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Evaluation of *Galdieria sulphuraria* and *Chlorella vulgaris* for the Bioremediation of Produced Water

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Abstract: Produced water (PW) is the largest waste stream generated by the oil and gas industry. Traditional treatment of PW burdens the industry with significant expenses and environmental issues. Alternatively, microalgal-based bioremediation of PW is often viewed as an ecologically safe and sustainable platform for treating PW. Moreover, the nutrients in PW could support algal growth. However, significant dilution of PW is often required in algal-based systems due to the presence of complex chemical contaminants. In light of these facts, the current work has investigated the potential of cultivating *Galdieria sulphuraria* and *Chlorella vulgaris* in PW using multiple dilutions; 0% PW, 5% PW, 10% PW, 20% PW, 50% PW and 100% PW. While both algal strains can grow in PW, the current results indicated that *G. sulphuraria* has a higher potential of growth in up to 50% PW (total dissolved solids of up to 55 g L^{-1}) with a growth rate of 0.72 ± 0.05 g L^{-1} d^{-1} and can achieve a final biomass density of 4.28 ± 0.16 g L^{-1} in seven days without the need for additional micronutrients. Additionally, the algae showed the potential of removing 99.6 ± 0.2% nitrogen and 74.2 ± 8.5% phosphorus from the PW.

Keywords: microalgae; *Galdieria sulphuraria*; *Chlorella vulgaris*; growth; biomass; nitrogen; phosphorus; salinity; thermophilic; bioremediation; produced water

Citation: Rahman, A.; Pan, S.; Houston, C.; Selvaratnam, T. Evaluation of *Galdieria sulphuraria* and *Chlorella vulgaris* for the Bioremediation of Produced Water. *Water* **2021**, *13*, 1183. https://doi.org/10.3390/w13091183

Academic Editor: Pei Xu

Received: 18 March 2021
Accepted: 22 April 2021
Published: 25 April 2021

1. Introduction

In oil and gas industry, the volume of produced water (PW) increases with oilfields' age. In the United States alone, onshore oil and gas extraction operations generate an estimated 24 billion barrels of PW annually, making it the largest waste stream associated with the upstream development of petroleum hydrocarbons [1]. Produced water has a complex composition, containing inorganic and organic components and dissolved and dispersed oils and grease components. In addition, there are heavy metals, dissolved gases, treating chemicals, radionuclides, scaling products, microorganisms, etc. [2]. Currently, most of the PW is reinjected into the disposal wells and it is more expensive to reinject than to treat the PW. The cost of treating one barrel of PW is 0.775 USD, whereas the reinjection cost is 0.75–80 USD per barrel [3]. According to the grand view research report, the global PW treatment market size is 5.8 billion USD in 2015 and is projected to expand at a compound annual growth rate of 6.0% through 2024 [4].

Since PW is composed of several harmful constituents, it can reduce potable water quality and affect soil fertility while being in contact with soil; hence, the deposition of PW without treatment may procure adverse effects to the environment [3,5]. However, it is noted that PW is a mixture of several components and contaminants whose concentration varies significantly from one oilfield to another [6]. Although different treatment technologies such as physical, chemical and membrane techniques have been introduced for PW treatment, they consume huge energy and often require large space [7,8].

Alternatively, microalgae-based bioremediation systems are an ecologically safer, more economical and efficient alternative for current physiochemical processes to treat heavily contaminated wastewaters [9,10]. Several studies have shown these algal-based systems' versatility for treating wastewaters ranging from municipal wastewaters to acid mine wastewaters [11,12]. Moreover, the biological treatment methods appear to be a less expensive way for reducing contaminants from polluted water [6]. Microorganisms such as bacteria, fungi and algae can be effectively used in biological treatments as the contaminants serve as the food for microorganisms [9]. Moreover, algae-based treatment technologies are a low cost, ecological and relatively novel approach to typical aerobic and anaerobic methods [9–11]. Additionally, algae have been reported to grow in adverse natural conditions and remove toxic contaminants by biosorption and bioaccumulation [11].

