas was nitrogen and short speed was 5mm/min.

Identification of fatty acids was carried out by comparing their retention times with those of standards.

Protein was estimated according to the method described by Hartree[10], which is a modification of the original folin-phenol method of Lowey *et al*,(1951).

Estimation of total free amino acids

The total free amino acids were estimated according to the method described by Ya and Tunekazu[11].

RESULTS

Growth data of *Chlorella salina* and *Nannochloropsis salina* are presented in Figures (1,2) respectively, showed the effect of different concentrations ranging from 0.5-2% of fermentation liquor on the growth of two microalgae as compared to the basal medium. The results recorded that by decreasing or increasing the concentration of the waste less or more than (1.0 and 1.75%), decreasing the growth of the *Chlorella salina* and *Nannochloropsis salina* respectively. This means that 1.0 and 1.75% concentrations of fermentation liquor were the most suitable concentrations for the maximum growth of *Chlorella salina* and *Nannochloropsis salina* respectively. The organisms recycled itself (3.5 and 2.8 times), of *Chlorella salina* and *Nannochloropsis salina* respectively, and the

Table1. Chemical constitutes of the *Chlorella salina* and *Nannochloropsissalina*under the optimum growth conditions

Parameter	*Chlorella salina*		*Nannochloropsissalina*	
	Basal	1.0% Waste	Basal	1.75% Waste
Protein (mg/ml)	0.224	0.254	0.225	0.335
Amino acid (µg/gm f.W.)	660	943	635	1121
Fatty acids (%)				
(C18:4)	0.030	0.230	1.100	1.540
(C20:5)	0.075	2.370	3.000	4.480
(C22:6)	2.040	2.500	0.140	4.000
Total Fatty acids	2.145	5.100	4.240	10.020

generation time was 2.0 days for two algae. In addition, the maximum growth rate of the studied algae observed at thirteen days.

Effect of temperatures on the growth of the tested algae

According to figures 3 and 4 results of culturing *Chlorella salina* and *Nannochloropsis salina* respectively, for thirteen days at different temperature (25, 30, 35°C) in the basal medium and the best concentration of fermentation liquor for each algae. These data show that the most suitable temperature for growth of *Chlorella salina* is 25°C and 30°C for the *Nannochloropsis salina.*

Effect of light intensities on the growth of the tested algae

Data obtained for the experiments concerning the effect of different light intensities (300, 600, 900, 1200, 1500 and 1800 Lux) on the growth of the tested microalgae for thirteen days are recorded in figures 5 and 6. These showed that increasing light intensities from 300 Lux, the growth parameter recorded increased gradually till light intensity of 1500 Lux where the parameter of growth was the best of all.

Effect of salinity grades on the growth of the tested algae

According to figures 7 and 8 results of culturing *Chlorella salina* and *Nannochloropsis salina* respectively, for thirteen days under different salinities (10, 20, 30, 35 and 45%). These data clearly showed that the growth rate was the best at salinity 30% in the both tested algae. The number of cells, growth rate, generation time and recycling number in case of basal medium were 11.30 x10^6, 0.48, 2.20 and 3.20 respectively, and in case of waste were 12.10 x10^6, 0.49, 2.00 and 3.50 respectively, to *Chlorella salina*. The same parameters in basal medium 11.80 x10^6, 0.48, 2.00 and 3.50 respectively, and in fermentation liquor waste were 13.40x10^6, 0.51, 1.90 and 3.70 respectively, to *Nannochloropsis salina.*

Some chemical constitutes of the tested algae

Results are recorded in table1. These experiment was carried out under the optimum

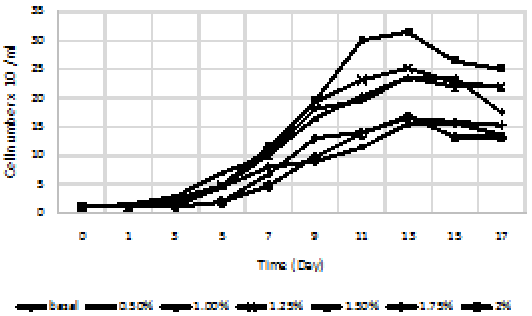

Fig. 1. Growth curves of *Chlorella salina*under different concentrations of fermentation liquorand basal medium

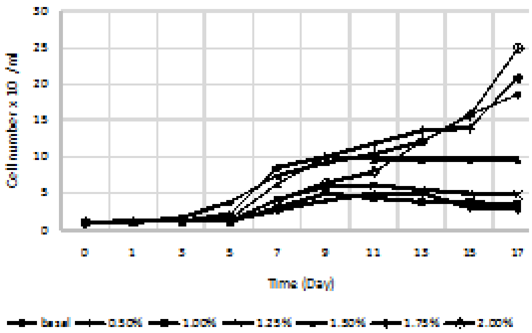

Fig. 2. Growth curves of *Nannochloropsissalina*under different concentrations of fermentation liquorand basal medium

