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Cultivation of hydrocarbon-tolerant microalgae in flowback wastewaters produced during hydrofracking of impermeable rocks

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Contributions: GAL: conceptualization, methodology, investigation, data care, writing- original draft preparation, writing- reviewing and editing, supervision. AC: data care, visualization, validation, writing- reviewing and editing. NTD: conceptualization, writing- reviewing and editing, supervision.

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Abstract

Huge amounts of Wastewaters (WWs) are produced yearly by the hydrofracking of impermeable rock formations for the extraction of oil or natural gas. Flowback Wastewaters (FWs) are characterized by high contents of inorganic contaminants and hydrocarbons thus representing a relevant threat for the environment. In this work three hydrocarbon-tolerant microalgae have been cultivated in flowback water generated during hydraulic fracturing to investigate their growth kinetics. All three strains could grow in FWs irrespective of the presence of oil hydrocarbons. Biomass productivity varied significantly among the strains. *Ochromonas danica* achieved a specific growth rate equal to 0.386 day^{-1} during the exponential phase and a maximum biomass productivity equal to 39 mg L^{-1} day^{-1} after 11 days of batch cultivation. *Scenedesmus dimorphus* was capable to grow in the FWs by achieving a biomass concentration

equal to 0.5 g L^{-1} after about 25 days of cultivation. On the contrary, *Prototheca zopfii* was strongly affected by the contaminants of FWs. Ultimately, this study demonstrated that specific strains of microalgae could thrive in FWs and thus represent suitable candidates to future research activity aimed to verify the possibility to bio-remediate these harmful WWs.

Introduction

Oil and gas industry frequently uses hydraulic fracturing technology to enhance oil and/or gas recovery from underground reservoirs. The collection of gases and/or oils is obtained by the injection of Fracking Fluids (FF) into geologic formations.[1] These FF contain mainly sand and several chemicals used to control pH, microbial growth, gelling, and corrosion.[2] This process generates Wastewaters (WW) of great concern due to their potential adverse impact on ecosystems at ground and surface water, and soil level. Flowback Wastewater (FW), made of hydraulic FF and formation brines, represents 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.[3]

Its composition in terms of various inorganic and organic compounds is relative to the geological formation fractured. In particular, the presence of hydrocarbons and the extremely high Total Dissolved Solids (TDS) content in FW makes the handling and treatment of this stream very challenging.[4]

The impact that FWs can have on the environment and public health depends on multiple variables such as number and types of wells, local geology, hydrology, proximity to freshwater

sources, existence of water treatment facilities, and availability and location of deep-disposal wells. Handling methods for the final disposal of fracking waters are limited to deep well injection, and reuse and/or recycling for agricultural and industrial purposes including reuse in fracturing operations after treatment.[5] The choice of the suited treatment technology is often limited by the high cost associated to the management options available to the fracking industry.

Extremophilic microalgae are capable to thrive and survive in harsh media such as highly contaminated WWs.[6–8] Along these lines, biological treatment of FWs using algae has been explored during the last decade.[9–11] Thermogravimetrical analysis on the algal biomass and the amount of lipids accumulated in the cells reveal that the biomass obtained after microalgae growth in FFs can be potentially exploited as feedstock in the energy sector for the production of biodiesel or for other commercial applications (*i.e.* bioplastics, biofertilizers).[12]

Recently, effect of algae growth in FW on residual water quality has been reported.[13] The latter study highlighted the possibility of some microalgae strains to thrive in FW and their ability to remove some of the inorganic contaminants. In particular, algae were able to reduce TDS by 65%, nitrate by 100% and boron by 95%. For a better understanding of FWs as potential algae growth media and WW remediation it is fundamental to investigate the chemical composition of these WWs as well as the optimal strain selection.

Hence, considering the harsh chemical composition typical of this type of water stream three microalgae strains were cultivated in a FW. The main goal of this study was to fill the scientific knowledge gap in the field by investigating the potential of these strains to use FW as growth medium and producing biomass.

Materials and Methods

Inoculum, culture medium and wastewater preparation

The algae strains examined in this study, *Scenedesmus dimorphus* (UTEX 1237), *Prototheca zopfii* (UTEX 1438), and *Ochromonas danica* (UTEX 1298) were obtained from the culture collection of the University of Texas at Austin, USA.[14] From UTEX official website more details on the composition of the culture maintenance media can be found. The strains were maintained in 50 mL glass tubes at room temperature and were illuminated during the 12h light period/day by two 32 W white fluorescent tubes providing a Photosynthetic Photon Flux Density (PPFD) of 40 μmol m^{-2} s^{-1}.

