Nutrients and organic matters removal of ospitals wastewater by microalgae

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Abstract .Global water contamination by different sorts of pollutants become an emerging environmental concern. The study aims to examine the capacity of Chlorella sp and Anabaena sp simultaneous, remediate hospital wastewater at 22°C and 32°C on gradient concentration of Nutrients and organic matters 5%, 25% and 50%, the parameters measured on the day 1, 4, 10 and 14 of planting them in lab bioreactors. The results showed greater vitality of Chlorella sp and Anabaena sp to remove pollutants at both temperatures at 50% concentration. At 22°C Anabaena sp showed more removal efficiency of PO₄ than Chlorella sp at 100% and 82%, respectively, while for NO₃ both almost have the same ability at 99% and 94% sequentially. It was similar to COD when the removal percentage was 94% for both of them. At 32°C the results illustrated different pattern of removal for NO₃ and COD but, it was same for PO₄. Chlorella sp has better removal proportion for NO₃ at 76% and COD at 93% compare to Anabaena sp for NO₃ and COD at 54% and 92%. However, Anabaena sp removes 69% and Chlorella sp 56% of PO₄ at this temperature. Our study indicates that the microalgae performed great ability to remove and capacity of growth in this type of wastewater which is appearing to play a key role in biodegradation process through high removal efficiency and low environmental impact.

1. Introduction

The use of algae cultures in wastewater treatment extended to about 75 years ago specially Chlorella and other species[1]. USA, Australia and other countries shows significant interest in developing and advance use of algae in treating wastewater, that’s come as a result of a combination of large-scale biological knowledge with an engineering system for getting high-value outcomes [1, 2]. In recent years there are more attention focused on using microalgae in a new biological system of wastewater treatment [3]

Microalgae can overcome on some main limitation of fungi and bacteria in term of using them in wastewater treatment because they need carbon source and other elements in a balance of stoichiometric for pollutant degradation [2]. Among many wastewater treatment applications, biosorption using microalgae proposed as a win-win paradigm biorefinery [4]. The fascinating approach of microalgae as bio-treater has gotten huge attractive due to their photosynthetic abilities, converting solar energy and producing biomass [1]. Bioreactors techniques in wastewater treatment using microalgae cultivation is considered to be optional sustainable strategic removing contamination method [5].
Microalgae has been used in wastewater treatment system for many reasons include reduced both chemical oxygen demand COD, removing N and/or P and for heavy metals. [1]. The ability of microalgae has been proven to treat organic materials [4]. Wastewater can be treated and clean by microalgae [6]. Microalgae needs to consume around (45-60% microalgae dry weight) of Nitrogen and phosphorus as they need it to synthetic progress as well as other macro nutrients [7], and heavy metal through the adsorption mechanism [8]. *Chlorella sp* was widely used in wastewater treatment application as it has proved the capacity of reduce COD and removing N and P on different retention period [9]. With regard to *Anabaena sp* so it has also the ability to remove nutrients, organic materials as well as absorbing heavy metals [10].

Microalgae has the ability to switch their metabolism between autotrophic and heterotrophic which is called “Mixotrophic” according to carbon source and nutrient availability [11]. The unique features make them promising choice for efficient wastewater treatment [2].

With regard to biomass microalgae can be classified as largest worldwide producers. Therefore, using microalgae overweight benefits compares to other techniques (i)Higher photosynthetic fixation (ii) Able to combine leftover nutrients from the wastewater stream in their growing process. (iii) capable to manage and grow up in extreme adverse conditions (v) mitigating greenhouse gases emitted mainly CO₂ [7].

Hospitals wastewater typically consists numerous chemical pollutants which are acting as toxic elements [12]. The hospital wastewater has complex considerable loads of organic, inorganic pollutants and pharmaceutical agents about 10-100 times more than regular domestic wastewater [8]. In addition, it has a content noticeable antibiotic concentration 1000 times more than domestic wastewater and they are becoming main limitation of biological wastewater system (covenantal activities sludge CAS) [12].

