ISSN No.: 2454- 2024 (online)

International Journal of Technical Research & Science

INVESTIGATION THE GROWTH OF GREEN ALGAE NANNOCHLOROPSIS SP. ON DISTILLERY EFFLUENT AND ITS PHYSICO-CHEMICAL PROPERTIES ANALYSIS

Su Win Aye[1], Phyu Phyu Win[1]
E-mail id: suwinaye5@gmail.com
[1]Applied Chemistry Research Department, Department of Research and Innovation (DRI), Ministry of Education, Myanmar
[2]Chemical Technology Research Department, Department of Research and Innovation (DRI), Ministry of Education, Myanmar

Abstract- The wastewater effluent from distillery industries directly discharges to the natural environment which causes various adverse effects on soils, water bodies, air and health. The aim of the current study was to cultivate Nannochloropsis sp. microalgae in wastewater originate from distillery factory and analyze for physico-chemical parameters such as reduction of COD, removal of total phosphorous, total nitrate and at the same time the production of biomass which could be extracted for total lipid content. At the end of treatment, Nannochloropsis sp. reduced 94.17% of COD, 82.43% of removal of total phosphorous and 87.15% of removal of total nitrate. The production of biomass yield was 3.6 gL^{-1} and the total lipid content was extracted about 24.74% of the biomass dry weight. Therefore, microalgae culture offers an effective solution for wastewater treatments, because they provide a tertiary bio-treatment coupled with the production of potentially valuable biomass, which can be used for different purposes. It is also strongly a powerful technique for the removal of pollutants from wastewater effluent.
Keywords: biomass, effluent, Nannochloropsis sp.,physico-chemical parameters

1. INTRODUCTION

Distillery industries are the agro-based industries with high organic and inorganic contains which are high strength based and difficult to disposed. Now a day's distillery industry is the major source of water pollution in our country. Basically distillery industry is related with fermentation industry which is a biological process in which micro-organism to produce different types of alcoholic products. If distillery industry uses fermented material then its wastes contain large amount of organic and inorganic when it mixes with water then it causes water pollution. The waste which is obtained from distillery industry when it mix with water then it will have extremely high BOD value and high COD, high chlorides and sulphates, dissolved solids, brown yellowish colour with bad odour[1].
In economic capital of Myanmar, Yangon, nearly about 3500 factories including brewery and distillery present which releases a thousand liter of waste, such huge amount of waste will cause serious hazard to environment, hence treatment of distillery waste should be essential. Among them, one of the distillery factory located in Shwe Pyi Thar Industrial zone (1), Yangon used water 40,000- 50,000 gallons/ 24 hours. In this factory, total yield of distillery was produced 8,000 gallons/ 24 hours and total amount of wastewater was released 30,000 gallons/ 24 hours in 2014-2015. The chemical oxygen demand (COD) of discharge was about 12864 mgL^{-1} in this distillery factory. The results of chemical oxygen demand (COD) and other indirect measurement of the amount of organic compounds in wastewater showed levels higher than the standard specifications. The higher the level of organic compounds, the higher the amount of oxygen microbes require to break them down and the higher the chance of damage to the surrounding ecosystem.
Wastewater is any water that discharging from domestic residences and industrial effluent that containing poisons solids. There are numerous processes that can be used to clean up waste waters depending on the type and extent of contamination. Most wastewater is treated in industrial-scale wastewater treatment plants (WWTPs) which may include physical, chemical and biological treatment processes[2].
Recently, algae have become significant organism for biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic and inorganic toxic substances and radioactive matters in their cells/bodies[3]. Biological wastewater treatment systems with micro algae have particularly gained importance in last 50 years and it is now widely accepted that algal wastewater treatment systems are as effective as conventional treatment systems. These specific features have made algal wastewaters treatment systems and

significant low-cost alternatives to complex expensive treatment systems particularly for purification of municipal wastewater[4].