Among recent studies, Hopkins et al. reported *Cyanobacterium aponinum*, *Parachlorella kessleri* as potential candidates for cultivation in brackish to saline PW [13]. The group isolated *C. aponinum* and *P. kessleri* in a polyculture in PW from an oil and gas production facility located in the Permian Basin of southwestern New Mexico [13]. The polyculture primarily contained *C. aponinum*, *P. kessleri* and several species of a halotolerant bacteria. Additionally, a few photoautotrophic species such as *Nannochloropsis salina* and *Dunaliella tertiolecta* have also shown their potential to thrive in brackish to hypersaline PW-based media, as reported by Graham et al. [14]. In its native status, PW contains most of the components needed for algal growth (i.e., N, P, trace metals, dissolved carbon dioxide, etc.) at various concentrations [9]. However, salinity and oil content in PW can impede the growth of algae [15]. Oil layers in PW may hinder algal growth, either through the toxicity of the free oil components and/or by obstructing the light required for algal photosynthesis [15]. However, in a comprehensive study, Godfrey concluded that the process of cultivating algae in PW is a reasonable substitute to freshwater and expensive chemicals [16]. In the current study, we screened two algal species for their growth and bioremediation potential using PW. The algal species include *Galdieria sulphuraria* and *Chlorella vulgaris*. *G. sulphuraria* is a thermophilic mixotrophic alga and *C. vulgaris* is a green alga known for its high resistance against rough conditions, such as its ability to grow in various wastewater discharge conditions [17–19].

2. Materials and Methods

2.1. Sub-Culturing of Algae

The isolates of unicellular red alga, *G. sulphuraria* CCMEE 5587.1, obtained from the Culture Collection of Microorganisms from Extreme Environments (University of Oregon), were assessed in this study. The strain was grown in Cyanidium medium (CM) inside an incubator (Percival, IA, USA) at 42 °C with 24 h of continuous illumination (~4000 lux) [20]. Cultures were streaked onto agar plates and single colonies were picked to start axenic cultures from culture plates to CM, scaling up the volume to 1 L Erlenmeyer flasks. The CO_2 concentration inside the incubator was maintained at ~3%. The following macro and micro level constituents were used to prepare CM: $(NH_4)_2SO_4$, 1.32 g L^{-1}; KH_2PO_4, 0.27 g L^{-1}; NaCl, 0.12 g L^{-1}; $MgSO_4 \cdot 7H_2O$, 0.25 g L^{-1}; $CaCl_2 \cdot 2H_2O$, 0.07 g L^{-1}; Nitch's trace element solution, 0.5 mL; $FeCl_3$ (solution = 0.29 g L^{-1}), 1.0 mL. The pH of the media was adjusted to 2.5 with 10 N H_2SO_4.

C. vulgaris (UTEX number 395) was purchased from the UTEX Culture Collection of Algae, University of Texas, Austin, TX, USA. The strain was grown in an incubator (Percival Scientific Inc., Perry, IA, USA) at 28 °C with 16/8 h light/dark cycle at an illumination of ~4000 lux. *C. vulgaris* was cultured in Bold's Basal Medium (BBM), which was prepared with several modifications of 3N–BBM+V [21,22]. The composition of the modified 3N–BBM+V medium was: 430 µmol L^{-1} K_2HPO_4, 1.3 mmol L^{-1} KH_2PO_4, 300 µmol L^{-1} $MgSO_4 \cdot 7H_2O$, 2.94 mmol L^{-1} $NaNO_3$, 128 µmol L^{-1} $CaCl_2 \cdot 2H_2O$, 430 µmol L^{-1} NaCl, 132 µmol L^{-1} EDTA, 18 µmol L^{-1} $FeSO_4 \cdot 7H_2O$, 185 µmol L^{-1} H_3BO_3, 4.91 µmol L^{-1} $ZnCl_2$, 1.17 µmol L^{-1} $MnCl_2 \cdot 4H_2O$, 1.01 µmol L^{-1} $CuSO_4 \cdot 5H_2O$, 280 nmol L^{-1} $CoCl_2 \cdot 6H_2O$ and 794 nmol L^{-1} Na_2MoO_4. The average pH of the prepared BBM was 6.7 ± 0.2. New genera-

tions were prepared by adding 30 mL of previous generation culture to 120 mL of BBM in a 500 mL borosilicate Erlenmeyer flask.

