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Purification of Reverse Osmosis Concentrate Using *Chlorella Vulgaris*

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KEYWORDS
Chlorella vulgaris, reverse osmosis concentrate (ROC), GC-MS, FTIR

ABSTRACT:
The current study focuses on the application of microalgae, specifically *Chlorella vulgaris*, for treating reverse osmosis concentrate (ROC) from wastewater treatment plants. The study examines the effectiveness of *C. vulgaris* in removing nutrients, heavy metals, and other compounds from ROC. The ROC samples were collected from water purification plants and *C. vulgaris* was utilized in the treatment of ROC. The physicochemical properties of the wastewater, such as pH, alkalinity, dissolved oxygen, total solids, and heavy metal concentrations, were analyzed before and after treatment with *C. vulgaris*. The results showed that *C. vulgaris* was able to remove significant amounts of nutrients, heavy metals, from the ROC. The study also conducted GC-MS and FTIR analyses to identify the compounds present in the ROC before and after treatment. Overall, the research demonstrates the potential of microalgae as an effective and sustainable solution for treating wastewater and recovering valuable resources.

Introduction

Over the past few decades, there has been a tremendous global expansion of industrialization and urbanisation in response to the growing population. These advancements have led to a significant increase in water contamination. Approximately 80% of wastewater is being released into water bodies without adequate pre-treatment, mostly due to the fast expansion of the population and economic development (WWAP, 2017; Corcoran et al., 2010; WWAP, 2012; UN-Water, 2015). Consequently, there has been a shortage of water for agricultural, industrial, and domestic purposes in various regions worldwide (van Vliet et al., 2017; Gleick & Palaniappan, 2010). Moreover, projections indicate that by 2050, approximately 5 million individuals will experience a significant scarcity of water as a result of both climate change and the pollution-induced contamination of freshwater, ultimately leading to an increased demand for water. Therefore, implementing recycling and purification methods for wastewater is an essential approach to address the growing global need for fresh and uncontaminated water.

Reverse Osmosis (RO) is the widely accepted and proven process used for water reclamation. This method has been utilised for the purpose of desalination, the generation of drinkable water, and in the treatment of tertiary wastewater (Subramanian & Jacangelo, 2013; Joo & Tansel, 2015; Wang et al., 2017; Morillo et al., 2013). RO technology has been extensively employed since 1970 to address water scarcity. Reverse Osmosis use a semi-permeable membrane to purify water, yielding two outputs: permeate, which is the pure water, and concentrate, which comprises the salts and chemicals extracted from wastewater (Mauguin and Corsins, 2005). The properties of the concentrate are influenced by the quality of the feed water, as well as the pretreatment and cleaning methods employed (Chelme-Ayala et al., 2009; Greenlee et al., 2009; Squire et al., 1997; Watson, 1990). The concentration of the components in the permeate is twice as high as that in the feed water (Chelme-Ayala et al., 2009). Reverse osmosis (RO) rejects organic chemicals, such as Endocrine Disrupting chemicals (EDCs), ions, salts, cosmetics, and biological components. As a result, the concentrate produced by RO includes a high concentration of these harmful substances (Umar et al., 2014).

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The primary obstacle and disadvantage linked to reverse osmosis (RO) technology is the management and elimination of ROC (Comstock and al., 2011; Vander Bruggen et al., 2003 and Greenlee et al., 2009). The discharge of ROC into water bodies without previous treatment leads to eutrophication and degradation of water quality because to its high nitrogen and phosphorus content (Li et al., 2010a). Likewise, the existence of dissolved organic and inorganic substances in ROC renders them extremely poisonous when discharged into the environment without adequate treatment. Therefore, in order to achieve environmental preservation and sustainable development, it is essential to appropriately address the issue of ROC.

While there are physical, chemical, and biological methods for treating ROC, each strategy has limitations that impede their use in RO therapy. The physical approach entails the occurrence of membrane fouling and necessitates the use of substantial energy, rendering it economically impractical (Morillo et al., 2013; Umar et al., 2016; King et al., 2020; Eversloh et al., 2015; Maeng et al., 2018). The efficacy of the bacterial-based biological approach for treating ROC is compromised by an imbalance in the carbon-to-nitrogen ratio and the stringent operational requirements (Wang et al., 2016). Likewise, the chemical approach may lead to secondary contamination as a result of introducing potent compounds (Umar et al., 2016).