Wastewater treatment which is applied to improve or upgrade the quality of a wastewater involves physical, chemical and biological processes in primary, secondary or tertiary stages. It is well known that algae have an important role in self-purification of organic pollution in natural waters. Moreover, many studies revealed that algae remove nutrients especially nitrogen and phosphorus, heavy metals, pesticides, organic and inorganic toxins, pathogens from surrounding water by accumulating and/or using them in their cells[5]. Microalgae feed on inorganic nutrients and several species are capable of removing nitrogen and phosphorus from the effluents[6].

Nannochloropsis species represents a genus of marine microalgae with high photosynthetic efficiency and can convert carbon dioxide to storage lipids. The use of algal species (Nannochloropsis sp.) in wastewater treatment in aquaculture could offer the combined advantage of removing nutrients and biomass production at the same time providing an interesting opportunity[7]. The potential environmental benefits of microalgae, such as CO_2 mitigation and bioremediation of waste water by removing large amounts of nutrients and heavy metals, have attracted much attention recently. The cultivation of Nannochloropsis in waste water for nitrogen utilization as well as flue gas for CO_2 supply has also been investigated in several studies. These environmentally beneficial applications may be coupled with lipid production, which represents a possible direction toward cost-effective production of lipids by Nannochloropsis[8].

2. RESEARCH MATERIALS AND METHODS

2.1 Microalgae Strain and Cultivation

The species selected for cultivation was a strain of Nannochloropsis sp. purchased from Department of Fisheries, Ministry of Agriculture, Livestock and Irrigation, Myanmar.

The Nannochloropsis sp. microalgae was inoculated in a 10 liters glass bottle containing standard f/2 medium (pH = 7). The cultures were maintained at room temperature (25- 27ºC) on a fluorescent light with a light: dark photoperiod of 12 h: 12 h for 12 days cultivation. Sterile air was aerated into the glass bottle through an air sparger at the bottom of the glass.

2.2 Sampling and Analysis of Distillery Effluent

The distillery effluent samples were collected from Shwe Pyi Thar Industrial zone (1) located in Yangon, Myanmar. Sample of distillery effluent was collected in a clean glass or plastic container (the lid, seal and bottle was rinsed with boiling water before use) and stored below 4°C in order to any contamination before perform in this experiment. The standard procedure for physico- chemical properties of sample effluent was used to analysis the reduction of chemical oxygen demand (COD), biological oxygen demand (BOD), removal of nutrients, total suspended solids (TSS), total dissolved solids (TDS), metal ions and pH.

2.3 Determination of Microalgae Growth

The Nannochloropsis sp. culture was not grown in the original distillery effluent in this experiment. Therefore, firstly, the effluent samples were experimented to different sets using tap water; (1:1), (1:2), (1:3); (effluent: tap water) respectively, mixed the mixture of microalgae to observe the microalgae growth. From all experiments proved that the cultivation of microalgae inside the diluted effluent (1:3) more efficient than other culture medium. Then the Nannochloropsis sp. was cultured with (1:3) dilution of effluent after adjusting pH 7 to 7.4 and cultivated in 2 liters flask bottles with necessary nutrients especially nitrate and phosphate by adding as standard f/2 medium ratio for 12 days treatment. All of the treatments were cultured at room temperature (25±2 °C), continuously aerated with air mixture under fluorescent light with an intensity of 100, 300 μmol photons/m^2 /s and 12: 12 (day: night) photoperiod. All the experiments samples were prepared in triplicates in this study.

2.4 Physico-Chemical Characteristics of Distillery Effluent

All the following parameters were determined in distillery effluent by Nannochloropsis sp. during the treatment time (12 days).

COD and BOD were determined according to the Standard Methods for the Examination of water and wastewater.

Total phosphorus was determined by phosphate testing kit HI713 in order to the adaption of the standard methods for the examination of water and wastewater (20^{th} edition), ascorbic acid method by HANNA Instruments, Romania. And total nitrate was performed by adaption of the cadmium reduction method by using nitrate testing kit HI96786 by HANNA Instruments, Romania.

The total suspended solids (TSS) and total dissolved solids (TDS) were analysed by the Standard Methods for the Examination of water and wastewater. Then, the metal ions (Mg, K, Fe, Zn, Cu and Mn) were examined standard method of physical and chemical for water used in industry by using atomic absorption spectroscopy (AAS400).

