Nutrient Enrichment of Wastewater Generated during Hydraulic Fracturing with Animal Wastewater to Enhance Microalgae Growth

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Hydraulic fracturing technology widely used for recovery of oil and gas from tight shale formations generate millions of gallons of wastewater. This study examined viability of algal remediation of the hydraulic fracturing wastewater (HFWW). Our previous studies have shown that although Oklahoma native algae strains can grow in HFWW, cell growth was constrained by the low nutrient concentrations in HFWW. Hence, the goal of this study was to examine the effect of nutrient supplementation of HFWW with another nutrient rich wastewater on microalgae growth. An Oklahoma, USA, native algae strain, *Picochlorum oklahomensis*, was selected for the study because of its high biomass productivity in wastewater. This study demonstrated that *P. oklahomensis* can grow in HFWW. Supplementation of the HFWW with animal wastewater enhanced biomass productivity and lipid content of the biomass. Chemical compositions of the wastewater before and after algae growth were significantly different indicating substantial amount of contaminant removal.

1. Introduction

Hydraulic fracturing, also known as fracking, is a process during which water containing various chemicals is injected at high pressure into tight geologic formations holding oil and/or gas. The latter process induces fractures through which oil or natural gas can easily flow to the surface enhancing oil and gas recovery. Fracking process utilizes large volumes of freshwater depleting scarce resources, generating wastewater (WW) and creating environmental pollution. The liquid waste produced during the fracking operations is of great concern due to its potential adverse impact on ground and surface water, and soil. There are two main WW streams generated during hydraulic fracturing and oil and gas production, flowback water (FW) and produced water (PW). FW is the injected fluid mixed with the formation water that returns to the surface within the first few days following the initial fracturing of a shale (Lutzu et al., 2019a and 2019b). PW is the water stream that comes to the surface mixed with oil and gas during the production period. It represents the most concerning waste stream due to its larger production volume than FW and its potentially harmful chemical composition. PW may contain metals, radioactive compounds and other chemicals including Na, K, Cl⁻, Br⁻, Ca, Ba, Sr, Ra, and U, and organic chemicals such as solvents, biocides, and scale inhibitors (Maguire-Boyle and Barron 2014). Extremely high total dissolved solids (TDS) content in PW, in some cases exceeding 300.000 mg L^{-1}, makes the handling and treatment of this stream very challenging (Estrada and Bhamidimarri 2016). Handling methods for the final disposal of fracking waters are limited to deep well injection, and reuse and recycling for agricultural and industrial purposes including reuse in fracturing operations after treatment. Microalgae-based technologies are today recognized to be a valid alternative for the treatment of several typologies of WWs while producing biofuels and capturing CO_2 (Concas et al., 2013; 2016; and 2019). However, only recently microalgae were studied using FW and PW as a potential growth medium for algal biomass production and contaminant removal (Lutzu and Dunford 2019a, 2019b). Although microalgae can

grow in FW, biomass production is constrained by the limited nutrient (K and N) availability in the WW. Animal wastewater (AW) is a rich source of nutrients needed for algae cell growth (Lutzu et al., 2020a). Hence, it can be used to supplement fracking WW to enhance algae growth. This study examines the potential use of AW as an additive to improve algal biomass production in hydraulic fracturing wastewater (HFWW). Considering that the chemical composition of FW and PW are significantly different (Ferrer and Thurman 2015), and that fracking generates much PW than FW, PW was chosen as the algae growth medium in this study. An Oklahoma native microalgae strain, *Picochlorum oklahomensis* (PO), was grown in PW, AW and their mixtures. The effects of the WW source on the algal biomass growth profile, contaminant removal efficiency and the biomass characteristics were investigated.

2. Material and Methods

2.1 Inoculum, culture medium and wastewater preparation

PO was obtained from the culture collection of the National Center for Marine Algae and Microbiota (NCMA), Boothbay, Maine (2016). The detailed chemical composition of the culture media used to maintain algae can be found on the UTEX (2012) and NCMA official websites. PW used in this study was collected from the wells operating in southeast, OK, USA. The WW samples were filtered using a filter paper disk (#1, Whatman, UK), and then sterilized at 121°C, 0.1 MPa for 20 min in an autoclave prior to microalgae cultivation. The AW, which was collected from a swine farm at the Oklahoma State University, Stillwater, OK, was first filtered through a sand filter in order to remove suspended solids. Then, the filtered WW was autoclaved at 121°C for 20 min.

