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Evaluation the efficiency of *Scenedesmus obliquus*, *Chlorella vulgaris* and a mixture of two algae in municipal wastewater treatment

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Abstract: This experiment was conducted over a 15-day period to determine efficiency of two algae species, *Scenedesmus obliquus*, *Chlorella_vulgaris*, either singularly or in combination, in nutrient absorption from municipal wastewater. Algal cell counting was performed daily, but water nitrate, nitrite, phosphate, ammonia, chlorophyll a, algal biomass, BOD and COD levels were determined every other day. At the end of the experiment, water phosphate in treatments C0, M50 and S100 was zero. Also, water nitrate levels reached zero, only in treatment M50 and all groups of treatment S. The maximum algal biomass was observed in treatment S0 (0.6 gL^{-1}; $p<0.05$) at the end of the experiment. Algal biomass of the treatment S groups was significantly lower than that of the other treatments (0.2 gL^{-1}; $p<0.05$). The maximum chlorophyll a with significant difference was observed in treatment S0. COD and BOD content showed constant trends among the treatments. The lowest COD and BOD was observed in treatments S and C respectively (4.1 mgL^{-1}; $p<0.05$; 1.3 mgL^{-1}; $p<0.05$). Overall, *Sc. Obliquus* was more efficient than *Ch. vulgaris i*n biomass production, chlorophyll a concentration and nutrient absorption.

Keywords: *Scenedesmus obliquus*, *Chlorella vulgaris,* phosphate, municipal wastewater

Introduction

Huge amounts of anthropogenic municipal wastewater are produced in the residential regions. Water and detergents utilization in routine life, human activities like construction and agriculture, discharge huge amounts of nitrogen and phosphorus to the aquatic ecosystems. Water nutrient enrichment and eutrophication may affect the ecosystem structure and function. Algae are photosynthetic organisms which use sun light to convert carbon (carbon dioxide) into other organic materials and release oxygen into atmosphere (Huzisige and Ke, 1993; Gebre, 2017). It is necessary to monitor aquatic ecosystems nitrogen and phosphorus and prevent violation of the suitable water criteria. Employ of micro-algae with high wastewater treatment potential is a biological method to suppress water nutrients such as nitrogen and phosphorus (Zhang et al., 2017; De la Noiie and Proulx, 1988). Wastewater treatment by micro-algae has some advantages such as nitrogen and phosphorus recycling, prevention of subsequent pollution and biomass production which can be applied for biofuel, cosmetic materials, hygienic materials and food production (Voltolina, *et al.,* 2004; Yalcin , *et al*., 2006). *Chlorella vulgaris* is a green single-cell alga with worldwide distribution; whereas, *Scenedesmus* is a green non-motile alga (Lee, *et al.*, 2001; Li, *et al.*, 2010). Micro-algae are superior to macro-algae and higher plants, because they grow rapidly and are capable to adapt natural and artificial conditions, which this adaptation is accompanied with increased protein, carbohydrate and lipid synthesis (Rasmussen, *et al.*, 2008). Green algae have outstanding ability to adapt in a variety of aquatic ecosystem (Solisio, *et al.*, 2008; Brodie *et al*., 2017).

Ch. vulgaris and *Sc. obliquus* are rapid growing organisms, resistant to handling in rearing systems, easy and inexpensive to produce, capable to tolerate a wide range of temperature and pH; also they absorb nutrients rapidly and even are able to absorb nitrogen and phosphorus at very low ambient concentrations (Li, *et al.*, 2010; Abolhasani *et al*., 2018; Jung *et al*., 2017). This experiment has been conducted on different concentrations of municipal wastewater, because water nutrient shortage may inhibit algae growth and efficiency (Kong, *et al.,* 2010).

The aim of this study was to determine growth and efficiency of *Ch. vulgaris* and *Sc. obliquus* to remove nutrients from different concentrations of municipal wastewater, and to determine interaction of the two algae when are mixed to treat the wastewater.

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Materials and Methods

The algae were cultured under static system condition in liquid culture medium Z_8 according to Lavens and Sorgeloos (1996). 500-ml flasks filled with 250 ml culture medium were used for this experiment, under sterile condition and at 23°C±2 temperature. Municipal wastewater of Gorgan City was obtained from the main refinery at the final stage. The wastewater was first filtered with filter paper and then autoclaved to ensure lack of microbial load. This experiment was conducted with 9 treatments and 4 replications, which include: *Chlorella* 0, 50, 100 (C0, C50, C100), *Scenedesmus* 0, 50, 100 (S0, S50, S100) and *Chlorella*-*Scenedesmus* mixture 0, 50, 100 (M0, M50, M100). Three concentrations of the wastewater were used: 50% (1 part of sterile distilled water was mixed with 2 parts of the wastewater), 100% (1 part of sterile distilled water was mixed with 1 part of the wastewater), and 0% (no dilution was performed); initial nutrient concentrations of each medium are presented in Table 1.

Tab. 1: Initial concentration of nutrients in each treatment (mgL^{-1}).

Compounds	0%	50%	100%
Nitrite	58.2	38.9	32.3
Phosphate	52.2	24	21.3
Nitrate	116.4	107	69.15
Ammonia	2.05	1.55	0.95

Algae inoculation into each replication of the treatments was conducted in a constant manner. For this, 24000 cells were inoculated into each 500-l flasks filled with 250 ml of autoclaved wastewater. In the mixture treatment, 12000 cells from each algae species were inoculated to the medium (Voltolina, *et al*., 2004). Dry matter and chlorophyll a were measured every other day according to Permila and Rao (1997) and Aminot and Ray (1999). Cell counts was daily made by hemocytometery method (Martınez *et al.*, 2000). Measurement of nutrients (phosphorus and nitrate) was conducted every other day using Wagtech kits (UK) and photometer apparatus. BOD_5 was determined every other day according to Gonzales *et al.* (1997). Specific growth rate (SGR) and doubling time (DT) are two important factors indicating reproduction rate of algae in culture medium. SGR and DT were determined according to the following formulas (Omori and Ikeda, 1984):

$SGR = (LnN_2 - LnN_1)/t$

N_2: cell count in the second sampling; N_1: cell count in the first sampling, t: time between the two sampling.