FW samples were collected from oil-producing wells operating in Okarche, OK, USA. The samples were stored at room temperature in plastic buckets prior to the experiments no more than 3 months. Subsequently, they were subjected to the pre-treatment procedure shown in Figure 1 in order to remove suspended oil, Volatile Organic Compounds (VOCs), ensure axenic conditions and reduce dark coloring. The WW samples were first treated with an oil skimmer (Mighty Mini SS2, Abanaki Corporation, OH, USA) for 3h to separate the oil layer at the surface of the liquid from the water and placed in a separatory funnel overnight to obtain a full separation of the two phases. Then the lower phase was collected and boiled at 60°C for 1 h while stirring continuously. Finally, the water sample was filtered through a filter paper disk (#1, Whatman, UK), and subsequently sterilized for 20 min at 121°C and 0.1 MPa with an autoclave (Hirayama, HVE-50, Ramsey, MN, USA) before microalgae cultivation.

Algae cultivation

As cultivation systems, 2 L glass reactors (indicated as PBRs) were used, which were placed inside a closed chamber under controlled conditions, as described in our previous works.[9,10] Briefly, the PBRs were sealed with a GL45 3-port cap equipped with tube adapters (CPLabSafety, Novato, CA, USA). The gas (air and CO_2 mixture) was bubbled through a sterile syringe filter (Argos Technologies, Elgin, IL, USA). A polypropylene check valve (VWR Science, Bristol, CT, USA) allowed air leaving the PBR and prevented the reverse flow. The PBRs were kept in a closed growth chamber maintained at 23 ± 4°C. Twelve 23 W cool white fluorescent bulbs (Osram Sylvania, Wilmington, MA, USA), installed on the ceiling of the growth chamber, were the light source providing a PPFD of 85 ± 4 μmol m^{-2} s^{-1} measured by a quantum meter (model QMSW-SS, Apogee Instruments Inc., Logan, UT, USA). The lights in the growth chamber and the gas flow through the PBRs were controlled based on a 12:12 h cycle. The gas flow rate was 20 mL min^{-1} and the concentration of CO_2 (industrial carbon dioxide, Airgas, Stillwater, OK, USA) in the air (Grade D breathing air, Airgas, Stillwater, OK, USA) was 2% v v^{-1}. 1.2 L and 0.1 g L^{-1}were set as initial working volume of the PBR and cell concentration, respectively. After the cultivation, which lasted one month, the culture biomass was separated by centrifugation (at 9722 g for 10 min) and the liquid phase used for WW analysis.

Characterization of microalgae growth pattern

The Optical Density (OD) at 680 nm was used to monitor microalgae growth as reported in detail elsewhere.[10] Cell concentration based on dry weight X (g_{dw} L^{-1}) was performed according to the procedures reported elsewhere.[15]

The maximum biomass productivity (ΔX) was expressed as:

$$\Delta X_{dw} = \frac{X_{\max} - X_0}{t_{\max} - t_0} \quad (1)$$

where t_0 represents the initial time of the cultivation period. A pH-meter (model AR20, Fisher Scientific, Waltham, MA, USA) was utilized to monitor the culture pH.

Wastewater quality

The standard analytical water quality methods proposed by the American Public Health Association were taken into account to analyse the chemical composition of the WW samples prior microalgae cultivation.[16] Water samples were centrifuged at 9722 g for 10 min and glass microfiber filters (GF/CTM, Whatman, UK) were used to filter them before performing chemical tests.

Quantitative evaluation of algae growth using a logistic model

The following equation, based on the so-called logistic growth model, was used for the evaluation:

$$X = \frac{X_{\max}}{1 + \exp\left[-\mu_{\max}\left(t - t_i\right)\right]} \quad (2)$$

where $X_{max}(\mathrm{g\,L^{-1}})$ represents the maximum algae concentration achieved during the growth, $\mu_{max}\,(\mathrm{day^{-1}})$ is the maximum specific growth rate while $t_i\,(\mathrm{day})$ is the so-called time of inflection, i.e the time when the instantaneous growth rate starts to decrease. Logistic models have been widely applied in the literature to capture the growth behaviour of microalgae strains. [17,18] Model parameters were evaluated by tuning their values to obtain the best agreement between model simulations and experimental data. It should be noted that the fitted value of X_{max} could be different from the actual maximum value of biomass concentration observed experimentally.

