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Purification of Reverse Osmosis Concentrate using *Chlorella vulgaris*

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ABSTRACT

The present investigation centres on the utilisation of microalgae, particularly *Chlorella vulgaris*, for the remediation of reverse osmosis concentrate (ROC) derived from water treatment facilities. The study investigates the efficacy of *C. vulgaris* in eliminating nutrients, heavy metals, and other substances from ROC. An analysis was conducted on the physico-chemical characteristics of the wastewater, including pH, alkalinity, dissolved oxygen, total solids, and concentrations of heavy metals. This analysis was performed both before and after treatment with *C. vulgaris.* The findings demonstrated that *C. vulgaris* effectively eliminated substantial quantities of nutrients and heavy metals from the ROC. The study additionally performed GC-MS and FTIR tests to ascertain the composition of the ROC both prior to and during treatment. Collectively, the research showcases the capacity of microalgae to serve as a proficient and enduring remedy for wastewater treatment and the retrieval of important resources.

Key words: *Chlorella vulgaris, Reverse osmosis concentrate (ROC), GC-MS, FTIR*

Introduction

In recent decades, global industrial growth and urbanization, driven by population expansion, have led to a significant rise in water pollution, with approximately 80% of wastewater discharged untreated into water bodies (WWAP, 2017; Corcoran *et al*., 2010; UN-Water, 2015). This has exacerbated water scarcity for agricultural, industrial, and domestic use (van Vliet *et al*., 2017; Gleick and Palaniappan, 2010) and forecasts indicate that nearly 5 million people may face severe water shortages by 2050 due to climate change and pollution (van Vliet *et al*., 2017). Consequently, recycling and purification of wastewater using Reverse Osmosis (RO) has become essential. RO effectively removes harmful compounds and produces purified water (permeate) and a concentrated waste (concentrate) containing pollutants like Endocrine Disrupting Chemicals (EDCs) (Subramanian and Jacangelo, 2013; Umar *et al.,* 2014). The main challenge is the management of Residual Organic Concentrate (ROC), which can lead to eutrophication if not treated (Li *et al*., 2010a). Current treatment methods-including physical, chemical, and biological-have limitations, such as membrane fouling and potential secondary contamination (Morillo *et al*., 2014; Umar *et al*., 2016). In contrast, microalgae offer a promising solution, effectively extracting nutrients from ROC while producing valuable biofuels (Maeng *et al*., 2018; Zhang *et al*., 2019). This study focuses on using ROC to cultivate *Chlorella vulgaris*, assessing its potential in nutrient, heavy metal, and substance removal, alongside its impact on ROC physico-chemical properties.

Materials and Methods

Collection of ROC

The ROC samples were obtained from a mineral water facility situated in Patteeswaram, Thanjavur District, Tamilnadu, India. The samples were collected and brought to the laboratory for storage at a temperature of 4 °C.

Microalgae cultivation

The microalgae species, *Chlorella vulgaris*, were acquired from the National Repository for Microalgae and Cyanobacteria (NRMC), Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamilnadu, India (Voucher No. 103). The microalgae were grown and kept in ATCC medium: 824 ASN-III media.

Chlorella vulgaris culture and its maintenance

C. vulgaris samples were cultured on ATCC agar at 25 °C with a 12-hour light/dark cycle. The microalgae were later transferred to liquid medium for identification using traits from "Biology of the Algae" by Palmer. The resulting cultures were used for ROC treatment.

Experimental setup

Chlorella vulgaris microalgae were grown in a controlled environment with specific conditions: room temp of 34±1 °C, 65% humidity. 20 liters of wastewater samples were used. Algae were mixed with ATCC medium and monitored for 20 days. Data analysis was done three times (Taiwo, 2016).

Physico-chemical evaluation of ROC

The physicochemical properties of ROC were analyzed before and after treatment with *Chlorella vulgaris* microalgae. Tests included pH, alkalinity, DO, EC, TDS, TS, TSS, BOD, COD, phosphate, and nitrate. Methods outlined in the APHA (2012) manual were used for analysis. Spectrophotometry measured phosphate and nitrate, potentiometry measured pH and EC, and volumetric techniques quantified COD, BOD, and DO. Heavy metals were analyzed using UV-spectrophotometry three times as per Manivasakam (2005).

Xenobiotics removal efficiency

The calculation of the removal efficiency (RE) of pollutants by *C. vulgaris* was performed using the formula proposed by Taiwo *et al.* (2016).

$$RE\ (\%) = (Ci-Cf)/(Ci\ X\ 100)$$

Where, Ci = concentration of element in untreated wastewater. Cf = concentration of element in treated wastewater.

Statistical analysis

Descriptive statistics on physico-chemical variables, heavy metals, and elements were analyzed. Pearson's correlation examined relationships using IBM SPSS v25. Significant associations were found ($p < 0.01$).