$DT = \log^2_e/SGR$

This experiment was conducted as a completely randomized design using factorial method with two factors: algae type (*Chlorella*, *Scenedesmus* and mixed) and wastewater concentration (0, 50 and 100 percent). To monitor organic materials removal over time, regression was used; whereas, to find relationship among the measured parameters, Pearson correlation test was used. The analyses were performed in SPSS 17 software, and graphs were drawn using Excel 2013.

Results

As phosphate and nitrate are the major nutrients in wastewater, efficiency of the algae for these compounds' uptake was investigated during the experiment. The effect of algae type, wastewater concentration, time and interactions among them on phosphate uptake was as follow (Tab. 2).

Tab. 2: F-value for the effect of algae, wastewater concentration and their interaction on phosphate removal.

Time (Day)	Alga	Con.	Alga×Con.
2	417.29**	177.74**	81.7*
4	915.41**	231.65**	598.7**
6	201.17**	213.67**	206.78**
8	171.38**	174.57**	9.43
10	120.58**	93.32**	89.3*
12	74.17**	55.59**	139.58**
14	49.34**	40.61**	121.1**

Con.: Concentration ** $P<0.01$; * $P<0.05$

Algal type and wastewater concentration had significant effect on phosphate uptake in the culture medium during the experiment, but their interaction was not significant at day 8.

According to the results (Tab. 3), phosphate and nitrate concentrations significantly decreased during the experiment. Wastewater phosphate was completely depleted in M0, M50 and S100. The maximum phosphate concentration was observed in C50 group (2 mgL^{-1}). S100 treatment had the lowest phosphate during the experiment; but C0 treatment had the highest phosphate during the first 8 days of the experiment, and C50 treatment had the highest phosphate in the last days of the experiment. On the other hand, the mixed algae treatment had the higher rate of phosphate uptake.

According to the Table 4, effect of the algae type, wastewater concentration, time and their interactions on nitrate uptake was as follow at different time.

Tab. 3: Phosphate concentration in different treatments during the experiment (mean ± SD).

Treatment	Day							
	0	2	4	6	8	10	12	14
C0	50.4^{Aa} (±0.47)	40.4^{bA} (±0.6)	27^{cA} (±0.63)	24^{dA} (±0.68)	13^{eA} (±0.67)	5.4^{fC} (±0.53)	2.4^{Gc} (±0.49)	0^{hF}
C100	21.3^{aC} (±0.37)	9^{Be} (±0.21)	8.1^{bE} (±0.21)	7.8^{bD} (±0.15)	5.7^{cD} (±0.43)	4.8^{dD} (±0.41)	4^{eA} (±0.54)	1.6^{fB} (±0.12)
C50	24^{aB} (±0.38)	22.5^{aC} (±0.42)	16^{bC} (±0.29)	10^{cC} (±0.1)	8^{dC} (±0.5)	6^{eB} (±0.57)	3^{fB} (±0.5)	2^{fA} (±0.16)
M0	50.4^{aA} (±0.47)	13.2^{bD} (±0.25)	10.8^{cD} (±0.23)	4.2^{dF} (±0.13)	2.5^{eF} (±0.31)	2.2^{eF} (±0.34)	1.8^{eD} (±0.24)	1^{eC} (±0.08)
M100	21.3^{aC} (±0.37)	7.2^{bF} (±0.17)	4.8^{cG} (±0.13)	3.1^{eG} (±0.1)	2.4^{fF} (±0.3)	2.4^{fF} (±0.35)	0.8^{gF} (±0.09)	0.04^{hE} (±0.013)
M50	24^{aB} (±0.38)	10.5^{bE} (±0.2)	6.5^{cF} (±0.15)	5.5^{cE} (±0.11)	2.9^{dE} (±0.34)	1^{eG} (±0.28)	0.8^{eF} (±0.09)	0^{eF}
S0	50.4^{aA} (±0.47)	30.3^{bB} (±0.5)	22.2^{cB} (±0.47)	12^{dB} (±0.14)	10^{eB} (±0.41)	7.2^{fA} (±0.59)	1^{gF} (±0.1)	0.8^{gD} (±0.01)
S100	21.3^{aC} (±0.37)	5.1^{bG} (±0.14)	2.8^{cH} (±0.1)	2.3^{dH} (±0.1)	2^{dF} (±0.21)	1.12^{eG} (±0.2)	0.65^{fG} (±0.07)	0^{gF}
S50	24^{aB} (±0.38)	22^{bC} (±0.42)	15^{cC} (±0.26)	9^{dC} (±0.12)	8^{dC} (±0.5)	3.5^{eE} (±0.32)	1.03^{fE} (±0.1)	0.72^{fD} (±0.001)

Lowercase letters show significant difference within each row and uppercase letters within each column (α=0.05).

Tab. 4: F-values of effect of algae type, concentration and their interaction on nitrate uptake.

Time (Day)	Alga	Con.	Alga×Con.
2	354.5^{**}	200.41^{**}	130.91^{**}
4	152.33^{**}	161.98^{**}	91.23^{*}
6	320.89^{**}	355.85^{**}	145.76^{**}
8	103.96^{**}	89.87^{**}	85.88^{**}
10	92.69^{**}	103.98^{**}	73.18
12	95.49^{**}	90.44^{**}	75.95^{**}
14	76.87^{**}	87.4^{**}	34.21

Con.: Concentration ** P<0.01; * P<0.05

Similar to phosphate, nitrate uptake was significantly affected by algal type and wastewater concentration, interaction and wastewater concentration had no significant effects at days 10 and 14.

