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Growing Algae in Produced Water generated during Oil and Gas Production using Hydraulic Fracturing Technique

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Hydraulic fracturing technology is widely used for recovering natural gas and oil from tight oil and gas reserves. Large volumes of wastewater are generated during this process. Produced water is considered wastewater by the regulators, hence, need to be treated or disposed accordingly. This study examines algal treatment of produced water. Eleven microalgae strains were examined. Wastewater quality before and after algae treatment was evaluated. Algal biomass grown in produced water was characterized for its volatile matter, fixed carbon and ash contents. The experimental results indicate that microalgae can grow in produced water. The chemical composition of the algal biomass obtained in produced water was strain specific. Cyanobacteria SP47 exhibited the highest high heating value and fixed carbon among the strains examined in this study. About 60 % total dissolved solids, 100 % nitrate and phosphate, over 65 % boron reduction in produced water could be achieved. This study has demonstrated that algal treatment of produced water can significantly reduce the costs and the adverse environmental impact of hydraulic fracturing technology.

1. Introduction

Oil and gas production in low-permeability and unconventional reservoirs is often stimulated using hydraulic fracturing technique during which a fluid containing chemical additives, and a solid propping agent (proppant) is pumped under pressure into a reservoir containing trapped oil or gas. High hydrostatic pressure creates new fractures and links the fissures that are already present in the reservoir and proppant holds open the cracks allowing oil and gas freely flow into the well ([Gallegos and Varela, 2015](#_ENREF_16)). The large volume of water used and the amount of wastewater (WW) generated during this process are great concerns due to their potential adverse effects on the availability of freshwater resources, harmful environmental impact. Disposal techniques currently used in industry and the lack of information on the ultimate fate of this WW are problematic. There are three streams of water that have potential adverse environmental impacts through surface spills and underground injection: fracking fluid, flowback (FWW) and produced water (PWW). FWW returns to the surface following the fracturing process. More water may be produced along with oil and gas after the flowback period is over and well is in production. The water generated during gas/oil production is referred to as PWW. FWWs and PWWs can contain elements such as sodium, potassium, chloride, bromide, calcium, barium, strontium, radium and uranium, and have total dissolved solids (TDS) concentrations exceeding 300,000 mg L−1 ([Estrada and Bhamidimarri, 2016](#_ENREF_11)). Current WW management options (deep-well injection, reuse and recycling, treatment on-site, surface disposal or spreading) are challenging due to their high cost and damaging environmental impacts (Acharya et al, 2011). Most of the algal treatment studies focused on animal (Mezzanotte et al., 2018) and industrial WW ([Lutzu et al., 2016](#_ENREF_29)). Two previous studies ([Racharaks et al., 2015](#_ENREF_38); [Wood et al., 2015](#_ENREF_47)) examining algae growth in FWW did not examine the effect of algae growth on residual water quality. In a recent study, we reported the effect of growing algae in FWW on residual water quality ([Lutzu and Dunford, 2017](#_ENREF_28)). Considering that chemical composition of FWW and PWW can be significantly different, and volume of the PWW generated during oil and gas production is much larger than that of FWW, examining algal remediation of PWW is imperative. Hence, this study examines for the first time growth profile of Oklahoma native microalgae in PWW collected from a well operating in Oklahoma, USA, as a new alternative treatment technology to reduce cost and minimize the environmental impact of WW management and treatment. Inorganic chemical content of WW was analyzed before and after algae growth. Algal biomass grown in PWW has been characterized using a thermogravimetric analysis (TGA) method.

* 1. Material and methods

**2.1 Inoculum and culture medium preparation**

Microalgae strains investigated in this study were obtained from the culture collection of Algae at the University of Texas at Austin and the National Center for Marine Algae and Microbiota, Boothbay, Maine. The strain identification numbers, media used to prepare inoculum as well as the location where the strains were isolated are reported in Table 1. The detailed chemical composition of the culture media used to maintain algae can be found on the UTEX and NCMA official websites. All the strains were grown in the media that were recommended by UTEX and NCMA. Culture maintenance conditions were as follows: room temperature, a photosynthetic photon flux (PPF) of 40µmol m−2 s−1 provided by two 32 W white fluorescent tubes (F32T8/SP65/ECO, General Electric Company, Fairfield, CT), and a light/dark photoperiod of 12 h.