Results

Before treatment with *C. vulgaris*, the pH of ROC was 6.85±0.18. After 20 days, pH increased to 7.47.98±0.07, while alkalinity rose from 155.80±3.92 to 230.20±0.94. Dissolved oxygen initially rose to 8.26±0.06 mg/l in 24 hours with no change in ROC levels. By the 20th day, DO was 9.44±0.14 mg/l. Microalgae had a significant impact on total dissolved solids (TDS) levels in the ROC, lowering TDS to 2774.00±8.49 mg/l after 20 days. Total suspended solids (TS) decreased to 2945.00±0.24 mg/l after microalgae treatment, with *C. vulgaris* removing 43.40% of TS. The untreated river water had TSS of 1635.40±18.29 mg/l, which dropped to 170.67±0.24 mg/l post-treatment, a removal efficiency of 89.56%.

Assessing the removal of BOD and COD using *C. vulgaris*

Electrical conductivity of the ROC also decreased to 6.86±0.11 mS cm^{-1} after 20 days.The elimination of

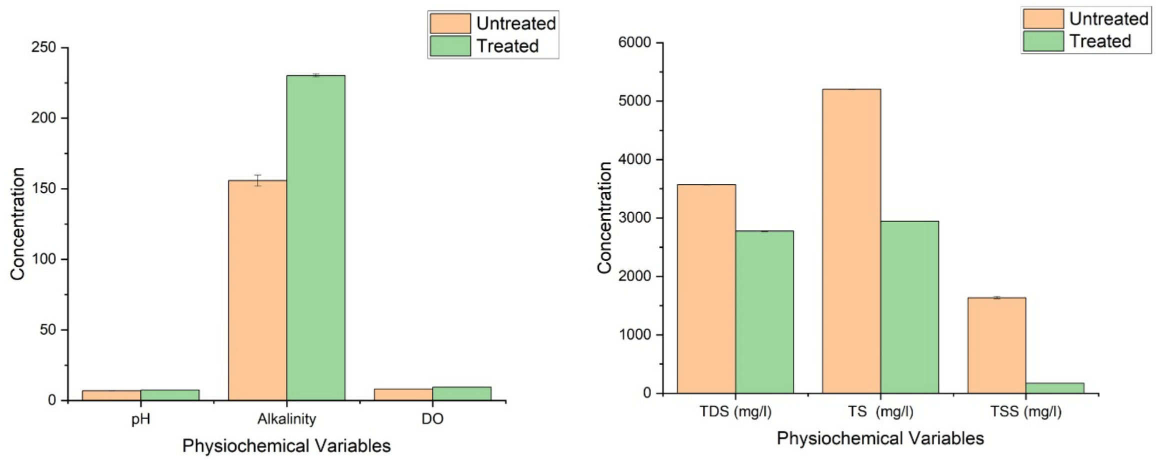


Fig. 1. Effect of *C. vulgaris* on pH, alkalinity dissolved oxygen, TDS, TS and TSS of ROC

BOD experienced a substantial improvement, rising from 2.12% to 45.59% after a 20-day treatment with micro algae. Following a 20-day treatment period, the initial Biochemical Oxygen Demand (BOD) was effectively decreased to 99.33± 1.25 mg/l. The elimination of BOD experienced a substantial rise, rising from a small 1.89% to an astounding 32.79% (Fig. 2).

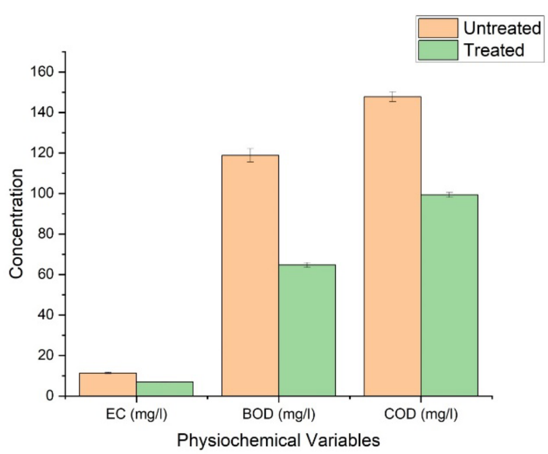


Fig. 2. Effect of *C. vulgaris* on eliminating the EC, BOD and COD in ROC

Exploring the absorption of nutrients by *C. vulgaris*

The 4th table presents results of research on nitrate removal from ROC using *C. vulgaris*. Initial nitrate content was 51.60±3.50 mg/l. After 20 days of treatment, nitrate reduced to 16.87±0.21 mg/l with an efficiency of 67.31%. Phosphate decreased from 8.43±0.28 mg/l to 6.79±0.01 mg/l, efficiency of 19.45%. Chloride reduced from 85.00±6.72 mg/l to 78.95%. Calcium eliminated by 56.08% (Fig. 3).