2.5 Microalgae Harvesting

After 12 day cultivation and treatment, the microalgae were harvested by centrifugation method and collected the pellet microalgae biomass and then stored at refrigerator.

2.6 Measurement of Microalgae Biomass

Samples for biomass analysis were taken in the start of the trial, at 2, 4, 6, 8, 10 and 12 days after the start of the experiment. The biomass of microalgae cells were calculated based on the difference between weights and expressed the dry weight unit in gL^{-1}.

2.7 Extraction of Total Lipid Content

The total lipids were extracted according to the Bigogno's method [9].

3. RESULTS AND DISCUSSION

3.1 Characteristics of Original Distillery Effluent

Some characteristics of original distillery effluent were as follow. The TDS of effluent was 7876 mgL^{-1} and the TSS value was 1112 mgL^{-1}. The colour of the effluent was brown yellowish and the odour was offensive. The concentration of total nitrate of effluent was about 284.02 mgL^{-1} whereas the concentration of total phosphorous was 17.52 mgL^{-1} in original effluent. The level of COD and BOD of effluent were 12864 mgL^{-1} and 6193 mgL^{-1} respectively. Measurement of physico- chemical characteristics of effluent showed higher level than the standard specifications. Excessive amount of waste effluent which is not properly treated and directly drained out to water sources which hazardously affecting the quality of water hence to overcome this problem treatment of waste effluent from distillery industry must be required. Moreover, the composition of metal ions such as Mg, K, Fe, Zn, Cu and Mn of effluent were 351.52, 216.32, 9.20, 4.41, 0.109 and 3.03 mgL^{-1} respectively. And the pH value of effluent was 3- 4.

3.2 Elimination of Chemical Oxygen Demand (Cod)

The elimination of COD from distillery effluent was tested by adding sufficient nutrients: (1) effluent adding extra nitrate (2) effluent adding extra phosphate (3) effluent adding extra nitrate and phosphate at the start day of the treatment by using Nannochloropsis sp. microalgae to the diluted medium (1:3). By adding extra nitrate, Nannochloropsis sp. could eliminate 68.75% of COD in effluent after 6 day treatment, phosphate was 73.09% and nitrate and phosphate was 67.25% respectively. So the elimination of COD was efficiently declined with adding phosphate nutrient during 6 days treatment. Then, after 12 days treatment by Nannochloropsis sp., the elimination of COD by adding nitrate was 93.25%, phosphate was 94.17% and nitrate and phosphate was 93.37% respectively. Treatment of effluent in the current experiment by microalgae Nannochloropsis sp. induced significant reduction in COD with increasing treatment time. Therefore, Nannochloropsis sp. could eliminate COD by adding extra phosphate nutrient more than nitrate nutrient showed in Figure.3.1.