All chemicals were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO, USA) or VWR (Radnor, PA, USA); deionized water (DIW) was the solvent unless otherwise specified.

2.2. Produced Water

The produced water for this work was collected from an oil and gas facility located in the Permian Basin, United States. The specific characteristics of the PW are presented in Table 1.

Table 1. Specific characteristics of produced water. Values represent the average ± SD of n = 5 technical replicates.

Parameter	Unit	Measured Values (Sample Size, n = 5)
pH	–	6.71 ± 0.05
Density	kg m^{-3}	1075 ± 3
Total Solids (TS)	mg L^{-1}	111,876 ± 1119
Total Suspended Solids (TSS)	mg L^{-1}	674 ± 246
Total Dissolved Solids (TDS)	mg L^{-1}	111,202 ± 1046
Electrical Conductivity (EC)	mS cm^{-1}	122.42 ± 0.43
NH_3–N	mg L^{-1}	452 ± 8

2.3. Experimental Design

G. sulphuraria and *C. vulgaris* were evaluated in five different experimental media compositions with 5% PW, 10% PW, 20% PW, 50% PW and 100% PW. Sterile deionized water was used to dilute PW for these experiments and respective standard media for the algae were used as the controls. Inoculum algae for the experiment were grown in 1 L Erlenmeyer flasks as described in Section 2.1. At the beginning of the experiment, the inoculum was centrifuged at 2000× *g* for 10 min at 25 °C (accuSpin 400 centrifuge, Fisher Scientific, Waltham, MA, USA) and the supernatant was carefully discarded without any exploitation of the biomass, which was resuspended in the five media compositions and the standard media (controls). Each media composition was transferred in sextuplicate in 16 mm borosilicate glass tubes with a working volume of 6 mL. The tubes were partially closed with plastic caps and sealed with parafilm to reduce evaporation. The tubes were then placed in the outer rim of a Tissue Culture Roller Drum Apparatus (New Brunswick Scientific, Enfield, CT, USA), rotating at 16 rpm. The roller drum was housed inside an incubator (Percival Scientific Inc., Perry, IA, USA) maintained at alga-specific culture temperature and light/dark cycle as described in Section 2.1. The incubator's CO_2 level was kept constant at 2–3% (vol/vol).

Since the PW used in the current study contains NH_4–N, as reported in Table 1, the initial concentration of ammoniacal nitrogen varied depending on the PW percentage used in the experiments. In contrary to the initial NH_4–N concentration, the initial NO_3–N and PO_4–P for all the PW experiments were attempted to maintain at the respective standard media level by adding:

i. the same salts as the standard media;
ii. the same amount of salts, as presented in Table 2.

Addition of N and P in PW for maintaining high biomass density and, consequently, a more efficient bioremediation process has been suggested by researchers in previous studies [9].

2.4. Analytical Techniques

Ammonia nitrogen, nitrate nitrogen and phosphate–phosphorus were measured using HACH DR 3900 (HACH, Loveland, CO, USA) spectrophotometer with different standard

HACH vials or powder for corresponding parameters and measurement range. Hanna pH meter (HI 5522, Hanna Instruments, Woonsocket, RI, USA) was used for pH and conductivity measurement of the PW. Biomass density was quantified in terms of the optical density (OD) measured with HACH DR 3900 spectrophotometer (HACH, Loveland, CO, USA). For *G. sulphuraria*, the biomass density was evaluated in terms of "ash–free dry weight" (g AFDW per L) correlated to OD at 750 nm with an equation developed and reported elsewhere by Pan et al. [17].

Table 2. NO_3–N and PO_4–P concentrations in respective standard media and the corresponding salts.

