Determination of the Growth Rates of Spiroplina and Chaetoceros Algae in Urban Waste Sewage and their Capability to Deplete Nitrate and Phosphate Content in the Sewage

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ABSTRACT: The application of cyanobacterial and diatom cultures for the treatment of industrial effluents has been well recognized. In this study aimed to evaluate the effect of urban sewage on growth of *Spiroplina plantensis* and *Chaetoceros muelleri*. The experiment was conducted in 6 treatments as a growth medium. Result showed that in treatment 5 maximum cell densities was \((565\times10^3\pm237.7)\) at day7 thus treatment 5 has best condition for growth *S. plantensis* and in treatment 3 maximum cell density was \((825\times10^4\pm92)\) at day13.Treatment 5 has best condition for growth *C. muelleri*. Total chlorophyll a, contents (µg/l) recorded in *S. Plantensis* and *C. muelleri* was highest at treatment 3(0.21±0.07) and treatment 4(0.23±0.10) respectively. In present investigation, both the algal species can be good potential to growth in urban sewage. The urban sewage removal efficiency of *C. muelleri* was higher as compare to *S. plantensis* which can be recommended for phytoremediation purpose. © JASEM

http://dx.doi.org/10.4314/jasem.v20i3.24

Keywords: Spiroplina; Chaetoceros; Nitrate and phosphate; Chlorophyll a; Urban sewage; Depletion.

The pollution increase, industrialization and rapid economic development, are cause of decrease the availability and quality of water resources, in many areas worldwide. Nowadays, uncontrolled discharge from industrial sectors or agriculture discharged to aquatic ecosystems and contaminated total aquatic environment which, not only cause toxic effect on human, via accumulation in aquatic animals, through food chain but also affect biodiversity (Lavajoo et al. 2015). Wastewater generated from urban or industry sewage sources contain high concentrations of organic matter, nitrogen and phosphorus, and causes eutrophication in receiving water. Urban sewages contain nutrients (which have been identified as the main causes leading to eutrophication in natural waters) must be treated before being discharged into water bodies (Mallick, 2002). Instances of the effect of sewages on microalgae growth are arrested cell division, inhibited growth rate, restrained enzyme activity and reduced photosynthesis (Chen et al., 2009; Baumann and Morrison, 2009). Compared to other aquatic organisms in marine environment, unicellular microalgae exhibit highest resistant to sewages and highly recommended as bio-indicator for the assessment of marine pollution (Rijstenbeil et al., 1994; Kapkov and Belemenkina, 2003; Kapkov and Belemenkina, 2007). The use of several microalgae cultures in wastewater treatment has a major advantage that allows effective utilizing of nutrients (De la Noue et al.,1992). Microalgae culture systems can be employed in different processes, such as wastewater treatment and production of animal food (De la Noue and Proulx, 1988). The wastewater treatment by microalgae and cyanobacteria is known (De la Noue et al., 1993; De la Noue and Proulx, 1988; Oswald, 1988). In fact, traditional wastewater treatment process required high operation cost to provide suitable condition for aerobic bacteria to effectively consume organic components in polluted water. However, microalgae provide an efficient low-cost approach to treat wastewater (Lananan et al., 2014; Nasir et al., 2015). Recent studies showed that many algal species, especially *Chlamydomonas, Scenedesmus* and *Chlorella* decreased nutrients under light condition (mixotrophy) and they are also capable of heterotrophic growth on simple molecules, such as acetate, glucose and organic acids in the dark (Laliberte and De la Noue, 1993). It has been suggested that a Microalgae have been offered as bioremediation treatment to decrease \((NH_4)^+, NO_3^-\) and \(PO_4^{3-}\) nutrients (Mallick, 2002). The presence of high concentrations of ammonia and urea in urban sewages inhibit algal growth and physiological activity (Przytocka-Jusiak, 1976). Therefore, in the present study the growth rates and amount of nitrate and phosphate absorption of *Spiroplina plantensis* and *Chaetoceros muelleri* on urban wastewater were determined.