Nitrate was completely depleted at day 12 in S50 group and at the end of experiment in M50, S0 and S100 groups, but C0 group had the highest nitrate at the end of the experiment (18.9 mgL^{-1}). M100 treatment had the lowest nitrate during the first 6 days of the experiment; S50 treatment had the lowest value from the day 6 until the end of the experiment; and the highest nitrate was observed in C0 treatment throughout the experiment (Tab. 5).

Tab. 5: Nitrate values in different treatments during the experiment (mean ± SD).

Treatment	Day							
	0	2	4	6	8	10	12	14
C0	116.4^{aA} (±2.76)	83.52^{bA} (±1.12)	79.74^{cA} (±2.1)	70.74^{dA} (±2.72)	59.4^{eA} (±2.12)	36.7^{fA} (±1.98)	30.48^{gA} (±1.45)	18.9^{hA} (±1.13)
C100	69.15^{aC} (±1.67)	44.52^{bD} (±1.01)	35^{cD} (±1.4)	28.44^{dC} (±1.31)	26.9^{dB} (±1.73)	21.66^{eB} (±1.25)	16.89^{fB} (±0.97)	5.64^{gB} (±0.9)
C50	107^{aB} (±1.98)	51.15^{bB} (±1.08)	45.85^{cC} (±1.7)	18.6^{dE} (±1.21)	10.72^{eE} (±1.34)	7.55^{fC} (±1.09)	3.53^{gC} (±0.12)	0.65^{hC} (±0.09)
M0	116.4^{aA} (±2.76)	48.98^{bC} (±1.04)	46.5^{cB} (±1.7)	34.08^{dB} (±1.52)	13.02^{eD} (±1.38)	4.7^{fD} (±1.01)	1.94^{gD} (±0.1)	0.36^{gC} (±0.05)
M100	69.15^{aC} (±1.67)	11.16^{bF} (±0.7)	9.96^{cG} (±0.9)	9.18^{cG} (±0.91)	7.2^{dG} (±1.01)	4.65^{eD} (±1.01)	1.42^{fD} (±0.09)	0.27^{fC} (±0.01)
M50	107^{aB} (±1.98)	33.45^{bE} (±0.9)	14.85^{cF} (±1.12)	12.85^{dF} (±1.1)	8.85^{eF} (±1.08)	1.06^{fF} (±0.9)	0.18^{fE} (±0.01)	0^{fC}
S0	116.4^{aA} (±2.76)	53.3^{bB} (±1.07)	29.76^{cE} (±1.37)	24.48^{dD} (±1.23)	21.24^{eC} (±1.43)	3.1^{fE} (±0.98)	0.58^{gE} (±0.03)	0^{gC}
S100	69.15^{aC} (±1.67)	18.06^{bF} (±1)	12.8^{cG} (±1.1)	5.61^{dH} (±0.8)	3.72^{eH} (±0.9)	2.3^{fF} (±0.94)	1.15^{fE} (±0.08)	0^{fC}
S50	107^{aB} (±1.98)	49.85^{bC} (±1.03)	10.65^{cG} (±1.01)	9.98^{cG} (±0.93)	5.4^{dH} (±0.98)	1.51^{eF} (±0.67)	0^{fE}	0^{fC}

Lowercase letters show significant difference within each row and uppercase letters within each column (α=0.05).

Figure 1 shows that treatments C50 and C100 (89% and 92% respectively) removed lower phosphate percentage than the other treatments; also, treatments C0 and C100 (83% and 91%) removed lower nitrate percentage than that of the other treatments. C0 group removed significantly lower nitrate percentage than C100; both groups nitrate removal percentages were significantly lower than that of the other treatments. Treatments C0, M50 and S100 had significantly higher phosphate removal percentages than that of the other treatments. Overall, *Ch. vulgaris* efficiency in nitrate and phosphate removal was lower than the other treatments ($P<0.05$).

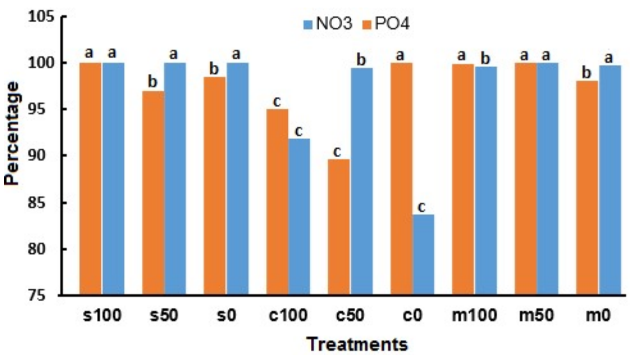

Fig. 1: Percentage of nitrate and phosphate removal in different treatments at the end of the experiment. Different letters show significant difference among the treatments (α=0.05).

With regard to Figure 2, the treatments efficacy in phosphate uptake was relatively similar, when mixed algae are used. Among the *Chlorella* treatments, C0 group was significantly more efficacious to remove phosphate from culture media (Fig. 3). Phosphate uptake in *Scenedesmus* groups was very fast, as the phosphate of medium approximately reached zero at the day 10.

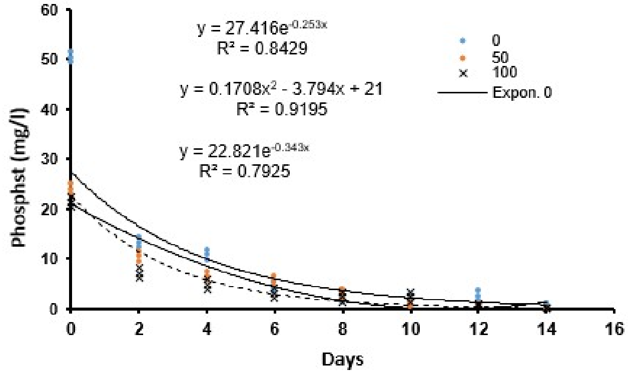

Fig. 2: Phosphate removal trend in mixed algae treatment.

