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Potential of Fungal-Microalgal Species in the Environmental Biotechnology

Muzhda Q. Qader[*1], Yahya A. Shekha[1]

[1]*Environmental Science and Health Department, College of Science, Salahaddin University, Erbil, Kurdistan Region, Iraq.*

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ABSTRACT

Biotechnology involves the provision of services to society by microbial communities. By using microorganisms to detoxify contaminated water, and environmentally friendly solutions to the treatment of wastewater. Isolates of fungi Aspergillus niger, Candida albicans and microalgal isolates Tetradesmus nygaardi and Scenedesmus quadricauda were efficiently utilized to treat wastewater. The wastewater sample was taken from Erbil wastewater channel near Dhahibah village, the sample was analyzed for physicochemical parameters such as pH, EC, TDS, BOD5, PO4, NH4, NO3, and NO2 (every three day) during 21 days of experiment. Results revealed that a mixed culture of S. quadricauda with T. nygaardi were shows the best removal of PO4, NH4, NO3 and NO2 which was 94.94%, 90.73%, 88.23%, 93.84% respectively. Whereas a mixed culture of T. nygaardi with A. niger showed the best removal of BOD5 (94.9%), the highest reduction in electrical conductivity and total dissolved solid were recorded by mix culture of A. niger with C albicans which was 81.65% and 81.52%. During the treatment period chlorophyll-a concentration reached the highest value of (1.052, 1.005 and 2.521 mg. l-1). Statistically, there were a significant difference ($p \leq 0.05$) between the control and all microbial strains for all wastewater tested variables.

Keywords: Microbiota remediation; Microalgae; Aspergillus niger; Water quality; Filamentous fungi; Biotechnology.

1. Introduction

Water is one of the most important natural resources on our planet. In recent years, fast population growth, , rapid urbanization, industrialization and The excessive use of pesticides and fertilizers have contaminated the already limited freshwater resources[1]. Water covers over 73 percent of the earth's surface. It is the basic component of the lithosphere and atmosphere and it is essential for all living things to survive[2]. Two-thirds of the water on earth is covered by ice caps and glaciers, and only 3% of that water is freshwater. It means that only 1% of the water on earth is available to sustain diverse plant and animal ecosystems in sources including, rivers, lakes, and groundwater[3]. Every year, millions of people worldwide, especially in developing nations, die from a water-borne disease[4].

Natural factors including geology, climate, and hydrology, as well as a variety of anthropogenic factors like the discharge of municipal and agricultural drainage and industrial sewage water, have an effect on the water quality[5]. Before using water for any of its intended uses, including agricultural, recreational, drinkable, and industrial water uses, its quality must be established. Water quality is described in terms of its physical, biological, and chemical properties[6].

* Corresponding author
E-mail address: mzhda.qadir@su.edu.krd (Instructor).
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The environment affects the water quality, which is determined using the water's physicochemical parameters[7].Yeasts have received much attention recently since they are one of the most active extracellular enzyme producers and can be cultured using inexpensive substrates[8]

It's not a new idea to use microalgae for wastewater treatment, and several researchers have found methods to take advantage of the algae's rapid growth and nutrient-removal abilities. Although other nutrient stripping activities, such as ammonia volatilization and phosphorus precipitation as a result of the algae's high pH, also occur, the nutrient removal is attributable to the algae's assimilation of nutrients[9].

Carbon, nitrogen, and phosphorus are nutrients that microbes need to support their metabolic activities[10, 11]. The appropriate nutrient concentration in any system is affected by several factors, such as microalgae species, present environmental conditions (light/temperature), the nature of the pilot system, and so on[12].

Fungi can be found in water, soil, the sea, as well as on the body of organisms. In different habitats, different populations of yeast can be found, and changes in the community reflect changes in the environment[13]. It has been suggested that the fungus *Rhodotorula* sp., *Candida* sp., *Trichosporon* sp., and

Cryptococcus sp. acts as indicators of water contamination [14]. Additionally , yeasts are highly biodegradable to a variety of resistive and organic poisons[13].

At the end of the 1970s[15], developed a system for yeast wastewater treatment. Since then, yeast's unequaled effectiveness in the treatment of various wastewaters has attracted attention on a global scale. Technology for treating yeast wastewater has been resulted in the discovery that yeast can manufacture lipids[16], glycolipids and enzymes[17]. The importance and efficiency of fungal and microalgal strains in breaking down pollutants and utilizing them as a food source to improve the quality of Erbil wastewater is aim of this study.

2. Methods and Materials

2.1 Collection of wastewater and experimental set-up

The domestic wastewater samples which are used in this study have been collected. The described experiments were carried out using two fungal genera and two different genera of algae. The most dominant species such as Aspergillus niger (ATCC 16404), Candida albicans (ATCC 10231)[18], Tetradesmus nygaardi (MZ801740) and Scenedesmus quadricauda (MZ801741) were utilized as test organisms for wastewater treatment. On a horizontal orbital shaker at 150 rpm and 27 ± 1 °C, experiments were conducted in batch reactors using Erlenmeyer glass flasks.