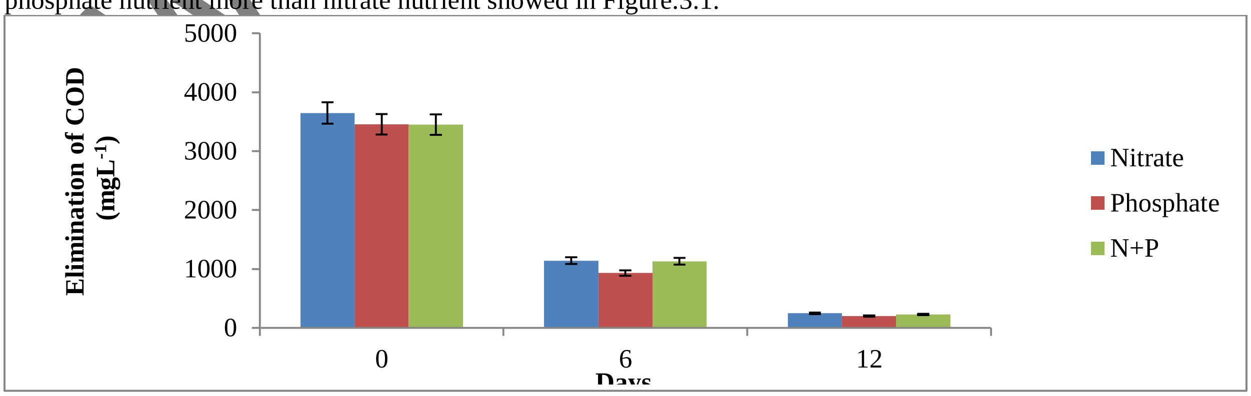

Fig.3.1. Elimination of COD by Nannochloropsis sp. after 12 Day Treatment

3.3 Removal of Total Phosphorous

The concentration of total phosphorous from effluent was removed by Nannochloropsis sp. demonstrated in Fig. 3.2. The original total phosphorous of effluent was 17.52 mgL^{-1} and so it was showed that Nannochloropsis sp. microalgae slightly consume the nutrients from the effluent. After 6 days cultivation, total phosphorous removal was slightly increased in all supplement nutrients respectively. The last 12 day treatment, the concentration of total phosphorous in effluent was 82.43% in nitrate, 25.26% in phosphate and 46.87% in nitrate and phosphate nutrients. The maximum removal of total phosphorous concentration was 82.43% at extra nitrate nutrient supplement.

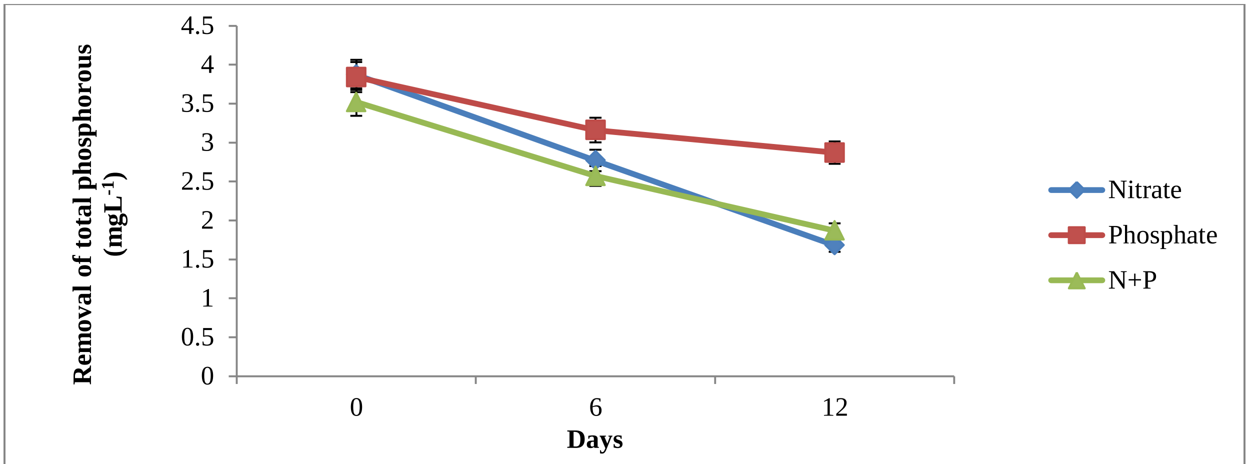

Fig.3.2 Removal of Total Phosphorous by Nannochloropsis sp. After 12 Day Treatment

3.4 Removal of Total Nitrogen

Removal of total nitrogen from distillery effluent by Nannochloropsis sp. treatment in nitrate adding medium was removed 85.36% from the media, 89.47% in phosphate adding medium and 87.15% in nitrate and phosphate adding medium respectively at the end of treatment. The Figure.3.3 proved to be highly efficient in removing total nitrogen in all supplement nutrients at very short period of time. The concentration of total nitrogen by adding nitrate and phosphate nutrient was nearly similar to by adding phosphate nutrient, the highest percentage of removal of total nitrogen from the media.