Table 1: Collection site and culture maintenance media for the microalgae strains investigated in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Identification number | Media\* | Collection site |  |
| *Aphanocapsa* sp. | UTEX SP23 | A+ | Great Salt Plains, OK, USA |  |
| *Geitlerinema amphibium* | UTEX SP27 | A+ | Great Salt Plains, OK, USA |  |
| *Geitlerinema carotinosum* | UTEX SP28 | A+ | Great Salt Plains, OK, USA |  |
| *Komvophoron* sp. | UTEX SP33 | A+ | Great Salt Plains, OK, USA |  |
| *Phormidium kuetzingianum* | UTEX SP38 | BG11+1% NaCl | Great Salt Plains, OK, USA |  |
| *Pseudoanabaena* sp. | UTEX SP46 | A+ | Great Salt Plains, OK, USA |  |
| *Pseudoanabaena* sp. | UTEX SP47 | A+ | Great Salt Plains, OK, USA |  |
| *Pseudoanabaena* sp. | UTEX SP48 | A+ | Great Salt Plains, OK, USA |  |
| *Botryococcus braunii* | UTEX LB572 | WSE | Cambridge, UK |  |
| *Aphanothece* sp. | CCMP2555 | BG11 | Great Salt Plains, OK, USA |  |
| *Picochlorum oklahomensis* | CCMP2329 | MAS | Great Salt Plains, OK, USA |  |

\*MAS = Modified Artificial Seawater, WSE = Waris Soil Extract

* + 1. Wastewater collection and characterization

The WW sample used for the experiments was classified as PWW and collected in 2014 from a well operating in Cumberland, OK, USA. The samples were stored at room temperature in plastic buckets prior to the experiments. The WW sample was filtered using a filter paper disk (#1, Whatman, UK), and then sterilized at 121 °C for 20 min in an autoclave (Hirayama, HVE-50, Ramsey, MN, USA) prior to its use.

2.3 Algae Cultivation

The algae strains examined in this study belonged to two main classes, Cyanophyceae (SP23, SP27, SP28, SP33, SP38, SP46, SP47, SP48 and CCMP2555), and Chlorophyceae (LB572, CCMP2329). LB572 and CCMP2329 are unicellular spherical green algae, SP23 and CCMP2555 are unicellular spherical cyanobacteria, while all the others are filamentous cyanobacteria cells. The algae were cultivated in 2 L glass reactors (PBR) (Kimble Chase Life Science and Research Products LLC, Vineland, NJ), sealed with a GL45 3-port cap equipped with tubing adapters (CPLabSafety, Novato, CA). The PBR were kept in a closed growth chamber with inner dimensions of 118.8 cm x 58.4 cm x 76.9 cm (length x height x width). The growth chamber was maintained at 23 ± 4 °C. Twelve 23 W cool white fluorescent bulbs (Osram Sylvania, Wilmington, MA), installed on the ceiling of the growth chamber, were used as light source providing a PPF of 85 ± 4 µmol m−2 s−1 measured by a quantum meter (model QMSW-SS, Apogee Instruments Inc., Logan, UT). Cultures were subjected to a 12:12 h cycle in which 12 h was light with aeration while the other 12 h was dark without aeration. The aeration rate was 20 mL min-1 and the concentration of CO2 (industrial carbon dioxide, Airgas, Stillwater, OK) in the air (Grade D breathing air, Airgas, Stillwater, OK) was 2 % v/v. The initial cell concentration of the culture medium was 0.1 g L-1 and the working volume of the PBR was 1.2 L.