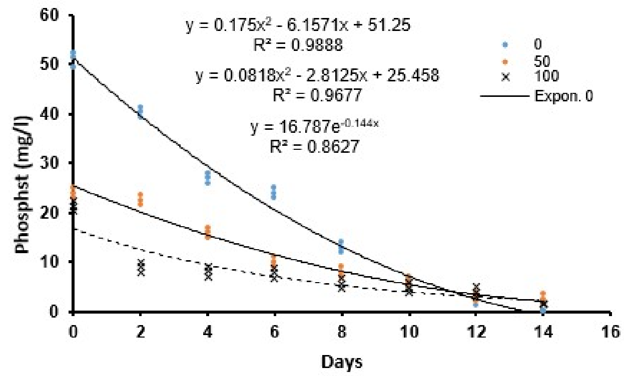

Fig. 3: Phosphate removal trend in *Chlorella* treatment.

Phosphate removal was faster in concentration 0, compared to the other concentrations, and increase in concentration slowed down the phosphate removal (Fig. 4).

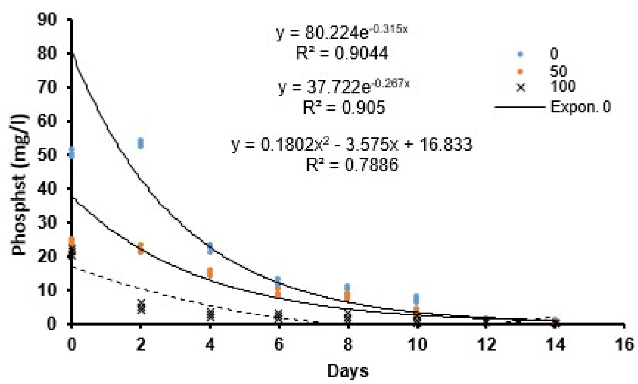

Fig. 4: Phosphate removal pattern in *Scenedesmus* treatment.

Nitrate removal from the media was very fast. Nitrate removal was faster in concentration 0, compared to the other concentrations, and increase in concentration slowed down nitrate removal (Fig. 5). Nitrate uptake in *Chlorella* treatments was lower than the other treatments, and the lowest level was observed in C0 group (Fig. 6). Nitrate uptake in *Scenedesmus* was very fast, and the fastest uptake was observed in S0 group (Fig. 7).

Algae biomass is directly related to medium nutrient concentration. As shown in Table 6, algae biomass in *Chlorella* treatments showed no significant change during the experiment; whereas, in *Scenedesmus* and mixed treatments algae biomass significantly changed during the experiment. The highest biomass was observed in S0 treatment (0.6 gL^{-1}); whereas, the lowest biomass was observed in the *Chlorella* treatment (C0, C50, C100; 0.2 $g\ L^{-1}$). Biomass in C100 treatment was 0.1 $g\ L^{-1}$ during the first 8-day of the experiment.

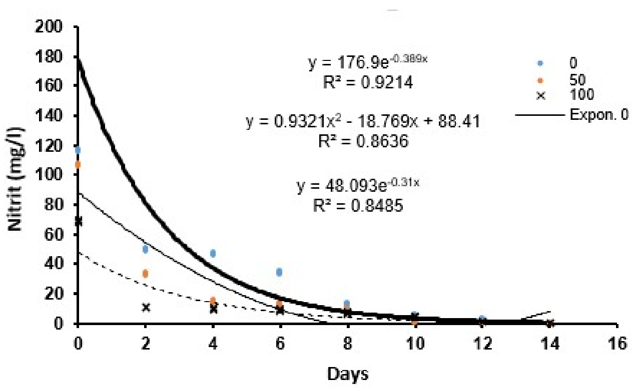

Fig. 5: Nitrate removal pattern in mixed algae treatment.

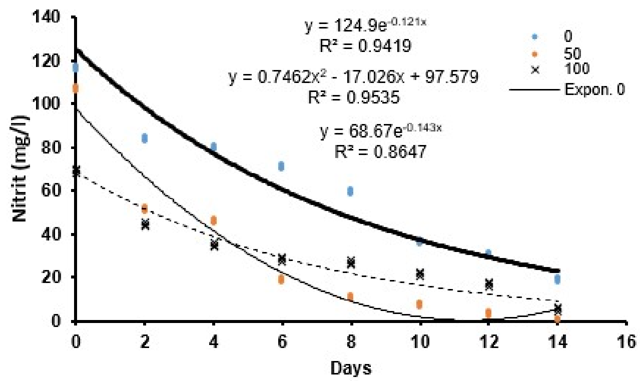

Fig. 6: Nitrate removal pattern in *Chlorella* treatment.

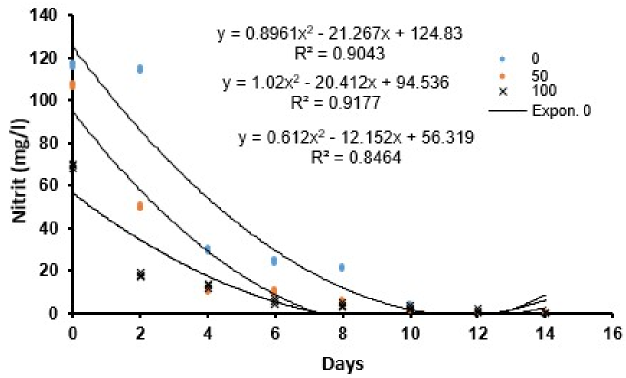

Fig. 7: Nitrate removal pattern in *Scenedesmus* treatment.