Microalgae have acquired pace as a beneficial and efficient solution for treating ROC, surpassing conventional physical, chemical, and biological approaches. Microalgae therapy may effectively recover nutrients, like as nitrogen and phosphate, from ROC. In addition, the microalgae efficiently utilise the nutrients received from the ROC to produce biomolecules, including carbohydrates, proteins, and lipids, through the processes of photosynthesis and nutrient recycling. These biomolecules are then used for the generation of biofuel (Maeng et al., 2018; Zhang et al., 2019; Chang et al., 2020; Zhu et al., 2019). The concentration of nitrogen in ROC typically ranges from 40 to 70 mg/L, phosphorus from 10 to 30 mg/L, and organic compounds from 30 to 70 mg/L. These components have an impact on the growth of microalgae (Li et al., 2011) and create a favourable environment for their growth and the subsequent removal of nutrients (Minhas et al., 2016). The study focuses on the application of ROC for the cultivation of microalgae, namely *C.vulgaris*, and its effectiveness in removing nutrients, heavy metals, and other materials from the ROC. An assessment was conducted to determine the impact of *C. vulgaris* on the physicochemical parameters of ROC.

Materials and Methods

Collection of ROC

The samples of ROC were collected from a mineral water plant situated in Patteeswaram, which is located in the Thanjavur District of Tamilnadu, India. The specimens were obtained in containers that had been pre-treated with acid, subsequently fixed with HNO_3, and then transported to the laboratory for storage at a temperature of 4ºC.

Microalgae cultivation

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

Chlorella vulgaris culture and its maintenance

The *C. vulgaris* samples were introduced onto an ATCC agar medium. The inoculated plates were maintained at a temperature of 25ºC in a controlled environment, namely a culture chamber. The chamber was furnished with a white fluorescent light source that functioned on a 12-hour cycle alternating between light and darkness. The growth was examined at regular intervals. After the harvest, the microalgae colonies were then moved to a liquid media supplied by the American Type Culture Collection (ATCC). The identification of the algae was carried out on the basis of their morphological and cultural characteristics. To identify the algae, Palmer's book "Biology of the Algae" was used (Palmer, 1997). The obtained algal cultures were then used to treat ROC.

Experimental setup

The experiment entailed cultivating *Chlorella vulgaris* microalgae in ROC, subject to controlled climatic conditions, namely a constant room temperature of 34±1ºC and a relative humidity of 65%. Twenty liters of wastewater samples were collected using clean and labeled bowls having a capacity of 35 liters. The experimental setup involved the combination of 100 ml of ATCC medium with 0.15g of the algae species *C. vulgaris* in the wastewater. Control was maintained in the absence of growth media and microalgae. The experimental approach and data analysis were performed three times. The algal development was sustained for a period of 20 days, which is comparable to 480 hours (Taiwo, 2016).

Physico-chemical evaluation of ROC

In order to remove suspended particles and bacteria, the ROC samples underwent filtration using a Whatman membrane filter with a pore size of 0.45µm. The physicochemical properties of ROC, such as pH,

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alkalinity, Dissolved Oxygen (DO), Electrical conductivity (EC), Total Dissolved Solids (TDS), Total Solids (TS), Total Suspended Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), phosphate, and nitrate, were analysed before and after treatment with the microalgae species *Chlorella vulgaris*. The analysis was performed using the prescribed protocol specified in the APHA (2012) manual. The spectrophotometric approach was used to quantify the nutrient levels, specifically phosphate and nitrate, in ROC. The potentiometric approach was used to measure the pH and electrical conductivity (EC). The quantification of Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Dissolved Oxygen (DO) was performed utilising volumetric analytical techniques. The concentrations of heavy metals (HMs), such as iron, cadmium, zinc, copper, chromium, mercury, and lead, were quantified using a digital UV-spectrophotometer, following the analytical procedure outlined by Manivasakam (2005). The analysis was performed three times.

