Algal Research 11 (2015) 165–168

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Biomass and phycocyanin production from cyanobacteria dominated biofilm reactors cultured using oilfield and natural gas extraction produced water

Jonathan L. Wood [a,*], Charles D. Miller [a], Ronald C. Sims [a], Jon Y. Takemoto [b]

[a] Utah State University, Department of Biological Engineering, 4105 Old main Hill, Logan, UT 84322-4015, United States
[b] Utah State University, Department of Biology, 5305 Old Main Hill, Logan, UT 84322-4105, United States

ARTICLE INFO

*Article history:*
Received 25 February 2015
Received in revised form 23 May 2015
Accepted 23 June 2015
Available online xxxx

*Keywords:*
Cyanobacteria
Oil and gas extraction wastewater
Produced water
Phycocyanin
Rotating Algal Biofilm Reactor

ABSTRACT

The production of cyanobacterial biofilm biomass and phycocyanin from Rotating Algal Biofilm Reactors utilizing undiluted produced water from oil and natural gas extraction as a medium was demonstrated in this study. Oil and natural gas extraction produced water is the largest waste stream generated by these industries and may provide an abundant source of non potable water for the culture of cyanobacteria and phycocyanin. In the present study, a unialgal cyanobacteria isolate from the Logan City, Utah Wastewater Treatment Facility was shown to exhibit an areal ash free dry weight biomass productivity of 4.8 ± 0.7 g/m$^2$-day when cultured in produced water medium. The cyanobacterial biofilms yielded an areal phycocyanin productivity of 84.6 ± 9.3 mg/m$^2$-day with a maximum crude extract purity of 0.23 ± 0.03. The utilization of produced water for the production of cyanobacterial biofilm biomass and associated high value products could provide significant economic and bioremedial advantages to current produced water disposal technologies.



## 1. Introduction

Produced water is the largest waste stream generated by the hydrocarbon recovery industry [1]. Largely unsuitable for discharge, this wastewater is generally recycled or reinjected into disposal wells. Often, however, produced water is disposed of in large ponds for holding/evaporation. The large volumes of produced water held in open ponds represent a waste that is expensive to transport and dispose. Utilization of this produced water as an algal growth medium has the potential to remove chemicals from the produced water, generate useful algae biomass and other valuable products, and minimize the large volumes of freshwater resources required for algae culture.

The Rotating Algal Biofilm Reactor (RABR) is a novel algal biofilm reactor platform that utilizes a semi-submerged rotating drum with attached growth substrata and an integrated harvesting apparatus [2,3]. Algal growth in occluded waters is possible as the RABR rotates in and out of the water exposing the biofilm culture to light, nutrient, and gas exchange. The resulting algal biofilms may be harvested and dewatered with reduced operation costs when compared with traditional suspended culture [4]. Utilization of the RABR for the growth of algal biofilms may address the need for the economic treatment of produced water using immobilized biological films [1]. Cyanobacteria dominated biofilms have been shown to tolerate heavy oil pollution and degrade petroleum components [5,6]. Many Oscillatoriales in particular have been implicated in facilitating petroleum degradation directly and indirectly through oil droplet emulsification and the creation of oxic/anoxic zones within a biofilm [7]. Additionally, the resulting algal biofilms may be used to generate a variety of useful bioproducts including biofuel feedstock, high value chemicals, pharmaceutically active compounds, animal feed, and bioplastics [4,8].

Phycocyanin is a major blue phycobiliprotein pigment found in cyanobacteria that has many potential applications in cosmetics, foods, medicine, and biotechnology [9–11]. Widely used as a label for immunoassays and fluorescence diagnostics, phycocyanin contains covalently bound phycocyanobilin chromophores that have highly specific and intense fluorescent properties [12]. Production and accumulation of phycocyanin by cyanobacteria varies greatly and is regulated by many environmental factors including light intensity, temperature, and nutrient availability [13–16]. The degree of phycocyanin purity is dependent on cellular yields, lysis methods, pH of extraction solvents, the use of cold temperatures and low light to reduce degradation, and the co-extraction of contaminants [17–19]. Production of high value phycocyanin is currently dominated by the outdoor culture of *Arthrospira platensis* in open ponds and raceways [9]. This style of cyanobacteria culture generally requires large volumes of prepared growth medium, and expensive harvesting and drying operations. The RABR growth platform coupled with utilizing produced water as a growth medium may reduce the costs of phycocyanin production. The