Tab. 6: Biomass in different treatments during the experiment (mean ± SD).

Treatment	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
C0	0^{bB} (±0.01)	0.1^{aA} (±0.01)	0.2^{aB} (±0.02)	0.2^{aB} (±0.01)	0.2^{aD} (±0.01)	0.2^{aE} (±0.01)	0.2^{aE} (±0.01)
C100	0^{cB} (±0.01)	0^{cC} (±0.01)	0.1^{bC} (±0.01)	0.1^{bC} (±0.01)	0.1^{bE} (±0.01)	0.2^{aE} (±0.01)	0.2^{aE} (±0.01)
C50	0^{dB} (±0.01)	0.1^{cB} (±0.01)	0.2^{bB} (±0.02)	0.2^{bB} (±0.01)	0.3^{aC} (±0.02)	0.2^{bE} (±0.01)	0.2^{bE} (±0.01)
M0	0.1^{dA} (±0.01)	0.1^{dB} (±0.01)	0.2^{cB} (±0.02)	0.2^{cB} (±0.01)	0.3^{bC} (±0.02)	0.4^{aC} (±0.03)	0.4^{aC} (±0.02)
M100	0.1^{bA} (±0.01)	0.1^{bB} (±0.01)	0.3^{aA} (±0.02)	0.3^{aA} (±0.02)	0.3^{aC} (±0.02)	0.3^{aD} (±0.02)	0.3^{aD} (±0.02)
M50	0.1^{cA} (±0.01)	0.1^{cB} (±0.01)	0.3^{bA} (±0.02)	0.3^{bA} (±0.02)	0.4^{aB} (±0.03)	0.4^{aC} (±0.03)	0.4^{aC} (±0.02)
S0	0.1^{dA} (±0.01)	0.2^{cA} (±0.02)	0.2^{cB} (±0.02)	0.2^{cB} (±0.01)	0.5^{bA} (±0.03)	0.6^{aA} (±0.04)	0.6^{aA} (±0.03)
S100	0.1^{dA} (±0.01)	0.1^{dB} (±0.01)	0.2^{cB} (±0.02)	0.3^{bA} (±0.02)	0.3^{bC} (±0.02)	0.5^{aB} (±0.04)	0.5^{aB} (±0.03)
S50	0.1^{cA} (±0.01)	0.1^{cB} (±0.01)	0.2^{bB} (±0.02)	0.2^{bB} (±0.01)	0.2^{bC} (±0.01)	0.4^{aC} (±0.03)	0.4^{aC} (±0.02)

Lowercase letters show significant difference within each row and uppercase letters within each column (α=0.05).

Overall, the *Scenedesmus* treatments had signific-antly higher biomass compared to the other treatments, at the end of the experiment ($P<0.05$). However, the *Chlorella* treatments had significantly lower biomass from the first day of the experiment. The mixed treatments' biomass was intermediate with the highest biomass in M0 and M50, and the lowest in M100.

Chlorophyll a values are shown in Table 7. Chlorophyll of the *Scenedesmus* treatments was significantly higher than the other treatments at the last days (14^{th}) of the experiment. Also, chlorophyll concentration in the treatments M was higher than those of the C group. Overall, the highest chlorophyll value was observed at the day 10 in M50 treatment (4.97 mgL^{-1}), but the lowest value was related to C100 at the day 2 (2.91 mgL^{-1}). Mean chlorophyll value at the day 10 was higher than the other sampling days in all groups. The lowest chlorophyll values were observed in the *Chlorella* treatments at all the sampling days. Among the *Chlorella* treatments, C100 treatment had the lowest chlorophyll value. Overall, S0 treatment had significantly higher chlorophyll at the end of the experiment ($P<0.05$); whereas, C100 treatment had the lowest chlorophyll. During the experiment, M50 treatment had the highest value ($P<0.05$). Chlorophyll value in M50 treatment is correlated to algae cell graph, which shows the highest number a t the day 8, and increase in algae cell number has resulted in biomass augment in M50 treatment.

Tab. 7: Chlorophyll a values in different treatments during the experiment (mean ± SD).

Treatment	Day						
	2	4	6	8	10	12	14
C0	1.6^{gE} (±0.67)	3.5^{bD} (±0.5)	2.83^{eC} (±0.58)	3.1^{cC} (±0.49)	3.9^{aC} (±0.81)	2.91^{dD} (±0.54)	2.65^{fB} (±0.55)
C100	1.5^{gE} (±0.62)	2.91^{bE} (±0.45)	2.89^{cC} (±0.6)	2.96^{bC} (±0.47)	3.16^{aD} (±0.61)	2.46^{dD} (±0.42)	2.13^{eB} (±0.45)
C50	1.55^{fE} (±0.63)	3.12^{bD} (±0.46)	2.98^{cC} (±0.61)	3.04^{bC} (±0.51)	3.27^{aD} (±0.65)	2.65^{dD} (±0.46)	2.2^{eB} (±0.47)
M0	1.7^{eC} (±0.71)	4.25^{aB} (±0.65)	3.57^{bB} (±0.76)	3.64^{bB} (±0.76)	3.43^{cC} (±0.67)	3.42^{cB} (±0.78)	2.83^{dB} (±0.59)
M100	1.88^{fB} (±0.74)	3.94^{bC} (±0.61)	3.02^{dB} (±0.65)	3.71^{cB} (±0.78)	4^{aB} (±0.88)	2.98^{dD} (±0.57)	2.23^{eB} (±0.48)
M50	1.77^{cC} (±0.72)	4.85^{aA} (±0.72)	3.27^{cB} (±0.72)	3.68^{bB} (±0.77)	4.97^{aA} (±0.91)	3.53^{bB} (±0.81)	2.45^{cB} (±0.51)
S0	2.22^{eA} (±0.81)	4.4^{bB} (±0.67)	4.33^{bA} (±0.88)	4.4^{bA} (±0.85)	4.87^{aA} (±0.95)	3.78^{cA} (±0.83)	3.17^{dA} (±0.64)
S100	1.84^{fB} (±0.71)	3.88^{bC} (±0.58)	3.23^{dB} (±0.64)	3.71^{cB} (±0.78)	4.28^{aB} (±0.9)	3.3^{dC} (±0.71)	2.37^{eB} (±0.49)
S50	1.9^{fB} (±0.82)	4.41^{bB} (±0.67)	3.43^{cB} (±0.7)	3.77^{cB} (±0.79)	4.64^{aB} (±0.93)	3.1^{dC} (±0.68)	2.27^{eB} (±0.49)

Lowercase letters show significant difference within each row and uppercase letterswithin each column (α=0.05).