Microalgae can be grow in hospitals wastewater although, it includes some undesired compounds like heavy metals, toxins, pharmaceuticals and other pollutants [6]. Hospitals wastewater consists high concentration of phosphorus and nitrogen originate from detergents and pharmaceuticals [13]. Microalgae has the capacity to grow in an environment with high concentration of nutrients in wastewater discharge [14]. So they can be extremely attractive for cheap, sustainable, and eco-friendly treatment approach [15]. Conventional treatment method do no remove phosphorus from effluent so that these high concentration could cause eutrophication if it used for agriculture rather than other purposes[13]. Despite using microalgal species in wastewater treatment in removing nutrients and pollutants has become widespread [16]. since 1957 [17]. It still faces many challenges [13].

As such the objective of this study are to investigate that efficiency of *Chlorella sp* and *Anabaena sp* microalgae species in nutrients and organic matters removal from real contaminated hospital wastewater.

### 2. Materials & Methods

#### 2.1. Algae strains and cultures co

Two of microalgae available in Iraqi environment have been used in the research study. The pure strain of *Chlorella sp* and *Anabaena sp* obtain from the ministry of sciences and technology. Microalgae culture through using Chu-10 media, that has been proven successful growth for many algae species [10, 18].
2.2. Wastewater sampling and bioreactor preparation

Hospitals wastewater samples have taken from Kirkuk province – Iraq, then they sterilized by using Autoclave at 121°C and 1 kg/cm for 15 min and store in refrigerator to use them for bio-reactor later on. The bio-reactor prepared by design isolated continuous air system on reactors, each reactor consists of 100 ml, the reactors content different concentration of wastewater 5%, 25% and 50% as in the table (1).

| The bioreactor symbol | Percentage of wastewater | Percentage of microalgae solution |
|-----------------------|--------------------------|----------------------------------|
| 5%                    | 95%                      | 5%                               |
| 25%                   | 75%                      | 25%                              |
| 50%                   | 50%                      | 50%                              |

2.3. Nutrients and organic matters analyses

Liquid supernatant filtered samples of wastewater were taken from bio-reactors on the 1,4,10,14 retention time (days) (heavy metals) respectively of the experiment collected for analyses. COD, NO₃ and PO₄ have tested according to (APHA 2001)

3. Results and Discussion

3.1. Chemical Oxygen Demand (COD)

The results of COD removal shown in the figure (1&2)

![Anabaena sp COD Reduction at 22°C and 32°C](image)

*Anabaena sp* shows the higher COD removal proportion for both 22°C and 32°C in the 4th measurements. After more than 24 hrs. of running the bio-reactor the removal was varied between 8% to 22%, except Con 50% at 32 the removal was more than 40%. The results on the 2nd measurement shows sharp increase in removing organic matters reaching between 50% to 80%. However, Con 5% and 25% at 22°C showed a gradual increase pattern of removing organic materials at 30% and 25%
receptively. The 3rd measurement on the day 10 of retention time has the same pattern of the 2nd measurement but, with more removal percentage between 80% to 90%. The last day of the experiment shows high removal of all concentrations and temperature to be between 88% to 94%. The trend of wastewater concentration 5% and 25% at 22°C showed a dramatic increase of organic matters removal while reaching to a removal point equal to another wastewater proportion in the reactor.

The results of Anabaena sp indicate that it has the ability to adapt and remove pollutants at high temperature [19]. In addition, it has the capacity to enter the exponential growth phase at such relatively high temperature [20]. The majority of pollutants removal in stability phase after Anabaena sp will adapt to the concentration and bio-reactor conditions [12]. Anabaena sp has the capacity to grow up and consume different organic matter concentration at a wide range of temperature [21]. That, however, indicate the ability of Anabaena sp to eliminate organic matters from hospital wastewater [1].