2.2.1 Mold culture Aspergillus niger preparation

The fresh culture of selected fungi in this study has been sub-cultured individually onto slants containing solidified Sabouraud's Dextrose Agar (SDA) and *A. niger* was incubated for 7 days at 25 ± 1°C. *A. niger* spores have been suspended from each culture slant in 10 ml of sterile distilled water. To get similar cell counts of 3×10^4 CFU/ml, spore suspensions have been diluted with double-sterile water.

2.2.2. Yeast culture Candida albicans preparation

The fresh culture of *C. albicans* have been sub-cultured individually onto slants containing solidified SDA Sabouraud's Dextrose Agar and incubated for and 24 hrs. at 37 ± 1°C, yeast cell have been suspended in 10 ml sterile distilled water. Yeast cells have been diluted with double-sterile water to obtain similar cell counts of 2×10^4 CFU/ml.

2.2.3. Microalgae cultivation

The cells of *Scenedesmus quadricauda* 6.8×10^4CFU/ ml. and *Tetradesmus nygaardi* 7.2×10^4 CFU/ ml. were cultured in BG11 broth medium with light-emitting diode (LED) lights in distilled water at room temperature, a constant pH of (7.5 ± 0.3), and a constant temperature of (25 ± 1°C) , and at constant light intensity (3000 lux) 16hrs light and 8hrs dark for 21 days[19].

2.3 Analytical methods

The wastewater samples were analyzed for pH, electrical conductivity (EC), phosphate (PO_4), ammonia (NH_4), nitrate (NO_3), nitrite (NO_2), and biological oxygen demand (BOD_5). To investigate the role of fungi and microalgae in the treatment of wastewater, pure mixed cultures of *Aspergillus niger* with *Candida albicans* (F1*F2), *Scenedesmus quadricauda* with *Aspergillus niger* (A1×F1), *Scenedesmus quadricauda* with *Candida albicans* (A1×F2) and *Tetradesmus nygardi* with *Scenedesmus quadricauda* (A1×A2), were used. At the beginning of each series of experiments, 1200 mL of wastewater with pre-cultured species as mentioned above were inoculated in the flasks. The experiment was conducted for a total of 21 days under controlled conditions (temperature of 27 ± 2 ºC). Samples were periodically (every three days) analyzed for physicochemical parameters using standard methods [20]. Data were analyzed by calculating the removal efficiency by comparing the metal concentration before and after treatment[21].

$$Removal\ \% = \left[\frac{Initial\ \text{conc.} - Final\ \text{conc.}}{Initial\ \text{conc.}}\right] * 100$$

2.4 Chlorophyll estimation

For estimation of chlorophyll *a* 10 ml of culture was taken from each flask of sample and centrifuged at 3000 rpm for 5 min and the supernatant was discarded, and the cell was suspended with 5 ml of diethyl ether. The absorbance value of supernatant was measured using UV-spectrometer at 660 nm and 643 nm [22].

Chlorophyll *a* = $(9.92 * A_{660}) - (0.77 * A_{643})$

2.5 Statistical analysis

Statistical analysis has been conducted for the data using a software program (SPSS version 26) and Excel spreadsheets. Two-way ANOVA (Analysis of variance). A post hoc test (multi comparisons Duncan test) was applied to determine significant differences at 5%. All data are expressed as mean ± SE[23].

3. Result & Discussion

The initial analysis of Erbil wastewater before the treatment (control) were measured as follows (Table1a &b, Fig. 1) for pH, EC, TDS, were 8.2, 823 µS/cm, 487.2 mg/l respectively. While, the mean values of BOD_5, PO_4, NH_4, NO_3 and NO_2 were 982 mg/l, 29.7 mg/l and 76.05 mg/l, 68.27mg/l and 21.78 mg/l respectively. The pH value of wastewater in all batches steadily increased reaching 8.65, 8.52, and 8.45 after the 21 days of treatment (Fig. 1). When the wastewater was treated with (F1×F2, A1×F1, A1×F2 and A1×A2), the pH was decreased initially, and then increased. It may be due the accumulation of organic acids and the efficiency of the microbial degradation. This is similar with the reports of Noorjahan *et al.* [24]. Carbon dioxide assimilation causes pH to rise, and it can even approach 10 when no CO2 is present[25, 26] . If bicarbonate is utilized as a carbon source, it can reach 11 or higher[27].