2.2 Algae Cultivation

PO was cultivated in 2 L (1.2 L working medium and at an initial cell concentration of 0.1 g L^{-1}) photobioreactors (PBR). More details on the PBR and the cultivation technique can be found in a previous publication (Lutzu et al., 2020b). After one month of cell growth, the cultures were centrifuged at 9722 g/RCF for 10 min. The liquid phase was separated and used for WW. The collected algal biomass was dried as is without further treatment.

2.3 Characterization of microalgae growth pattern

PO growth was monitored by measuring the absorbance of the culture at 680 nm for 30 consecutive days. The detailed procedure adopted to monitor algal growth was reported in Lutzu et al. (2020b). The cell concentration (dry weight V^{-1}), X_{dw} (g L^{-1}), specific growth rate (μ), doubling time (t_d) calculations were described in detail elsewhere (Zhou and Dunford 2017; Zhu and Dunford 2013). The time evolution of biomass productivity $\pi_X(t)$ $[\mathrm{mg\,L^{-1}d^{-1}}]$ was evaluated through the following equation:

$$\pi_X\left(t\right) = \frac{X_{dw}\left(t\right) - X_{dw}\left(t_0\right)}{t - t_0} \tag{1}$$

where the t_0 represent initial time of the cultivation period. The pH of culture suspensions was measured by a pH meter (model AR20, Fisher Scientific, Waltham, MA).

2.4 Lipid content determination

The lipids were extracted from PO biomass following the procedure described in Zhou and Dunford (2017). Lipid content determination was carried out at the end of the cultivation period (30 days) according to the procedure explained in a previous publication (Lutzu et al., 2020b). The batch lipid productivity $\pi_L(t_f)$ $[mg\,L^{-1}d^{-1}]$ was evaluated as:

$$\pi_L\left(t_f\right) = \pi_X\left(t_f\right)q_L\left(t_f\right) \tag{2}$$

where t_f $[d]$ represents the final cultivation time and $q_L\left(t_f\right)[\%wt]$ is the lipid content evaluated at the same time

2.5 Wastewater quality

The standard analytical water quality methods were used for testing (APHA 2005). At the end of PO growth period, the culture was centrifuged at 9722 g/RCF for 10 min and the supernatant was filtered through a glass microfiber filter (GF/C™, Whatman, UK) prior to the chemical testing. Chemical composition of PW and AW were analyzed before and after microalgae cultivation. All the relevant ions and chemicals were analyzed by either an ICP (Inductively Coupled Plasma) emission spectrometer or Flow Injection Autoanalyzer (FIA). Chemical Oxygen Demand (COD) of WW samples was determined according to the USEPA Method (1980).

2.6 Data Analysis

All PO growth experiments, and the analytical tests were carried out at least in duplicate with the mean values being reported. Means were compared by Tukey's HSD test at a 95% confidence interval. Statistical analyses of the data were performed using SAS 9.3 and SAS 9.2 (SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1 Initial Wastewater Composition

Two PW samples examined in this study (Table 1) were collected from the wells operating in Oklahoma, USA. The fracking fluids used in these wells contained proppant, friction reducer, biocide, scale inhibitor, breakers and a number of other ingredients. TDS content of PW-1 was within the range reported for PW generated in other states (Benko and Drewes 2008). The composition of PW-2 was similar to that of PW-1, except for a higher content of sulfate and nitrate, and a lower content of TDS, chloride and ammonium. TDS in both PW-1 and PW-2 were within the range reported by a National Alliance for Advanced Biofuels and Bioproducts report (NAABB, 2014) for wells in southwestern USA. The composition of the AW was significantly different than both PW-1 and PW-2. Lower TDS and COD and higher P contents in AW make it a good resource for blending PW and AW for enhancing PO growth.

