

Statement of Purpose

We are grateful for the opportunity that we have had to participate in the URCO process, as well as for the research experiences we have had as a result of the support. We had the opportunity to get a hands-on experience with the scientific method, and develop and test a hypothesis. As part of our project we met with industry to research current engineering applications and worked closely with faculty to develop a novel design to wastewater treatment. URCO provided the support and funds needed to get the research project “off of the ground” and get started working in the laboratory. As a result of the research and laboratory work we were able to prepare a manuscript for peer-review and eventual publication in Chemosphere, a refereed scientific journal (see attached article and RGS acknowledgement within).



Short Communication

Nutrient and suspended solids removal from petrochemical wastewater via microalgal biofilm cultivation

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HIGHLIGHTS

- Petroleum refining wastewater provided a suitable medium for biofilm microalgae.
- Biofilm microalgae removed significantly more nutrients than suspended microalgae.
- Biofilm microalgae removed significantly more solids than suspended microalgae.
- Microalgal biomass productivity was 10X greater in biofilm vs suspended growth.

ARTICLE INFO

Article history:

Received 21 November 2016

Received in revised form

13 January 2017

Accepted 20 January 2017

Available online 23 January 2017

Handling Editor: T Cutright

Keywords:

Petroleum refining wastewater

Rotating algae biofilm reactor

Open pond lagoon systems

Microalgae

ABSTRACT

Wastewater derived from petroleum refining currently accounts for 33.6 million barrels per day globally. Few wastewater treatment strategies exist to produce value-added products from petroleum refining wastewater. In this study, mixed culture microalgal biofilm-based treatment of petroleum refining wastewater using rotating algae biofilm reactors (RABRs) was compared with suspended-growth open pond lagoon reactors for removal of nutrients and suspended solids. Triplicate reactors were operated for 12 weeks and were continuously fed with petroleum refining wastewater. Effluent wastewater was monitored for nitrogen, phosphorus, total suspended solids (TSS), and chemical oxygen demand (COD). RABR treatment demonstrated a statistically significant increase in removal of nutrients and suspended solids, and increase in biomass productivity, compared to the open pond lagoon treatment. These trends translate to a greater potential for the production of biomass-based fuels, feed, and fertilizer as value-added products. This study is the first demonstration of the cultivation of mixed culture biofilm microalgae on petroleum refining wastewater for the dual purposes of treatment and biomass production.

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1. Introduction

Refining of petroleum is a large global industry (Wauquier, 1995), which produces large quantities of wastewater estimated at 33.6 million barrels per day (Diya'uddeen et al., 2011). Petroleum refining wastewater contains nutrients, solids, and organic compounds, providing for a waste that is costly to treat using traditional wastewater treatment methods (Knight et al., 1999).

Microalgae culture has demonstrated the ability to remove nitrogen, phosphorus, and organic compounds from petroleum refining wastewater (Antić et al., 2006; Chavan and Mukherji, 2010; Madadi et al., 2016); however, the major current biological

treatment strategy for petroleum wastewater consists of suspended growth processes. Suspended growth processes often have operational problems associated with proper settleability of sludge and sludge accumulation. These complexities can be avoided through the use of attached growth treatment processes. Research by Chavan and Mukherji (2008) indicated that biofilm growth processes show great potential for replacing suspended growth processes, but there has been limited research on treating petroleum wastewater.

Furthermore, many conventional wastewater treatment approaches do not produce value-added products that offset the cost of treatment operations. In contrast, the Rotating Algae Biofilm Reactor (RABR) has demonstrated the ability to effectively remove macronutrients and to produce a variety of bioproducts (Christenson and Sims, 2011; Ellis et al., 2012; Sathish et al., 2014).

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Successful demonstration of a RABR system for treatment of petroleum refinery wastewater would provide a novel approach to both wastewater treatment and bioproduct development. This study examined the effectiveness of RABR treatment for the production of microalgae-based biomass and the removal of nutrients, suspended solids, and organic species from petroleum refining wastewater.

2. Materials and methods

2.1. Biofilm growth conditions

One-liter RABRs (0.029 m² aerial footprint) were constructed and operated as described by Christenson and Sims (2012). Solid braid cotton rope was used as biofilm growth substratum. RABRs and open pond lagoons were artificially illuminated continuously using fluorescent lamps operated at $230 \pm 15 \mu\text{E m}^{-2}$ and temperature was maintained at $20 \pm 1 \text{ }^\circ\text{C}$. RABRs were inoculated with polyculture biofilm microalgae represented by a mixture of the wastewater derived cultures described by Fica and Sims (2016) and Wood et al. (2015).