![Figure (2): Chlorella sp COD Reduction at 22°C and 32°C](image)

Chlorella sp has almost a similar COD removal trend to Anabaena sp for both 22°C and 32°C in the 4th measurements. The first measurement shows begin of removal at both temperatures and all concentrations and they were in order the lowest removal proportion was on concentration 5% at 22°C while the highest belong to concentration 50% at 32°C from 18% to 40% COD removal. The 2nd measurement has the same trend as 1st one, but, with larger removal scale between lowest and highest COD removal percentage from of 30% to 70%. The 3rd measurement COD removal keeps increasing by Chlorella sp where the range of removal at this time between 80% to 90%, except the concentration 50% at 22°C stays on the same level of removal rate. The final day of the experiment shows higher removal of COD on around 93%±2.

The results of Chlorella sp that it has ability to remove organic matter at both temperatures. Also, it has the capacity to grow and uptake organic matters from hospital wastewater although there are some toxic that might impacts of its growth process so that it takes a time for adapting to these circumstances at the beginning of the experiment after that it’s beginning with exponential growth phase and remove organic pollutants [18], that could be the reason when concentration of 50% at 22°C and 32°C takes more time than others to adapt. Hospitals wastewater has wide range and high concentration of complex organic compounds, all of them have at least a carbon atom in their chemical structure [1, 22]. On the other hand, Chlorella sp can effectively remove organic substances from hospitals wastewaters even though in high concentration [23]. Microalgae uptake soluble carbon sources in wastewater treatment in the process of photosynthesis as a source for fixing CO₂ [24]. Some
microalgae are mainly autotrophic while others are heterotrophic depending only on organic carbon as the main source. However, mixotrophic can use both regimes in the growth process [15, 24]. Some Chlorella sp species are mixotrophic[21].

3.2. Phosphorus PO₄

The results of PO₄ removal shown in the figure (3&4)

![Anabaena sp PO₄ Reduction at 22°C and 32°C](image)

*Figure (3): Anabaena sp PO₄ removing at 22°C and 32°C*

Anabaena sp shows the general increase trend of removing PO₄. on the 1st day of the experiment consume between 18% to 40% of PO₄ in the bio-reactors were 18% remove by wastewater concentration 5% at 22°C and 40% by concentration 50% at 32°C. The bio-reactors 2nd measurement at 22°C illustrates liner raise of removal for the three concentrations while, 32°C shows sharp increase. The 3rd measurement express reverse pattern when measurement at 22°C kept the liner increase, reaching to 85% to 90%, while 32°C shows slight increase to be around 94%±2% of removal compare to 22°C. The 4th measurement shows a different pattern when concentrations at 22°C kept increase removing PO₄ and exceeding all the concentrations of bio-reactors at 32°C at 97±2. However, concentrations of bio-reactors at 32°C shows decline pattern in removing PO4 from the bio-reactors.

The results indicate that removing phosphorus from the bio-reactors mainly control of the many factors. The adaptation phase was short period and begin by reducing phosphorus concentration than its enter to exponential growth phase to consume phosphorus [18]. Various factors impact on microalgae activities in treating wastewater, including temperature and nutrients variable concentration [16]. Temperature play a key role on removal rate[25, 26]. So that it’s clear that the bio-reactors at 22°C shows more sustainable liner removal slope while at 32°C the removal rates were much faster. the growth at 32°C is declining with reduction of phosphorus supply as it consumes in previous growth phases of the algae [16]. The continuous growth of microalgae and wastewater remediation require supplementation of essential macro nutrients [21]. Microalgae acclimated mechanism to extreme conditions like high concentration has been explained through physiological adaptation [2, 8].
Chlorella sp has shown a different PO₄ removal pattern compared to Anabaena sp. The first measurement of the experiment shows PO₄ removal proportion between 16% to 40%, then increase to 24% to around 80%. The 3rd measurement shows slight changes when the concentration of 25% at 22°C sharp increase in removal while concentration of 5% and 25% at 32°C shows the very limited removal rate. The 4th measurement shows decline in removal percentage of concentration 5% and 50% lower than 3rd measurement. However, the other concentrations reach to higher removal proportion than 3rd measurement. The results illustrated in figure (4).