2.4 Characterization of microalgae growth pattern

Cell growth was monitored by measuring the absorbance (ABS) of the culture at 680 nm by a spectrophotometer (model DU 520, Beckman Coulter, Brea, CA) for 30 consecutive days. Distilled water without algae cells was used as the blank for the ABS readings. A regression equation describing the relationship between dried biomass concentration and ABS was also calculated. Dry biomass concentration was determined gravimetrically as follows; a) about 5 mL of culture (V) was drawn from the reactors, b) the sample was passed through a pre-weighted glass microfiber filter (GF/CTM 55 mm diameter, Whatman, UK) and filter paper and the biomass retained on the filter were dried at 105 °C overnight to a constant weight, c) the filter paper was previously dried in a forced-air oven (model F Air 2.3 CF, VWR Science, Radnor, PA) at 105 °C for 2 h and then cooled to room temperature in a desiccator and weighed using an analytical scale (model A-160, Denver Instrument, Bohemia, NY). The cell concentration (dry weight), Xdw (g L−1), average biomass productivity (∆X), specific growth rate (μ), and doubling time (td) were calculated according to Zhou and Dunford (2017a).

2.5 Algal biomass characterization

The algal biomass was characterized by a TGA method ([Zhu and Dunford, 2013](#_ENREF_50)). In summary the weight loss of the heated biomass at different stages corresponded to the moisture (M), the volatile matter (VM), the fixed carbon (FC), and the ash (A) content of the sample. The higher heating value (HHV), MJ Kg-1 (dry basis), was calculated according to [Parikh et al (2005](#_ENREF_36)).

2.6 Wastewater quality

The chemical composition of the PWW was analyzed before and after microalgae cultivation. The water quality tests were performed by the Soil, Water and Forage Analytical Laboratory (SWFAL) at the Oklahoma State University. The standard analytical water quality methods were used for testing. At the end of the algae growth period, the culture was centrifuged at 8000 rpm for 10 min (Sorval RC-5C Plus, Ramsey, MN) and the supernatant was filtered through a glass microfiber filter (GF/CTM, Whatman, UK) prior to the chemical testing. The Chemical Oxygen Demand (COD) of the WW samples was determined with the USEPA Method (USEPA, 1980). A vial heater reactor (model DBR200, HACH, Loveland, CO) was used for the COD tests. Distilled water was used as blank for the ABS readings. A calibration curve generated using the standard solutions was used for determining the COD of the WW samples.

2.7 Data Analysis

All algae growth experiments and analytical tests were carried out at least in duplicate with the mean values being reported. Statistical analyses of the data were performed using SAS 9.3 and SAS 9.2 (SAS Institute Inc., Cary, NC). The regression equations for dry biomass concentration vs. ABS, μ, td, and proximate analysis by TGA were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).

* 1. **Results and Discussion**

**3.1. Produced water composition**

The water sample for algae growth was collected from a well operating in southeast Oklahoma, USA. According to the Frac Focus Chemical Disclosure Registry (2018), the well was fractured in April 2013 and fracking operation lasted 4 days. Hydraulic fracturing fluid used in this well contained crystalline silica as proppant, a propriety formulation friction reducer, glutaraldehyde as biocide, methanol, nonylphenol ethoxylate, and sodium perborate tetrahydrate as scale inhibitor and a number of other non-MSDS (Material Safety Data Sheet) ingredients. The water sample was received in our lab in January 2014. The well was in production at the time of the sampling, hence, the water collected were PWW not FWW. TDS content of water used for algae growth was 25,014 mg L-1 (Table 2) which was within the range reported for PWW generated in other states ([Benko and Drewes, 2008](#_ENREF_4)). The water was alkaline, pH 8.5, and contained very high concentration of chloride, 13,492 mg L-1 but had lower sodium and mineral content than those reported in PWW in different regions in USA. ([Cluff et al., 2014](#_ENREF_7)). The concentration of nutrients needed for algae growth such as nitrogen (N) and phosphorous (P) was quite low which is typical for the WW generated during hydraulic fracturing.

