Iranian Journal of Fisheries Sciences 19(4) 1780-1794 2020
DOI: 10.22092/ijfs.2018.117674

Bioflocculation technique for microalgal harvesting and wastewater nutrient recovery

Madkour A.G.M.E.[1*]; Ibrahim H.A.H.[2]; El-Sayed W.M.M.[3]; El-Moselhy K.M.[1]

Received: June 2017 Accepted: December 2017

Abstract

Fungal assist microalgal cultivation is getting substantial considerations due to the highly efficiency of bioflocculation process without demanded of using chemicals and little bit energy input. Therefore, Fungi pelletization-assisted microalgal cultivation were applied for improving algal harvesting process and nutrient recovery as step wise for wastewater treatment. Two strains of marine microalgae (*Nannochloropsis salina* and *Chlorella salina*) and three species of marine fungi (*Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus fumigatus*) were implied to investigate the effect of fungi-algae pelletization process on wastewater recovery. After 48 hour of cultivation, the highest flocculation efficiency (98.9%) was recorded by *A. fumigatus* assist *C. salina*, while the lowest percentage (85.9%) recorded by *A. niger* assist *N. Salina*. The highest nutrient removal percentage was for nitrite by *A. niger* assist *C. salina* /tap water (98.4%) followed by phosphate *A. fumigates* assist *N. salina*/sea and tap water (90.9%), then ammonia by *A. fumigates* assist *N. salina*/seawater (89.5%). The bioflocculation process is a promising for algal harvesting techniques and wastewater nutrient recovery.

Keywords: Wastewater treatment, Fungi/algae assist, Bioflocculation, Nutrient recovery, Microalgal harvesting

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

1-Marine Pollution Department, National Institute of Oceanography and Fisheries, Red Sea, Egypt.

2-Microbiology Department, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

3-Marine Microbiology Department, National Institute of Oceanography and Fisheries, Red Sea, Egypt.

*Corresponding author's Email: ag.madkour@niof.sci.eg

Introduction

Wastewater as a liquid waste discharged from several sources involved human, agriculture and industry activities. It has a wide range of potential biological contaminants and chemical concentrations. The increasing human activities and growing of urban populations results in increased amount of municipal wastewater (Pescod, 1992). To applied microalgae for treatment of wastewater is a traditional solution and many investigators have developed techniques for utilizing the high rate of algal growth and its ability for nutrient recovery. The nutrient recovery is the ability of microalgae for utilizing nutrients presented in wastewater as main carbon and nitrogen sources. Moreover, some nutrients could be removed by volatilization or precipitation due to the high pH produced by the algae. For ideal recovery of nutrients from wastewater, the microalgal strains should be grown healthy under controlling factors (Doran and Boyle, 1979; Hammouda *et al*., 1995). The healthy growth rate of microalgae is induced by physical factors (light and temperature), chemical factors (nutrients and carbon dioxide); and biological factors (controlling of predators) as well as considering of the bioreactor design, mixing and dilution rate (Benemann, 1997; Gultom and Hu, 2013).

On the other side, the flocculation is a technique by which the algae aggregate and formed flocs (a pellet like structures). Under normal condition, algal cells didn't produce flocs due to negative charges on its surfaces (González-Fernández and Ballesteros, 2013; Pires *et al*., 2013). Other microorganisms like bacteria and fungi can induce flocculation process (Lee *et al*., 2013; Muradov *et al*., 2015), specially that have positive charges on its surfaces. Filamentous fungi are promising as bioflocculating agents because it has ability for aggregation and high microalgal trapping efficiencies. Moreover, fungal self-aggregation has investigated for numerous filamentous strains and illustrated by coagulative and non-coagulative mechanisms (Zhang and Hu, 2012; Zhou *et al*., 2013; Xia *et al*., 2014).

Microalgae consider as a promising for biological treatment of wastewater and nutrient recovery (Coward *et al*., 2013; Garg *et al*., 2014). Immobilization techniques have been investigated for enhancing the biological treatment of wastewater (González-Fernández and Ballesteros, 2013). Calcium alginate used for immobilizing microalgae and keep the algal cells viability for long time. Moreover, the formation of alginate beads would rise the cost of the immobilization process (Schenk *et al*., 2008). For previous reasons, the aggregation of fungi and microalgal (bioflocculation process) are a vital process for harvesting of microalgae and nutrient recovery.

However, the exploiting of aggregation of fungi and microalgal cells phenomena and their effect on nutrient recovery is still inapplicable. Therefore, we will try to exploiting the process of fungal pelletization-assist algae (bioflocculation technique) for harvesting algal biomass and investment the pelletized fungi–algae symbiosis system as immobilized cells

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

to treat wastewater by improving nutrient removal.

Materials and methods

Collection of wastewater

The domestic wastewater samples were collected in sterile polyethylene bottles from Hurghada City, Red Sea, Egypt, during summer 2015. Samples were then transported immediately in icebox to the laboratory.

Analytical methods

The wastewater samples were filtered through 0.45 μm cellulose filter paper and analyzed for ammonia, phosphate and nitrite before and after treatment using the standard techniques described by American Public Health Association (Association, 1995). The biochemical composition of biomass were analyzed via standard procedures described in (Pádua et al., 2004); the carbohydrate, protein and lipid contents were estimated by the method of Dubois (Dubois *et al.*, 1956), method of Lowry (Lowry *et al*., 1951) and method of Bligh and Dyer (Bligh and Dyer, 1959), respectively.