RABRs and open pond lagoons were operated in continuous flow after an initial three-week period of batch operation to acclimate the biofilm to the wastewater. RABRs were operated in parallel triplicate groups with 24-hr and 48-hr hydraulic retention times (HRT), and duplicate open pond lagoons operated at a 36-hr HRT.

RABR biomass productivity was quantified by mechanically scraping the biofilm from the substratum, followed by determination of dry-weight as described by Wood et al. (2015). Open pond growth lagoon biomass productivity was quantified as the change in TSS concentration in the wastewater with time.

2.1.1. Wastewater characteristics

Wastewater was obtained from a refining industry in northern UT. Wastewater was collected from the influent wastewater stream immediately upstream of the API (American Petroleum Institute) Separator. Wastewater was dilute and homogenous and did not contain distinct oil and water phases. Wastewater characteristics are given in Table 1. All studies were performed using a single preserved sample to ensure constant uniformity of the influent wastewater.

2.2. Effluent wastewater sampling and analysis

Effluent wastewater from each RABR and lagoon unit was sampled weekly for 12 weeks. Analysis of COD, nitrogen, and phosphorous was performed on filtered samples using HACH reagent sets (Loveland, CO). TSS was measured according to Method 2540B (APHA, 2005).

Table 1

Petroleum refining wastewater composition used as the medium for microalgae cultivation.

Influent Wastewater Constituent	Influent Constituent Concentration
Total Nitrogen (N)	25 mg/L
Total Phosphorus (P)	1.8 mg/L
Total Suspended Solids (TSS)	39 mg/L
Chemical Oxygen Demand (COD)	163 mg/L
Diesel Range Organics (DRO)	22.2 mg/L
Gasoline Range Organics (GRO)	2.42 mg/L
pH	8.0

3. Theory

RABR wastewater treatment involves a partially submerged rotating drum, with appropriate growth substratum, that allows attached phototrophic microalgal biofilms to be exposed to two growth environments: 1) nutrients in the wastewater, and 2) light and carbon dioxide in the atmosphere. As the biofilm grows, nutrients from the wastewater can be converted to biomass in the biofilm. The biofilm is then removed, through mechanical scraping, allowing for the removal of nutrients from the overall treatment system and for the production of bioproducts from the harvested biomass. General RABR operation is fully described by Christenson and Sims (2012) and laboratory scale operation is described by Fica and Sims (2016) and Wood et al. (2015).

4. Results and discussion

Continuous-flow RABR treatment units demonstrated statistically significant reduction of nitrogen, phosphorus, and TSS compared to the open pond growth lagoon treatment (Fig. 1). Effluent concentrations from the 24-hr HRT RABR and 48-hr HRT RABR systems were decreased from the influent concentrations by 18.1 mg/L (72.4%) and 17.7 mg/L (70.8%) for nitrogen, 0.90 mg/L (50%) and 1.00 mg/L (55.6%) for phosphorus, and 20.9 mg/L (53.6%) and 23.9 mg/L (61.3%) for TSS, respectively. Whereas the open pond growth lagoon decreased effluent concentrations of nitrogen and phosphorus by 3.47 mg/L (13.9%) and 0.34 mg/L (18.9%) respectively, and increased TSS by 18.3 mg/L (46.9%). Both RABR groups demonstrated statistically larger reductions of nitrogen, phosphorus, and TSS compared to open pond lagoons, however no statistical difference in removal was found between 24-hr HRT and 48-hr HRT RABRs.