In the first days of the experiment (until day 6), cell count in *Chlorella* group (C0 and C50) was higher than the other groups. In the middle of the experiment (until day 10) M treatments (M0 and M50) had the highest cell count. At the end of the experiment (the day 10), S treatments (S0, S100 and S50) had the highest cell count. During the whole period of the experiment, the highest cell count was observed at the day 8 in M50 treatment (in M group, total number of *Chlorella* and *Scenedsmus* cells was counted). Among the *Scenedesmus* treatments, S0 treatment had the highest cell count at the day 12, whereas, among the *Chlorella* treatments, C) treatment had the highest cell count. Overall, the *Scenedesmus* treatments showed an increasing pattern, but *Chlorella* treatments showed increasing pattern in the first days of the experiment and decreasing pattern in the last days of the experiment (Tab. 8). Totally, cell count of M50 treatment and that of S50 treatment were highest at the day 8 and the end of the experiment among the treatments respectively ($P<0.05$).

BOD_5 showed decreasing pattern during the study period (Tab. 9). During the experiment, *Chlorella* group had higher BOD than the other treatments; among which C50 treatment had higher BOD than the other treatments. *Scenedesmus* treatments had the lowest BOD among the treatments; M treatments had intermediate BOD, which was similar to that of the *Chlorella* group in the first days of the experiment. This pattern continued until the last day of the experiment, but it was significantly lower than that of the *Chlorella* treatment. There was no significant difference in BOD among the *Scenedesmus* treatments, but they had lower BOD than the other treatments ($P>0.05$). The *Chlorella* treatment had similar BOD, which was significantly higher than those of S and M treatments ($P<0.05$).

COD was similar to BOD_5 between groups during the study period (Tab. 10). SGR of the *Chlorella* group was significantly lower than the other treatment. *Scenedesmus* treatments had the highest SGR,

Tab. 8: Algae cell number (x0.00001) in different treatments during the experiment (mean ± SD).

Treatment	Day						
	2	4	6	8	10	12	14
C0	1.1^{bA} (±0.47)	12^{aA} (±1.12)	14.24^{bC} (±1.76)	1.24^{bD} (±0.21)	0.98^{cF} (±0.12)	1.4^{bD} (±0.61)	0.72^{cC} (±0.11)
C100	1.13^{aA} (±0.48)	1.07^{bD} (±0.56)	0.84^{bA} (±0.12)	1.12^{aD} (±0.14)	0.9^{bF} (±0.1)	0.22^{cD} (±0.07)	0.25^{cC} (±0.09)
C50	0.87^{cB} (±0.31)	9.78^{bB} (±1.06)	11.74^{aA} (±1.32)	0.67^{cE} (±0.01)	0.53^{cF} (±0.09)	0.63^{cD} (±0.08)	0.56^{cC} (±0.1)
M0	1.12^{gA} (±0.47)	6.68^{cC} (±0.94)	7.72^{bB} (±1.21)	14.49^{aA} (±1.22)	12.66^{eB} (±2.13)	2.18^{dC} (±0.71)	1.57^{fB} (±0.43)
M100	0.98^{cB} (±0.35)	2.29^{aD} (±0.71)	1.22^{bC} (±0.23)	1.57^{bD} (±0.24)	1.59^{bE} (±0.23)	1.21^{bC} (±0.53)	2.17^{aB} (±0.67)
M50	1.04^{fD} (±0.4)	3.86^{cD} (±0.82)	10.49^{aA} (±1.25)	32.74^{dB} (±2.35)	27.1^{bD} (±2.98)	2.72^{dC} (±0.75)	2^{eB} (±0.63)
S0	0.59^{eC} (±0.21)	2.32^{dD} (±0.72)	2.93^{dD} (±0.29)	3.97^{cB} (±0.89)	10^{bC} (±1.75)	12.12^{aC} (±1.43)	4.49^{cA} (±1.1)
S100	0.46^{eC} (±0.16)	1.11^{dE} (±0.58)	1.32^{cE} (±0.24)	2.2^{bC} (±0.65)	2.3^{bD} (±0.46)	2.4^{bC} (±0.75)	2.83^{aB} (±0.93)
S50	0.36^{gC} (±0.12)	1.17^{fE} (±0.6)	1.7^{eE} (±0.35)	2.87^{dC} (±0.71)	4.33^{cD} (±0.78)	4.76^{bB} (±0.89)	5.87^{aA} (±1.23)

Lowercase letters show significant difference within each row and uppercase letters 0 within each column (α=0.05).

Tab. 9: BOD_5 value in different treatments during the experiment (mean ± SD).