The pH is affected by the absorption of nitrate and phosphate by algae. Assimilation of nitrate ions raises pH, while assimilation of ammonium ions lowers pH to as low as (3)[22, 28]. Remediation of electrical conductivity with (F1×F2, A1×F1, A1×F2 and A1×A2), were 81.68%, 81.40%, 80.86% and 80.50% respectively, after 21st days of wastewater treatment (Fig. 2). The reduction percent for TDS for all microbial cultures were (81.52, 81.11, 81.84 and 80.5%). During the present study, a pure mixed culture of *A. niger* with *Candida* spp. showed the highest removal of EC and TDS from wastewater. The removal of EC and TDS, showed by order, F1×F2> A1×F1 > A1×F2 > A1×A2.

Table 1 a: Effect of microbial mixed strains on some wastewater parameters during 21 days of treatment. Data represented as Mean ± SE.

Microbes	Time	pH	EC	TDS	BOD$_5$	PO$_4$	NH$_4$	NO$_3$	NO$_2$
F1×F2	1day	8.2±0.3^{a}	823±2.7^{a}	487.2±2.5^{a}	982±2.8^{a}	29.7±0.2^{a}	76.05±0.2^{a}	68.27±0.2^{a}	21.78±0.2^{a}
	3day	7.71±0.3^{a}	540±2.7^{c}	319.2±2.5^{d}	725±2.8^{c}	19.7±0.2^{c}	45.04±0.2^{c}	59.43±0.2^{c}	20.8±0.2^{ab}
	5day	7.90±0.3^{a}	451±2.7^{def}	267.4±2.5^{f}	650±2.8^{de}	17.8±0.2$^{c\text{-}f}$	37.89±0.2^{cde}	50.16±0.2^{e}	18.16±0.2^{cd}
	7day	8.30±0.3^{a}	400±2.7^{ghi}	221.0±2.5^{hij}	625±2.8^{ef}	16.9±0.2$^{e\text{-}h}$	33.08±0.2^{efg}	45.38±0.2^{f}	16.42±0.2^{e}
	9day	8.50±0.3^{a}	365±2.7^{i}	203.0±2.5^{j}	475±2.8^{ij}	15.4±0.2$^{g\text{-}k}$	32.04±0.2$^{e\text{-}i}$	40.16±0.2^{g}	14.18±0.2^{fg}
	11day	8.40±0.3^{a}	241±2.7$^{j\text{-}m}$	146.0±2.5^{klm}	425±2.8^{jkl}	14.3±0.2$^{i\text{-}l}$	25.42±0.2$^{g\text{-}l}$	26.50±0.2^{h}	10.22±0.2^{j}
	13day	8.50±0.3^{a}	212±2.7$^{k\text{-}p}$	123.0±2.5$^{m\text{-}r}$	375±2.8^{lm}	11.4±0.2^{mn}	24.90±0.2$^{h\text{-}m}$	24.09±0.2^{hij}	6.400±0.2^{l}