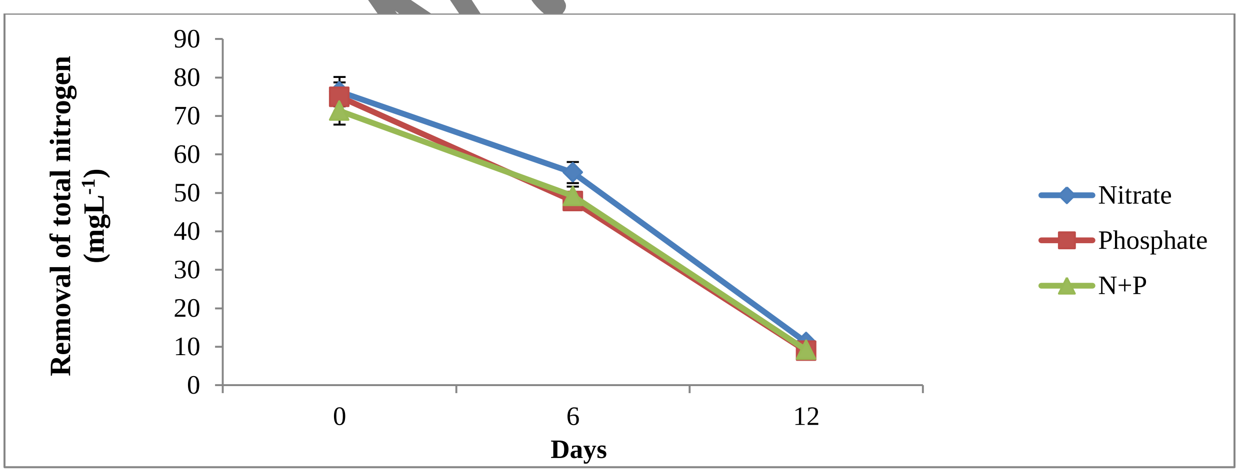

Fig.3.3 Removal of Total Nitrogen by Nannochloropsis sp. After 12 Day Treatment

3.5 Measurement of Microalgae Biomass

Nannochloropsis sp. was cultivated in 2 liters of bottle flasks (1:3 dilutions) of effluent medium by adding extra nutrients at the start of the treatment. The growth curve of the Nannochloropsis sp. in effluent medium was demonstrated in Figure.3.4. Results showed that the growth of Nannochloropsis sp. in phosphate adding medium was higher growth rate than in nitrate adding medium at the exponential phase. At the twelfth day of cultivation, the highest biomass yield of Nannochloropsis sp. with phosphate approached to 3.6 gL^{-1} whereas 3.2 gL^{-1} in nitrate and phosphate medium respectively. And the biomass of microalgae in nitrate medium reached nearly 2.8 gL^{-1}.