Data analysis

For the sake of reproducibility experiments and analytical tests were carried out at least in duplicate, typically in triplicate; SAS 9.3 (SAS Institute Inc., Cary, NC, USA) was used to perform the statistical analyses of the data. Microsoft Office Excel program (Excel 2016 Ink, Microsoft, USA) was adopted to calculate the regression equations correlating dry biomass concentration to OD, and to μ. All the data are reported as mean of values.

Results

FW sample was collected from a well producing oil at the time of sampling. In general, order of the relative abundance of the ions in FW were as follows: Na^+, HCO_3^-, Cl^-, SO_4^{2-}, Br^-, K^+, Ca^{2+}, and Mg^+ (Table 1). Raw WW was subjected to a pre-treatment procedure shown in Figure 1 to remove oil, VOCs, ensure axenic conditions and reduce dark colouring.

Figure 2 shows the time evolution of microalgae concentration grown in the pre-treated WW, it can be observed that the three strains grown in FW (Figure 2) showed low values of biomass concentration ($X_{max} < 0.5$ g L^{-1}). The experimental data were captured quite well by the logistic model when using the parameter values reported in Table 2.

It can be seen that the strain UTEX 1237 had not yet achieved the steady state when the experiment was interrupted thus showing a capability to further grow at the end of cultivation. The growth of this strain, characterized by a rate equal to 0.095 day^{-1} (Table 2), was well simulated by the model since the 95% confidence bands were very narrow and the adjusted r-squared equal to 0.989. The strain UTEX 1298 showed the best initial growth rate, *i.e.* 0.386 day^{1} (Table 2), but the stationary phase was quickly achieved after 10 days of cultivation probably due to the complete consumption of the controlling substrate.

Finally, the strain UTEX 1438 was characterized by the worst growth rate in the group (0.087 day^{-1}) as well as by the worst simulation by the logistic model. It can be observed that the strain UTEX 1298 was capable of growing with a rate much higher than those of the other two strains. The same strain achieved also the maximum biomass concentration turning out to be definitively the most suited to be cultivated in pre-treated fracking waters. UTEX 1298 was the strain characterized by both the highest biomass productivity and maximum biomass productivity, which were more than doubled compared to UTEX 1237 and UTEX 1438 (Figure 3).

Discussion

Some of the ions reported in Table 1 are metabolized by microalgae cells to sustain their growth. On the other hand, when many of them exceed certain values, the FW became toxic for most of the aquatic living forms.[19] In particular, the presence in FW of free oil, dissolved solids, suspended solids, metals, organics and VOCs may prevent the growth of the cells.

Oil removal is thus a crucial operation when aiming to use the WW for growing microalgae since in open ponds it could form a biofilm, which hinders the diffusion of atmospheric CO_2 within the culture thus inhibiting photosynthesis. Moreover, organic compounds could attract heterotrophic bacteria that compete with algae or use it as organic substrate. The aim of the filtration procedure (Figure 1) was to remove suspended solids, which increase liquid turbidity and limit light penetration within the culture thus potentially avoiding photosynthesis to take place. It is worth noting that among these solids not only inorganic particles could be present but even bacteria that would compete with algae.

Finally, in the boiling section (at 60 °C) the VOCs are removed to limit the possibility of heterotrophic bacteria to grow. No significant amounts of VOCs were separated in the boiling section. Thus, the filtration section is crucial when using WWs while its presence in the eventual pre-treatment plant of FW should be evaluated case by case. As final step, the autoclaving section ensured axenic culture media. The pre-treatment procedure was effective in reducing dark colour of the medium probably due to VOCs evaporation. The latter is a further outcome of the proposed pre-treatment procedure since dark colouring increases OD of the medium hindering the effective light diffusion within the culture and impeding the photosynthesis. On the other hand, intensive FW treatment implies commercial desalination technologies based on

membrane separation. To avoid membrane fouling some steps such as coagulation, softening, adsorption and filtration are required as pre-treatment. Unfortunately, this approach exacerbates the costs of purification system and increases waste creation.[20] It should be remarked that the proposed pre-treatment is necessary if the purpose is to recycle fracking WWs as a growth medium.