Determination of microalgae biomass productivity

The assessment of microalgae biomass productivity was performed using a UV-Visible spectrophotometer at a specific wavelength of 680 nm, which acted as a measure of microalgae concentration. The experimental protocol described in Kumar et al. (2015) was adhered to for this objective. A graph was constructed using pre-existing biomass concentration values (mg/ml). Standard solutions were generated using microalgae at concentrations ranging from 1 mg/ml to 10 mg/ml. The absorbance of the reference microalgae solution was measured at a precise wavelength of 680 nm.

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).

$$\text{RE (\%)} = \frac{Ci - Cf}{Ci\ X\ 100}$$

Where,

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

Statistical analysis

Descriptive statistics, such as means and standard deviations, were computed for the physicochemical variables, heavy metals (HMs), and elements. A Pearson's correlation analysis was conducted to investigate the associations between these variables. The statistical studies were conducted using the IBM software SPSS, specifically version 25. The correlation observed was considered statistically significant with a significance level of $p < 0.01$.

Results

Before treatment with micro algae, *C. vulgaris*, the initial pH of the ROC was measured at 6.85±0.18. During the 20-day period of micro algae cultivation, there was a steady rise in the pH of ROC, reaching a value of 7.47.98±0.07. There was a significant increase in alkalinity with levels rising from 155.80±3.92 to 230.20±0.94. After 24 hours of treatment, there was an initial increase in dissolved oxygen (DO) levels, measuring at 8.26±0.06 mg/L. Following this rise, there was no significant change in the concentration of reverse osmosis concentrate (ROC). The DO concentration at the end of the 20th day measured 9.44±0.14 mg/L (Table 1).

Table.1: Effect of *C. vulgaris* on pH, alkalinity and dissolved oxygen content of ROC

Duration of Treatment (h)	Water Samples/Physicochemical variables		
	pH	**Alkalinity (mg/l)**	**DO (mg/l)**
T1 (0 h)	6.85±0.18 ^{c}	155.80±3.92 ^{i}	8.14±0.15 ^{d}
T2 (24 h)	6.89±0.02 ^{c}	156.63±0.48 ^{gh}	8.26±0.06 ^{d}
T3 (48 h)	6.94±0.02 ^{c}	163.07±1.14 ^{ef}	8.28±0.02 ^{d}
T4 (72 h)	6.88±0.12 ^{c}	168.37±2.83 ^{e}	8.34±0.04 ^{d}
T5 (96 h)	6.99±0.01 ^{cb}	177.93±2.19 ^{d}	8.57±0.01 ^{c}
T6 (120 h)	7.27±0.12 ^{ab}	182.95±1.21 ^{d}	8.66±0.04 ^{c}
T7 (240 h)	7.38±0.13 ^{a}	197.37±1.47 ^{c}	8.88±0.02 ^{b}
T8 (360 h)	7.44±0.06 ^{a}	217.23±3.28 ^{b}	8.96±0.03 ^{b}
T9 (480 h)	7.47±0.07 ^{a}	230.20±0.94 ^{a}	9.44±0.14 ^{a}

Microalgae had a noticeable effect on the concentration of TDS in ROC, resulting in a decrease in TDS (2774.00±8.49 mg/L) by the 20th day of microalgae treatment. After 20 days, an apparent reduction in the

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concentration of TS of ROC was observed (2945.00±0.24 mg/L) with the application of micro algae treatment. The ROC analysis revealed that *C. vulgaris* was able to remove 43.40% of TS. The TSS of untreated ROC was determined to be 1635.40± 18.29 mg/L. At the conclusion of the micro algae treatment, the TSS concentration measured 170.67± 0.24 mg/L, demonstrating an impressive removal efficiency of 89.56%. As the micro algae continued to grow, the EC of ROC decreased. By the end of the 20th day (480 h), the EC of ROC was measured to be 6.86± 0.11 mS cm−1, showing a removal percentage of 0.11% (Table 2).