Growth Media.	NO_3–N mg L^{-1}	PO_4–P mg L^{-1}	NO_3–Salt	PO_4–Salt
CM	–	~66	–	KH_2PO_4
BBM	~42	~54	$NaNO_3$	K_2HPO_4, KH_2PO_4

$$Y = 0.4775{*}X - 0.0163;\ R^2 = 0.9967;\ X = \text{ OD value at 750 nm}$$

For *C. vulgaris*, the biomass density was evaluated in terms of "ash–free dry weight" (g AFDW per L), which was correlated to OD at 680 nm with the following two equations: respectively for glass tube measurement and cuvette measurement.

$$Y = 0.4685{*}X - 0.0277;\ R^2 = 0.9996;\ X = \text{ OD value at 680 nm (for glass tube)}$$

$$Y = 0.4314{*}X - 0.014;\ R^2 = 0.9997;\ X = \text{ OD value at 680 nm (for cuvette)}$$

Growth rate, in unit of "g AFDW per L per day", for each experiment was calculated for the exponential growth phase of *G. sulphuraria* and *C. vulgaris*. All the experiments were carried out in sextuplicate and analytical measurements were carried out in at least triplicates. The averaged data were presented with error bars equal to one standard deviation. The standard deviations were calculated using the Microsoft Excel software program (version 16.0, Redmond, WA, USA). The variations in the final biomass density, growth rate, N (both NH_4–N and NO_3–N) and P removal efficiency were analyzed using one–way analysis of variance (ANOVA).

3. Results

3.1. Algae Biomass Production in PW

The growth curves and growth rates for both *Galdieria sulphuraria* and *Chlorella vulgaris* are presented in Figure 1 and Table 3, respectively. *G. sulphuraria* and *C. vulgaris* showed promising growth at certain PW dilutions. For example, *G. sulphuraria* grew substantially at 5%, 10%, 20% and 50% PW (Figure 1a). The biomass density of *G. sulphuraria* at these experiments surpassed that of the standard media. Moreover, the maximum growth was obtained at 20% PW with a biomass density 3 times higher than that of the standard media. Additionally, the maximum growth rate 0.72 ± 0.05 g L^{-1} d^{-1}, calculated for the exponential growth phase of *G. sulphuraria* between day 0 and day 5, was observed for the same treatment, as presented in Table 3. In contrast, *C. vulgaris* grew significantly in 5%, 10% PW experiments and tended to grow in 20% PW experiments (Figure 1b). However, only the treatment at 5% PW resulted into a biomass density and growth rate similar to that of the standard media, as can be seen from Figure 1b and Table 3. No significant growth at lower dilutions can be attributed to the presence of some heavy metals in the PW, as indicated by the high TDS of PW.