In a previous study carried out under similar growth conditions used in this study all the thirteen strains examined produced X_{max} between 0.2 g L^{-1} and 1 g L^{-1}[19]. It should be pointed out that only two of the thirteen strains analyzed were green algae while the others were cyanobacteria. In our study, UTEX 1237, 1438 and 1298 attained a X_{max} of 0.5 g L^{-1}, 0.5 g L^{-1} and 0.42 g L^{-1}, respectively, which were similar to the values reported for the two green algae *Tetraselmis striata* (0.4 g L^{-1}) and *Picochlorum oklahomenis* (0.8 g L^{-1}).

The negative value of the inflection time computed for the strain UTEX 1438 (Table 2) has the physical meaning that the time derivative of biomass concentration started to decrease already from the first instants of cultivation. As already mentioned, the values of X_{max} computed by the model were slightly different from those experimentally measured and provide an inference whose validity should be further evaluated through specific experiments.

Conclusions

Three microalgae strains were able to grown in a harsh WW, such as FW, generated during a fracking process. The concentration of contaminants in original WW was decreased by a four-step pre-treatment to the point that microalgae can survive and grow in WW. Relatively low

biomass productivities were mainly due to the low availability of macronutrients. *Scenedesmus dimorphus* resulted the best performing strain.The experimental data were captured quite well by the proposed logistic model when using the parameter values This study generated the basic experimental data needed for optimization of an integrated process involving WW pre-treatment and subsequent algae growth, technical and economic evaluation and process scale up. The biomass obtained after the growth of these strains in FW can be potentially exploited as feedstock for biodiesel production or for other commercial applications.

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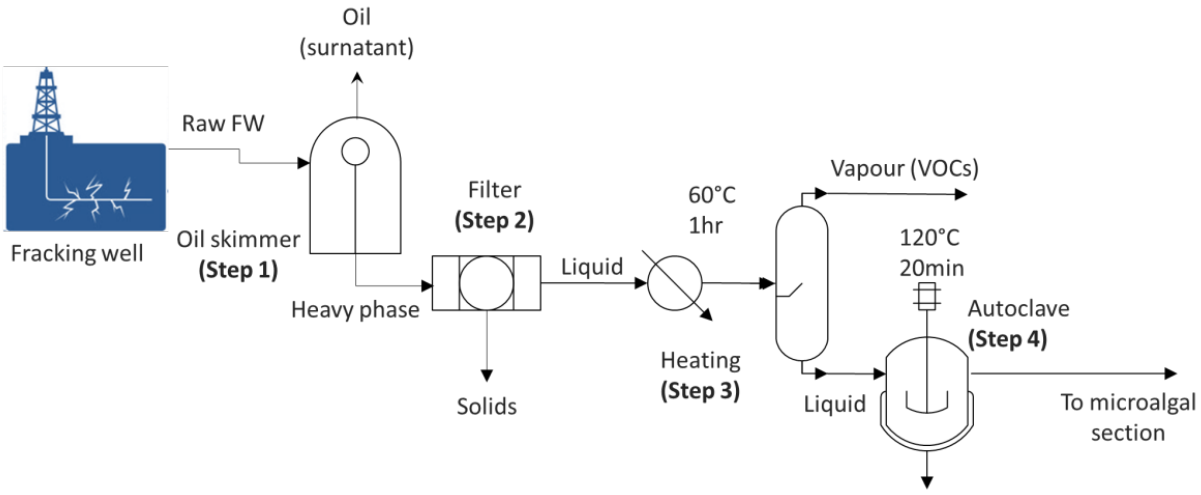

Figure 1. Scheme of the fracking wastewater pre-treatment.