	15day	8.40±0.3^{a}	193±2.7$^{m\text{-}s}$	118.0±2.5$^{n\text{-}s}$	300±2.8^{no}	8.40±0.2^{op}	18.03±0.2$^{l\text{-}o}$	20.88±0.2^{klm}	2.820±0.2^{o}
	17day	8.45±0.3^{a}	185±2.7$^{o\text{-}s}$	105.0±2.5$^{p\text{-}t}$	200±2.8^{pq}	6.80±0.2^{pq}	17.23±0.2$^{l\text{-}p}$	20.48±0.2^{lmn}	2.760±0.2^{o}
	19day	8.48±0.3^{a}	170±2.7$^{p\text{-}s}$	100.0±2.5^{rst}	175±2.8^{qr}	2.80±0.2^{r}	10.30±0.2^{opq}	17.99±0.2$^{n\text{-}q}$	2.440±0.2^{opq}
	21day	8.55±0.03^{a}	151±2.7^{s}	90.00±0.8^{t}	100±2.8^{r}	2.10±0.2^{r}	7.740±0.2^{q}	10.04±0.02^{s}	2.380±0.02^{opq}
A1×F1	3day	7.65±0.3^{a}	543±2.7^{c}	324.0±2.5^{cd}	575±2.8^{fg}	18.4±0.2^{cde}	38.02±0.2^{cde}	57.83±0.2^{c}	20.2±0.2^{b}
	5day	7.90±0.3^{a}	465±2.7^{d}	270.6±2.5^{f}	500±2.8^{ih}	16.3±0.2$^{e\text{-}i}$	36.53±0.2^{def}	49.79±0.2^{e}	18.06±0.2^{cd}
	7day	8.50±0.3^{a}	404±2.7e$^{\text{-}i}$	240.0±2.5^{ghi}	375±2.8^{lm}	15.5±0.2$^{g\text{-}k}$	29.90±0.2$^{f\text{-}j}$	44.57±0.2^{f}	16.06±0.2^{e}
	9day	8.60±0.3^{a}	360±2.7^{i}	210.0±2.5^{j}	325±2.8^{mn}	14.1±0.2^{jkl}	22.10±0.2$^{j\text{-}n}$	40.562±0.2^{g}	13.78±0.2^{gh}
	11day	8.50±0.3^{a}	248±2.7^{jkl}	148.0±2.5^{klm}	250±2.8^{op}	13.0±0.2^{lm}	24.24±0.2$^{h\text{-}n}$	24.9±0.2^{hi}	9.88±0.2^{jk}
	13day	8.60±0.3^{a}	227±2.7$^{j\text{-}o}$	132.0±2.5$^{l\text{-}p}$	200±2.8^{pq}	10.2±0.2^{no}	23.87±0.2$^{i\text{-}n}$	22.89±0.2i$^{\text{-}l}$	6.06±0.2^{lm}
	15day	8.30±0.3^{a}	199±2.7$^{l\text{-}s}$	122.0±2.5$^{m\text{-}r}$	150±2.8^{qrs}	7.10±0.2^{pq}	16.99±0.2$^{m\text{-}p}$	20.08±0.2^{mn}	2.60±0.2^{op}
	17day	8.42±0.3^{a}	191±2.7$^{n\text{-}s}$	110.0±2.5$^{p\text{-}t}$	100±2.8^{stu}	5.80±0.2^{q}	16.37±0.2^{nop}	19.67±0.2^{mno}	2.34±0.2^{opq}
	19day	8.51±0.3^{a}	176±2.7$^{p\text{-}s}$	103.0±2.5$^{q\text{-}t}$	75.0±2.8^{tu}	2.60±0.2^{r}	9.630±0.2^{pq}	16.46±0.2^{q}	2.04±0.2^{opq}
	21day	8.62±0.3^{a}	153±2.7^{rs}	92.00±2.5st	50.0±2.8^{t}	2.00±0.2^{r}	7.280±0.2^{q}	9.230±0.2^{s}	1.9±0.2^{opq}