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Table.2: Effect of *C. vulgaris* on eliminating the solid content in ROC

Duration of Treatment (h)	Water Samples/Physicochemical variables		
	TDS (mg/l)	**TS (mg/l)**	**TSS (mg/l)**
T1 (0 h)	3568.60±5.75 ^{a}	5203.40±5.11 ^{a}	1635.40±18.29 ^{a}
T2 (24 h)	3564.00±2.94 ^{a}	4942.33±0.33 ^{a}	1378.33±0.33 ^{b}
T3 (48 h)	3457.33±0.94 ^{b}	4794.67±0.21 ^{b}	1337.34±0.21 ^{c}
T4 (72 h)	3344.00±2.83 ^{c}	4648.33±0.29 ^{c}	1304.33±0.29 ^{d}
T5 (96 h)	3238.00±0.82 ^{d}	4304.33±0.42 ^{d}	1066.33±0.42 ^{e}
T6 (120 h)	3128.00±2.45 ^{e}	4135.33±0.17 ^{e}	1007.33±0.17 ^{f}
T7 (240 h)	2908.33±1.70 ^{f}	3509.00±0.34 ^{f}	600.67±0.34 ^{g}
T8 (360 h)	2898.67±5.25 ^{g}	3349.00±0.68 ^{g}	450.33±0.68 ^{h}
T9 (480 h)	2774.00±8.49 ^{h}	2945.00±0.24 ^{h}	170.67±0.24 ^{i}

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

There was a significant enhancement in the removal of BOD, increasing from 2.12% to 45.59% after 20 days of micro algae treatment. After 20 days of treatment, the initial BOD was successfully reduced to 99.33± 1.25 mg/L. There was a significant increase in the removal of BOD, going from a mere 1.89% to an impressive 32.79% (Table 3).

Table.3: Effect of *C. vulgaris* on EC, BOD and COD of ROC

Duration of Treatment (h)	Water Samples/Physicochemical variables		
	EC (mg/l)	**BOD (mg/l)**	**COD (mg/l)**
T1 (0 h)	11.36±0.30 ^{a}	118.85±3.31 ^{a}	147.80±2.48 ^{a}
T2 (24 h)	10.87±0.14 ^{b}	116.33±1.25 ^{a}	145.00±0.82 ^{a}
T3 (48 h)	10.17±0.03 ^{c}	117.67±1.25 ^{b}	143.33±2.05 ^{ab}
T4 (72 h)	9.68±0.02 ^{d}	109.33±1.25 ^{b}	139.00±1.41 ^{b}
T5 (96 h)	9.37±0.04 ^{e}	103.83±0.62 ^{c}	132.00±1.63 ^{c}
T6 (120 h)	8.84±0.05 ^{f}	100.33±1.25 ^{c}	127.00±1.63 ^{c}
T7 (240 h)	8.18±0.02 ^{g}	85.00±0.82 ^{d}	119.00±1.41 ^{d}
T8 (360 h)	7.74±0.08 ^{h}	73.00±1.63 ^{e}	112.33±2.05 ^{e}
T9 (480 h)	6.86±0.11 ^{i}	64.67±1.25 ^{f}	99.33±1.25 ^{f}

Exploring the absorption of nutrients by *C. vulgaris*

The table 4 presents the findings of the study on the removal of nitrate, a nutrient, from the ROC by C.vulgaris. Before the treatment of ROC with micro algae, the nitrate concentration in the ROC was measured at 51.60± 3.50 mg/L. *C. vulgaris* proved to be highly effective in removing nitrate, with a final concentration of 16.87± 0.21 mg/L after 20 days of treatment. *C. vulgaris* was able to achieve a nitrate removal rate of 67.31%. Prior to the treatment of ROC with micro algae, the phosphate concentration in the ROC was measured at 8.43± 0.28 mg/L. At the conclusion of the treatment period (20 days), the phosphate concentration measured 6.79± 0.01 mg/L. *C. vulgaris* was able to remove 19.45% of phosphate. Before treatment with micro algae, the ROC exhibited a chloride concentration of 85.00± 6.72 mg/L. *C. vulgaris* was able to achieve a chloride removal rate of 78.95%. *C. vulgaris* was able to remove 56.08% of calcium.

