Title: Algal Remediation of Waste Water Produced during Hydraulic Fracturing

Authors' Names and Affiliations: Nurhan Turgut Dunford, Professor, Oklahoma State University, FAPC Room 103, Stillwater, OK, 74078

Phone: 405-744-7062

E-mail address: Nurhan.Dunford@okstate.edu

Institution, and department: Oklahoma State University, Department of Biosystems and Agricultural Engineering

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Students: (Include number of students supported by the project during the project period in the table below.)

Student Status	Number	Disciplines
Undergraduate		
M.S.		
Ph.D.		
Post Doc	1	Biology
Total		

Principal Investigators: Nurhan Turgut Dunford, Professor, Oklahoma State University, Department of Biosystems and Agricultural Engineering

Publications: (Two manuscripts are in preparation)

1) Nan Zhou; Nurhan Turgut Dunford. ***In Press***. Characteristics of Green Microalgae and Cyanobacteria Isolated from Great Salt Plains. Trans. ASABE.
2) Nurhan Turgut Dunford. Lipid Profile of Oklahoma Native Microalgae Strains and Chemical Composition of the Bio-oil Produced by Pyrolysis of the Algal Biomass. 2017 American Oil Chemists' Society Annual Meeting and Industry Showcases, April 30–May 3, 2017, Rosen Shingle Creek, Orlando, Florida, USA.
3) Nurhan Turgut Dunford; Giovanni Lutzu. Algal Wastewater Remediation. 37th Annual Oklahoma Governor's Water Conference and Research Symposium, Norman, OK. October 22-12, 2016.

4) Nurhan Turgut Dunford. Algal Treatment of Wastewater Generated during Animal and Natural Gas Production. 2. Alg Technology Symposium, Seferihisar, Izmir, Turkey, May 24-27, 2016.
5) Giovanni Antonio Lutzu; Nurhan Turgut Dunford. Growing Oklahoma Native Microalgae Strains in Waste Water Generated during Hydraulic Fracturing for Natural Gas Production. Oklahoma Clean Lakes and Watersheds Conference, Stillwater, OK, March 29-30, 2016.

Problem and Research Objectives:

Problem statement: Oklahoma is one of the largest natural gas and oil producing states in the country. The oil and gas industry utilizes fracking technology widely and generate large volumes of wastewater (frac water). Frac water contains high concentration of inorganic salts and other organic and inorganic pollutant. The current wastewater disposal methods are costly and adversely affect underground water sources. Development of new technologies for frac water remediation and reuse is critical for the long-term sustainability of this industry and most importantly for protection of the environment, safety of the citizens and conservation of diminishing water resources.

Microalgae are ubiquitous photosynthetic microorganisms that are found both in marine and freshwater environments. They have a great potential to produce not only biomass as feedstock for renewable fuels, high-value products, food, and feed applications but also to provide a viable solution to the problem of environmental pollution. Microalgae can grow in wastewater and absorb contaminants, hence, produce biomass while cleaning of wastewater.

Objectives: The main objective of this project was to answer the following question: Can microalgae cultivation be used to treat wastewater produced during hydraulic fracturing (frac water)? The ultimate goal is to use frac water for algal biomass production while removing contaminants. The target is to clean up the water to a level that it can be re-used for irrigation or in industrial operations. The specific objectives of the proposal are as follows: 1) determine the types and concentrations of the contaminants and micro and macro nutrients present in water collected from fracking facilities operating in different regions of OK; 2) identify Oklahoma native algae strains that are capable of growing in frac water; 3) examine the contaminant removal in monocultures of microalgae; 4) examine the chemical composition of the algal biomass produced in frac water.

Methodology:

Objective 1: Both flow back and produced water samples were obtained from fracking sites operating in OK (see Table 1).

TABLE 1: Types and location of wastewater samples examined in the study.