* Corresponding author at: Department of Biological Engineering, Utah State University, 4105 Old Main Hill, Logan, UT 84322-4105, United States.
*E-mail address:* jlwood@mail.bradley.edu (J.L. Wood).

http://dx.doi.org/10.1016/j.algal.2015.06.015
2211-9264/

aims of this study were to determine the growth of cyanobacteria dominated biofilms using produced water as a growth medium, and to determine the production of phycocyanin by the resulting biofilms.

## 2. Material and methods

### 2.1. Organism isolation and characterization

Cyanobacteria used in this study were obtained from harvesting the upper layer of a mixed culture algal biofilm from pilot scale RABRs operated at the Logan City, Utah municipal wastewater treatment facility, a 460 acre (1.86 $km^2$) open lagoons system [2]. A unialgal biofilm forming culture was obtained on cotton rope in produced water supplemented with ACS grade 3.0 g/L $NaNO_3$, 0.5 g/L $K_2HPO_4$, and 50 mg/L cycloheximide [20].

To characterize the unialgal isolate, referred to hereafter as Logan Lagoons Cyanobacteria 2 (LLC2), a crude cell lysate was used as template DNA for 23S plastid ribosomal DNA primers previously described by Sherwood and Presting [21]. The PCR mixture contained 5 μL of 10x PCR buffer, 8 μL of 25 mM $MgCl_2$, 2 μL containing 2 mM of each deoxynucleotide triphosphate, 1 μL dimethylsulfoxide, 1 μL each of 50 mM forward and reverse primers (Eurofins Genomics, Huntsville, AL), 0.5 μL *Taq* DNA polymerase (Fermentas, Pittsburgh, PA), and 2 μL DNA template for a 50 μL reaction volume. PCR amplification was performed using the following conditions: 2 min denaturation step at 95 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 54.4 °C, and 1 min at 72 °C followed by a final elongation at 72 °C for 10 min. PCR products were purified after agarose gel electrophoresis using Qiagen QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and sequenced by the Utah State University Center for Integrated Biosystems (Logan, UT). The resulting sequence chromatograms were examined for readout noise using 4Peaks (Netherlands Cancer Institute, Amsterdam, The Netherlands). Forward and reverse 23S sequences were then aligned using BLASTn and then compared against NCBI's nucleotide collection database, http://blast.ncbi.nlm.nih.gov (Table 1).

### 2.2. Growth conditions

Rotating Algal Biofilm Reactors (RABRs) (8.9 cm dia. × 17.8 cm L) and associated 1 L working volume acrylic tanks were constructed and physically operated as described by Christenson and Sims [2]. The bioreactors were constructed with 3/16 in. dia. (0.476 cm dia.) solid braid cotton rope and operated in previously aerated produced water amended with 3.0 g/L $NaNO_3$ and 0.5 g/L $K_2HPO_4$ [22]. Produced water used in this study was obtained from the Southern Cross disposal facility near Baggs, Wyoming.

A 1000 W sodium vapor lamp coupled with a 24% transmittance neutral density filter (Rosco, Sun Valley, CA), provided 220 μmol photons $m^{-2}$ $s^{-1}$ of photosynthetically active radiation to the upper most surface of the RABR units over a 14 h on:10 h off light cycle. The water temperature within the tanks averaged 20 ± 2 °C. A 1 *g* centrifuged wet weight inoculum, previously grown in produced water, was added to each RABR cotton rope substrata 15 min before beginning rotation of the reactors.