**3.2 Algae growth**

The reasons for selecting the Oklahoma native strains for this study have been previously reported by Zhou and Dunford (2017a). As shown in Table 3, µ were quite low because of the limitations of the growth conditions, i.e. limited nutrient availability, mainly low N and P, and low (0.02 g L-1) initial biomass concentrations used in this study. SP47 was the fastest growing species when cultivated in PWW (µ = 0.48 day-1).The same strain also performed relatively well in FWW resulting in higher µ among the strains examined in the study ([Lutzu and Dunford, 2017](#_ENREF_28)). The µ for this species in PWW was also similar to that obtained in standard growth medium (Zhou and Dunford, 2017a). Xmax in PWW varied between 1.11 and 2.49 g L-1. The best performing strain was SP33, a halotolerant (0-5 % NaCl) blue-green filamentous alga. Differences in Xmax among the Cyanobacteria strains were significant, while those for the Chlorophyceae were not. Cell growth and biomass characteristics of SP38, SP46, SP47, SP48 and CCMP2329 cultured in the standard media (Table1) have been reported in other publications ([Zhou and Dunford, 2017b](#_ENREF_49)). Previously reported Xmaxfor CCMP2329 grown in MAS medium under similar growth conditions used in the present study, 2.1 g L-1 ([Zhou and Dunford, 2017a](#_ENREF_48)), and PWW, 1.87 g L-1 (Table 3), were not significantly different (p > 0.05). It is important to note that although the strains used in both studies were the same, *P. oklahomensis,* Zhu and Dunford study ([Zhu and Dunford, 2013](#_ENREF_50)) was carried out using the cultures obtained from UTEX (UTEX B2795) while the same strain used in this study was from NCMA, CCMP2329

Table 2: Chemical composition of wastewater before and after algae growth

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| % Reduction after algae cultivation | | | |  |
| Irrigation water parameters PWW 2555 SP27 SP28 SP38 SP46 SP47 2329 | | | |  |
| Calcium (mg L-1) | 101 26 24 23 39 NR NR NR |  | | |  |
| Magnesium (mg L-1) | 36 NR NR NR 12 NR NR NR |  | | |  |
| Potassium (mg L-1) | 179 38 27 30 60 NR NR NR |  | | |  |
| Nitrate-N (mg L-1) | 0.2 100 100 100 100 NR NR NR |  |  |  |
| Chloride (mg L-1) | 13,492 28 33 35 64 NR NR NR |  | | |  |
| Sulfate (mg L-1) | 18 NR NR NR NR NR NR NR |  | | |  |  |
| Boron (mg L-1) | 114 40 40 39 67 1 5 2 |  | | |  |  |
| Bicarbonate (mg L-1) | 868 61 69 61 76 46 48 44 |  | | |  |  |
| Carbonate (mg L-1) | 77 NA 70 NA NA NA NA NA |  | | |  |  |
| pH  EC (µmhos cm-1) | 8.5 6 NR 7 9 6 6 7  37,900 26 30 30 57 NR NR NR |  |  |  |
| Ammonium (mg L-1) | 86 54 66 57 97 47 52 31 |  |  |
| ICAP\_P (mg L-1) | 0.01 30 35 35 61 NR NR NR |  |  |
| TDS (mg L-1) | 25,014 26 30 30 57 NR NR NR |  |  |
| COD (mg O2 L-1) | 1,764 24 24 32 69 NR NR NR |  |  |

ICAP\_P = phosphorus measured by Inductively Coupled Argon Plasma emission spectrophotometer; TDS = Total Dissolved Solids; NR = No reduction; NA = Not available because carbonate was measured by titration method which is good for the pH range of 8.3-4.5 and pH of the sample after algae growth was higher than 8.3.

Table 3: Effect of culture media on the growth characteristics of microalgae strains.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strains | µ (day-1) | td (day) | Xmax (g L-1) ∆X (mg L-1 day-1) |  |
| SP23 | 0.08e | 5.60b | 1.64b 100.0d |  |
| SP27 | 0.34c | 1.90e | 1.66b 119.0c |  |
| SP28 | 0.23d | 3.04d | 2.25a 96.0d,e |  |
| SP33 | 0.21d | 3.83c | 2.49a 83.0f,g |  |
| SP38 | 0.25d | 4.10c | 1.35b 51.0i |  |
| SP46 | 0.21d | 3.63c | 2.17a 92.0e,f |  |
| SP47 | 0.48b | 1.43e | 1.96a 169.0b |  |
| SP48 | 0.18d | 5.64b | 1.11b 90.0f |  |
| LB572 | 0.08e | 9.21a | 1.63b 63.0h |  |
| CCMP2555 | 0.23e | 2.90d | 1.50b 63.0h |  |
| CCMP2329 | 0.35c | 4.31b | 1.87a 280.0a |  |

µ: specific growth rate, td:doubling time, Xmax:maximum biomass concentration, ∆X: average biomass productivity.