Table 1 b: Effects of microbial mixed strains on some wastewater parameters during 21 days of treatment. Data represented as Mean ± SE.

Microbes	Time	pH	EC	TDS	BOD$_5$	PO$_4$	NH$_4$	NO$_3$	NO$_2$
A1×F2	1day	8.2±0.3^{a}	823±2.7^{a}	487.2±2.5^{a}	982±2.8^{a}	29.7±0.2^{a}	76.05±0.2^{a}	68.27±0.2^{a}	21.78±0.2^{a}
	3day	7.8±0.3^{a}	552±2.7^{c}	325.8±2.5^{cd}	700±2.8^{cd}	19.0±0.2^{cd}	44.26±0.2^{c}	58.63±0.2^{c}	19.98±0.2^{b}
	5day	8.0±0.3^{a}	479±2.7^{de}	273.0±2.5^{ef}	600±2.8^{efg}	17.2±0.2$^{d\text{-}g}$	43.81±0.2^{cd}	49.39±0.2^{e}	17.82±0.2^{d}
	7day	8.6±0.3^{a}	430±2.7^{fgh}	242.4±2.5^{gh}	500±2.8^{hi}	16.5±0.2$^{e\text{-}h}$	31.98±0.2$^{e\text{-}h}$	44.98±0.2^{f}	14.90±0.2^{f}
	9day	8.7±0.3^{a}	372±2.7^{i}	216.0±2.5^{ij}	450±2.8^{ijk}	15.0±0.2$^{h\text{-}l}$	26.71±0.2$^{g\text{-}k}$	40.16±0.2^{g}	12.86±0.2^{hi}
	11day	8.5±0.3^{a}	257±2.7^{jk}	151.0±2.5^{kl}	400±2.8^{kl}	14.1±0.2^{jkl}	24.71±0.2$^{h\text{-}m}$	26.50±0.2^{h}	9.340±0.2^{jk}
	13day	8.7±0.3^{a}	236±2.7$^{j\text{-}n}$	138.0±2.5l$^{\text{-}o}$	250±2.8^{op}	11.2±0.2^{mn}	24.20±0.2$^{h\text{-}n}$	23.29±0.2^{ijk}	5.180±0.2^{mn}
	15day	8.4±0.3^{a}	214±2.7$^{k\text{-}p}$	125.0±2.5$^{l\text{-}r}$	200±2.8^{pq}	8.30±0.2^{op}	17.16±0.2$^{l\text{-}p}$	20.6±0.2^{lmn}	2.420±0.2^{opq}
	17day	8.5±0.3^{a}	202±2.7$^{l\text{-}r}$	116.0±2.5st	150±2.8^{qrs}	6.20±0.2^{q}	16.49±0.2$^{m\text{-}p}$	20.1±0.2^{mn}	2.040±0.2^{opq}
	19day	8.6±0.3^{a}	188±2.7$^{n\text{-}s}$	107.0±2.5$^{p\text{-}t}$	125±2.8^{rst}	2.70±0.2^{r}	9.750±0.2^{opq}	16.9±0.2^{pq}	1.860±0.2^{opq}
	21day	8.6±0.3^{a}	159±2.7^{qrs}	93.33±2.5st	75.0±2.8^{tu}	2.20±0.2^{r}	7.220±0.2^{q}	9.47±0.2^{s}	1.80±0.2^{opq}
A1×A2	3day	7.9±0.3^{a}	572±2.7^{b}	362.2±2.5^{c}	650±2.8^{e}	18.6±0.2$^{c\text{-}f}$	30.61±0.2$^{e\text{-}j}$	55.42±0.2^{d}	19.78±0.2^{c}
	5day	8.1±0.3^{a}	447±2.7$^{d\text{-}g}$	315.0±2.5^{e}	625±2.8^{efg}	16.9±0.2$^{e\text{-}h}$	30.03±0.2$^{g\text{-}k}$	47.79±0.2^{f}	17.30±0.2^{e}
	7day	8.6±0.3^{a}	439±2.7^{hi}	255.4±2.5^{fg}	550±2.8^{gh}	16.0±0.2f$^{\text{-}i}$	24.96±0.2$^{h\text{-}m}$	44.57±0.2^{f}	14.02±0.2^{fg}
	9day	8.7±0.3^{a}	393±2.7^{j}	225.0±2.5^{hij}	500±2.8^{hi}	14.8±0.2$^{h\text{-}i}$	23.90±0.2$^{i\text{-}n}$	39.35±0.2^{g}	12.24±0.2^{i}
	11day	8.4±0.3^{a}	273±2.7^{jkl}	166.0±2.5^{k}	475±2.8^{ij}	13.5±0.2^{jkl}	23.04±0.2$^{j\text{-}n}$	24.09±0.2^{hij}	9.120±0.2^{k}
	13day	8.5±0.3^{a}	245±2.7$^{k\text{-}p}$	144.0±2.5^{lmn}	450±2.8^{ijk}	10.8±0.2^{n}	20.15±0.2$^{k\text{-}n}$	21.7±0.2$^{j\text{-}m}$	4.360±0.2^{n}
	15day	8.3±0.3^{a}	217±2.7$^{k\text{-}q}$	130.0±2.5$^{l\text{-}q}$	400±2.8^{kl}	7.60±0.2^{pq}	16.39±0.2^{nop}	19.3±0.2^{mno}	2.06±0.2^{opq}
	17day	8.4±0.3^{a}	207±2.7$^{k\text{-}q}$	124.0±2.5$^{m\text{-}r}$	325±2.8^{mn}	5.90±0.2^{q}	16.15±0.2^{nop}	17.3±0.2^{opq}	1.72±0.2^{opq}

	19day	8.5 ± 0.3^{a}	$193\pm2.7^{m-s}$	$110.0\pm2.5^{p-t}$	250 ± 2.8^{op}	2.50 ± 0.2^{r}	9.290 ± 0.2^{pq}	14.05 ± 0.2^{r}	1.48 ± 0.2^{pq}
	21day	8.5 ± 0.3^{a}	160 ± 2.7^{qrs}	95.00 ± 2.5^{st}	101 ± 2.8^{stu}	1.50 ± 0.2^{r}	7.0400 ± 0.2^{q}	8.030 ± 0.2^{s}	1.34 ± 0.2^{q}