Fungal strains

Three marine fungal strains were collected from laboratory of marine microbiology at National institute of Oceanography and fisheries (NIOF), Red Sea Branch, Egypt. The strains were isolated and characterized by the microbiology lab stuff using Lactophenol Cotton Blue techniques (Domsch *et al*., 1980). They were identified as; *Aspergillus niger*, *Penicillium chrysogenum* and *Aspergillus fumigatus*. The harvesting spores were re-cultivation at 25 °C for 5-7 days on potato dextrose agar plates (PDA) containing 20 g L^{-1} glucose. 10 mL of sterile saline solution were used for collection of spores, and used for inoculation.

Preparation of fungal pellets

Solutions of spore suspension (1.5-2.0 $\times 10^7$ spores/l) were growing at 28°C in potato dextrose broth (PDB) at 150 rpm for 72 h (Atlas, 2010). Before mixing with microalgal strains, flocs were washed for three time using sterile saline solution, followed by sterile algal media.

Microalgal strains

Marine microalgal **s**trains (*Nannochloropsis salina* and *Chlorella salina*) were obtained from Aquaculture Laboratory, NIOF Red Sea Branch, Egypt. The microalgae were cultivated on F/2 medium under light/dark at 100 rpm and 25°C, lighting using fluorescent light (50 μmol photons m^{-2} s^{-1}) with a 16 h/8 h light/dark cycle at 25°C (Guillard and Ryther, 1962; Salim *et al*., 2011).

Screening of fungi assist algae for flocculation process

For screening the ability of strains for flocculation process, three marine fungal strains were applied with two marine microalgal strains (Vandamme *et al*., 2015). Fungal flocs grown on 100 ml of PDB were added to 100 ml algal cell suspension (collected by centrifugation at 6,000 g, then washed twice and suspended with sterilized saline solution), and the mixtures were shaken at 100 rpm for 24 h. Optical

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

Density (OD750) were analyzed at zero time and 24 h. The excess of liquid removed using a Pasteur pipette and the pellets washed with sterile saline solution followed by algal medium. All of biologically experiments replicated at least three times. Flocculation efficiency (FE) calculated based on changes in OD and cell numbers of uncaptured algal cells in the co-cultivation media at time 0 and 24 h later according to the following formula:

$$FE\% = \frac{A - B}{A} \times 100$$

Where A=OD and/or cell number at time 0; B=OD and/or cell number after 24 h. The morphology of the fungal and algal cells and co-cultivation pellets observed under bright field conditions using Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany) and images were picked with EC2 digital camera.

Wastewater treatment

Wastewater samples were spin down to eliminate large particles then filtered by Whatman filter paper and autoclaved at 121°C for 1hr. the samples were allowed to cool at room temperature and stored at 4°C for farther work. The concentrations of nitrite, phosphate and ammonia were evaluated. The concentration of other inorganic nitrogen in the wastewater, such as NO_3, was very low and not reported. Wastewater diluted to 50% with tap water and seawater for experiments. The fungal/algal pellets produced on PDB were harvested by filtration, and wet pellets were added to wastewater (approximately, 1 g L^{-1} DW). The mixtures incubated at 150 rpm for 48 h.

Effects of wastewater dilution on treatment process by fungal/algal pellets

Microalgae were grown in the mixture of municipal wastewater and seawater as well as tap water. Their ratios were 25%, 50% and 75% (v/v), respectively. All cultures were stationary culture without aeration.

Results

Pretreated wastewater samples (physically treated wastewater, wastewater diluted with seawater, and wastewater diluted with tap water) used in this study were analyzed for nutrient content (Table 1). The obtained data revealed ranges 0.156-0.174, 27.352 - 53.459 and 11.962 - 13.929 mg L^{-1} for nitrite, phosphate and ammonia, respectively. These results showed that the dilution with seawater and tap water decreased the concentration of each nutrient.

Table 1: Nutrient analysis of wastewater used in the current investigation.

Sample	Nitrite (mg L^{-1})	Phosphate (mg L^{-1})	Ammonia (mg L^{-1})
Wastewater before treatment	0.245	66.734	46.162
Wastewater after physical treatment	0.174	53.459	13.929
Wastewater plus seawater	0.156	27.352	11.962
Wastewater plus tap water	0.159	29.768	12.482

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

In the present study, flocculation efficiency (percentage) was evaluated at 0, 24, and 48 h. The obtained results showed that the efficiency of flocculation increased periodically with time (Table 2). At 24 h, the highest efficiency (83.2%) was recorded by *A. fumigatus* assist *N. salina*, while the lowest efficiency (57%) was observed by *A. niger* assist *C. Salina* assist. While at 48 h, the highest efficiency (98.9%) was in the case of *A. fumigatus assist C. salina*, and the lowest percentage (85.9%) was by *A. niger* assist *N. salina* (Figs. 1, 2).

Table 2: Flocculation efficiency (FE %) achieved by fungi assist microalgal species.