Table 1: Chemical composition of wastewater used in this study

	PW-1	PW-2	AW	A/P-2 1:1	A/P-2 1:2	A/P-2 1:5
Sodium	8.596	5.024	145	2.695	3.536	4.222
Calcium	101	109	24	48	56	62
Magnesium	36	19	32	23	18	14
Potassium	179	125	423	228	219	168
Nitrate-N	0.2	231	36	n.d	n.d	7.2
Chloride	13.492	6.836	251	3.090	4.157	5.155
Sulfate	18	1.064	94	788	972	1.219
Boron	114	78	1	31	42	55
Bicarbonate	868	530	687	805	801	661
Carbonate	77	n.d	112	149	71	75
Ammonium	86	17	86	54	38	14
ICAP_P	0.01	1.2	24	13	8	5
TDS	25.014	15.642	1.835	8.474	10.487	12.810
BOD5	n.d	9.5	5.9	7	7.6	10.3
COD	1.764	1.700	186	461	1.104	877
pH	8.5	8	9	8.9	8.6	8.7

Note: PW-1: Produced water sample 1, PW-2: Produced water sample 2, AW: Animal wastewater, nd: Not detected, ICAP: Inductively Coupled Argon Plasma.
All the values are expressed in terms of mg L^{-1}. All data were obtained as means from at least two experiments.

3.2 PO growth in produced water, animal wastewater and produced water mixtures

PO was originally isolated in the area of the Great Salt Plains (GSP), Northwestern Oklahoma (Kirkwood et al. 2008), an extremely hard environment for most life forms, because of the wide temperature and salinity fluctuations. µ of PO in PW-1 and PW-2 were lower than µ achieved for the same strain in regular media such as MAS and f/2 (Zhu and Dunford 2013; Mayers et al. 2018). The latter results are due to the limited nutrient availability, mainly low N and P in PW. The highest µ for PO, 0.35 day^{-1}, was achieved in PW-1 which had a N/P ratio of 15, close to the standard ratio of 20. However, PO performed less better than in AW and its mixtures. In particular, X_{max} in PW-1 was 1.90 g L^{-1} which was higher than X_{max} achieved in PW-2 for PO (Table 2). Previously reported X_{max} for PO grown in regular media under similar growth conditions used in the present study were not significantly different (Zhou and Dunford 2017). A previous study reported that PO produced significantly lower X_{max} in FW (Lutzu and Dunford 2019a) as compared to that in PW-1 and PW-2. It was also shown that algae growth in HFWW can be significantly improved by nutrient supplementation (Zhou and Dunford 2017). To further test our hypothesis that PO can grow in PW and nutrient supplementation of PW enhances algal cell growth, PO was cultivated in PW-2 supplemented with growth nutrients by mixing it with AW at the ratios of 1:1, 1:2, and 1:5 by volume (Table 1). PW-2 rather than PW-1 was selected for the AW supplementation study because of the lower TDS and X_{max} obtained in PW-2 as compared to those in PW-1.

Table 2: Growth characteristics of PO cultivated in AW, PW-1, PW-2 and different AW/PW-2 concentrations

Growth medium	μ (day^{-1})	t_d (day)	X_{max} (g L^{-1})
PW-1	0.35±0.02	4.30±1.59	1.90±1.00
PW-2	0.15±0.01	2.90±0.19	1.20±1.40
AW	0.16±0.07	4.30±1.62	2.70±0.36
AW/PW-2(1:1)	0.18±0.01	3.20±1.20	2.40±0.44
AW/PW-2(1:2)	0.18±0.01	2.10±0.26	2.00±0.39
AW/PW-2(1:5)	0.15±0.01	2.70±1.30	1.80±1.40

Note: μ: specific growth rate, t_d: doubling time, X_{max}: maximum biomass concentration. PW-1: Produced water sample 1, PW-2: Produced water sample 2, AW: Animal wastewater. All data were obtained as means from at least two experiments/measurements.