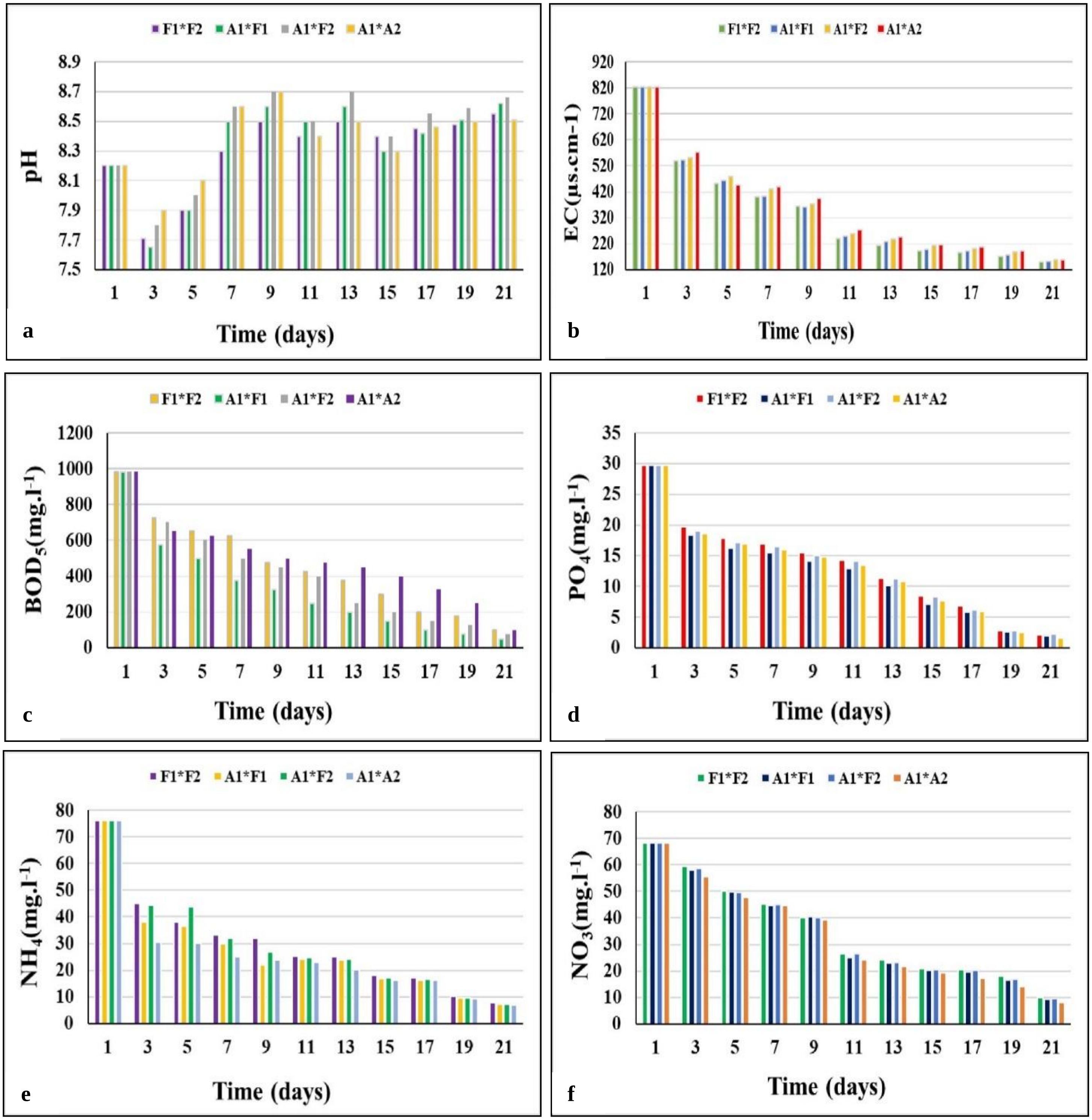
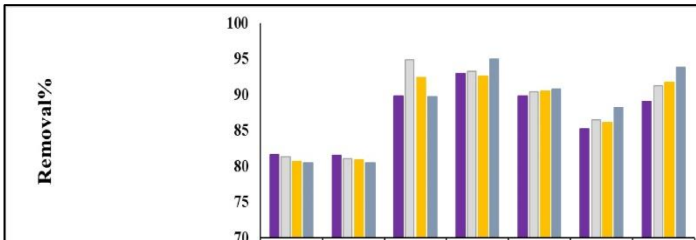


Figure 1: Effect of algal and fungal strains on: (a) pH value of wastewater. (b) Electrical conductivity of wastewater. (c) Biological oxygen demand of wastewater. (d) Phosphate content of wastewater. (e) Ammonium content of wastewater. (f) Nitrate content of wastewater.

Statistically, significant differences ($P \leq 0.05$) were observed between control treatment with all other treatments, especially treatment F1×F2 showed lower values of TDS (169.2 mg. l^{-1}). This may be due to that the fungi mixture act as synergism to reduce amount of dissolved salts. Same trend of variations also observed for EC variable (Table 1a & b). BOD_5 measurement is one of the main indicators used in water pollution assessment to evaluate the effects of wastewaters on receiving water bodies[29]. BOD_5 in the present study was reduced when wastewater treated with F1×F2 (89.81%), A1×F1 (94.9%), A1×F2 (92.36%) and A1×A2(89.71%) respectively after 21 days (Fig. 2). A mixed culture of A1×F1 and A1×F2, shows the best reduction capacity of BOD_5 during wastewater treatment.



	EC	TDS	BOD5	PO4	NH4	NO3	NO2
A.niger*C.albicans(F1*F2)	81.65	81.52	89.81	92.92	89.81	85.29	89.07
S.quadricauda*A.niger(A1*F1)	81.4	81.11	94.9	93.26	90.42	86.47	91.27
S.quadricauda*C.albicans(A1*F2)	80.68	80.84	92.36	92.59	90.49	86.11	91.73
S.quadricauda*T.nygaardi(A1*A2)	80.55	80.5	89.71	94.94	90.73	88.23	93.84

Wastewater tested variables

Figure 2: Percent removal of wastewater tested variables by microbial cultures.