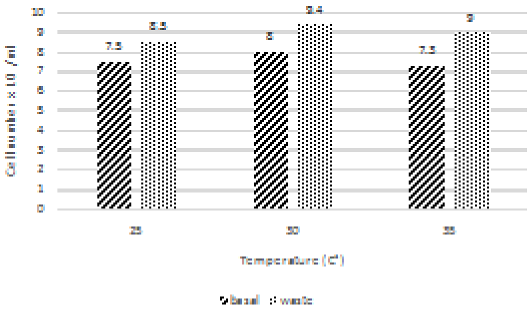

Fig. 3. Effect of different temperatures on the growth of *Chlorella salina*

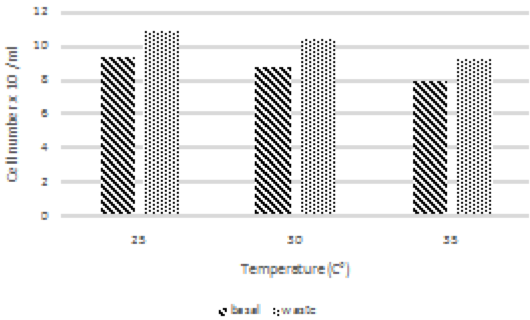

Fig. 4. Effect of different temperatures on the growth of *Nannochloropsissalina*

growth conditions and in the best concentration of fermentation liquor at 1.0% in case of *Chlorella salina* and 1.75% in *Nannochloropsis salina.* The total protein content in case of waste is higher than in basal medium in both tested algae. It increased by 1.48 times in *Nannochloropsis salina.* The free amino acids have the same trend of protein, in *Nannochloropsis* the amino acid is 1121µg/gm fresh weight cultivated in waste medium, while it is 635 µg/gm fresh weight cultivated in basal medium i.e. nearly of the half content. From the retention times recorded in the chromatographs, it appears that the quantity of the three more essential fatty acids (C18:4) parinaric acid, (C20:5) eicosapentaenoic acid and (C22:6) docosahexaenoic acid differed greatly in the two organism whether in the basal medium or in the waste. However the quantity of these fatty acids were greater in organisms cultured in the waste rather than in the basal medium. More over the quantity of these acids were higher in *Nannochloropsis salina* than in *Chlorella salina* whether in the waste or in the basal medium. The quantity of the C20:5 and C22:6 in the *Chlorella salina* were 2.3 fold compared to basal medium while in *Nannochloropsis salina* it increased 2.7 times more than the basal medium. In the waste, *Nannochloropsis salina* contained two times higher of these two acids rather than that of *Chlorella salina.* At the same time in the basal medium, *Nannochloropsis salina* contained 1.5 times higher than that of *Chlorella salina,* which indicate that *Nannochloropsi ssalina* contained eicosapentaenoic acid and docosahexaenoic acid more than *Chlorella salina,* which could be used as a diet for larvae of fishes.

DISCUSSION

The importance of microalgae as the essential food of almost all the larval forms of fishes. However, the isolation, maintenance and mass culture of these organisms are a pre-requisite in the hatchery system throughout the world. Green algae do have some beneficial aspects since, the unicellular and colonial forms of these algae are

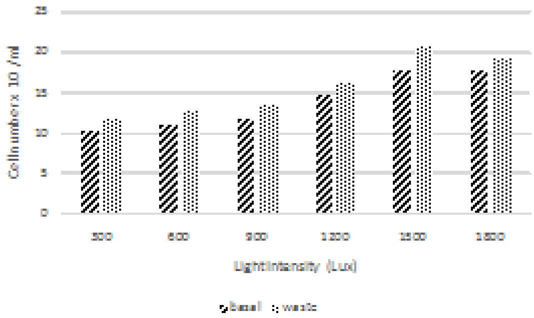

Fig. 5 . Effect of different light intensities on the growth of *Chlorella salina*

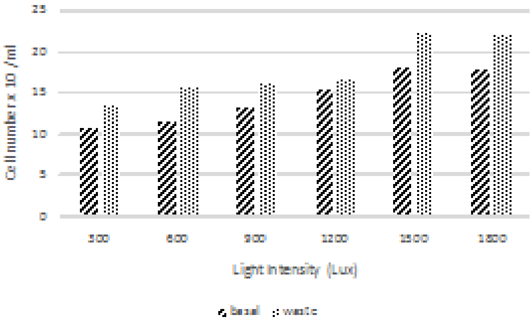

Fig. 6. Effect of different light intensities on the growth of *Nannochloropsissalina*