*Chlorella sp.* Green microalgae [1, 7]. It is a common single cell microalgae used in wastewater treatment application, that has the ability to grow up and remove high and low concentration of nutrients (N and P) composition [27]. Phosphorus plays a pivotal role in algal growth metabolism [24]. Phosphorus is regularly exists in wastewater as inorganic anionic types, [21]. It is demonstrated luxury and rapid phosphorus removal ability that through synthesized and accumulate polyphosphates in its tissues [9]. Phosphorus reduction in the first 24 h of the experiment mainly assumingly associated with adsorption on the cells surface, with no biological activities to uptake process [28]. Moreover, it considers to be the adaptation phase when for it inside the bio-reactor. *Chlorella sp.* showed an optimal temperature 22°C±2, the average temperature was more necessary for growth than the day or night temperature range[8, 28]. The temperature impacts on higher metabolic activities and hence nutrients uptake rates can be indicated for rising temperatures, reaching its peak when cultivation temperature is almost reach to the optimal degree. On the other hand, rise in temperature could lower the solubility of some nutrients in wastewater[24]. *Chlorella sp* has potential capacity to remove phosphorus from wastewater, therefore it gained considerable attention [29]. The removal of phosphorus and nitrate are associated through cellular metabolism [27]. Microalgae consider to be an effective method for remediating, hospital wastewater in high concentration of phosphorus and nitrogen [30]

### 3.3. Nitrate NO₃

The results of NO₃ removal shown in the figure (5&6)

*Anabaena sp* shows ability to remove NO₃ around 90% of total NO₃ in this experiment. The first day the removal rate was between 20% to 30%. Then the removal increases reaching to 3rd measurement. The 4th measurement shows decline in the concentrations at 32°C while the concentrations at 22°C kept increasing. As in the figure (5).
The results indicated that *Anabaena sp* has the ability to remove a high percentage of nitrate [29]. The adaptation phase made the algae cope with bio-reactor conditions and will adapt. The high concentration of nitrogen could impact on overall removal performances mainly in adapting growth phase [31]. *Anabaena sp* Cyanophyta can use and cope up with high concentration of nitrate and grow up in a rich-nutrients environment [29]. Nitrogen as Nitrate (NO$_3$) is the most favorable source of nitrogen after nitrogen as ammonia, so it can use it till depleted. Because of low energy require to absorb the process [32]. NO$_3$ consider to be one of most common principle nitrogen forms in wastewater that can be uptake by *Anabaena sp*. The sharp increase of NO$_3$ removal by *Anabaena sp* at the exponential growth phase could be because, linked with their metabolic activities [29]. Microalgae needs mainly constitutes four elements include carbon, phosphorus, nitrogen and sulfur[30]

For *Chlorella sp* has express high ability to remove NO$_3$ through experiment retention time. The 1$^{st}$ measurement the NO$_3$ removal was between 17% to 32%. Then more to increase NO$_3$ removal range taken places in 2ed and 3$^{rd}$ measurements at 40% to 75% and 77% to 95% respectively. The 4$^{th}$ measurement shows decline in the concentration of 25% and 50% at 22$^\circ$C while, the remain concentration stays at the removal rate reaching to 82% to 97%. As in figure (6)
4. Conclusion:

The results of our study match with Gomez [32], as well as with Abdel-Raouf [1] when they indicated that Chlorella sp has the ability to eliminate nutrients (NO$_3$ and PO$_4$) from industrial wastewater at more than 50.2% and 85% receptively. Microalgae could not remove nitrogen without the presence of phosphorus or conversely in wastewater treatment process because both of them are consider to be essential elements for algae growth [32]. The high concentration of NO$_3$ enhanced algae performance for more removal rate of exponential growth phase [2], for that the sharp rise in removal proportion began after the 4$^{th}$ day of the experiment lunched. In addition, Chlorella sp could use nitrate as a primary nitrogen source for nitrogenous metabolism [9]. The self-adapting features of microalgae marked as beneficial for wastewater treatment due to frequent changes in conditions [33]. Microalgae consider to be a low cost approach for nutrients removing [1].

5. References

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