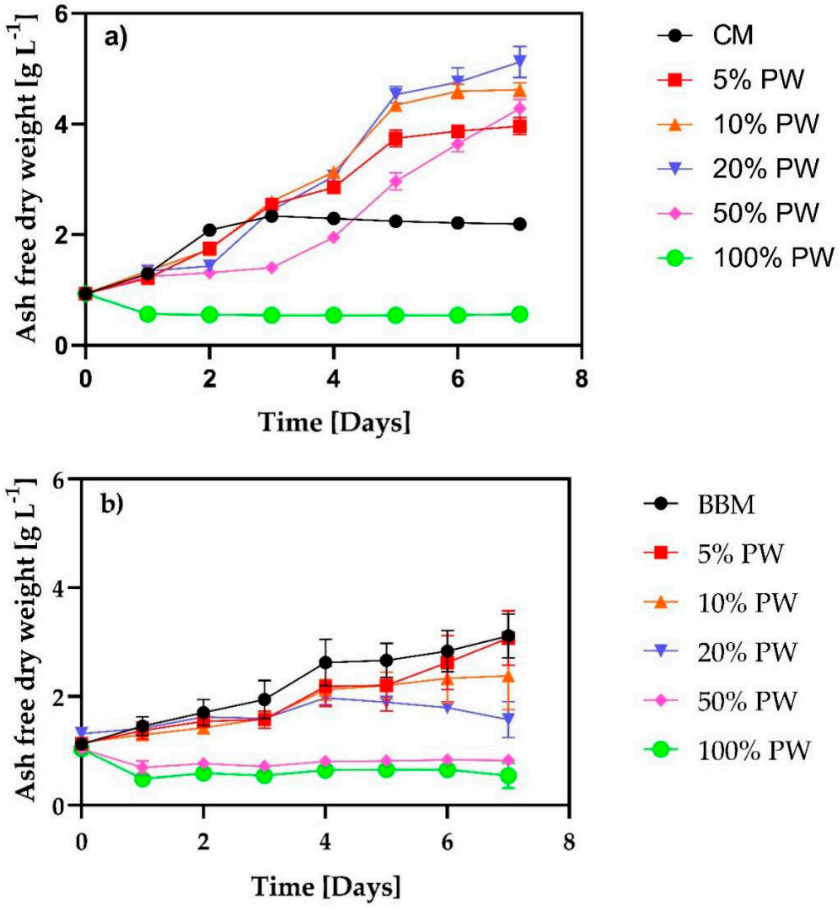

Figure 1. Growth curve for (**a**) *Galdieria sulphuraria*; (**b**) *Chlorella vulgaris*. Data points represent the average ± SD of n = 6 biological replicates.

Table 3. Growth rates of *G. sulphuraria* and *C. vulgaris*. Values represent the average ± SD of n = 6 biological replicates.

Experiment	Growth Rate (g L^{-1} d^{-1})	
	G. sulphuraria	*C. vulgaris*
Standard Media	0.47 ± 0.04	0.37 ± 0.17
5% PW	0.56 ± 0.05	0.37 ± 0.14
10% PW	0.68 ± 0.03	0.24 ± 0.07
20% PW	0.72 ± 0.05	0.17 ± 0.04
50% PW	0.72 ± 0.05	0.00 ± 0.00
100% PW	0.00 ± 0.00	0.00 ± 0.00

3.2. Nitrogen and Phosphorus Removal by Algae

The removal of NH_4–N and PO_4–P by *G. sulphuraria* in CM and different PW dilution experiments over 7 days are presented, respectively, in Figure 2a and Figure 2b. The produced water used in the current experiment contains NH_4–N, as reported in Table 1. Therefore, the initial concentration of ammoniacal nitrogen varied depending on the PW percentage in the experiments, as can be noticed from Figure 2a. Nevertheless, all the NH_4–N were removed by *G. sulphuraria* at 5%, 10%, 20% and 50% PW. Therefore, almost 100% removal efficiency was observed for these experiments. These findings are strongly corroborated by the growth and biomass density of *G. sulphuraria* at these PW percentages, as were discussed in Figure 1a. The initial NH_4–N concentration (~302 mg L^{-1}) at 50% PW was the closest to that (~292 mg L^{-1}) of the CM. Interestingly, the former resulted in

99.6 ± 0.2% removal efficiency of the ammoniacal nitrogen while the latter achieved only 44.4 ± 8.5% removal.

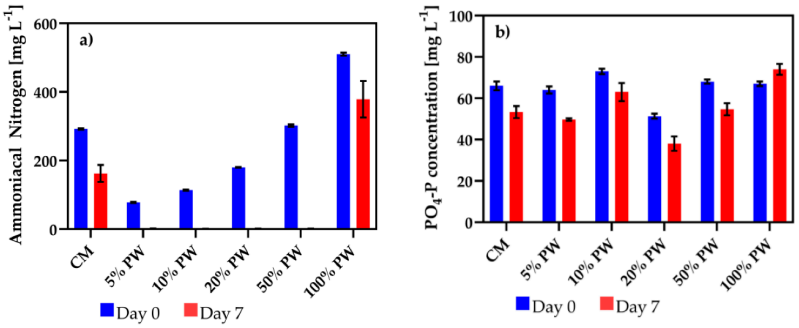

Figure 2. (**a**) NH_4–N removal and (**b**) PO_4–P removal; by *G. sulphuraria* in CM and different PW dilution experiments over 7 days Data points represent the average ± SD of n = 3 biological replicates.

In contrast to the initial NH_4–N concentration, the initial PO_4–P concentrations for all the PW dilution experiments were maintained to the CM level by externally adding KH_2PO_4 to the experiments, as was presented in Table 2. According to Figure 2b, it is evident that PO_4–P remained in excess at all the PW dilution experiments, including the CM.