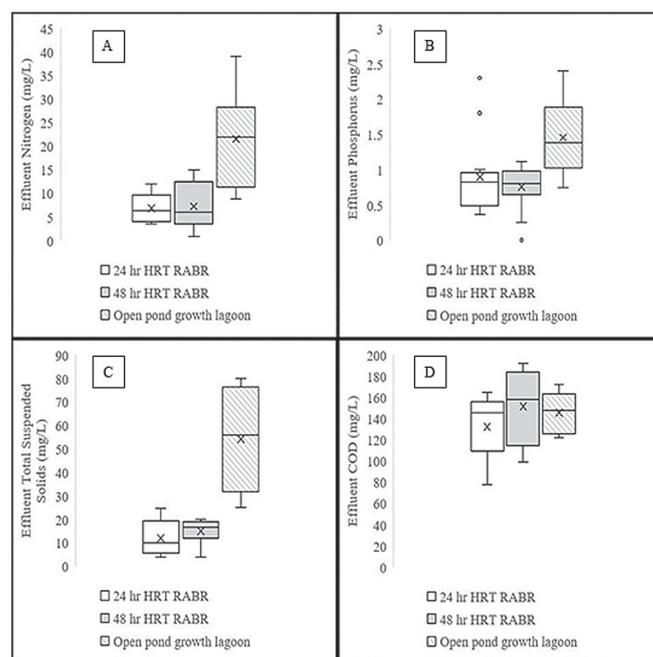


Fig. 1. (A–D) Effluent wastewater characteristics. Fig. 1A Effluent nitrogen concentration. Fig. 1B Effluent phosphorus concentration. Fig. 1C Effluent total suspended solids concentration. Fig. 1D Effluent COD concentration. The sample median is represented by a horizontal line through the box, the mean of the sample is denoted with an x, the lower and upper bounds of the boxes represent the 25th and 75th percentile of sample, whiskers represent maximum and minimum values, and dots represent outliers.

To effectively compare biomass productivity of the RABR and the open pond growth lagoon systems, biomass productivity was determined per unit area of aerial footprint. Dry biomass was produced at an average rate of $4.11 \text{ g m}^{-2} \text{ d}^{-1}$ across all RABR groups, compared to $0.4 \text{ g m}^{-2} \text{ d}^{-1}$ for the open pond lagoon. Visual microscopy indicated that the microalgae community of the RABR system was dominated by filamentous cyanobacteria while open pond lagoon microalgae was largely composed of green microalgae species. Overall, the RABR system demonstrated a significantly larger biomass productivity than the open pond growth lagoon system, and therefore, demonstrated a significantly larger potential to produce biomass-based, value-added products.

In order to perform a mass balance on nitrogen and phosphorus in the system, biofilm was assumed to follow the molar C:N:P ratio given by the Redfield Ratio for microalgae of 106:16:1 (Hillebrand and Sommer, 1999). Biomass productivity of $4.11 \text{ g m}^{-2} \text{ d}^{-1}$ correlates with a removal of $17.5 \text{ mg L}^{-1} \text{ d}^{-1}$ nitrogen and $2.4 \text{ mg L}^{-1} \text{ d}^{-1}$ of phosphorus. Nitrogen removal calculated from the mass balance using the Redfield Ratio closely matches the observed nitrogen removal. This correlation suggests that nitrogen removal was most significantly due to biomass growth and not sorption or solids removal. Calculated phosphorus removal, however, did not closely match observed values. This discrepancy was not unexpected due to the large variation in C:P ratios observed in cyanobacterial cultures (Hessen et al., 2005).

The RABR technology did not perform differentially from the open pond lagoons in the removal of chemical oxygen demand (COD) (Fig. 1D). Due to the availability of carbon dioxide as the carbon source for the phototrophic biofilm and the recalcitrant nature of the organic substances in petroleum refining wastewater, a significant reduction in COD due to heterotrophic activity of the biofilm was not expected nor observed.

All statistical analysis was conducted using ANOVA and t-testing. P-values less than 0.005 were considered to be statically significant.

5. Conclusions

Microalgae was successfully cultivated on petroleum refining wastewater using both biofilm and suspended open pond systems. Significant reductions in nutrients and suspended solids concentrations were statistically significantly greater using the biofilm (RABR) system compared to the open pond growth lagoon system. Additionally, the RABR system exhibited greater biomass productivity than open pond lagoons, which represents biomass that can be utilized to produce value-added products. This study demonstrated the application of mixed culture microalgae in Rotating

Algae Biofilm Reactors (RABRs) as a novel approach in the treatment of petroleum refining wastewater that also provides a biomass feedstock for the production of downstream bioproducts.

Acknowledgements

Financial support was provided by The State of Utah Science Technology and Research (USTAR) Initiative (121212-E25771), Utah State University Office of Research and Graduate Studies, and WesTech Engineering Incorporated.

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