Treatment/Time Fungal and microalgal assist	24 h FE (%)	48 h FE (%)
A. niger assist *N. salina*	59.3	85.9
A. niger assist *C. salina*	57.0	88.8
P. chrysogenum assist *N. salin*	73.6	95.9
P. chrysogenum assist *C. salin*	72.9	95.1
A. fumigates assist *N. salina*	83.2	98.4
A. fumigates assist *C. salina*	82.2	98.9

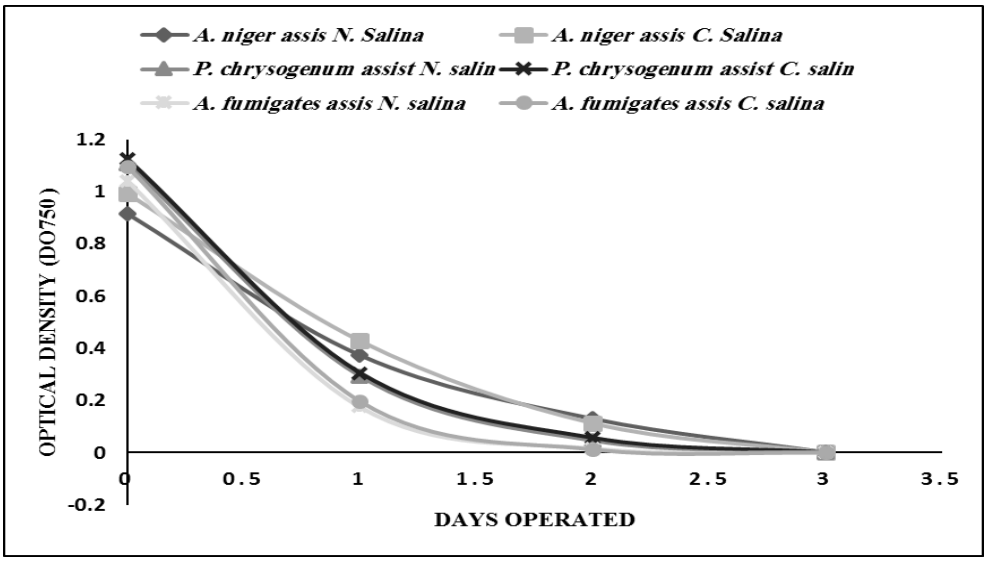

Figure 1: Time course of optical density during flocculation process.

After 48 hour, the removal percentages (%) of nutrients (nitrite, phosphate and ammonia) in different water samples under investigation using different fungi assist microalgae were estimated and data are presented in Fig. 3. The obtained results revealed that the highest removal percentage was for nitrite by *A.niger* assist *C. salina* /tap water (98.4%) followed by phosphate *A. fumigates* assist *N. salina*/sea and tap water (90.9%), then ammonia by *A. fumigates* assist *N. salina* /seawater (89.5%). On the other side, the lowest removal(%) was of phosphate by *P. chrysogenum* assist *N. salin* diluted with sea water (26.2%) followed by Ammonia *A. niger* assist *N. salin*a/tapwater (27.7%), then Nitrite by *P. chrysogenum* assist *N.*

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

salina/tapwater (71.7%). Moreover, the *A. fumigatus* assist *C. salina* and *A. fumigatus* assist *N. salina* assist are considered the most effective biofloc rather than the others. Therefore, removal percentages of nutrients in different diluted wastewaters after treatment using *A. fumigatus* assist *C. salina* and *A. fumigatus* assist *N. salina* assist were furthermore studied.

The concentrations of nutrients in different diluted wastewaters were determined and then the removal percentages (%) of nutrients were calculated after treatment via different fungal and microalgal species assists (Table 3). Data exhibited that the removal of nitrite ranged from 72.7 to 93.4% while the phosphate removal percentages ranged from 72.7 to 98.8% and removal of ammonia ranged from 66.1 to 97.7%. Therefore, the most nutrient removed was phosphate (98.8%) at 25% dilution with tap water in the case of *A. fumigatus* assist *N. salina* assist.

The biochemical composition of different biomasses in the different cases was determined comparatively to controls. In different controls (Table 4-A), the highest protein content in fungi was of *P. chrysogenum* (53.1%), followed by *A. fumigatus* (51.4%) then *A. niger* (35.4%). The highest protein content in microalgae was of *C. salina* (39.8%) then *N. salina* (37.9%). The highest carbohydrates content in fungi was of *A. niger* (17.2%) followed by *P. chrysogenum* (12.7%) then *A. fumigatus* (11.3%). The highest carbohydrates content in microalgae was of *N. salina* (25.1%) then *C. salina* (22.8%). The highest lipid content in fungi was of *A. fumigatus* (35.6%) followed by *P. chrysogenum* (25.6%) then *A. niger* (23.6%). The highest lipid content in microalgae was of *C. salina* (27.7%) then *N. salina* (19.7%).

In different treatments by all assists with seawater and tap water (Table 4-B), the highest protein content in assists was of *A. niger* assist *C. salina*/seawater (39.7%). The highest carbohydrates content in assists was of both *A. niger* - *N. salina*/tapwater and *A. niger* assist *C. salina*/tapwater (40%). While, the highest lipid content in assists was of *A. niger* assist *N. salina*/seawater (25.3%). In different dilutions of the potent assist (*A. fumigates* assist *C. salina* and *A. fumigates* assist *N. salina*) with seawater and tap water (Table 4-C), the highest protein content was of *A. fumigates* assist *C. salina*/seawater, 75 % dilution (39.2%). The highest carbohydrates content was of *A. fumigates* assist *C. salina*/seawater, 50% dilution (37.5%). While, the highest lipid content was of *A. fumigates* assist *C. salina/* tapwater, 75% dilutions (26.7%).