The removal of NH_4–N, NO_3–N and PO_4–P by *C. vulgaris* in BBM and different PW dilution experiments over 7 days are presented, respectively, in Figure 3a, Figure 3b and Figure 3c. Again, PW used in the current experiment contains NH_4–N, as reported in Table 1. Therefore, the initial concentration of ammoniacal nitrogen varied depending on the PW percentage in the experiments, as shown in Figure 3a. Almost all the NH_4–N was removed by *C. vulgaris* at 5%, 10% and 20% PW with respective removal efficiency of 99.1 ± 0.5%, 99.9 ± 0.1% and 91.3 ± 4.3%. In the case of 50% and 100% PW, no significant amount of NH_4–N was removed. These findings are corroborated by the growth of *C. vulgaris* at these PW dilutions, as was discussed in Figure 1b.

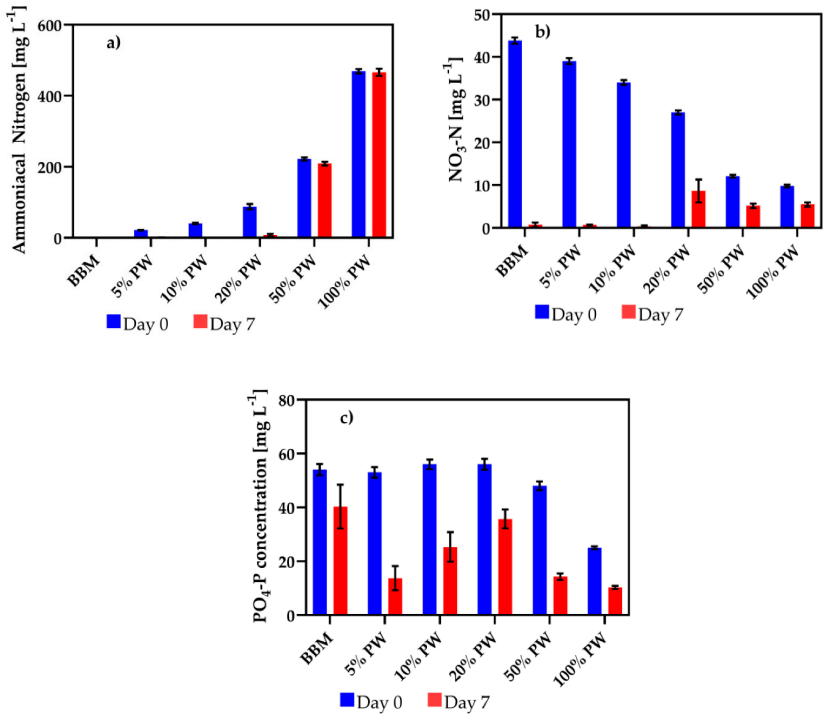

Figure 3. (**a**) NH_4–N removal, (**b**) NO_3–N removal and (**c**) PO_4–P removal; by *C. vulgaris* in BBM and different PW dilution experiments over 7 days Data points represent the average ± SD of n = 3 biological replicates.

Contrary to the initial NH_4–N concentration, the initial NO_3–N and PO_4–P for all the PW dilution experiments were attempted to maintain at the BBM level by adding corresponding standard media salts to the experiments as presented in Table 2. Although the same amounts of salts were added to each experiment, the measured NO_3–N and PO_4–P concentrations at Day 0 were different, as can be observed from Figure 3b,c. We attribute these variations to some undetermined chemical complexations (especially in the case of NO_3 salt), indicated by the gradual decrease in the measured initial concentrations as the PW percentage in the experiments increased, between the added salts and the PW constituents. However, this uninvestigated fact was disregarded following the experimental protocols described in the experimental design section of the study. Similar to NH_4–N, almost all the NO_3–N were removed by *C. vulgaris* at 5% and 10% with respective removal efficiencies of 98.2 ± 0.3% and 98.9 ± 0.6%, as can be seen from Figure 3b. However, in the case of 20%, 50% and 100% PW, 68.0 ± 9.9%, 57.2 ± 4.3% and 43.9 ± 4.8% NO_3–N were removed, respectively. From Figure 3c, it is evident that PO_4–P remained in excess at all the PW dilution experiments, including the BBM. Nevertheless, the maximum removal efficiency 74.2 ± 8.5% was achieved at 5% PW, which further indicated the maximum growth reached by *C. vulgaris* at this PW dilution, as discussed previously. We believe the lower availability of initial NO_3–N, in addition to higher metal concentrations, at higher PW percentages have negatively impacted algal growth at higher PW percentages.