SITE	COUNTY	WASTE TYPE
ElReno	Canadian	Flow back
Cumberland	Marshall	produced
Okarche – JR Burton	Kingfisher	produced
Okarche – Alig	Kingfisher	produced
Okarche – Santana	Kingfisher	produced
Okarche - Carol	Kingfisher	Flow back
Okarche – Judy	Kingfisher	Flow back
Okarche - Dorothy	Kingfisher	Flow back
Okarche – Judy	Kingfisher	produced

Good Laboratory Practices (GLP) for safe handling, storage and disposal practices were followed and students and employees working on the project were trained on GLP. Standard Operating Procedures (SOP) were prepared and followed throughout the project execution. The water samples were analyzed for their chemical composition using standard/official analytical protocols. The samples were analyzed for conductivity, TDS (total dissolve solids), pH, iron, aluminum, copper, boron and other heavy metals, COD (chemical oxygen demand), BOD (Biological oxygen demand).

Objective 2) The following microalgae strains were examined in this project: SP19, SP20, *Nannochloropsis oculata*, *Botryococcus brunii*, *Duneliella Tertiolacta*, *Picochlorum oklahomensis*, SP38, SP44, SP46, SP47, SP48, SP50, SP1, SP11, SP22, SP23, SP27, SP28, SP29, SP30, SP31, SP33, SP25. The screening study was carried out in 1 L sterile flasks placed in an environment controlled growth chamber which were maintained at 20^{o}C, 85 µmol/m^{2}/sec illumination, 12 h light-12 h dark period, 20 mL/min gas bubbling with 5% CO_2-95 % air (v/v) mixture. The growth performance of the strains were monitored through the analysis of biomass concentration in the medium and optical density measurement at 780 nm. Algae growth tests were carried out in microfiltred and autoclaved wastewater to maintain monocultures.

Objective 3) The most productive algae strain identified in objective 2 were cultivated in flow back and produced water samples. The produced biomass was separated from the growth medium (wastewater) by centrifugation after cells reached stationary growth phase. Supernatant (residual waste water after biomass removal) was analyzed for the compounds/parameters listed in objective 1. Contaminant removal efficiency was calculated for each compound/parameter as follows: Contaminant removal efficiency = [(Concentration of the contaminant in wastewater prior to algae cultivation - Concentration of the contaminant in wastewater after algae cultivation and biomass removal)/ Concentration of the contaminant in wastewater prior to algae cultivation]*100.

Objective 4) The biomass produced in objective 2 and 3 were characterized for its chemical composition. Ash, mineral and heavy metal composition and content, volatile matter, fixed carbon, high heating value of the biomass samples were determined by using standard analytical methods and Thermogravimetric Analysis (TGA).

Principal Findings and Significance:

Selected Results

Data in Table 2 clearly demonstrates the effect of culture growth medium and strain type on microalgae growth parameters and biomass production efficiency. In general, maximum biomass concentration obtained in flow back water was lower than (< 1 g dry biomass/L culture medium) that is obtained in standard media. Standard growth media are optimized for cell survival and contain all the essential nutrients for algae growth. The culture banks, UTEX and CCMP, from which the algae strains examined in this study were purchased, use standard media to maintain their culture collection.

A few strains performed similar in flow back water and standard medium, i.e. SP22. Microalgae utilizes nitrogen and phosphorous as nutrients for growth. Frac water is poor in nutrients (Table 3). To test our hypothesis that ***low biomass concentration in frac water is due to the limited nutrient availability to the cells***, a series of experiments were carried out using growth media enriched in nutrients. The experimental results support our hypothesis. Biomass concentrations in nutrient supplemented growth medium were 6.6 (SP50) and 21 (UTEX 2164) times higher than those obtained in frac water without supplementation (Table 2).

Biomass produced by *diatoms*, SP1 and CCMP2525, grown in flow back water had significantly ($p < 0.05$) higher ash content than that of filamentous, SP31, and unicellular, SP22, green microalgae (Table 4). As expected, strains with high ash content had low HHV (Higher Heating Value). The highest HHV, 21 MJ/kg, was measured in biomass produced by SP31. Biomass from the latter strain also had the highest VM (volatile matter), 79.9%, and lowest fixed carbon, 1.7%. Low ash, high VM and HHV are desirable in biomass to be used as feedstock for bioproduct manufacturing.