Although μ of PO in AW, PW-2 and their mixtures were similar, t_d and X_{max} were significantly higher in AW as compared to the other water samples examined in this study (Table 2). Supplementation of PW-2 with AW significantly improved algal biomass production. Optimum AW/PW-2 mixture ratio was 1:1 by volume. Under the latter conditions, X_{max} doubled as compared to that obtained in PW-2 only. At higher mixture ratios X_{max} decreased significantly. A similar trend was reported for two marine strains, *Nannochlorpsis oculata* and *Isochrysis galbana* (Ammar et al. 2018). In Figure 1a it is shown that biomass productivity $\pi_X(t)$ achieves a maximum after cultivation times ranging from 2 to 7 days depending on the specific medium being considered. The highest instantaneous productivity, i.e. about 170 mg L^{-1} d^{-1} was obtained after 2 days of cultivation by using the medium AW/PW-2(1:5). However, a good productivity, i.e about 150 mg L^{-1} d^{-1} was achieved even when using the PW-2 alone.
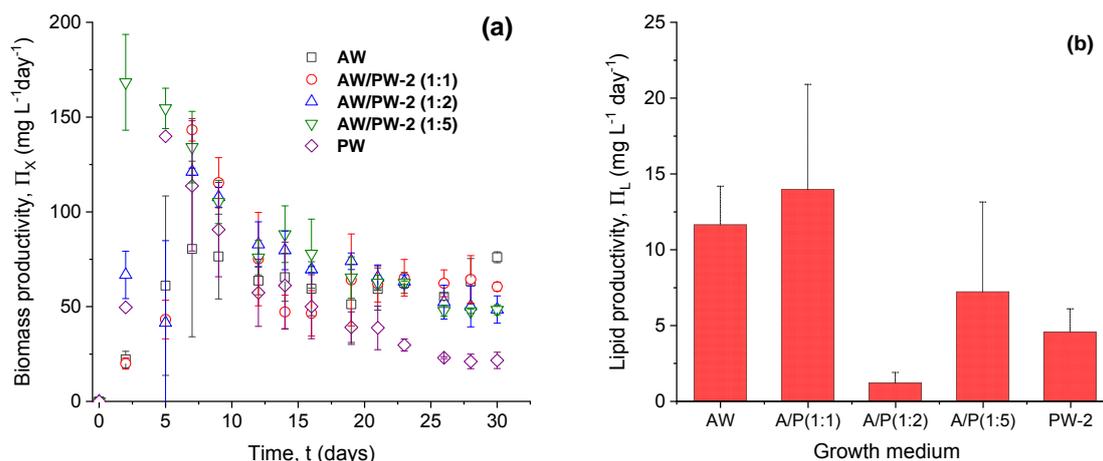

Figure 1. Time evolution of biomass productivity (a) and final batch productivity (b) with the different media.

While the initial productivities are very high it should be noted that biomass cannot be harvested after a so short cultivation time because biomass concentration in the reactor is very low and the withdrawal of algae could lead to reactor washout. Therefore, the most important values of biomass productivities are those obtained for longer periods of time. As it can be observed (cf. Figure 1a), after cultivation times greater than 7 days productivity starts to decrease with a different gradient depending on the specific medium adopted. Then, at the end of cultivation, the higher biomass productivity was attained using the AW medium ($\sim$ 75 mg L^{-1} d^{-1}) and the AW/PW-2 (1:1) one ($\sim$ 60 mg L^{-1} d^{-1}). Accordingly, the best performing media were the ones with a higher content of AW. In fact, the latter may introduce significant amount of organic substrate to the culture medium and promote bacteria proliferation. In our study, a relatively high inoculum amount, 0.1 g L^{-1}, was used and WW samples were autoclaved prior to algae inoculation under sterile conditions. Hence, under the experimental conditions used in this study, the amount of bacteria in the produced biomass is expected to be not significant. The current study established that microalgae can grow in PW and the chemicals present in PW do not have a significant inhibitory effect on cell growth. Hence, PW could be a potential WW source for algal biomass production.