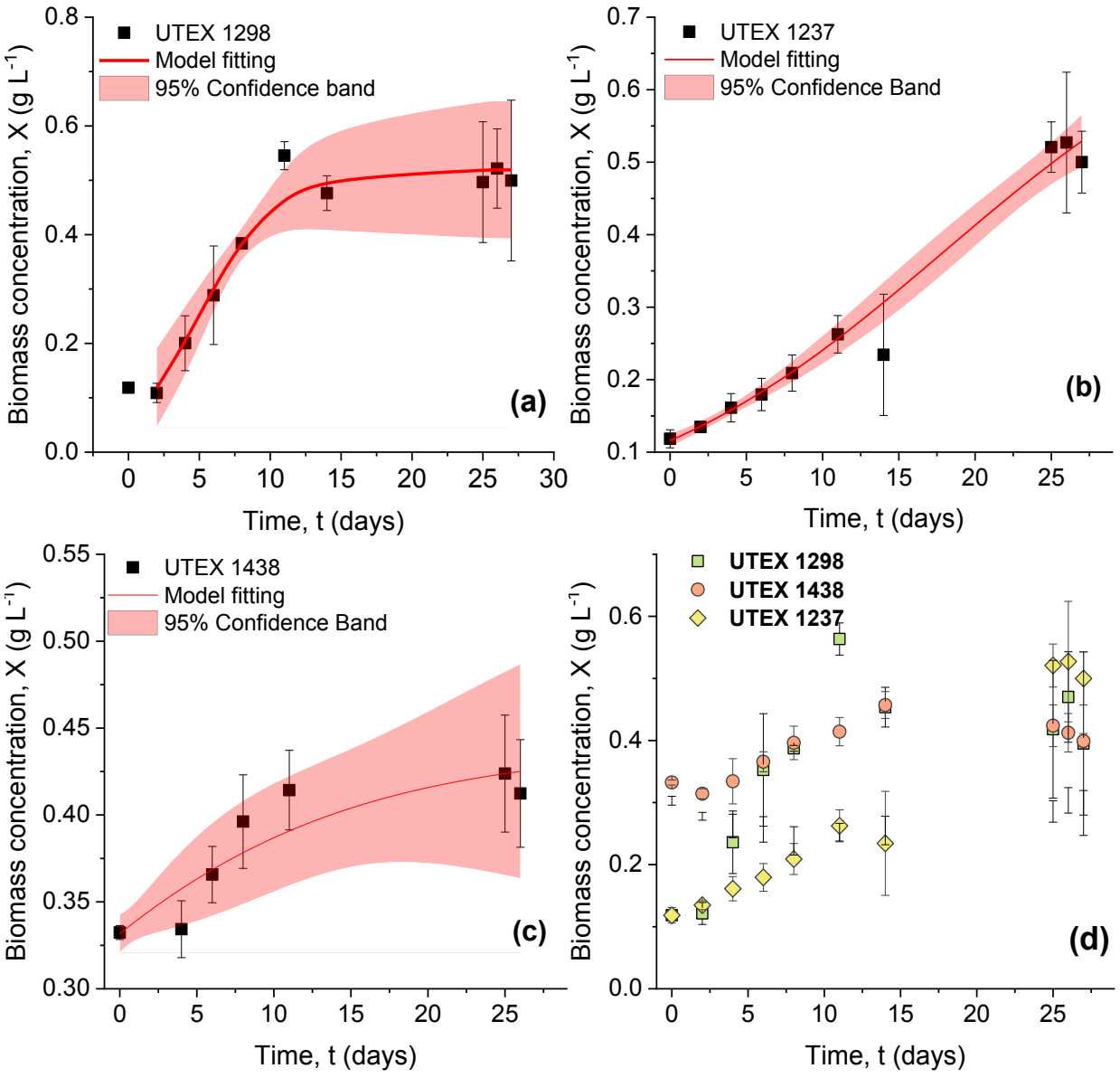

Figure 2. Experimental and simulated evolution of biomass concentrations of the strains UTEX 1298 *Ochromonas danica* (a), UTEX 1237 *Scenedesmus dimorphus* (b) and UTEX 1438 *Prototecha zopfii* (c). Comparison of all experimental data (d).