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

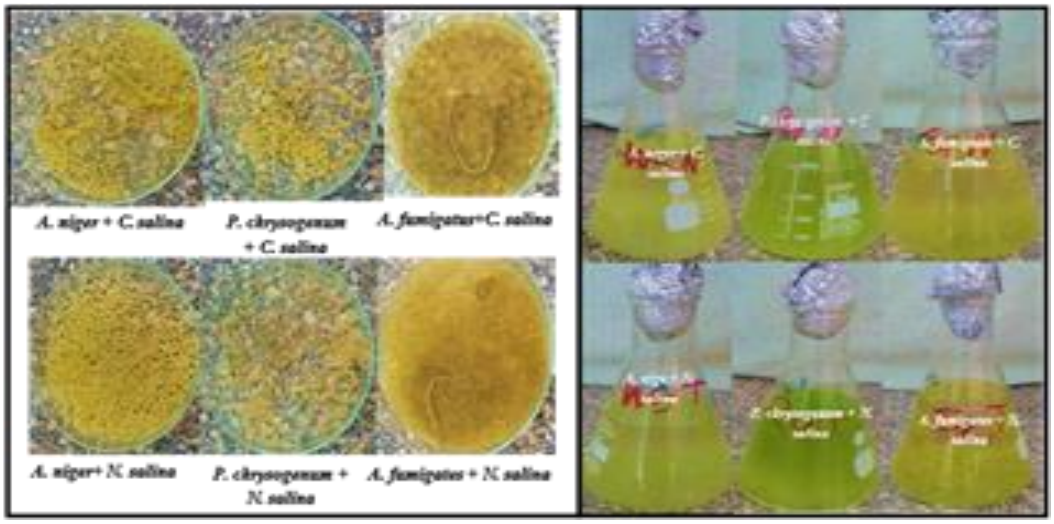

Figure 2: Macrographs showing the flocculation process via fungi assist microalgal species.

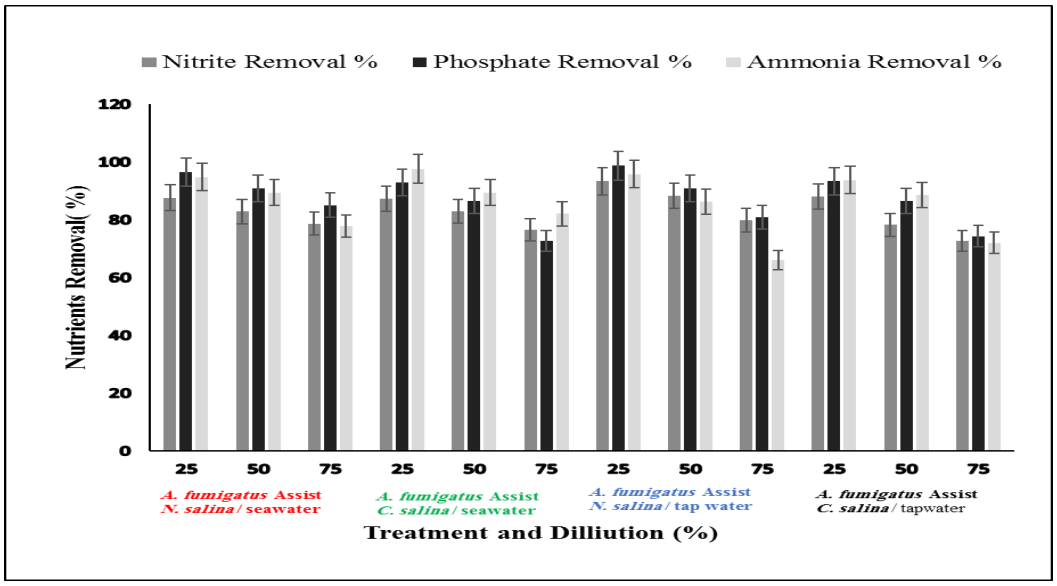

Figure 3: Removal percentage (%) of nutrients of wastewater using *Aspergillus fumigates* assist microalgal species.

Table 3: Concentrations (mg L^{-1}) of nutrients in wastewaters after treatment using *Aspergillus fumigatus assist Nannochloropsis salina and Chlorella salina* assist.

Treatment	Dilution %	Nitrite	Phosphate	Ammonia
		Conc. (mg L^{-1})		
A. fumigatus Assist N. salina / seawater	25	0.019	0.9 49	0.620
	50	0.026	2.478	1.254
	75	0.033	4.069	2.640
A. fumigatus Assist N. salina / tap water	25	0.0105	0.350	0.5041
	50	0.0185	2.683	1.691
	75	0.0320	5.672	4.231
A. fumigatus Assist C. salina / seawater	25	0.0196	1.915	0.2726
	50	0.0265	3.6564	1.2668
	75	0.0364	7.457	2.1331
A. fumigatus Assist C. salina / tap water	25	0.0189	1.961	0.764
	50	0.0345	3.975	1.417
	75	0.0434	7.5973	3.4717

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

Table 4-A: Biochemical composition of control biomass under the current investigation.

Sample No.	Total protein (%)	Total carbohydrates (%)	Total lipids (%)	Other (%)
N. salina	37.9	25.1	19.7	17.3
C. salina	39.8	22.8	27.7	9.7
A. Niger	35.4	17.2	23.6	23.8
P. chrysogenum	53.1	12.7	25.6	8.6
A. fumigatus	51.4	11.3	35.6	1.7

Table 4-B: Biochemical composition of different biomasses under the current investigation.