The variations in the final biomass density, growth rate, N and P removal efficiency were analyzed using ANOVA and the results are presented in Table 4. These results indicate that there is a significant difference ($p < 0.05$) among different *G. sulphuraria* and *C. vulgaris* experiments in terms of final biomass density, growth rate, N and P removal efficiency.

Table 4. ANOVA statistical evaluation test to analyze the variations in final biomass density, growth rate, N and P removal efficiency in different experiments using *G. sulphuraria* and *C. vulgaris*.

	Sum of Squares	Degree of Freedom	Mean Square	F	*p*-value	$\alpha = 0.05$
			G. sulphuraria experiments			
Biomass Density (g L^{-1})						
Between Groups	80.93	5	16.09	618.35	1.57×10^{-24}	$p < 0.05$, there is a significant difference
Within Groups	0.63	24	0.03			
Total	47.71	29				
Growth Rate (g L^{-1} d^{-1})						
Between Groups	3.51	5	0.70	544.86	1.80×10^{-21}	$p < 0.05$, there is a significant difference
Within Groups	0.03	21	0.00			
Total	2.28	26				
NH_4–N Removal (%)						
Between Groups	16,990.40	5	3398.08	110.69	1.30×10^{-9}	$p < 0.05$, there is a significant difference
Within Groups	368.38	12	30.70			
Total	17,358.78	17				
P Removal (%)						
Between Groups	2597.68	5	519.54	27.33	3.65×10^{-6}	$p < 0.05$, there is a significant difference
Within Groups	228.15	12	19.01			
Total	2825.83	17				
			C. vulgaris experiments			
Biomass Density (g L^{-1})						
Between Groups	37.01	5	7.40	45.92	3.63×10^{-13}	$p < 0.05$, there is a significant difference
Within Groups	4.84	30	0.16			
Total	20.33	35				
Growth Rate (g L^{-1} d^{-1})						
Between Groups	0.68	5	0.14	35.33	1.06×10^{-11}	$p < 0.05$, there is a significant difference
Within Groups	0.12	30	0.00			
Total	0.39	35				
NH_4–N Removal (%)						
Between Groups	31,685.05	4	7921.26	1292.35	1.61×10^{-13}	$p < 0.05$, there is a significant difference
Within Groups	61.29	10	6.13			
Total	38,549.13	14				
NO_3–N Removal (%)						
Between Groups	8884.93	5	1776.99	74.65	1.29×10^{-8}	$p < 0.05$, there is a significant difference
Within Groups	285.65	12	23.80			
Total	9170.58	17				
P Removal (%)						
Between Groups	5466.69	5	1093.34	14.78	8.99×10^{-5}	$p < 0.05$, there is a significant difference
Within Groups	887.49	12	73.96			
Total	6354.18	17				