Means with the same superscript letters in a column are not significantly different (Tukey’s HSD test, p > 0.05).

Hence, the difference in Xmax in two different media might not be uniquely due to the difference in chemical composition of the growth media. SP38 and SP47 showed an opposite trend and produced about three time as much biomass in PWW, 1.35 g L-1 and 1.96 g L-1, as compared to the standard media. It is possible that SP38 and SP47 are capable of metabolizing some of the organic matter that may be present in the PWW. There are studies indicating that several algae strains are capable of metabolizing some of the petroleum hydrocarbons (Carpenter et al., 1989). Cyanobacteria examined in this study can be grouped in two groups based on their Xmax in PWW. One group including SP28, SP33, SP46, and SP47 exhibited a Xmax greater than 1.9 g L-1 while the other group (SP23, SP27 and CCMP2555) had a lower Xmax, about 1.5 g L-1. The species *Pseudoanabaen*a, *Komvophoron*, *Geitlerinema* and *Phormidium* can live as single filament or agglomerated in clusters or in fine mucilaginous colonies. Single filaments show facultative motility that allow them to swim in the medium like green algae do with the use of flagella. Examination of the algae cells under microscope revealed that SP38 and SP48 had a tendency to form cell clumps when no mixing was applied, while SP28, SP33, SP46 and SP47 tended to stay as single filaments or in small colonies. Therefore, higher ∆X of SP28, SP33, SP46 and SP47 in PWW could be partly due to their motility and reaching to the nutrients more efficiently than the other filamentous strains. A previous study ([Lutzu and Dunford, 2017](#_ENREF_28)) reported that SP23, SP27, SP28, SP46, SP47, SP48, CCMP2555 and CCMP2329 produced significantly lower Xmax in FWW as compared to that in PWW. Our results confirm the significant effect of both culture growth medium chemical composition and the strain type on biomass production efficiency. Most importantly, the current study demonstrated that chemicals in PWW do not have a significant inhibitory effect on cell growth. Hence, PWW could be a potential WW source for algal biomass production.

**3.3 Biomass Characteristics**

Chemical composition of biomass produced by algae is a critical factor in choosing a strain for commercial cultivation. A TGA method was used to determine M, VM, FC, Ash and HHV contents of the samples (Table 4). The VM contents of the algal biomass generated in PWW ranged from 61.9 % to 83.9 % which was similar to the range reported in the literature for algae ([Soria-Verdugo et al., 2017](#_ENREF_42)). SP23 and SP47 had the lowest and highest FC contents, respectively. High VM and FC contents are desirable attributes for producing pyrolysis oil from biomass. The Ash contents of all the samples were relatively low. This aspect is very important, since high Ash content in the biomass reduces its value for most conversion processes. Other drawbacks of high Ash content biomass are high processing costs, poor combustion, and limited biomass disposal options. LB572 and SP38 had the highest Ash contents among the strains examined in this study. Both strains are green algae and filamentous Cyanobacteria, respectively. As reported in the previous section, SP38 tends to form cell clumps while LB572 cells live in colonial association via the production of a complex extracellular matrix. Both strains are non-motile and tend to form large colonies and/or filaments which could entrap salt. Considering the high salt concentration of PWW used for algae growth in this study, a significant portion of the Ash in the biomass is expected to be on the cell surface rather than internalized. Ash content of the biomass can be reduced by washing the samples after harvest. For example, the biomass produced by SP46 grown in standard medium, A+, contained about 15.2 % Ash as harvested ([Zhu and Dunford, 2013](#_ENREF_50)). Washing the biomass with deionized water reduced the A content to 2.5 % indicating that a significant portion of the Ash was on the biomass surface, not internalized by the cells. It is important to point out that the biomass produced by the same strains in FWW had significantly higher Ash content ([Lutzu and Dunford, 2017](#_ENREF_28)) than that in PWW. The HHV values were similar to those of biomass grown in regular media, and in some cases it was even higher. SP47 and SP48 had the highest HHV, 20.0 MJ Kg-1. SP38 and LB572 had relatively low HHV due to high Ash content in their biomass. These values were within the range, 15-25 MJ Kg-1, recommended for biomass to be considered for biofuel production ([Orosz and Forney, 2008](#_ENREF_35)).