Sample No.	Total Protein (%)	Total Carbohydrates (%)	Total Lipids (%)	Other
A. niger + N. salina/seawater	27.3	23.2	25.3	24.2
A. niger + N. salina/tap water	17.2	40	17.7	25.1
A. niger + C. salina/seawater	39.7	33	15.3	12
A. niger + C. salina/tap water	17.2	40	15.7	27.1
P. chrysogenum + N. salina/seawater	32.7	22.2	19.9	25.2
P. chrysogenum + N. salina/tap water	21.4	35.4	13.6	29.6
P. chrysogenum + C. salina/seawater	26	21	18.7	34.3
P. chrysogenum + C. salina/tap water	21.4	35.4	17.6	25.6
A. fumigatus + N. salina/seawater	34.2	26.7	25	14.1
A. fumigatus + N. salina/tap water	29.1	31.8	16.5	22.6
A. fumigatus + C. salina/seawater	38.4	37.4	13.6	10.6
A. fumigatus + C. salina/tap water	36	32.1	21.1	10.8

Table 4-C: Biochemical composition of different biomasses in different dilutions of wastewaters under the current investigation.

Treatment	Dilution %	Total protein (%)	Total carbohydrates (%)	Total lipids (%)	Other
A. fumigatus assist N. salina/seawater	25.0	17.5	24.0	18.3	40.2
	50.0	34.3	26.7	24.9	14.1
	75.0	38.9	33.9	26.0	1.2
A. fumigatus assist N. salina/tap water	25.0	16.0	20.2	12.5	51.3
	50.0	29.1	31.2	16.5	23.2
	75.0	31.8	33.2	19.3	15.7
A. fumigatus assist C. salina/seawater	25.0	12.6	33.1	9.7	44.6
	50.0	38.4	37.5	13.6	10.5
	75.0	39.2	36.4	15.5	8.9
A. fumigatus assist C. salina/tap water	25.0	26.8	21.9	9.4	41.9
	50.0	36.0	32.1	21.1	10.8
	75.0	32.0	36.9	26.7	4.4

Discussion

The bioflocculation of marine microalgal strains (*N. salina* and *C. salina*) with marine filamentous fungi (*P. chrysogenum*, *A. niger* and *A. fumigatus*), was evaluated as a promising effective process for microalgal cells harvesting and nutrient recovery.

The evaluation of bioflocculation process provides a vital process for algal harvesting without adding any chemical flocculants. It can be occurred under normal conditions of cultivation, and allows algal farmers to reuse the cultivation medium without any additional treatment. Both microalgal and fungal cells could serve as accurate bioremediation agents for nutrient

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

recovery from wastewater (Price *et al*., 2001; Schenk *et al*., 2008; Christenson and Sims, 2011; de Boer *et al.*, 2012; Duong *et al*., 2012; Sing *et al*., 2013; Singh and Singh, 2014).

The current work evaluated the ability of *A. fumigatus* assist *C. Salina*, *A. fumigatus* assist C. *Salina* , *P. chrysogenum* assist *C. Salina*, *P. chrysogenum* assist *N. Salina* and *A. niger* assist *C. Salina* and *A. niger* assist *C. Salina* to be grown on and uptake nutrients (nitrite, phosphate and ammonia) from municipal waste water. The properties of the collected municipal wastewater were measured and summarized in Table 1. The dilution of wastewater with either seawater or tap water 50/50 % (v/v) were decreased the concentration of nitrite by 9.92, 8.31%, phosphate by 48.83, 44.31% and ammonia by 14.12, 10.38%, respectively. However, the order of the nutrient concentration was in the following order; phosphate >ammonia > nitrite.

Microalgal harvesting efficiency was determined by the decreases in optical densities after 24 and 48 h of co-cultivation with fungal beads. At 24 h, flocculation efficiency % ranged between 56.9 to 83.2%. These percentages are considered good if we want to save time, effort and possibilities. It can be notice that at 24 h, *A. niger assis C. salina and A. niger assist N. salina* recorded the lowest flocculation efficiency % (59.3, 56.9%) but it recorded a high results at 48 h (85.9, 88.7%), so it is advisable to use after 48 h. At 48 h percentage efficiency of flocculation raised at all assists. Flocculation efficiency percentage of all assists was in the order of *A. fumigatus* assist *C. salina* > *A. fumigatus* assist *N. salina* > *P. chrysogenum* assist *N. salina* > *P. chrysogenum* assist *C. salina* > *A. nigar* assist *C.salina* > *A. nigar* assist *N. salina*. *A. fumigatus* assist *N. salina* and *A. fumigatus* assist *C. salina* showed the highest flocculation efficiency percentage at both 24 and 48 h and nutrient removal percentage for phosphate and ammonia, so it was chosen in the different dilutions with seawater and tap water.

Aerobic or anaerobic biological degradation is the basic method for wastewater treatment however; the inorganic compounds such as nitrate, ammonium and phosphate ions are still presented. Those compounds lead to eutrophication in ponds and cause undesired microalgal blooms (Sawayama *et al*., 1998). Microalgal cultures offer a vital solution for wastewater treatments because of microalgal ability to employ inorganic nitrogen and phosphorous for their growth (Oswald, 1988a; Oswald, 1988b; Richmond, 1986) and heavy metals recovery (Rai et al., 1981). Current study, ammonia removal percentage ranged between 27.7 and 89.5%, after 2-days cultivation. Tam and Wong (1990) were mentioned algae cultivation could remove ammonia from wastewater by consumption of NH_4-N and NH_3 stripping (Tam and Wong, 1990). The NH_3 stripping take place only under high pH, high of temperature excessed than 27°C (Reeves, 1972), and in the presence of high amounts of urea in the wastewater (Matusiak *et al*., 1976). Since the urea was not dominant in the used wastewater and probably the