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Table 4. Nutrient and element concentration of ROC during microalgae treatment

Duration of Treatment (h)	Organic and inorganic elements (mg/l)			
	Nitrate (NO_3^-)	Phosphate (P)	Calcium (Ca)	Chloride (Cl)
T1 (0 h)	51.60±3.50 ^{a}	8.43±0.28 ^{a}	68.60±4.88 ^{a}	85.00±6.72 ^{a}
T2 (24 h)	48.73±0.54 ^{b}	8.38±0.03 ^{a}	60.64±1.63 ^{a}	75.53±3.40 ^{a}
T3 (48 h)	45.73±0.54 ^{c}	8.35±0.37 ^{ab}	57.53±0.29 ^{b}	62.01±1.25 ^{b}
T4 (72 h)	41.27±0.33 ^{d}	8.28±0.03 ^{a}	51.90±1.57 ^{b}	59.99±2.87 ^{c}
T5 (96 h)	38.57±0.39 ^{e}	7.98±0.03 ^{bcd}	46.95±1.06 ^{c}	50.05±2.49 ^{c}
T6 (120 h)	32.83±0.25 ^{f}	7.87±0.03 ^{abc}	42.71±0.80 ^{cd}	34.24±2.05 ^{d}
T7 (240 h)	26.77±0.05 ^{g}	7.35±0.02 ^{cde}	38.01±0.90 ^{ef}	26.00±4.32 ^{e}
T8 (360 h)	21.90±0.37 ^{h}	7.04±0.02 ^{de}	33.57±0.42 ^{fg}	19.50±3.30 ^{f}
T9 (480 h)	16.87±0.21 ^{i}	6.79±0.01 ^{f}	30.13±0.37 ^{h}	17.89±3.56 ^{f}

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

The concentration of zinc in the untreated ROC was measured to be 19.43± 1.85 mg/L. After 20 days of micro algae growth, the concentration of zinc decreased to 4.47±0.29 mg/L, resulting in a removal efficiency of 22.24%. After 20 days of micro algae growth, the concentration of iron decreased to 4.04± 0.01 mg/L. The treatment with *C. vulgaris* resulted in a removal efficiency of 10.75%. After 48 hours of treatment, the micro algae were able to fully absorb copper, resulting in a complete removal efficiency of 100% within the same time frame (Table 5).

Table 5. The heavy metal concentration of ROC during microalgae treatment

Duration of Treatment (h)	Heavy metals (mg/l)		
	Iron (Fe)	Zinc (Zn)	Copper (Cu)
T1 (0 h)	4.56±0.14 ^{a}	19.43±1.85 ^{a}	0.02±0.00 ^{a}
T2 (24 h)	4.54±0.02 ^{a}	17.83±0.21 ^{a}	0.01±0.00 ^{a}
T3 (48 h)	4.51±0.01 ^{ab}	15.57±0.21 ^{b}	-
T4 (72 h)	4.44±0.03 ^{abc}	14.57±0.33 ^{bc}	-
T5 (96 h)	4.39±0.01 ^{abc}	13.53±0.25 ^{cd}	-
T6 (120 h)	4.33±0.02 ^{bcd}	12.57±0.34 ^{ef}	-
T7 (240 h)	4.27±0.01 ^{cd}	10.80±0.29 ^{f}	-
T8 (360 h)	4.19±0.01 ^{de}	7.4±0.08 ^{g}	-
T9 (480 h)	4.07±0.01 ^{f}	4.47±0.29 ^{h}	-

Analysis of compounds using GC-MS for identification

Prior to treatment

Table 6 displays the major compounds that were identified through GC-MS analysis prior to the treatment of microalgae, *C. vulgaris*. Upon analysing the GC-MS chromatogram (Fig.1), it is evident that there are four distinct peaks with retention times of 14.599, 21.369, 22.686, and 25.572. It was found that the compound Decanedioic acid, bis(1,2,2,6,6-pentamethyl-4- piperidinyl) ester accounted for a significant portion of 75.59% of the area. The other compounds identified in the research had varying percentages of presence in ROC. These compounds include dimethyl ester of 1,4-Benzenedicarboxylic acid, 4,439;- methylenebis- Benzenamine, and 1,12-di(2-nitro-3-ethoxyphenoxy) Dodecane, with percentages of 1.15%, 3.47%, and 19.79% respectively. 7.2.6.2