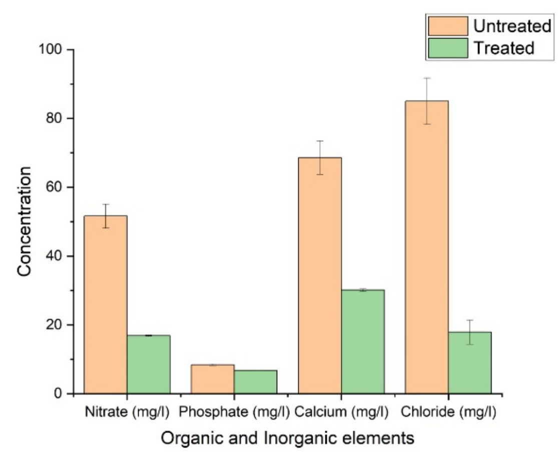


Fig. 3. Nutrient and element concentration of ROC during microalgae treatment

Investigating the potential of *C. vulgaris* in removing heavy metals

The initial zinc concentration in untreated ROC was 19.43± 1.85 mg/l, decreasing to 4.47±0.29 mg/l after 20 days of microalgae growth with a removal efficiency of 22.24%. After 20 days, iron decreased to 4.04± 0.01 mg/l with a clearance efficiency of 10.75% using *C. vulgaris* . Within 48 hours, microalgae completely removed copper, achieving a 100% removal efficiency (Fig. 4).

Analysis of compounds using GC-MS for identification

Table 6 identifies chemicals in *C. vulgaris* before

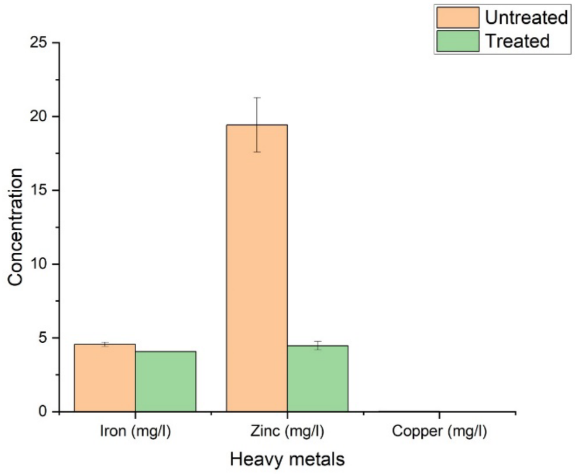


Fig. 4. The heavy metal concentration of ROC during microalgae treatment

treatment. Decanedioic acid ester was the most prevalent at 75.59%. After treatment, Table 7 shows compounds with 1-4 Benzene dicarboxylic acid, dimethyl ester being dominant at 73.99%. Other compounds make up 5.80-7.34%.

Analysis using FTIR

Before treatment, the study identified specific chemical groups in untreated wastewater through distinct stretching vibrations, including hydroxyl, H-bonded OH, methylene C-H, isocyanate, and amide groups. Polysulfides and aliphatic iodo compounds were also detected at various wavelengths. After treatment with microalgae, there was a shift in wavelengths indicating the presence of OH groups in phenol and alcohol, symmetric stretching of methyl C-H, terminal alkyne, and stretching of alkenyl C=C. The treated wastewater showed a reduction in polysulfides and complete elimination of aliphatic bromo compounds, as well as a decrease in aliphatic iodo compounds.