Organic pollutants are broken down by microorganisms when they consume them for growth and reproduction. The microorganism obtains energy by catalyzing energy producing chemical reactions and this energy is utilized in the creation of the new cell. [30]. Fungal communities are essential components of activated sludge and serve as the main decomposers in wastewater treatment systems, due to the abundance and diversity of fungal biomass, which particularly plays a major role in the processes of organic matter biodegradation and nutrient cycling[31].

Fungi have a number of extracellular enzymes that can break down complex compounds by nonspecific oxidation processes[32]. For that reason many fungal species like *Aspergillus*, *Penicillium*, *Fusarium*, *Absidia*, and a variety of other fungal species have all been implicated to the removal of carbon and nutrient sources from wastewater[33, 34]. A suggested yeast as an indicator fungus for water pollution is *Candida* sp. has strong environmental resistance and it is highly biodegradable to a variety of poisons that are resistant and organic[13]. *Saccharomyces cerevisiae* shows the highest percentage reduction of 52.5 % for BOD_5[35].

Orthophosphate or organically bound phosphates are the main sources of phosphorus in natural water and wastewater[20]. Percentage of Phosphate reduction using (F1×F2, A1×F1, A1×F2 and A1×A2), were 92.92, 93.26, 92.59 and 94.94 % respectively after 21st days of wastewater treatment (Fig. 2). A mix culture of (A1×A2) resulted in highest 94.94 % of PO_4 removal after 21st days. Percentage of PO_4 reduction order in wastewater was a mixed culture of A1×A2> A1×F1 > F1×F2> A1×F2.

Thanh and Simard[36], demonstrated the capacities of seventeen fungal biomasses to remove phosphates (84.1 %). *Aspergillus terreus* have separately and successfully removed phosphorus (58 %)[37]. *Aspergillus niger* isolated from the Brazilian high phosphorus ore was used in order to remove PO_4. After 21 days, the removal percent were 13.8 and 33.2 %[38]. According to the study estimates, reduced phosphate values are a result of the element's use in many essential processes, such growth and reproduction, where it is necessary to build various energy-rich compounds like phospholipids and nucleic acids[39]. the findings of Kshirsagar[40], demonstrated that after 10 days of wastewater treatment, the percentage of phosphate reduction was 23.74 % for the control while it was 50.83, 52.57, 47.48 and 42.40 % for *Aspergillus terreus*, *Aspergillus niger*, *Rhizopus nigricans*, and *Cunninghamella* sp.

Nitrogen is a critical element for growth of fungi, they use to build many biomolecules, including amino acids, proteins, and vitamins[41]. They are often contributed to the formation of different cellular organelles. Both inorganic and organic nitrogen are essential for fungi growth and in determining nutrients transporting through the transportation system in the cytoplasmic[42]. Percentage of ammonia reduction with (F1×F2, A1×F1, A1×F2 and A1×A2), were 89.81, 90.42, 90.49, and 90.73% respectively after 21st days of wastewater treatment (Fig. 2). A mixed culture of (A1×A2), resulted in the highest 90.73% of NH_4 removal after 21st days, whereas percent reduction of nitrate, were 85.29, 86.47, 86.11, and 88.23% respectively (Fig. 2), that referred a mixed culture of (A1×A2), showed the highest removal of NO_3 from wastewater. The removal of nitrate showed by order, A1×A2 > A1×F1 > A1×F2 > F1×F2. Nitrite in the present study was reduced when wastewater treated with treatment (F1×F2), 89.07%, treatment (A1×F1) (91.27%), treatment (A1×F2) (91.73%) and treatment (A1×A2) (93.84%) respectively after 21st days (Fig. 2). A mixed culture of (A1×F1 and A1×F2), shows the best reduction capacity of nitrite during treatment.

Kadhim *et al*[43], revealed that reduction rates in nitrite was (87–97%) when *A. flavus*, *A. niger* , *A. terrues* used to treat wastewater. Travieso *et al*.[44], demonstrated that removal rates of organic nitrogen, ammonia, and total phosphorus of 90.2 %, 84.1 %, and 85.5 %, respectively.

The rate of PO4 removal was more than that reported by Wu et al.[45], who revealed that *Chlamydomonas* sp. removed 33%.of PO_4 in wastewater. Microalgae growth is believed to be essential for nitrogen removal through uptake, degradation, and sedimentation because nitrogen was required by microalga cells to synthesize proteins, nucleic acids, and phospholipids [46] moreover, the microalgal process of denitrification and nitrification[47].