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Table 6: Compounds identified from ROC using GC-MS before microalgae treatment

S. No	R. time	Area %	Compound name
1	14.599	1.15	1,4-Benzenedicarboxylic acid, dimethyl ester
2	21.369	75.59	(Decanedioic acid, bis(1,2,2,6,6-pentamethyl-4-piperidinyl) ester
3	22.686	3.47	Benzenamine, 4,4'-methylenebis-
4	25.572	19.79	Dodecane, 1,12-di(2-nitro-3-ethoxyphenoxy)-

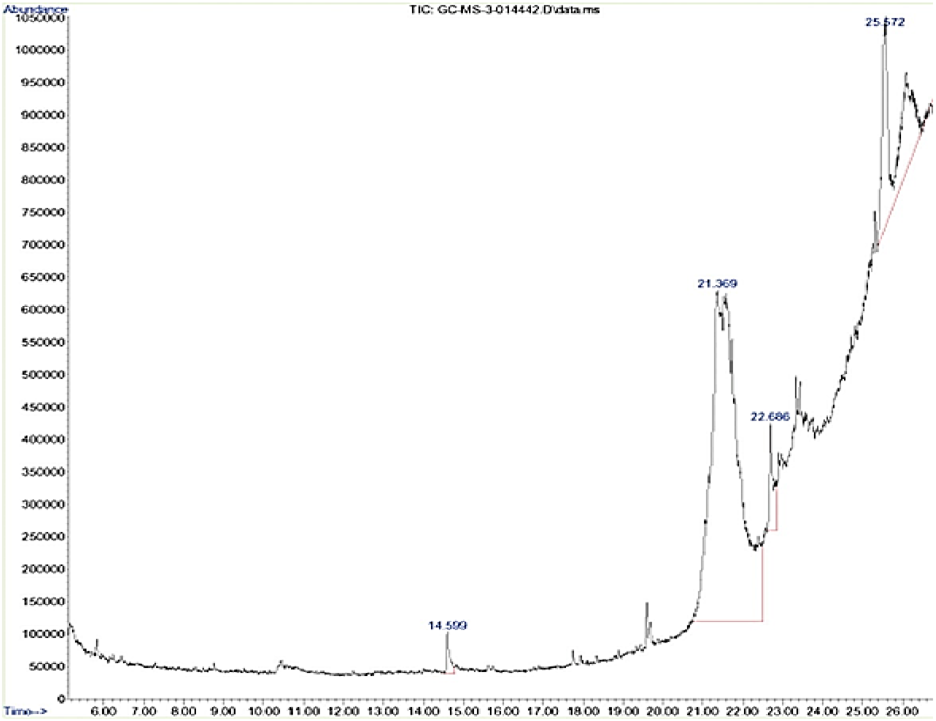

Fig: 1. GC-MS chromatogram of untreated Reverse Osmosis Concentrate

After treatment

The compounds identified through GC-MS analysis following treatment of microalgae, *C. vulgaris*, are presented in Table 7. Upon analysis, the GC-MS chromatogram (Fig.2) revealed the presence of five distinct peaks, each with their respective retention times of 12.452, 14.600, 15.732, 16.764, and 20.069. It was found that the compound 1-4 Benzene dicarboxylic acid, dimethyl ester accounted for a significant portion of the area, specifically 73.99%. Other compounds found in ROC included Pentafluorochlorodimethyl trisulfide, phthalic acid, 3-methylbenzyl dodecyl ester, cyclic octaatomic sulphur, and Pregna-1,4-diene-3,20-dione, 21-hydroxy-11.beta.,17-bis(trimethylsiloxy)-, bis(O-methyloxime) bis(trimethylsilyl) phosphate (ester). These compounds made up 5.80, 6.35, 6.52, and 7.34% of the mixture, respectively.