Discussion

The Reverse Osmosis (RO) method generates a con-

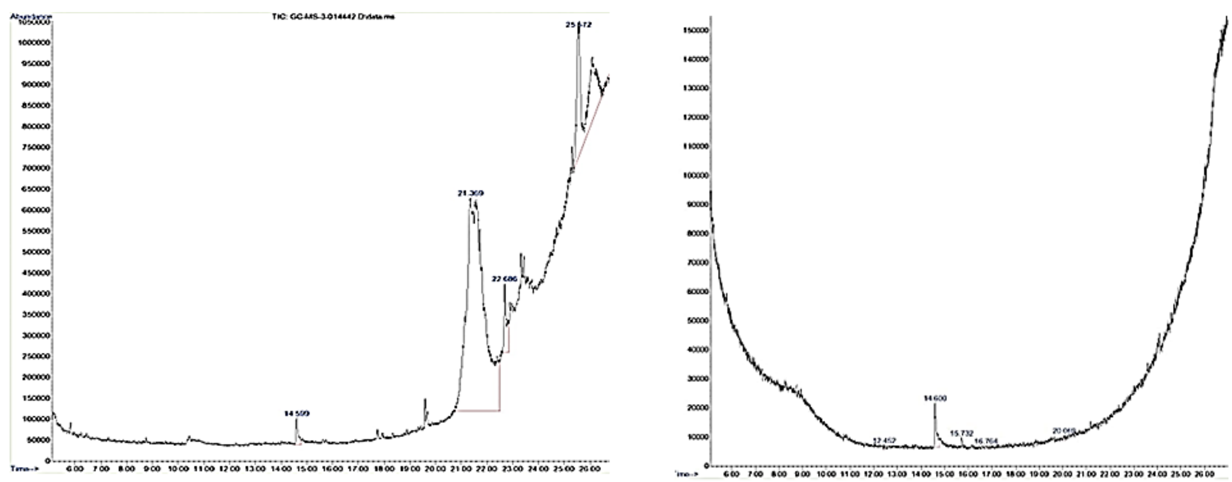
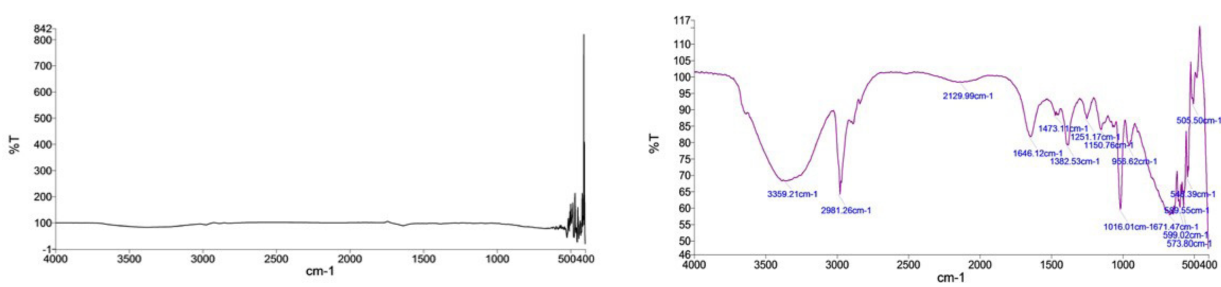


Fig. 5. GC-MSchromatogramof(a) untreated and (b) treated ROC



(a) Before treatment (b) After treatment

Fig. 6. FTIR analysis of untreated Reverse Osmosis Concentrate

centrate (ROC) with higher levels of dissolved organic and inorganic substances than the original water, which poses health and environmental risks if disposed of untreated, contributing to eutrophication (Umar *et al*., 2015; Zhong *et al*., 2019). Microalgae cultivation can reclaim nutrients from ROC, but harmful chemicals may inhibit microalgal growth (Sayre, 2010; Zhang *et al*., 2017). The optimal ROC concentration for microalgae biomass production is between 25% and 30%, with observed pH increases indicating photosynthetic activity (Matos *et al*., 2017; Ikehata *et al*., 2018). After 20 days, phosphate levels in ROC significantly dropped from 2.43 mg/l to 0.79 mg/l, with microalgae such as *C. vulgaris* showing efficient phosphate absorption (Mohensi *et al.,* 2020; Powell *et al*., 2011). Heavy metals like zinc, copper, chromium, and mercury were detected in ROC, with *C. vulgaris* achieving 100% copper removal in 48 hours (Chan *et al.,* 2014; Yao *et al.,* 2013). Calcium uptake by *C. vulgaris* reached 63.95% over 20 days, influenced by initial concentrations and pH (Wang *et al*., 2016). Chloride concentrations in ROC decreased despite higher detected levels, showing no growth impact on microalgae (Shen *et al*., 2019; Chang *et al*., 2021). Total Dissolved Solids (TDS) reduced significantly from 3568.60 mg/l to 2780 mg/l after treatment, while dissolved oxygen levels increased to 9.44 mg/l (Mohseni *et al*., 2020). GC-MS analysis identified 25 chemicals, dominated by Diethylmalonic acid, while FTIR confirmed reductions in certain compounds post-treatment. These results demonstrate the potential of microalgae for treating ROC and the importance of pH and salinity in enhancing nutrient absorption and microalgal growth (Mostafa *et al*., 2012; Chen and Oswald, 1998).

Conclusion

The present study analysis yields the following conclusions. Application of *C. vulgaris* to the ROC resulted in elevated levels of pH, alkalinity, and dissolved oxygen. The presence of microalgae significantly decreased the levels of solids, including total solids, dissolved solids, and suspended solids. The levels of nutrients, including chloride, nitrate, phosphate, and calcium, were observed to have reduced. Similarly, the microalgae *C. vulgaris* demonstrated efficient elimination of heavy metals from the ROC. Therefore, the research indicates that employing microalgae, specifically *C. vulgaris*, for wastewater treatment, specifically ROC, could serve as a viable alternative for eliminating xenobiotics.

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Conflict of Interest

The writers affirm that they possess no conflicting interests.

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