MATERIALS AND METHODS

*Test Species*: In this study the microalgae cultures of marine blue green algae (*Spiroplina plantensis*) and Diatom (*Chaetoceros muelleri*) were obtained from the phytoplankton culture laboratory, of institution Persian Gulf and Oman Sea Hormozgan in Iran. The
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Experimental Design: In the laboratory, the samples of sewage were filtered through a 25mm, 3µm glass microfiber filters (GF/C) mounted on a Millipore filtration unit and sterilized by autoclaving at 121°C for 15 minutes. The culture growth medium prepared with three replicates for conducting the experiments and the duration was 15 days under similar laboratory conditions at temperature (30°C ± 2°C) for Spiroplana plantensis and Temperature (25°C ± 2°C) for Chaetoceros muelleri. Light (2500 ± 500) lux for Spiroplana plantensis and Light (4500 ± 500) lux for Chaetoceros, optimum pH was between 8.0 and 11 for Spiroplana plantensis and for Chaetoceros muelleri was between 9.0 and 11, that adjusted by electronic pH meter (ELICO, Model LI 120) and with constant aeration.

Treatments A: (1) Spiroplana plantensis was cultivated in f/2 Medium based on (Guillard, 1975). (2) Spiroplana plantensis was cultivated in 80% of filtered seawater and 20% urban waste (3) Spiroplana plantensis was cultivated in 60% of filtered seawater and 40% urban waste (4) Spiroplana plantensis was cultivated in 40% of filtered seawater and 60% urban waste (5) Spiroplana plantensis was cultivated in 20% of filtered seawater and 80% urban waste (6) Spiroplana plantensis was cultivated in 100% urban waste. Total volume of culture and media was 200ml.

Treatments B: (1) Chaetoceros muelleri was cultivated in f/2 Medium based on (Guillard, 1975) (2) Chaetoceros muelleri was cultivated in 80% of filtered seawater and 20% urban waste (3) Chaetoceros muelleri was cultivated in 60% of filtered seawater and 40% urban waste (4) Chaetoceros muelleri was cultivated in 40% of filtered seawater and 60% urban waste (5) Chaetoceros muelleri was cultivated in 20% of filtered seawater and 80% urban waste (6) Chaetoceros muelleri was cultivated in 100% urban waste. Total volume of culture and media was 200ml.

Cell density and Growth Rate: The cell growth was monitored by measuring cell numbers by manual counting under the binocular light microscope. Growth rates were calculated as µ. day⁻¹ according to the following Formula: \[ \mu = (N_1/N_0)/t \] Where, N0 and N1 represent cell density at the start and the end of the growth period, and tare the time between measurements (in days).

Physicochemical analysis: Water quality analysis of ammonia and phosphorus (orthophosphate) determination were based on Phenate Method and Vanadomolybdo phosphoric Acid Method adapted from the Standard Method for the Examination of Water and Wastewater (APHA, 2005). Sampled water was clarified from the MA and EM biomass by centrifugation at 5000 rpm, 15 min to obtain clear supernatant which was subjected immediately to water quality analysis for the determination of ammonia and phosphorus. Nitrate and chlorophyll, were also measured by spectrophotometer UV/visible (Varian-carry 100) according to Manual of Oceanographic Observations and Pollutant Analysis Methods procedures (Marine environment assessment marine meteorology, 1999).

Statistical analysis: To test whether there was statistical difference among the cell density between Chaetoceros muelleri and Spiroplana plantensis in different days, we performed a Student’s t-test. The mean and standard deviation values of the triplicates for each treatment were calculated. The results were analyzed statistically by using Spss 17 software and graphical analyses were performed using Microsoft Office Excel.