4. Discussion

Since produced water is composed of several harmful constituents, including heavy metals, it is understood that high concentration PW has higher toxicity to algae. However, our current study indicated that the thermophilic algae, *G. sulphuraria*, can withstand the toxicity at an outstanding level. To the best of our knowledge, this is the first reported exploration for biomass production by *G. sulphuraria* using PW. In a similar study, Badrinarayanan reported that *Chlamydomonas reinhardtii*, a freshwater alga, can grow in 20 times diluted PW [23]. The same author reported that *Nannochloropsis* sp., a marine microalga, showed a higher growth and biomass production rate than that of *C. reinhardtii* at the same PW dilution. However, both the algae showed higher growth rates when grown along with the standard medium. Our current study observed *G. sulphuraria* to grow in up to 50% PW outperforming its growth in standard media. Moreover, before cultivating algae, Badrinarayanan pre-treated PW by centrifugation and vacuum filtration to remove the solids, whereas we used raw PW in its untreated form. Although pre-treated PW of Badrinarayanan had a close TDS value as our PW, the current finding indicates that PW can be utilized in its native state in the case of *G. sulphuraria*. It is noted that, although several dilution studies for cultivating algae using PW have been conducted previously, only a few studies reported the biomass density, growth rate and removal of N and P from the PW [12,24,25]. Our study further indicates that *G. sulphuraria* achieved a substantially higher growth rate than that of the previously reported ones by several other microalgae. Among the recent studies, Hopkins et al. cultivated *Dunaliella tertiolecta*, a marine species, in PW with a TDS range from 30 g L^{-1} to 210 g L^{-1} and reported growth rates between 0.009 g L^{-1} d^{-1} to 0.017 g L^{-1} d^{-1} [12]. In our case, *G. sulphuraria* showed 0.56 $\pm$ 0.05 g L^{-1} d^{-1} to 0.72 $\pm$ 0.05 g L^{-1} d^{-1} in PW with TDS range of 5 g L^{-1} to 55 g L^{-1} (i.e., 5% PW to 50% PW).

Unlike *G. sulphuraria*, *C. vulgaris* has been previously cultivated in PW. Among the available studies, Das et al. reported 92% removal of the total nitrogen (TN) in 15 days from the pre-treated PW by *Chlorella* sp. [9]. Our results indicated that *C. vulgaris* could achieve as much as 100% N removal at 5% and 10% PW in only 7 days. We attribute this finding to the higher amount of biomass production in our case which is 3.1 $\pm$ 0.5 g L^{-1} in 7 days, as compared to Das et al.'s 1.2 g L^{-1} after 15 days. In a similar study Calderón–Delgado et al. grew *C. vulgaris* in PW at 25%, 50%, 75% and 100% dilutions and reported a maximum growth rate of 0.252 $\pm$ 0.004 d^{-1} at 25% PW [25]. Therefore, it is evident that at greater dilutions, *C. vulgaris* could successfully utilize suitable organic compounds from PW. However, the PW's toxicity at higher PW percentages could not be ignored, as observed in the current study and reported previously in several other studies [6,9]. Nevertheless, when compared with *G. sulphuraria* the most significant findings from current study become further evident. *G. sulphuraria* in the current study achieved a final biomass density of 5.12 $\pm$ 0.28 g L^{-1} in 7 days with a growth rate of 0.72 $\pm$ 0.05 g L^{-1} d^{-1} that are several times higher than that of the *C. vulgaris* reported by any other studies [9,25].

5. Conclusions

The current study indicated that both *G. sulphuraria* and *C. vulgaris* efficiently took up the required nutrients (N and P) present in the PW. Additionally, the growth rate and the final biomass density indicated no further nutrient supplementation was necessary to improve biomass production. A growth rate of up to 0.72 $\pm$ 0.05 g L^{-1} d^{-1} was achieved by *G. sulphuraria* in PW with TDS as high as 55 g L^{-1}. In the case of *C. vulgaris*, the alga produced twice as much biomass in half as much time (i.e., 3.1 $\pm$ 0.5 g L^{-1} in 7 days, compared to 1.2 g L^{-1} after 15 days) as was reported previously. Nevertheless, lipid, carbohydrate and protein profiles need to be studied to further understand the bioremediation potential of *G. sulphuraria* and *C. vulgaris* in order to treat PW.

Author Contributions: Conceptualization, A.R., S.P. and T.S.; methodology, S.P.; software, A.R.; validation, A.R., T.S. and C.H.; formal analysis, A.R and S.P.; investigation, T.S.; resources, A.R., T.S.;

data curation, A.R., S.P.; writing—original draft preparation, A.R.; writing—review and editing, S.P., C.H., T.S.; visualization, A.R.; supervision, T.S.; project administration, T.S.; funding acquisition, T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Center for Midstream Management and Science (CMMS)—Lamar University.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors. The data are not publicly available due to the continuation of a follow-up study by the authors.

Acknowledgments: Support provided by The Center for Midstream Management and Science (CMMS) Lamar University and The Office of Undergraduate Research at Lamar University in undergraduate author's OUR fellowship is acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

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