Treatment	Day						
	2	4	6	8	10	12	14
C0	4.86^{aA} (±0.98)	2.77^{bA} (±0.86)	1.94^{cA} (±0.92)	1.86^{cB} (±0.85)	1.64^{dB} (±0.71)	1.68^{dB} (±0.71)	1.52^{eA} (±0.71)
C100	2.74^{aC} (±0.67)	2.24^{bB} (±0.8)	1.66^{cB} (±0.74)	1.94^{cA} (±0.87)	2^{cA} (±0.97)	1.78^{cB} (±0.74)	1.56^{cA} (±0.72)
C50	3.91^{aB} (±0.89)	3^{bA} (±0.88)	2.3^{cA} (±0.99)	2.42^{dA} (±0.98)	2.18^{eA} (±0.99)	1.8^{fB} (±0.8)	1.48^{gA} (±0.65)
M0	3.72^{aB} (±0.85)	2.9^{bA} (±0.89)	2.06^{cA} (±0.97)	2.32^{cA} (±0.96)	2^{dA} (±0.97)	2^{dA} (±0.84)	1.4^{eB} (±0.61)
M100	2.56^{aC} (±0.57)	2.2^{bB} (±0.79)	1.75^{cB} (±0.78)	1.96^{cA} (±0.88)	1.82^{cB} (±0.96)	1.62^{dB} (±0.68)	1.36^{dB} (±0.54)
M50	2.26^{aC} (±0.43)	2^{bC} (±0.79)	1.5^{cB} (±0.7)	1.6^{cB} (±0.7)	1.4^{dB} (±0.67)	1.2^{eC} (±0.5)	1^{fC} (±0.31)
S0	1.59^{aD} (±0.34)	1.2^{bD} (±0.61)	0.91^{cC} (±0.09)	1^{cC} (±0.13)	0.9^{cC} (±0.23)	0.8^{dD} (±0.24)	0.7^{dC} (±0.13)
S100	1.56^{aD} (±0.32)	1.2^{bD} (±0.61)	1^{cC} (±0.13)	1.08^{cC} (±0.14)	0.98^{cC} (±0.26)	0.94^{cD} (±0.31)	0.72^{dC} (±0.14)
S50	2.1^{aD} (±0.4)	1.92^{bD} (±0.74)	1.5^{cC} (±0.56)	1.72^{cB} (±0.74)	1.6^{cC} (±0.69)	1.2^{dD} (±0.5)	0.78^{eC} (±0.15)

Lowercase letters show significant difference within each row and uppercase letters within each column (α=0.05).

Tab. 10: COD value in different treatments during the experiment (mean ± SD).

Treatment	Day						
	2	4	6	8	10	12	14
C0	24.31^{aA} (±1.56)	13.88^{bA} (±1.54)	11.64^{bA} (±1.56)	9.3^{bB} (±1.23)	8.2^{cB} (±1.12)	8.4^{cB} (±1.24)	7.6^{cA} (±1.34)
C100	13.72^{aC} (±1.1)	11.2^{aB} (±1.34)	10^{bB} (±1.2)	9.7^{bB} (±1.27)	10^{bA} (±1.25)	8.9^{cA} (±1.28)	7.8^{cA} (±1.37)
C50	19.56^{aB} (±1.41)	15^{bA} (±1.64)	13.8^{bA} (±1.78)	12.1^{bA} (±1.87)	10.9^{cA} (±1.29)	9^{cA} (±1.29)	7.4^{dA} (±1.3)
M0	18.6^{aB} (±1.37)	14.5^{bA} (±1.58)	12.4^{cA} (±1.67)	11.6^{cA} (±1.76)	10^{cA} (±1.25)	10^{cA} (±1.34)	7^{dA} (±1.27)
M100	12.8^{aC} (±1)	11^{aB} (±1.32)	10.5^{aB} (±1.23)	9.8^{bB} (±1.28)	9.1^{bA} (±1.17)	8.1^{cB} (±1.22)	6.8^{dB} (±1.25)
M50	11.3^{aC} (±0.98)	10^{aB} (±1.23)	9^{bB} (±1.1)	8^{cC} (±1.11)	7^{dB} (±1.03)	6^{eC} (±1.08)	5^{fB} (±1.1)

Tab. 10: continued.

Treatment	Day						
	2	4	6	8	10	12	14
S0	7.95^{aD} (±0.9)	6^{bC} (±1.1)	5.5^{bC} (±0.76)	5^{bD} (±0.98)	4.5^{cC} (±0.87)	4^{cD} (±0.9)	3.5^{dC} (±1.06)
S100	7.8^{aD} (±0.9)	6^{bC} (±1.1)	6^{bC} (±0.98)	5.4^{cD} (±1.09)	4.9^{cC} (±0.9)	4.7^{cD} (±1.01)	3.6^{dC} (±1.07)
S50	10.5^{aC} (±0.99)	9.6^{aB} (±1.14)	9^{bB} (±1.1)	8.6^{bC} (±1.14)	8^{bB} (±1.08)	6^{cC} (±1.08)	3.9^{dC} (±1.09)

Lowercase letters show significant difference within each row and uppercase letters within each column (α=0.05).

however, M100 treatment had the highest SGR (2.98). DT showed inverse trend compared to SGR as the highest DT was related to *Chlorella* treatments and C50 (0.7) and the lowest DT was observed in *Scenedesmus* group (Fig. 8).

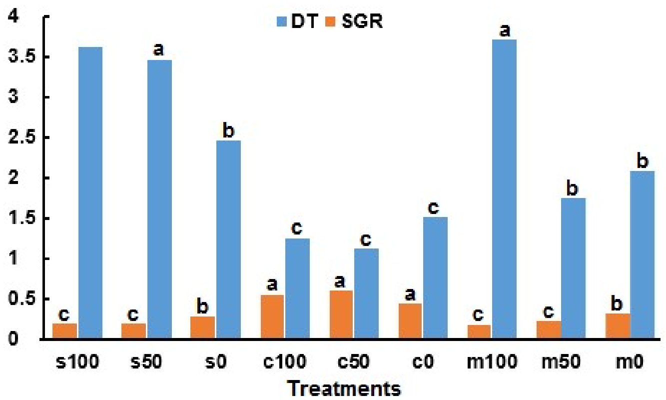

Fig. 8: SGR and DT in different treatments during the experiment. Different letters show significant difference during the experiment (α=0.05).