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

temperature never rising more than 27 °C the stripping process might not have been considering in the current work, and the messing of ammonia because of the absorption that performing with algae and/or physical absorbance by algae–fungi complex due to their unique structure. Lau *et al*. (1996) was investigate the nutrients removal by *Chlorella* sp. and mentioned that the nutrient removal efficiency was 86% for inorganic N (Lau *et al*., 1996). This result agrees with our result when we aggregate *C. salina* and *N. salina* with *A. fumigatus*/sea and tap water. Wang *et al*. (2010) examined the growth of a wild-type *Chlorella* sp. in domestic wastewaters collected from different stations of the treatment cycle of a domestic wastewater treatment plant (Wang *et al*., 2010). It was found that this algae strain grew best in the centrate due to it had high levels of COD, phosphorus and nitrogen, than the other three wastewater's stations. A total nitrogen removal rate of 82.8% and total phosphorus removal rate of 85.6% were observed in centrate during 10-day batch cultivation.

Phosphorus is macronutrient important for algal growth. The algae is utilized the Phosphorus as inorganic orthophosphate (PO_4^{-3}). The utilization of orthophosphate is a vital process that needs energy. The maximum algal removal of phosphate about 90.9%, this finding is a significant according to the results obtained from other work using domestic wastewater (Lau *et al*., 1995; Woertz *et al*., 2009; Wang *et al*., 2010;). So, using algae-fungi pellets for wastewater recovery is recommended rather than separate fungi or algae. Song *et al*. (2002) stated the rising of pH up to 8 causes flocs of algae and adsorption of inorganic phosphates (Song *et al*., 2002).

The *A. fumigatus* assist *N. salina* and *A. fumigatus* assist *C. salina* recorded the highest phosphorus removal. Since, marine microalgae *C. salina* as the immobilized cells of marine have been reported to be have a greater efficiency to utlize of phosphate within the few days of incubation in contrast to *C. salina* free cells. Sriram and Seenivasan (2012) stated similar result of waste water phosphate uptake to be 86% by *Chlorella* sp., these finding agree with our results, where phosphorous removal percent was 86.6 with *C. salina* / *A. fumigates* (Sriram & Seenivasan, 2012). Colak and Kaya (1988) reported that algae have ability to remove 50.2% of nitrogen and 85.7% of phosphorus from industrial wastewater and remove about 97.8% phosphorus from domestic wastewater (Colak and Kaya, 1988).

According to public health organization, nitrate itself is not toxic, but its turn into nitrite that a potential hazard (Sedlak, 1991). Because of nitrite inside the body, can oxidize iron (II) and for methaemoglobin, which binds oxygen less effectively than normal hemoglobin.

As in ammonia and phosphorus, *A. fumigats* assist *N. salina* and *A. fumigats* assist *C. salina* assist record high nitrite removal percentage, but *A. nigar* assist *N. salina*/sea and tap water & *A. nigar* assist *C. salina* /sea and tap water recorded the highest phosphorus removal.

In addition, we assessed the *A. fumigatus* assist *N. salina* and *A. fumigatus* assist *C. salina* have ability to recover the nutrients from diluted

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

wastewater. For these investigations, the wastewater was diluted up to 25%, 50% and 75% with tap water and seawater. After 48 h of *A. fumigatus* assist *N. salina* incubation in 25%, Wastewater diluted with tap water, NH_3 removal was 93.4% and PO_4 removal was 98.8%, while *A. fumigatus* assist *C. salina* incubation in 25%, wastewater diluted with seawater recorded the highest removal of ammonia (97.7%). Dilution of 25% (seawater or tap water) gave the highest removal of nutrients. In general, removal percentage reversed commensurate with increasing dilutions.

For instance, the using of microalgal cultures for nutrient recovery of wastewater via different strains as *Nannochloropsis* and *Chlorella* are high applicable as well as other microalgae because of their photosynthetic abilities, utilizing solar energy and turn it into biomasses and remove nutrients as phosphorus and nitrogen making eutrophication (Chevalier and de la Noüe, 1985).

Several works focus on nutrient recovery using microalgae. Generally, microalgae uptake nutrients to build their own biochemical composition. Thus, this work aimed to test the biochemical contents of fungi assist algae during nutrient recovery. However, the biochemical composition of collected biomass in the several trails was evaluation. In control samples, the highest protein content in fungi was of *P. chrysogenum* (53.1%), followed by *A. fumigatus* (51.4%) then *A. niger* (35.4%). Moreover, the protein content in microalgae N. *salina* and *C. salina* were 39.8, 37.9%, respectively. The highest carbohydrates content in fungi was of *A. niger* (17.2%) followed by *P. chrysogenum* (12.7%) then *A. fumigatus* (11.3 %). The highest carbohydrates content in microalgae was of *C. salina* (25.1%) then *N. salina* (22.8%). The highest lipid content in fungi was of *A. fumigatus* (35.6%) followed by *P. chrysogenum* (25.6%) then *A. niger* (23.6%). The highest lipid content in microalgae was of *N. salina* (27.7%) then *C. salina* (19.7%).

Three main contents represent the major carbohydrates of fungi cell involved branched monosaccharides; unbranched polymers of *N*-acetyl-D-glucosamine; and polymers of mannose associated with proteins turned into glycol proteins. Additionally, the cell walls contain proteins (about 25%) and minor amounts of lipid (about 7%) (Calderone and Scheld, 1987). The biochemical composition of microalgae varies related to species, growth stage, light exposure time and temperature (Harrison *et al*., 1977; Morris *et al*., 1983).