This study also examined chemical composition of the frac water samples collected from several wells operating in Oklahoma (Table 1). Most of the produced water samples had a dark oil layer (2% of the total sample weight) which was removed prior to microalgae growth experiments (Picture 1). Significant differences were observed in chemical composition of the samples. Table 3 shows examples of the water quality test results for flow back and produced water. Alkalinity and pH of the flow back water were slightly higher than those of the produced water, 1712 mg/L and 9 and 839 mg/L and 8.5, respectively. Boron, total dissolved solids (TDS) and chlorine contents of the produced water (114, 25000 and 13492, respectively) were significantly higher than those for the flow back water (30, 16,000 and 7065 mg/L, respectively).

Algae growth in frac water and subsequent biomass removal resulted in a significant decrease in the concentrations of many of the wastewater contaminants (Table 5). The drop in pH of the wastewater during algae growth is partly due to the CO_2 bubbling through the growth medium and carbonic acid formation. Nitrogen present in the wastewater samples was taken up and used as nutrient by algae cells resulting in 100% removal. About 65-70% reduction in TDS, over 90% reduction in alkalinity and boron content and 60-70% reduction in chlorine and sodium contents in wastewater were also achieved.

One of the long term goals of this project is to clean up frac water to a level that can be used for irrigation. Hence, our finding that some microalgae strains remove boron very efficiently, is particularly interesting. Boron is used with calcium in plant cell wall synthesis and is essential for cell division. Boron requirements are much higher for reproductive growth. However, the range between an optimum and a toxic application rate is very narrow. Boron levels above 0.5 ppm are considered high for plant growth. Most of the frac water samples tested in this study contains much higher boron concentrations, 30-150 ppm, than needed by plants. Hence, boron removal is critical for potential use of frac water for irrigation.

Although it is not part of this project, one of the PI's PhD students has been able to convert algal biomass produced in wastewater to bio-oil, bio-char and gas which can be further processed to obtain higher value products to be used in industrial applications.

Significance of the findings and conclusions

- This study demonstrated that frac water can be used for microalgae growth.
- Several Oklahoma native microalgae strains (i.e. CCMP 2329, SP28, SP33, SP46 etc.) were identified as high biomass producers in frac water.
- It appears that diatoms accumulate high amount of salt in their cells, consequently, lowering the energy content and value of the biomass for bioproduct development.
- Significant differences were found in chemical composition of flow back and produced water samples collected from wells operating in different regions of Oklahoma.
- Frac water samples had very high total dissolved solids, alkalinity and chlorine contents.
- Although concentrations of nitrogen and phosphorous which are nutrients needed for algae cell growth were low in frac water, several strains performed similar or better in frac water as compared to standard growth media optimized for cell growth. *This could be due to the utilization of hydrocarbons present in frac water by some algae strains. However, this hypothesis needs to be further examined and supported by experimental data.*
- About 60-100% reduction in the concentration of sodium, TDS, alkalinity, chlorine, nitrogen, iron, copper and boron in frac water could be achieved after microalgae growth and biomass removal, supporting the potential of algal remediation of frac water.

- Further research on optimization of the algae growth conditions to maximize biomass productivity and contaminant removal is needed for enhancing technical and economic feasibility of this technology for large scale wastewater remediation.
- A better understanding of kinetics and mechanism of algal contaminant removal from frac water is critical for further exploration of the potential commercial applications of this technology.

Table 2: Effect of growth media on the growth characteristics of selected microalgae strains *.

Species	**SP1[1]**	**SP19[1]**	**SP22[1]**	**SP22[2]**	**SP31[1]**	**SP50[1]**	**SP50[2]**	**SP50[3]**	**2164[1]**	**2164[2]**	**2164[3]**	**2605[1]**	**2525[1]**
μ (day^{-1})	0.11	0.03	0.08	0.48	0.17	0.05	0.38	0.13	0.10	0.3	0.13	0.12	0.44
t_d (day)	6.1	29.3	8.5	1.44	4.8	12.6	1.84	5.2	7.0	2.2	5.3	8.1	3.5
X_{max} (g L^{-1})	0.37	0.43	0.39	0.38	0.67	0.33	0.72	2.2	0.30	1.2	6.3	0.29	0.48
ΔX (mg L^{-1} day^{-1})	12.9	45.0	11.0	25.3	54.0	11.0	51.2	123	26.5	80	424	36.0	43.3

*μ: specific growth rate, t_d: doubling time, X_{max}: maximum biomass concentration, ΔX: average biomass productivity.