Overall, S50, M100 and S100 treatments had significantly the highest DT and the lowest SGR during the experiment (P>0.05). However, the lowest DT was related to C50 and C100 (P<0.05). M0 and M50 treatments' DT was between the *Chlorella* and *Scenedesmus* groups, but had significantly higher DT and lower SGR than the *Chlorella* group. C0 treatment was similar to M0 and M50, but the other *Chlorella* treatments were significantly lower than the other treatments.

Discussion

The results showed that *Chlorella*, *Scenedesmus* and mixed treatments had the highest phosphate uptake at 0%, 100% and 50% wastewater dilution, respectively, which phosphate was completely depleted. Phosphate uptake rate was higher in the first days compared to the last days of the experiment. All *Scenedesmus* groups and M50 treatment completely depleted the medium nitrate, but the highest nitrate was related to C0 treatment suggesting that *Chlorella* is more efficient in phosphate removal and *Scenedesmus* is more efficient in both nitrate and phosphate removal. However, M50 was the only treatment that completely depleted the medium phosphate and nitrate suggesting that the two algae mixture was more efficient among the treatments. Lee and Lee (2001) reported that phosphate removal rate by *Chlorella kessleri* is 28% with initial phosphate concentration of 10 mgL^{-1} in culture medium. However, Mallick *et al.* (2002) reported that *Phormidium bonheri* was capable to completely remove phosphate and nitrate with initial concentrations of 30 and 42 mgL^{-1} in culture medium. Similar to the our results, it was found that *Sc. obliquus* is capable to remove 97% of nitrogen and phosphorus within 14 days with initial concentration of 27.4 and 11.8 mgL^{-1} (Martinez *et al.*, 2000). *Scenedesmus obliquus* is able to remove 99.1% of wastewater nitrogen (NH_4-N) within 12 days with initial concentration of 3.8 mgL^{-1} (Yang *et al.*, 2011). In our study, *Chlorella* reproduced with a high rate in the first days of the experiment, but it showed declining trend after the day 8 suggesting lower tolerance of *Chlorella* to culture medium conditions compared to the two other groups. In the mixed algae group, increasing pattern started at the day 4 continued until the day 10th, but showed decreasing pattern in the last days. However, in *Scenedesmus* group an increasing pattern was observed during the experiment suggesting its high resistance to the culture medium conditions. The highest chlorophyll value was observed in S0 treatment; *Scenedesmus* and *Chlorella* had significantly the highest and the lowest chlorophyll values among all treatments had. M50 treatment had the highest chlorophyll due to synergistic effect. This treatment had the highest cell count at the day 8th suggesting correlation between cell count and chlorophyll content. According to Fig. 4 and 5, under constant conditions, each *Chlorella* cell has higher chlorophyll value than each *Scenedesmus* cell. The highest and the lowest algal biomass was found in *Scenedesmus* and *Chlorella* groups, respectively. The mixed treatment, although was close to *Scenedesmus* group, had significantly lower biomass than this group. It is suggested that culture medium nitrogen had more important role than phosphorus in algae biomass production; this explain weak biomass production in C0 treatment despite complete phosphate uptake. Ruiz-Marin *et al.* (2010) found that in wastewater as culture medium, growth rate of *Ch. vulgaris* (8 h) is higher than *Sc. obliquus* (20 h), which is in agreement with the present results. Also, they reported that *Sc. obliquus* is more resistance to various medium conditions than *Ch. vulgaris*, and *S. obliquus* had significantly higher chlorophyll-a at the end of the experiment (0.54 vs. 0.22 mgL^{-1}). Tam and Wong (2000) reported than high ammonium uptake by *C. vulgaris* is due to reaction between carboxyl group in the outer membrane and ammonium molecules. Chlorophyll and biomass of *S. obliquus* and *C. vulgaris* was found to be related energy intake (light) and self-shading in culture medium. Ruiz-Marin *et al.* (2010) reported that chlorophyll and biomass of *S. obliquus* was higher than *C. vulgaris* (0.56 vs. 0.31 g L^{-1}). Kaewsarn (2002) showed that *C. vulgaris* has low efficiency when cell count and wastewater concentration is low, compared to high cell count and wastewater concentration. At low cell count and wastewater concentration (nitrate = 90 mgL^{-1}) *C. vulgaris* removed 69% of nitrate and 58% of COD within 10 days. When cell count and wastewater concentration increased (nitrate = 178 mgL^{-1}) *C. vulgaris* removed 80% of nitrate and 63% of COD within 10 days suggesting that this alga is more efficient in wastewater with high concentrations.

Conclusion

It is concluded that mixture of the two algae results in high biomass and cell count and is more efficient in nitrogen and phosphorus uptake, because M50 was the only treatment that totally depleted the medium phosphate and nitrate at the end of the experiment. Other mixed treatments were also efficient in nutrient uptake and had no significant difference compared to *Scenedesmus* treatments. The highest biomass was observed in M100 at the day 10^{th} suggesting that these two algae have synergic effects; whereas, S0 treatment had the highest biomass and the *Chlorella* treatments had the lowest biomass. Also, algae mixture had synergic effect on chlorophyll value and cell count, as M50 treatment showed the highest cell count at the day 8^{th} which was significantly higher than the other treatments. Overall, *Scenedesmus* treatment, particularly S0 treatment, was more efficient in biomass production, chlorophyll value and nutrient uptake compared to *Chlorella* treatment. *Chlorella* treatment was superior to the other treatments in wastewater treatment, except for phosphate uptake, which C0 treatment completely depleted the medium phosphate content. Finally, it is suggested that change in wastewater concentration and algae type can improve municipal wastewater refining.

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