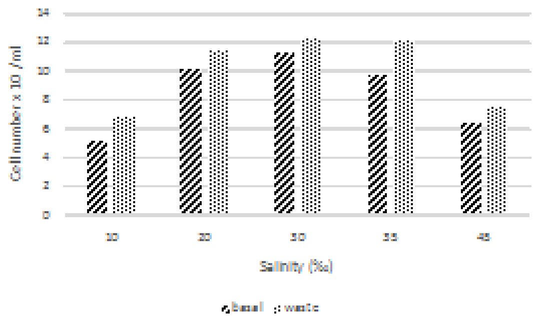

Fig. 7. Effect of different salinities on the growth of *Chlorella salina*

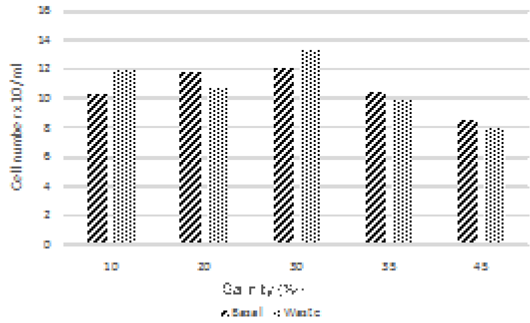

Fig. 8. Effect of different salinities on the growth of *Nannochloropsissalina*

members of aquatic food web beside they are also important oxygenators of the aquatic medium. Some such as *Chlorella* and *Nannochloropsis* have been utilized experimentally as a source of human food[3].

Growth parameter of *Chlorella* and *Nannochloropsis* cultured on different concentrations of the waste used (fermentation liquor) gave different results compared to the basal medium. Fermentation liquor as a supplemental medium for growth of *Chlorella* and *Nannochloropsis* gave best results compared to basal medium. Generation time for the basal medium after thirteen days was 2.5 days for both organisms. The same results were nearly obtained for *Nannochloropsis salina* at concentration 1.75% of fermentation liquor. However, for *Chlorella salina*, generation time reached 2.0 days at concentration 1.0% of waste indicating that fermentation liquor is more suitable for the growth of the two organisms rather than basal medium.

These algal cultures made of waste media saved the chemicals used in basal medium and decrease of lag phase in the presence of the organic waste is recorded, moreover, the increase of the growth yield of the organisms. The same results were obtained for *Dunaliella salina*[12,13].

The results of this study show the more suitable temperature, light intensity and salinity that could be considered as the most favorable ones for growth of the two organisms. Both organisms when cultured under different light intensities gave the maximum growth rate at light intensity of 1500 Lux, However, increasing light intensity more than this limit caused reduction in the growth. Many author[14,15,16] reported a similar result. It is clear that the best growth were obtained at moderate temperatures i.e. between 25°C and 30°C. However, the two organisms differed from each other. In this aspect, the optimum temperature for growth was 25°C for *Chlorella salina* and 30°C for *Nannochloropsis salina*. It was found by many authors that temperature above or less than 30°C may have a lethal effect on some algal species.[17,18,19]

Concerning the results obtained for the effect of different salinities on the growth of the studied algae. Both t he*Chlorella salina* and *Nannochloropsis salina* reached its maximal growth at 30‰. Similar results were also obtained by[20,21].

The total protein synthesized in *Chlorella salina* cultured in waste medium increased 1.5 times compared to the basal medium. In*addition*, the free amino acids were higher in the waste medium than in the basal medium, the ratio was 1:1.76 for the basal and waste medium respectively.

The same results were obtained for *Nannochloropsis salina,* where the ratio between the total protein in both basal and waste medium was 1: 1.13, while for amino acids it was 1: 1.42. It appear that *Chlorella salina* synthesized protein more than *Nannochloropsis salina* whether in the basal medium or in the waste. Wong and Ho[22], reported that *Chlorella* is efficient enough to convert nitrogen to protein and the produced protein can be highly used as food.

The polyunsaturated fatty acids of the omega-3 group, which were considered as an important component in the diet of cultivated marine organisms, were analyzed in both *Chlorella salina* and *Nannochloropsis salina* under optimal conditions of waste, salinity, temperature and light intensity. The quantity of these fatty acids were greater in two algae cultured in the waste medium rather than in the basal medium. Although protein was high in *Chlorella salina* than *Nannochloropsis salina.* These polyunsaturated fatty acids were high in *Nannochloropsis salina* than *Chlorella salina* under the same culture conditions. The quantity of eicospentaenoic acid (C20:5) and docosahexaenoic acid (C22:6) in *Chlorella salina* was 2.3 fold compared to the basal medium while in *Nannochloropsis salina* it was 2.7 times than the basal medium. Many authors concluded that marine unicellular algae could be regarded as a primary food source that supplies the essential polyunsaturated fatty acids; since fish cannot synthesize these polyunsaturated fatty acids[23,24,25].

Since *Chlorella salina* contained more protein than *Nannochloropsis salina* and at the same time *Nannochloropsis salina* contained the essential polyunsaturated fatty acids higher than *Chlorella salina*. We can suggest that for obtaining more valuable crops of fish a combination of the two algae could be applied as a diet of fishes for protein and polyunsaturated fatty acids supply.

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