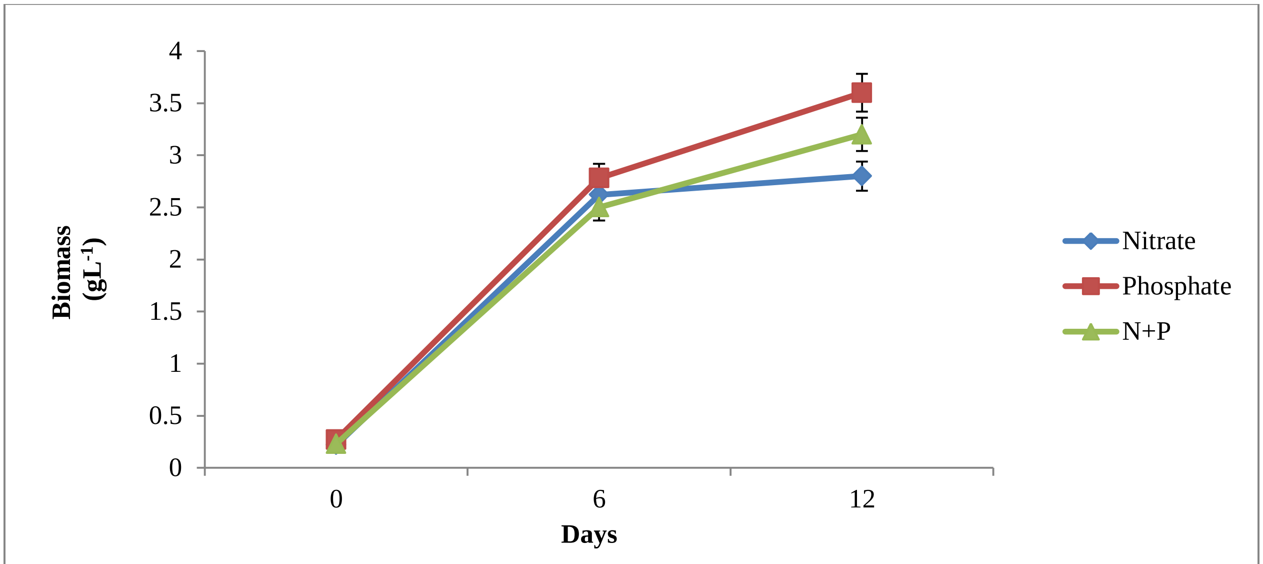

Fig.3.4 Biomass Determination by Nannochloropsis sp. After 12 Day Treatment

3.6 Extraction of Total Lipid Content

In Fig. 3.5. show that the total lipid contents for the microalgae in f/2 culture medium in this study was 25.05% of the dry weight .The lipid productivity for Nannochloropsis sp. in effluent by adding extra nitrate was 24.74%. It was showed that lipid content was nearly the same under nitrate addition condition and f/2 culture medium. Moreover, effluent adding extra nitrate and phosphate was demonstrated 20.01% of lipid content and 21.03% of total lipid extracted from adding extra phosphate in effluent by Nannochloropsis sp. after treatment. Nannochloropsis represents a genus of marine microalgae with high photosynthetic efficiency and can convert carbon dioxide to storage lipids mainly in the form of triacylglycerols.

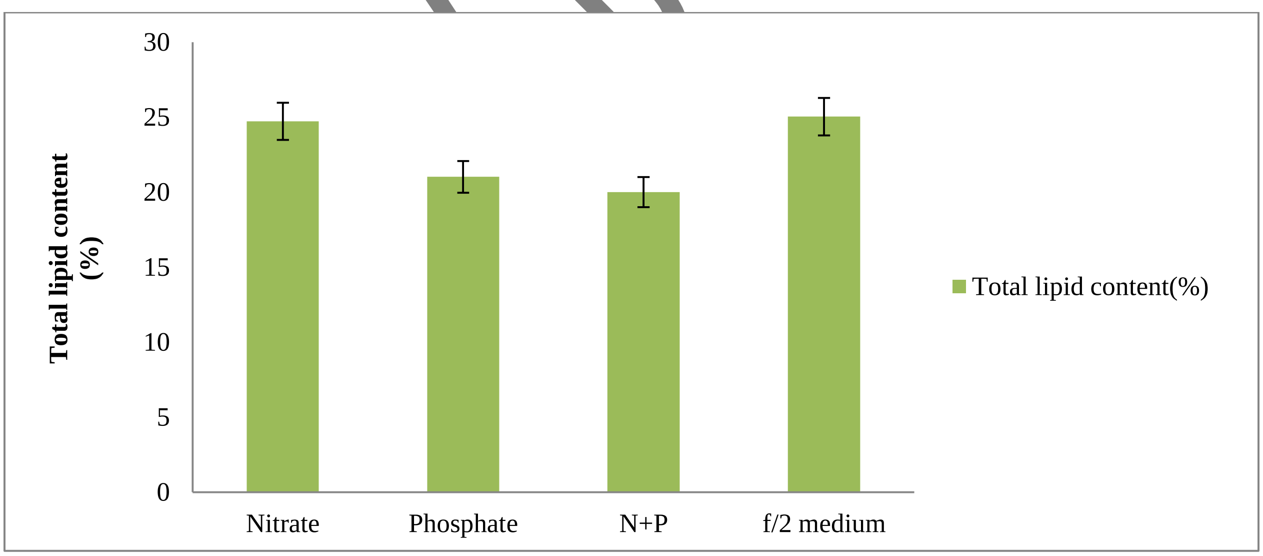

Fig. 3.5 Total Lipid Extraction by Nannochloropsis sp. After 12 Day Treatment

Some parameters of the treated effluent by Nannochloropsis sp. after 12 day cultivation with extra nutrients were seen in Table 3.1. The elimination of BOD from original effluent was above 97% in all nutrient supplements. And the pH value of the treated effluent was about 8. Therefore, the treated effluent from distillery factory could discharge safely to the environment.