RESULTS AND DISCUSSION

Growth of Spiroplana plantensis at different treatments: In the present study, the number of Spiroplana cells at different treatments was shown. In treatment 1 (100% f/2 Medium) cells appeared to reach their stationary phase after 11 days of cultivation and started to decline thereafter. In treatment 1 maximum and minimum cell density were \(588 \times 10^2 \pm 121\) and \(48 \times 10^2 \pm 8\) for day11 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density (P<0.05). All analyses were performed at 5% statistical significance level. In treatment 2 (80% f/2 Medium+ 20% urban waste) cells appeared to reach their stationary phase after 9 days of cultivation and started to decline thereafter. In treatment 2 maximum and minimum cell density were \(116 \times 10^2 \pm 16\) and \(52 \times 10^2 \pm 8\) for day 9 and day 15 respectively. In one-way analysis of variance (ANOVA), between days and cell density did not observed significant difference (P>0.05). All analyses were performed at 5% statistical significance level. In treatment 3 (60% f/2 Medium+ 40% urban waste) cells appeared to reach their stationary phase after 11 days of cultivation and started to decline thereafter. In treatment 3 maximum and minimum cell density were \(357 \times 10^2 \pm 92\) and \(54 \times 10^2 \pm 18.5\) for day 11 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density (P<0.05). All analyses were performed at 5% statistical significance level. In treatment 4 (40% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after
11 days of cultivation and started to decline thereafter. In treatment 4 maximum and minimum cell density were \((424.7 \times 10^{2} \pm 62.1)\) and \((60 \times 10^{2} \pm 6.1)\) for day 11 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level. In treatment 5 (20% f/2 Medium+ 60% urban waste) cells appeared to reach their stationary phase after 11 days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were \((565 \times 10^{2} \pm 237.7)\) and \((80 \times 10^{2} \pm 7.1)\) for day 7 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level (Fig.1) (Table.1).

### Table.1. Cell density of Spiroloina plantensis and Chaetoceros muelleri at the end of test period (day 15)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Spirolina ((\times 10^{2} \text{cell/ml}))</th>
<th>Mean</th>
<th>Std.</th>
<th>Chaetoceros ((\times 10^{4} \text{cell/ml}))</th>
<th>Mean</th>
<th>Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment1</td>
<td>275.90</td>
<td>181.46</td>
<td></td>
<td>324.02</td>
<td>250.97</td>
<td></td>
</tr>
<tr>
<td>Treatment2</td>
<td>83.07</td>
<td>27.78</td>
<td></td>
<td>539.66</td>
<td>234.47</td>
<td></td>
</tr>
<tr>
<td>Treatment3</td>
<td>180.71</td>
<td>100.76</td>
<td></td>
<td>664.95</td>
<td>236.74</td>
<td></td>
</tr>
<tr>
<td>Treatment4</td>
<td>264.71</td>
<td>161.86</td>
<td></td>
<td>424.16</td>
<td>205.67</td>
<td></td>
</tr>
<tr>
<td>Treatment5</td>
<td>333.0</td>
<td>222.51</td>
<td></td>
<td>475.88</td>
<td>328.17</td>
<td></td>
</tr>
<tr>
<td>Treatment6</td>
<td>320.61</td>
<td>202.18</td>
<td></td>
<td>305.80</td>
<td>252.74</td>
<td></td>
</tr>
</tbody>
</table>

Impact of different treatments and days on the growth of Spiroloina plantensis: To determination of best treatment condition and lowest time for highest Spiroloina plantensis growth we used of two-way analysis of variance (ANOVA). We observed a strong positive correlation \((R^2 = 0.82)\) between treatment conditions and times for Spiroloina plantensis growth (Fig.2).
Growth of Chaetoceros muelleri at different treatments: In treatment 1 (100% f/2 Medium) cells appeared to reach their stationary phase after 9 days of cultivation and started to decline thereafter. In treatment 1 maximum and minimum cell density were \((489 \times 10^4 \pm 121)\) and \((179 \times 10^4 \pm 8)\) for day 9 and day 5 respectively. In one-way analysis of variance (ANOVA), between days and cell density did not observe significant difference \((P>0.05)\). All analyses were performed at 5% statistical significance level.

In treatment 2 (80% f/2 Medium + 20% urban waste) cells appeared to reach their stationary phase after 9 days of cultivation and started to decline thereafter. In treatment 2 maximum and minimum cell density were \((639 \times 10^4 \pm 16)\) and \((439 \times 10^4 \pm 8)\) for day 9 and day 3 respectively. In one-way analysis of variance (ANOVA), between days and cell density did not observed significant difference \((P>0.05)\). All analyses were performed at 5% statistical significance level.

In treatment 3 (60% f/2 Medium + 40% urban waste) cells appeared to reach their stationary phase after 13 days of cultivation and started to decline thereafter. In treatment 3 maximum and minimum cell density were \((825 \times 10^4 \pm 92)\) and \((63 \times 10^4 \pm 18.5)\) for day 13 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level.