In different dilutions of the potent assist (*A. fumigatus* assist *C. salina* and *A. fumigates* assist *N. salina*) with seawater and tap water, the highest protein content was of *A. fumigates* assist *C. salina*/seawater, 75% dilution (39.2%). The highest carbohydrates content in fungi was of *A. fumigates* assist *C. salina*/seawater, 50 % dilution (37.4018%). The highest lipid content was of *A. fumigates* assist *C. salina*/tapwater, 75% dilution (26.7%). Phosphate and nitrogen are essential macronutrients for algal cells growth and metabolism. Nitrogen is a vital element for building cell nucleic acids and proteins. As an integral item of vital molecules like ATP, the energy

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

carrier in cells, phosphate is another very essential nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids. Limitation these elements maybe change the metabolic pathway of the organism. For instance, starvation of phosphorus or nitrogen changes the lipid metabolism to storage neutral lipid instead of synthesis lipid.

In briefly, the biochemical composition of harvested biomass (fungi assist algae) depended on recovery and uptake rates of nitrate, phosphate and ammonia. The fungi assist microalgae offer a promising technology to recovery macronutrients N and P supply from wastewater.

The current study supports algal-fungal association as a potential solution for harvesting of marine microalgae. Marine filamentous fungi, *A. fumigatus* provide highly efficient to harvesting of two strains of marine microalgae (*Nannochloropsis salina* and *Chlorella salina*). Regarding to the dilution of wastewater with seawater, the results illustrate that the dilution reduced the amount of freshwater required, thereby making the whole process economic. The additive and synergetic effects of *A. fumigates assist Nannochloropsis salina* and *Chlorella salina* pelletization on total biomass and lipid production were found for most of the microalgal strains can significantly improve total lipid yield. The bioflocculation technique will play a significant role on biofuel production from microalgae.

References

Association, A.P.H., 1995. Water Environment Federation. Standard methods for the examination of water and wastewater, 22 P.

Atlas, R.M., 2010. Handbook of microbiological media. Forth ed. CRC Press. 2040 P.

Benemann, J.R., 1997. CO_2 mitigation with microalgae systems. *Energy Conversion and Management*, 38, S475-S479.

Bligh, E.G. and Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

Calderone, R.A. and Scheld, W.M., 1987. Role of fibronectin in the pathogenesis of candidal infections. *Review of Infectious Diseases*, 9, 400-403.

Chevalier, P. and de la Noue, J., 1985. Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme and Microbial Technology*, 7, 621-624.

Christenson, L. and Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances*, 29, 686-702.

Colak, O. and Kaya, Z., 1988. A study on the possibilities of biological wastewater treatment using algae. *Doga Biyoloji Serisi*, 12, 18-29.

Coward, T., Lee, J.G. and Caldwell, G.S., 2013. Development of a foam flotation system for harvesting microalgae biomass. *Algal Research*, 2, 135-144.

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

de Boer, K., Moheimani, N.R., Borowitzka, M.A. and Bahri, P.A., 2012. Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption. *Journal of Applied Phycology*, 24, 1681-1698.

Domsch, K.H., Gams, W. and Anderson, T.H., 1980. Compendium of soil fungi. Vol. 1.ed. Academic Press (London) Ltd.

Doran, M.D. and Boyle, W.C., 1979. Phosphorus removal by activated algae. *Water Research*, 13, 805-812.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P. and Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356.

Duong, V.T., Li, Y., Nowak, E. and Schenk, P.M., 2012. Microalgae isolation and selection for prospective biodiesel production. *Energies*, 5, 1835-1849.

Garg, S., Wang, L. and Schenk, P.M., 2014. Effective harvesting of low surface hydrophobicity microalgae by froth flotation. *Bioresource Technology*, 159, 437-441.

Geddes, M., 1984. Limnology of Lake Alexandrina, River Murray, South Australia, and the effects of nutrients and light on the phytoplankton. *Marine and Freshwater Research*, 35, 399-415.

Gonzalez-Fernandez, C. and Ballesteros, M., 2013. Microalgae autoflocculation: an alternative to high-energy consuming harvesting methods. *Journal of Applied Phycology*, 25, 991-999.

Guillard, R.R. and Ryther, J.H., 1962. Studies of marine planktonic diatoms: I. Cyclotella Nana Hustedt, and Detonula Confervacea (CLEVE) Gran. *Canadian Journal of Microbiology*, 8, 229-239.

Gultom, S.O. and Hu, B., 2013. Review of microalgae harvesting via co-pelletization with filamentous fungus. *Energies*, 6, 5921-5939.

Hammouda, O., Gaber, A. and Abdelraouf, N., 1995. Microalgae and wastewater treatment. *Ecotoxicology and Environmental Safety*, 31, 205-210.

Harrison, P., Conway, H., Holmes, R. and Davis, C.O., 1977. Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum*, and *Thalassiosira gravida*. *Marine Biology*, 43, 19-31.

Lau, P., Tam, N. and Wong, Y., 1995. Effect of algal density on nutrient removal from primary settled wastewater. *Environmental Pollution*, 89, 59-66.

Lau, P., Tam, N. and Wong, Y., 1996. Wastewater nutrients removal by *Chlorella vulgaris*: optimization through acclimation. *Environmental Technology*, 17, 183-189.