Table 4: Chemical composition of biomass determined by thermogravimetric analysis (TGA).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strains | M | VM | FC Ash HHV |  |
| SP23 | 4.7e | 44.0d | 8.9e 42.4a 10.3d |  |
| SP27 | 4.1e | 47.1c,d | 7.1f 41.7a 10.4d |  |
| SP28 | 5.6d | 45.3d | 14.9d 34.2b  13.0c |  |
| SP33 | 7.2c | 49.1c,d | 15.8c 28.0c 13.9c |  |
| SP38 | 7.1c | 67.4a,b | 6.7f 18.7d 14.0c |  |
| SP46 | 5.0d,e | 44.0d | 5.6 45.4a 9.4d |  |
| SP47 | 7.2c | 54.1c | 24.2a 14.5 18.1a |  |
| SP48 | 10.6b | 53.8c | 8.9e 26.6c 12.7c |  |
| LB572 | 4.3 | 65.5b | 10.7e 19.5d 15.5b |  |
| CCMP2555 | 6.8c | 71.6a | 15.7c 5.9e 17.9a |  |
| CCMP2329 | 6.3c | 61.7b | 14.6d 17.5d 15.7b |  |

M: moisture, VM:volatile matter, FC:fixed carbon, HHV: high heating value.

Means with the same superscript letters in a column are not significantly different (Tukey’s HSD test, p > 0.05).

**3.4 Wastewater Chemical Composition after Algae Growth**

The ultimate goal of the algal treatment of PWW is to clean it up to a level that can be used for irrigation and/or in industrial applications while producing biomass that can be converted to bioproducts. Hence, in this study irrigation water quality parameters were used to evaluate the effect of algae growth on WW quality (Table 2). There was no detectable amount of nitrate and phosphate left in the medium after growing algae in PWW. Their complete removal is due to the algal cell uptake of this compound. A large portion of N in the form ammonium was also taken up by algae. SP38 was the highest consumer of ammonium, 97 %. For most of the cultures, pH of the medium dropped below 8 after algae growth. This can be explained by the CO2 enriched air feed to the culture, that can dissolve in water releasing H+ into the medium. Boron concentration is one of the quality parameters for irrigation water. Many plants are sensitive to high levels of Boron in soil and water (Brown et al., 2002). Boron concentration in PWW decreased significantly, 67 % reduction, after SP38 growth and biomass harvest. A 40 % reduction was achieved after cultivation with SP27, SP28 and CCMP2555. Boron requirement for algae growth does not seem to be general, and it is not well understood ([Fernandez et al., 1984](#_ENREF_12)). The strains examined in this study, SP27, SP28, SP38 and CCMP2555, are all Cyanobacteria. In general, the removal efficiency was specific to the algae strain. Furthermore, the contaminant removal efficiency depends on the amount of biomass present in the culture. Although a 40 % Boron removal was obtained by the strain with the higher biomass concentration (SP28), the best removal efficiency was achieved by the strain SP38, characterized by a lower biomass concentration. Therefore, further research is needed to clarify the pathways for Boron absorption and/or metabolism by these microorganisms.

* 1. Conclusions

The experimental results indicate that microalgae can grow in PWW. Cell growth is limited with the nutrient availability in the WW. The chemical composition of the algal biomass obtained in PWW was strain specific. SP47 exhibited the highest HHV and FC among the strains examined in this study. A significant amount of contaminant was removed from PWW by growing algae and harvesting the biomass. For example, about 60 % TDS, 100 % nitrate and phosphate, over 65 % Boron reduction in PWW could be achieved. This study has demonstrated that PWW can be a potential WW source for algal biomass production. However, further research is needed to demonstrate the technical and economic viability of algae growth in PWW in open pond systems.

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