In treatment 4 (40% f/2 Medium + 60% urban waste) cells appeared to reach their stationary phase after 9 days of cultivation and started to decline thereafter. In treatment 4 maximum and minimum cell density were \((523 \times 10^4 \pm 62.1)\) and \((195 \times 10^4 \pm 6.1)\) for day 9 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level.

In treatment 5 (20% f/2 Medium + 60% urban waste) cells appeared to reach their stationary phase after 13 days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were \((832 \times 10^4 \pm 237.7)\) and \((71 \times 10^4 \pm 7.1)\) for day 13 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level.

In treatment 6 (100% urban waste) cells appeared to reach their stationary phase after 15 days of cultivation and started to decline thereafter. In treatment 5 maximum and minimum cell density were \((529 \times 10^4 \pm 190.7)\) and \((58 \times 10^4 \pm 15.9)\) for day 15 and day 3 respectively. One-way analysis of variance (ANOVA), showed significant difference between days and cell density \((P<0.05)\). All analyses were performed at 5% statistical significance level.
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variance (ANOVA), showed significant difference between days and cell density (P<0.05). All analyses were performed at 5% statistical significance level (Fig.3) (Table.1).

Impact of different treatments and different days on the growth of Chaetoceros muelleri: Two-way analysis of variance (ANOVA) used for determination of best treatment condition and lowest time for highest Chaetoceros muelleri growth. We observed a strong positive correlation (R² = 0.70) between treatment conditions and times for Chaetoceros muelleri growth (Fig.4). The result of t-test suggested that in most of treatments (except treatment 3) at different days (except day15) did not have significant difference between both of algae (P>0.05) but there was significant difference between cell density in both of algae (P<0.05) (Fig.4) (Table.2).

Table.2. T-test analysis for comparison between C. muelleri and S. plantensis cell density in different treatments at 15 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day3</th>
<th>Day5</th>
<th>Day7</th>
<th>Day9</th>
<th>Day11</th>
<th>Day13</th>
<th>Day15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment1</td>
<td>s</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
</tr>
<tr>
<td>Treatment2</td>
<td>s</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>s</td>
<td>Ns</td>
</tr>
<tr>
<td>Treatment3</td>
<td>s</td>
<td>Ns</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Treatment4</td>
<td>s</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>s</td>
<td>Ns</td>
</tr>
<tr>
<td>Treatment5</td>
<td>s</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Treatment6</td>
<td>s</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>Ns</td>
<td>s</td>
<td>Ns</td>
</tr>
</tbody>
</table>

S: Significant difference at confidence interval 95% (p < 0.05).
NS: No significant difference at confidence interval 95% (P>0.05).
Fig. 4. *Chaetoceros muelleri* cell density in different treatments at 15 days

**Chlorophyll a**

The mean concentration of Chlorophyll a in different treatment in *Spirolina plantensis* and *Chaetoceros muelleri* were respectively (0.169±0.06) µg/l and (0.173±0.06) µg/l. The result of t-test between Chlorophyll a concentrations and two algae was showed no significant difference between them. Total chlorophyll a contents (µg/l) recorded in *Spirolina plantensis* and *Chaetoceros muelleri* were highest at treatment 3 (0.21±0.07) and treatment 4 (0.23±0.10) respectively (fig.5) (Table.3).

Fig. 5. Concentration of chlorophyll- a, in different treatments
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Table 3. Mean and standard deviation at the end of test period (day 15)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chlorophyll a (µg/l) Mean</th>
<th>Chlorophyll a (µg/l) Std.</th>
<th>Nitrate (mg/l) Mean</th>
<th>Nitrate (mg/l) Std.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirolina</td>
<td>0.16</td>
<td>0.04</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Chaetoceros</td>
<td>0.14</td>
<td>0.087</td>
<td>0.14</td>
<td>0.01</td>
</tr>
<tr>
<td>Treatment1</td>
<td>0.23</td>
<td>0.23</td>
<td>0.55</td>
<td>0.18</td>
</tr>
<tr>
<td>Treatment2</td>
<td>0.15</td>
<td>0.04</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td>Treatment3</td>
<td>0.21</td>
<td>0.07</td>
<td>0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>Treatment4</td>
<td>0.18</td>
<td>0.03</td>
<td>0.23</td>
<td>0.10</td>
</tr>
<tr>
<td>Treatment5</td>
<td>0.15</td>
<td>0.01</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>Treatment6</td>
<td>0.16</td>
<td>0.09</td>
<td>0.15</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Mean and standard deviation of three replicates are shown. Underline indicated maximum concentration.