Phosphorus has been successfully removed by *A. terreus* separately (58 %)[37]. *A. niger*, removed phosphorous by 13.8 and 33.2 %, respectively, after 21 days [48]. Yeast can convert the most of organic matter into nutritious, nontoxic single cell protein with high efficiency for wastewater treatment[49]. As a necessary element of growth, nitrogen is used by fungi to create a variety of biomolecules including amino acids, proteins, and vitamins, they are usually responsible for the formation cellular organelles[41].

Organic and inorganic nitrogen are both necessary for regulating the process of nutrient movement through the cytoplasmic vascular system and promoting fungal growth[42].

Chlorophyll-*a* concentration in all mixed batch cultures of (A1×F1, A1×F2, and A1×A2), were increased, the initial value of chlorophyll-*a*, were (0.30, 0.26 and 0.63 mg/l), then on day 21st reached the maximum (1.05, 1.005 and 2.5 mg. l^{-1}) respectively (Fig. 3). The maximum chlorophyll-*a* concentration was reported at the end of the treatment period, which could be due to algae's high nutrient consumption for metabolism and growth[50]. The highest biomass production (chlorophyll-*a*) was found at treatment (A1×A2) along 21 days of experiment, which was coincided with highest nutrient removal synergism effect of both microalgae raised chlorophyll *a* production to double compared to treatments (A1×F1 and A1×F2).

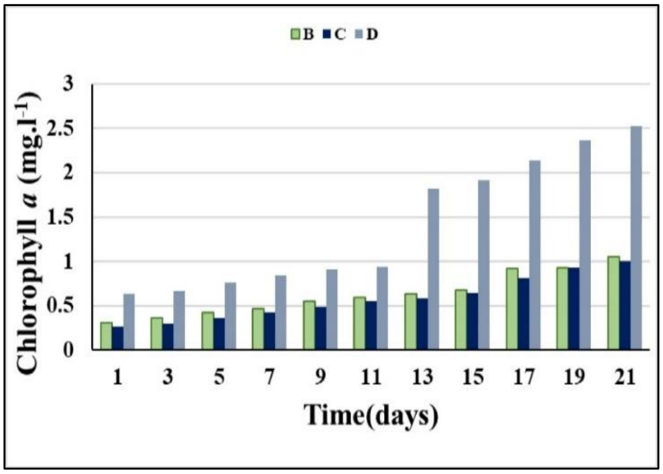


Figure 3: Micro-algal chlorophyll-a content during treatment of wastewater by using different microbial cultures.

The three primary factors that influence the growth of any microalgae are carbon, nitrogen, and phosphorus[51, 52]. They also depend on complex interactions between physical parameters such as light intensity[53, 54], pH[55], and temperature[56].

Although photosynthesis is the main source of energy for phytoplankton to produce sugar, microalgae still need additional nutrients to reproduce and growth[57]. Furthermore, as the algal cell increases, the rate of nutrients decrease, so algal biomass simply increases. Study has demonstrated that increasing and decreasing biomass is due mostly to the quantity of nutrients in the environment and then related to light. The decline in biomass in the environment can be attributed to the low nutritional environment because the light intensity in the environment was constant throughout the experiment[50]. These nutrients are typically phosphorus, nitrogen and iron, even if certain species also need trace elements [58, 59]. Furthermore according to Harker *et al.*[60], the highest concentration of chlorophyll *a* will be produced at the highest cell concentration or at the end of the exponential phase of growth, as chlorophyll a production shows a pattern of development similar to that of cell concentration.

Conclusion

Biotechnologies have been proposed for removing pollutants and recovering resources from wastewater simultaneously. Wastewater contains high levels of pollutants, causing serious harm to animals, humans, and the environment. Microalgae and fungi can absorb nitrogen, carbon, and phosphorus, making them potential wastewater treatment alternatives. The results revealed that pH value steadily increased in all bath cultures during the experiment. A mixed culture of *S. quadricauda* with *T. nygaardi* had the best removal of PO_4, NH_4, NO_3, and NO_2, while a mixed culture of *S. quadricauda* with *A. niger* seemed to have the best removal of BOD_5, and a mixed culture of *A. niger* with *C. albicans* had the highest reduction in electrical conductivity. Decreases in nutrients coincided with an increase in chlorophyll-*a* content with the highest algal biomass on the 21st day of the experiment 2.5 mg. l^{-1}. Generally, mix culture of *S. quadricauda* and *T. nygaardi* is most efficient treatment for all variables except for BOD_5, EC and TDS.

Conflict of interests

As for the requirements of the publishing policy, there is no potential conflict of interest for the authors.

Contribution

Muzhda Q. Qader, contributed to the laboratory analyzing of samples and writing the manuscript, statistical analysis and making final revision of the manuscript with supervising and encouragement of Yahya S. Ahmed.

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