Means with the same superscripts in a row are not significantly different (Tukey's HSD test, $P > 0.05$).

[1]Grown in flowback water

[2]Grown in UTEX/CCMP recommended media

[3]Grown in nutrient supplemented media

TABLE 3: Physical-chemical composition of flow back (FB-from El Reno, OK) and produced water (PW-from Cumberland, OK)

	FB	**PW**
Cations (mg L^{-1})		
Na	5111	8596
Ca	8	101
Mg	50	37
K	48	179
Anions (mg L^{-1})		
NO_3-N	39	0.2
Cl^-	7065	13492
SO_4^{2-}	21	18
B	30	114
HCO_3^-	1396	868
CO_3^{2-}	341	77
Trace elements (mg L^{-1})		
Zn	0.06	< DL
Cu	0.03	< DL
Mn	< DL	< DL
Fe	0.17	< DL
NH_4^+	NA	86
ICAP_P	NA	0.01
Derived values		
TDS (mg L^{-1})	16104	25014
SAR (%)	149	186
PAR (%)	0.8	2.3
RC (meq L^{-1})	30	9
SP (%)	98	98
HD (mg L^{-1})	224	403
ALK (mg L^{-1} as $CaCO_3$)	1712	839
pH	9	9
EC (µmhos cm^{-1})	24400	37900
COD (mg O_2 L^{-1})	1874	1764

Note: TDS = Total Dissolved Solids, SAR = Sodium Adsorption Ratio, PAR = Potassium Adsorption Ratio, RC = Residual Carbonates, SP = Sodium Percentage, HD = Hardness, ALK = Alkalinity, ICAP_P = Phosphorous by Inductively Coupled Argon Plasma, < DL = under detection limit, NA = not available

TABLE 4: Chemical composition of biomass produced water by selected microalgae strains grown in flowback water [determined by thermogravimetric analysis (TGA)]*.

STRAINS	***M***	***VM***	***FC***	***ASH***	***HHV***
SP1	7.8 ± 0.9	53.0 ± 0.7	3.7 ± 2.0	35.4 ± 3.6	10.1
CCMP2525	4.5 ± 1.6	39.1 ± 1.9	17 .0 ± 0.2	39.4 ± 0.1	12.4
SP31	6.9 ± 0.0	79.9 ± 0.7	1.7 ± 1.5	11.8 ± 0.8	21.0
SP22	10.9 ± 0.7	70.6 ± 4.4	6 .0 ± 6.2	12.5 ± 1.0	14.6

*M: Moisture (%), VM: Volatile matter (%), FC: Fixed carbon (%), Ash (%) and HHV: Higher heating value (MJ kg^{-1})

TABLE 5: Comparison of two algae strains for their contaminant removal efficiency. Data were collected from tests carried out with produced water. Components with no data indicate no reduction or increase (due to water evaporation during algae growth) in concentration.

	SP31	**UTEX 2525**
	(% reduction)	
Cations (ppm)		
Sodium	68	72
Calcium	-	-
Magnesium	-	46
Potassium	69	83
Anions (ppm)		
Nitrate-N	100	100
Chloride	65	69
Sulfate	-	-
Boron	97	98
Bicarbonate	88	88
pH	16	12
EC (µmhos/cm)	65	68
Trace elements (ppm)		
Zinc	-	-
Copper	33	100
Iron	100	100
Derived values		
TDS (ppm)	65	71
SAR (%)	72	69
PAR (%)	75	75
Sodium percentage (%)	6	2
Hardness (ppm)	-	21
Alkalinity (ppm as $CaCO_3$)	92	92
COD (mg O_2 L^{-1})	26	-

PICTURE 1: Oil layer separated from produced water samples.