Lee, A.K., Lewis, D.M. and Ashman, P.J., 2013. Harvesting of marine microalgae by electroflocculation: the energetics, plant design, and economics. *Applied Energy*, 108, 45-53.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951.

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193, 265-275.

Matusiak, K., Przytocka-Jusiak, M., Leszczyńska-Gerula, K. and Horoch, M., 1976. Studies on the purification of wastewater from the nitrogen fertilizer industry by intensive algal cultures. II. Removal of nitrogen from the wastewater. *Acta Microbiologica Polonica*, 25(**4**), 361-373.

Morris, R., McCartney, M. and Robinson, G., 1983. Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. *Journal of Experimental Marine Biology and Ecology*, 70, 249-262.

Muradov, N., Taha, M., Miranda, A. F., Wrede, D., Kadali, K., Gujar, A., Stevenson, T., Ball, A.S. and Mouradov, A., 2015. Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. Biotechnology for Biofuels, 8, 1-23.

Oswald, W., 1988. Micro-Algae and waste-water treatment. En Borowitzka, MA, and Borowitzka, LJ (Eds.), Micro-algal Biotechnology. Cambridge University Press. pp. 305-328

Oswald, W.J., 1988. Large-scale algal culture systems (engineering aspects). Micro-algal biotechnology. Cambridge University Press, Cambridge, pp. 357-394.

Padua, M.D., Fontoura, P.S.G. and Mathias, A.L., 2004. Chemical composition of *Ulvaria oxysperma* (Kutzing) bliding, *Ulva lactuca* (Linnaeus) and *Ulva fascita* (Delile). *Brazilian Archives of Biology and Technology*, 47, 49-55.

Pires, J., Alvim-Ferraz, M., Martins, F. and Simoes, M., 2013. Wastewater treatment to enhance the economic viability of microalgae culture. *Environmental Science and Pollution Research*, 20, 5096-5105.

Price, M.S., Classen, J.J. and Payne, G.A., 2001. *Aspergillus niger* absorbs copper and zinc from swine wastewater. *Bioresource Technology*, 77, 41-49.

Rai, L., Gaur, J. and Kumar, H., 1981. Phycology and heavy metal pollution. *Biological Reviews*, 56, 99-151.

Reeves, T.G., 1972. Nitrogen removal: a literature review. *Journal of Water Pollution Control Federation*, 44(**10**), 1895-1908.

Richmond, A., 1986. Handbook of microalgal mass culture. 1st ed. CRC Press. 536 P.

Salim, S., Bosma, R., Vermue, M.H. and Wijffels, R.H., 2011. Harvesting of microalgae by bio-flocculation. *Journal of Applied Phycology*, 23, 849-855.

Sawayama, S., Hanada, S. and Kamagata, Y., 2000. Isolation and characterization of phototrophic bacteria growing in lighted upflow anaerobic sludge blanket reactor. *Journal of Bioscience and Bioengineering*, 89, 396-399.

Sawayama, S., Rao, K. and Hall, D., 1998. Nitrate and phosphate ion removal from water by *Phormidium laminosum* immobilized on hollow

[DOR: 20.1001.1.15622916.2020.19.4.16.4]

fibres in a photobioreactor. *Applied Microbiology and Biotechnology*, 49, 463-468.

Schenk, P.M., Thomas-Hall, S.R., Stephens, E., Marx, U.C., Mussgnug, J.H., Posten, C., Kruse, O. and Hankamer, B., 2008. Second generation biofuels: high-efficiency microalgae for biodiesel production. *Bioenergy Research*, 1, 20-43.

Sedlak, R.I., 1991. Phosphorus and nitrogen removal from municipal wastewater: principles and practice. 2nd ed. CRC Press. 256 P.

Sing, S.F., Isdepsky, A., Borowitzka, M.A. and Moheimani, N.R., 2013. Production of biofuels from microalgae. *Mitigation and Adaptation Strategies for Global Change*, 18, 47-72.

Singh, A.P. and Singh, T., 2014. Biotechnological applications of wood-rotting fungi: A review. *Biomass and Bioenergy*, 62, 198-206.

Song, Y., Hahn, H.H. and Hoffmann, E., 2002. Effects of solution conditions on the precipitation of phosphate for recovery: A thermodynamic evaluation. *Chemosphere*, 48, 1029-1034.

Tam, N. and Wong, Y.S., 1990. The comparison of growth and nutrient removal efficiency of *Chlorella pyrenoidosa* in settled and activated sewages. *Environmental Pollution*, 65, 93-108.

Vandamme, D., Eyley, S., Van den Mooter, G., Muylaert, K. and Thielemans, W., 2015. Highly charged cellulose-based nanocrystals as flocculants for harvesting *Chlorella vulgaris*. *Bioresource Technology*, 194, 270-275.

Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J. and Ruan, R.R., 2010. Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. *Bioresource Technology*, 101, 2623-2628.

Woertz, I., Feffer, A., Lundquist, T. and Nelson, Y., 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering*, 135, 1115-1122.

Xia, C., Wei, W. and Hu, B., 2014. Statistical analysis and modeling of pelletized cultivation of *Mucor circinelloides* for microbial lipid accumulation. *Applied Biochemistry and Biotechnology*, 172, 3502-3512.

Zhang, J. and Hu, B., 2012. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. *Bioresource Technology*, 114, 529-535.

Zhou, W., Min, M., Hu, B., Ma, X., Liu, Y., Wang, Q., Shi, J., Chen, P. and Ruan, R., 2013. Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Separation and Purification Technology*, 107, 158-165.