Table 7: Compounds identified from ROC using GC-MS after microalgae treatment

S. No	R. time	Area %	Compound name
1	12.452	5.80	Pentafluorochlorodimethyl trisulfide
2	14.600	73.99	1,4-Benzenedicarboxylic acid, dimethyl ester
3	15.732	6.35	Phthalic acid, 3-methylbenzyl dodecyl ester
4	16.764	6.52	Cyclic octaatomic sulfur
5	20.069	7.34	Pregna-1,4-diene-3,20-dione, 21-hydroxy-11.beta.,17-bis(trimethylsiloxy)-, bis(O-methyloxime) bis(trimethylsilyl) phosphate(ester)

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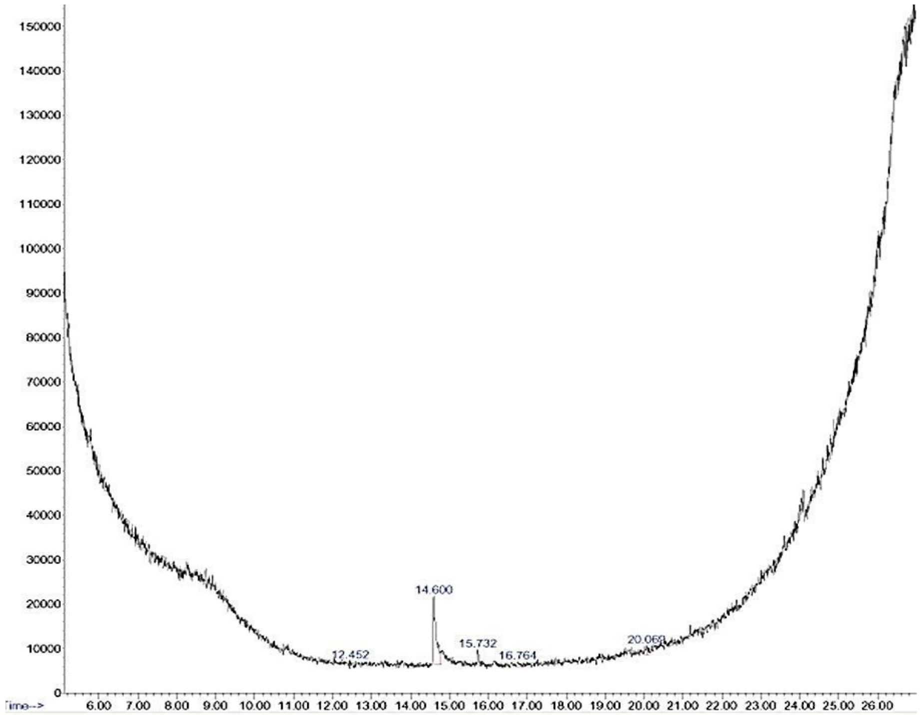

Fig: 2. GC-MS chromatogram of treated Reverse Osmosis Concentrate

Analysis using FTIR

Prior to treatment

In the analysis, it was observed that the untreated ROC exhibited significant stretching vibrations at specific wavelengths. These vibrations indicated the presence of certain chemical groups, such as hydroxyl group, H-bonded OH stretch, methylene C-H stretch, isocyanate (-N=C=O asymmetric stretch), and amide groups (Fig.3a). Upon analysis, it was discovered that the untreated wastewater contained polysulfides. These were identified by the presence of peaks at specific wavelengths, including 424.00 cm−1, 485.20 cm−1, 494.12 cm−1, 481.34 cm−1, 415.21 cm−1, 440.77 cm−1, and 471.18 cm−1. Peaks at 515.99 cm−1, 547.86 cm−1, 576.04 cm−1, 576.04 cm−1, 527.76 cm−1, 568.24 cm−1, and 516.99 cm−1 represent the aliphatic iodo compounds. 7.2.7.2