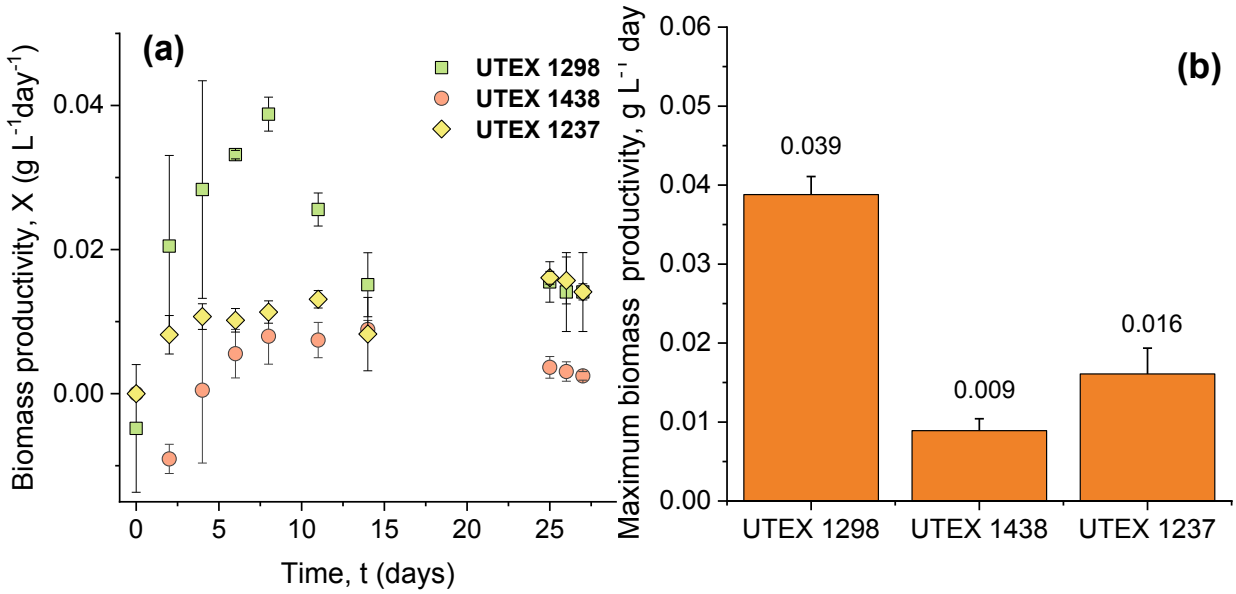

Figure 3. Time evolution (a) and maximum value (b) of biomass productivities of the three investigated strains

Table 1. Chemical composition of flowback water used after the different steps of pre-treatment.

Parameter	FW_1	FW_2	FW_3	FW_4
Sodium	2242	2229	2238	2358
Calcium	49.1	49.8	49.7	50
Magnesium	5.4	5.4	5.5	5.6
Potassium	53	53	53	56
Nitrate-N	0.2	0.1	0.1	0.1
Chloride	1405	1191	1247	758
Sulfate	1012.1	998.2	1002.9	1064.6
Boron	63.7	63.1	63.1	67.1
Bicarbonate	1577	1534	1580	1506
Carbonate	nd	27.5	nd	70.2
pH	7.4	8.5	7.8	8.7
EC (µmhos cm^{-1})	9340	9310	9490	9830
Zinc	0.08	0.08	0.08	0.08
Copper	0.06	0.06	0.06	0.21
Manganese	nd	nd	nd	nd
Iron	4.54	4.16	4.84	5.25
Ammonium	0.3	0.3	0.7	1.3
ICAP_P	0.28	0.22	0.27	0.62
TDS (ppm)	6344	6144	6263	6487
SAR (%)	81.1	80.1	80.4	84.3
PAR (%)	1.1	1.1	1.1	1.2
Residual carbonates (meq L^{-1})	23	23.1	23	24.1
Sodium percentage (%)	97.1	97.1	97.1	97.2
Hardness (ppm)	144.8	146.5	146.6	148

Alkalinity (ppm as CaCO3)	1292	1303	1295	1351

Notes: FW_1 = flowback after oil skimming, FW_2 = FW_1 + filtration, FW_3 = FW_2 + boiling, FW_4 = FW_3 + autoclave, ICAP_P: Total P determined by Inductively Coupled Argon Plasma ion chromatography method, TDS = Total Dissolved Solids, n.d.: Not detected. All the values are expressed in terms of mg L^{-1}. All data were obtained as means from at least two experiments.

Table 2. Relevant model parameter values found by fitting the experimental through the proposed model.

	UTEX 1237	**UTEX 1438**	**UTEX 1298**
X_{max} (g L^{-1})	0.753 ± 0.112	0.439 ± 0.045	0.519 ± 0.029
t_i (days)	17.957 ± 3.674	-12.891 ± 4.615	5.163 ± 0.435
μ_{max} (day^{-1})	0.095 ± 0.011	0.087 ± 0.056	0.386 ± 0.044
R^2_{adj}	0.989	0.828	0.9126