3.3 Lipid content of microalgae

In order to evaluate the possibility to exploit the produced microalgal biomass as a valuable product for energy or other commercial applications, the final lipid content in algal biomass grown in WW was assessed (cf. Lutzu et al., 2020b). When considering the algae growth in AW, the lipids accounted for about 15% wt of total biomass weight. When the AW/PW-2 mixtures are considered, by starting from the very low lipid content of the algal biomass grown in AW/PW-2 mixtures, i. e. about 3% wt, a significant increase to about 25% wt can be observed as the PW-2 fraction in the water mixture increased from 1:1 to 1:5, respectively. A similar value, i.e. about 25% wt, for the biomass lipid content was obtained when PW-2 was used in the growth experiments. This is probably because the lower content of nitrogen in PW can provoke N starvation phenomena that in turn, according to the literature can boost lipid synthesis in microalgae (Soru et al., 2019). Therefore, it can be concluded that by increasing the frac water content in the water mixture significant increases the lipid content in the biomass. This is probably due just to the fact that microalgae tend to accumulate lipids under stress, specifically under low nutrient availability. However, to evaluate the viability of the process, lipid productivity is a more indicative parameter, rather than weight percentage. In Figure 1b it can be noted that the higher lipid productivity, i. e. about 14 mg L^{-1} d^{-1}, was obtained with the medium AW/PW-2 (1:1). It is important to highlight that the addition of PW-2 led to an improvement of the lipid productivity with respect to the case where only AW was used. However, a further increase of PW-2 content led to a reduction of lipid productivity. This is because lipid productivity depends on both biomass productivity and lipid content (cf. Eq. 2) which react in opposite manner to N starvation. Thus, a too high content of PW-2 in the medium beside producing the increase of lipid content can inhibit biomass growth thus resulting in a reduction of the lipid productivity. AW/PW-2 (1:1) thus represents the optimal compromise between lipid content and biomass production.

3.8 Wastewater chemical composition after algae growth

Almost no nitrate or ammonia was left in the medium after growing PO in PW-2 and AW/PW-2 mixtures (cf. Figure 3). Over 99% N removal is due to the algal cell uptake of this compound.
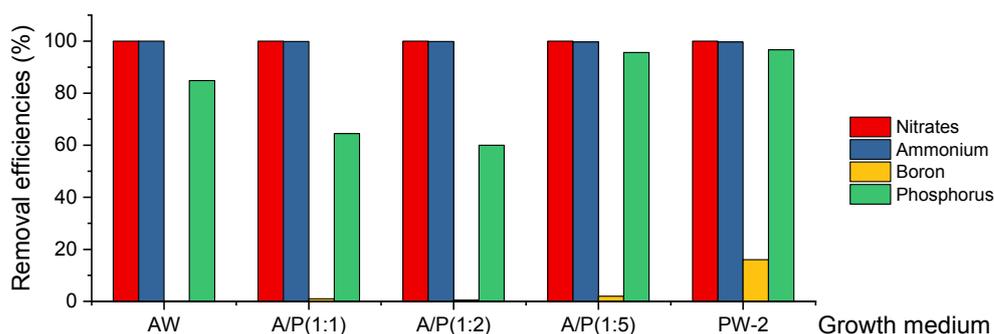

Figure 2. Effect of algae growth on the removal rate of relevant contaminants in the wastewaters

A large portion of P was also taken up from AW and PW-2 by algae. It is interesting to note that as the PW ratio increased in the medium, P removal increased, probably due to the lower amount of P availability in PW. The chemical composition of water determines its suitability for a given application such as irrigation. High concentrations of Cl⁻, Na and B in irrigation water or soil may cause toxicity problems in plants. Some plants are sensitive to high levels of B in soil and water. Excess B uptake by plant roots may result in complete plant growth inhibition in many agricultural regions (Brdar-Joanović 2020). Cultivation of PO in PW-2 resulted in a 16% B reduction while B uptake from AW and AW/PW-2 mixtures was significantly lower, <5%, probably due to the lower B concentrations in the latter water samples (Table 1).

4. Conclusions

This study investigated the growth profile of the microalgae strain PO in PW, AW and their mixtures. Cell growth in PW is limited with the nutrient availability. A significant increase in biomass concentration was achieved by nutrient (N and P) supplementation of PW by blending it with AW at 1:1 volume ratio. An inverse relationship was found between PO growth rate and final lipid content: as the PW content in the AW mixture increased the algal growth rate decreased while the final lipid content of the biomass increased. A significant amount of contaminants could be removed from WW by growing algae and harvesting the biomass (100% nitrate and ammonia, over 95% phosphate). Hence, PW and AW can be potential WW sources for algal biomass production, consequently minimizing the adverse environmental impact of WW generated during oil, gas and animal production.

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