Concentration of nitrate and phosphate in *Spirolina plantensis* and *Chaetoceros muelleri*: In this study the range of nitrate concentration in *Spirolina plantensis* and *Chaetoceros muelleri* were respectively between 0 mg/l to 0.56 mg/l and 0.25 mg/l to 5.0 mg/l. The results of one-way analysis of variance (ANOVA) for *Spirolina plantensis* and *Chaetoceros muelleri* suggested that there were no significant and significant difference in nitrate concentration in different treatments respectively. Also the range of phosphate concentration in *Spirolina plantensis* and *Chaetoceros muelleri* were respectively between 2 mg/l to 31.2 mg/l and 24.5 mg/l to 61.9 mg/l. The results of one-way analysis of variance (ANOVA) for *Spirolina plantensis* and *Chaetoceros muelleri* suggested that there were significant and no significant difference in phosphate concentration in different treatments respectively (Fig. 6, 7).

As we know application of cyanobacterial and diatom cultures for the treatment of industrial effluents has been well recognized. Microalgae culture suggests an interesting step for wastewater treatments, because they provide a tertiary biotreatment coupled with the production of potentially valuable biomass, which can be used for several purposes. The comparisons influence two factors (treatments and days) via one-way analysis of variance (ANOVA) in *Spirolina plantensis* was more than *Chaetoceros muelleri*. In *Spirolina plantensis* maximum cell density was the lowest in treatments 1 and 2. The important note in *Spirolina* sp. was in all treatments, cells appeared to reach their stationary phase at day 5 to day 11 then started to decline thereafter. In this study existence of nitrate and phosphate in treatments was one of the main factors on *Spirolina plantensis* growth at different days and the growth continued to stationary phase when enough nutrients were in media but growth decreased when the concentration of nutrients were low. Therefore we could suggest in lab conditions, the growth rate...
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...use of significant biomass. Other authors have reported the factors which could grow in wastewater and produce the great compatibility to alterations in environmental concentration of chlorophyll a, in the effluent (Karthikeyan et al., 2010). The concentration and inhibited at higher concentrations of Chaetoceros simplex the nutrient removal efficiency. The growth rate of the algal density, the better the growth and the higher nutrients to phytoplankton through nutrient limitation indicated Nitrogen was one of the chief limiting concentration of nutrients. Livingston et al. (2002) in reported papers, Lau et al. (1995) studied the higher the algal density, the better the growth and the higher the nutrient removal efficiency. The growth rate of Chaetoceros simplex was slightly enhanced with lower concentration and inhibited at higher concentrations of the effluent (Karthikeyan et al., 2010). The concentration of chlorophyll a, in Spirulina plantensis in treatment 2 and 5 presented exponential phase, due to increasing cell density in these treatments while Chaetoceros muelleri was not showed exponential phase in concentration of chlorophyll a. one of the main colures in microalgae is chlorophyll a, which have important role in photosynthesis and could used to indication for primary production (Strickland and Parson, 1972). The low concentration of nitrates in all treatments, suggested that there was no significant difference in nitrate concentration in different treatment in both of algae. Concentration of phosphate (mg/l) in both of algae in treatment 1(100% f/2 Medium) was higher than other treatments thus the reductions in urban waste phosphate in all algal treatments were significantly better than those without urban waste.

Conclusion: The phytoremediation was very efficient, cost effective and eco-friendly indicating that microalgae has vital role in the removal of different pollutants from wastewater. In present investigation both the algal species had very good potential to growth in urban sewage but the urban sewage removal efficiency of Chaetoceros muelleri was higher as compare to Spirulina plantensis which can be recommended for phytoremediation purpose.

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