Table-3.1 Physico-Chemical Characteristics of Distillery Effluent by Nannochloropsis sp. After 12 day Treatment

PARAMETERS	EFFLUENT	AFTER TREATMENT		
		NITRATE	PHOSPHATE	N+P
Colour	**Brown Yellowish**	**Yellow**	**Yellow**	**Yellow**
TDS (mgL^{-1})	7876	2580	2308	2670
TSS (mgL^{-1})	1112	663	694	604
BOD (mgL^{-1})	6193	13.70	14.90	10.80
Mg (mgL^{-1})	351.52	25.80	32.85	20.00
K (mgL^{-1})	216.32	86.60	136.70	68.90
Fe (mgL^{-1})	9.20	0.496	0.697	2.03
Zn (mgL^{-1})	4.41	0.326	0.579	0.500
Cu (mgL^{-1})	0.109	0.033	0.042	0.040
Mn (mgL^{-1})	3.03	0.040	0.102	0.0498
pH	3-4	8-9	8-9	8-9

CONCLUSION

In this experiment, the effluent from distillery factory was treated by Nannochloropsis sp. microalgae showed that the elimination of COD was 94.17% at phosphate adding medium. And the removal of total phosphorous and total nitrate from original effluent were 82.43% in nitrate adding medium and 87.15% in nitrate and phosphate adding medium respectively at the end of the treatment. At the twelfth day of cultivation, the highest biomass yield of Nannochloropsis sp. with phosphate adding medium approached to 3.6 g/L and the total lipid content for nitrate adding medium was extracted 24.74% of the dry weight. Therefore, Nannochloropsis sp. microalgae could contribute to wastewater effluent treatment and deposition with sufficient nutrients using distillery effluent as a medium.

ACKNOWLEDGMENT

Thanks are due to Director General of Department of Research and Innovation from Ministry of Education, Myanmar for providing research facility.

REFERENCES

[1] Atul Thakkar , Chemical Study on Distillery Effluent to Assess Pollution Load, International Journal on Emerging Technologies 4(2): 121-123, 2013.

[2] Sara AL-Rajhia, Nitin Raut, Fatma AL-Qasmi, Mariam Qasmi and Amal Al Saadi , Treatments of Industrials Wastewater by Using Microalgae, International Conference on Environmental, Biomedical and Biotechnology IPCBEE vol.41, 2012.

[3] Alp MT, Ozbay O, Sungur M.A. Determination of Heavy Metal Levels in Sediment and Macroalgae (Ulva sp. and Enteromorpha sp.) on the Mersin Coast 2011, 47- 55 , 2012.

[4] Palmer, C.M. A composite rating of algae tolerating organic pollution. J. Phycology, 78-82, 1969.

[5] Bulent Sen, Mehmet Tahir Alp, Feray Sonmez, Mehmet Ali Turan Kocer and Ozgur Canpolat, Relationship of Algae to Water Pollution and Waste Water Treatment, Chapter 14, 2013.

[6] Louise Lins de Sousa, Dominique Silveira da Hora, Emerson Andrade Sales and Louisa Wessels Perelo, Cultivation of Nannochloropsis sp. in Brackish Groundwater Supplemented With Municipal Wastewater as a Nutrient Source, Vol.57, n.2: pp. 171-177, March-April 2014.

[7] Sirakov, I. N. and K. N. Velichkova, Bioremediation of wastewater originate from aquaculture and biomass production from microalgae species -Nannochloropsis oculata and Tetraselmis chuii. Bulg. J. Agric. Sci., 20: 66-72, 2014.

[8] Xiao-Nian Ma, Tian-Peng Chen, Bo Yang, Jin Liu and Feng Chen, Lipid Production from Nannochloropsis, 2016.

[9] Bigogno C, Khozin-Goldberg I, Boussiba S, Vonshak A, Cohen Z, Lipid and fatty acid composition of the green oleaginous alga Parietochloris incisa, the richest plant source of arachidonic acid. Phytochemistry 60: 497–503. 2002.