**Table 1**
Most significant BLAST hits with 100% query coverage for 23S rDNA primer amplicon of unialgal isolate Logan Lagoons Cyanobacteria 2 (LLC2).

| Organism | % Identity |
|---|---|
| Uncultured algae L012 | 95 |
| *Nodosilinea* sp. FI22HA2 | 94 |
| *Nodosilinea epilithica* str. Kovacik | 94 |
| *Nodosilinea* sp. Rehakova 1960/20 | 94 |
| *Plectonema terebrans* CCAP 1463/4 | 94 |
| *Leptolyngbya* sp. PCC 7104 | 93 |
| Uncultured organism clone C6.12 | 90 |
| *Oscillatoria acuminate* PCC 6304 | 89 |

### 2.3. Biomass determination and phycocyanin extraction

Biofilms harvested from cotton rope substrata were lyophilized for biomass determinations, ash free dry weight (AFDW) analysis, and phycocyanin extraction. AFDW was performed using lyophilized material at 550 °C. AFDW and phycocyanin productivity were normalized to the areal view surface footprint of the operating reactor (0.0175 $m^2$) while yields were normalized to available growth surface area on substrata.

Phycocyanin extractions were performed by first re-suspending lyophilized powdered biomass in E-Pure deionized water and rehydrating the material for 15 min. The samples were then subjected to two freeze/thaw cycles with a subsequent 2 h extraction by agitation on a Thermolyne Speci-Mix rocker table (Thermo Fisher Scientific, Waltham, MA). Following centrifugation for 15 min at 12,000 *g*, the crude extract supernatant phase was collected and analyzed for phycocyanin concentration and extract purity.

### 2.4. Phycocyanin identification and quantification

Phycocyanin concentration in the crude extract was determined by the methods of Bennett and Bogorad [23]. Phycocyanin purity in extracts was measured as the ratio of the optical absorbances at 620 nm and 280 nm [24]. Phycocyanin (PC) yields were calculated as PC Yield (mg PC/g AFDW) = PC conc. (mg PC/mL) * extraction volume (ml) / AFDW of biomass (g).

SDS-PAGE was conducted on the crude phycocyanin extract of LLC2, Spirulina powder (Bio-Alternatives, Klamath Falls, OR) (positive control), *Chlorella vulgaris* UTEX 2714 (negative control), phycocyanin standard (AnaSpec, Fremont, CA), and a Bio-Rad Kaleidoscope Precision Plus protein standard ladder using a precast 10%–20% linear gradient Tris/HCl Ready Gel (Bio-Rad, Hercules, CA). Samples were boiled in water for 10 min in Laemmli sample buffer and β-mercaptoethanol. The resolved gel was rinsed and soaked for 5 min in a 20 mM zinc sulfate solution and phycocyanin subunit fluorescence was visualized over a 302 nm Benchtop 3UV Transilluminator lamp (UVP, Upland, CA) [25]. Subsequently, the gel was rinsed in deionized water and stained with Coomassie Blue G-250 (Bio-Rad, Hercules, CA).

### 2.5. Statistical analysis

All biomass and phycocyanin extraction experiments were conducted in triplicate with independent measurements. Error bars represent one standard deviation from the mean of the samples taken.

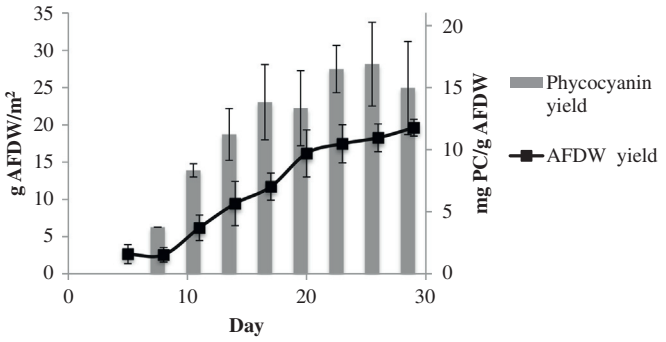


**Fig. 1.** Growth surface area AFDW and phycocyanin (PC) yields of harvested cyanobacterial biofilms grown in produced water (standard deviation shown, n = 3 for all measurements).