After treatment

Following the treatment with microalgae, a subtle change in the wavelength occurred, resulting in the emergence of peaks at 3360.25 cm−1, 2840.81 cm−1, 2160.06 cm−1, and 1646.43 cm−1. These peaks suggest the existence of the OH group of phenol and alcohol, the symmetric stretch of methyl C-H, the terminal alkyne (monosubstituted), and the stretch of alkenyl C=C, respectively. The FTIR spectrum of treated wastewater showed a significant reduction in polysulfides and complete removal of aliphatic bromo compounds. This was evident from the absence of peaks at 671.91 cm−1, 657.00 cm−1, and 609.00 cm−1 in the treated wastewater (Fig.3b). In addition, there was a noticeable decrease in peaks at 506.99 cm−1, 516.78 cm−1, 538.48 cm−1, and 546.10 cm−1 in the treated samples, indicating the removal of aliphatic iodo compounds.

Fig: 3. FTIR analysis of untreated Reverse Osmosis Concentrate

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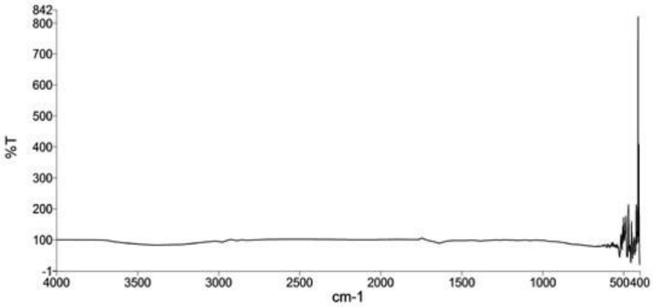
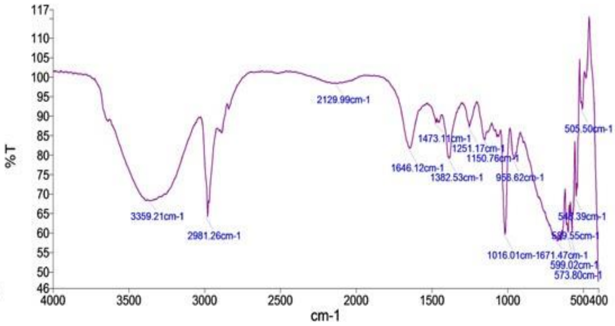


(a) **Before treatment**



(b) After treatment

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Biomass productivity

A standard graph was plotted to analyse the relationship between the absorbance read at 680 nm and the known concentration of algal biomass, in order to assess its productivity. The calculated linear regression equation is y = 0.0438x + 0.0086, and it has an R^2 value of 0.974 (Fig.4). Table 8 presents the rise in algal biomass concentration over the course of cultivation days.

Table 8: Microalgae biomass productivity concerning cultivation days

S.No	Cultivation period	Absorbance (680 nm)	Biomass (g/L)
1	0^{th} Day	0.07	1.000
2	1^{st} Day	0.11	1.200
3	2^{nd} Day	0.14	1.800
4	3^{rd} Day	0.17	2.200
5	4^{th} Day	0.20	2.800
6	5^{th} Day	0.24	3.400
7	10^{th} Day	0.33	4.200
8	15^{th} Day	0.37	5.100
9	20^{th} Day	0.42	5.800

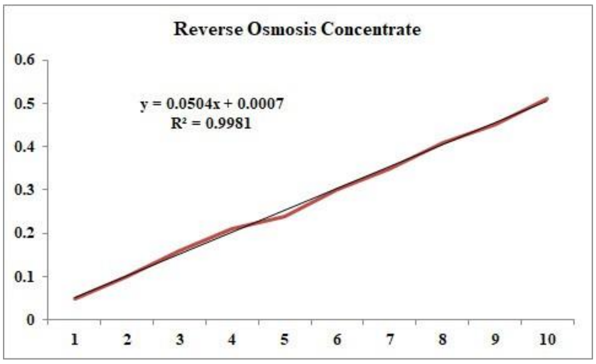

Fig: 4. Linear Regression of Biomass productivity

Table 7: Intra-specific relationship between microalgae biomass production and physico-chemical and chemical contaminants

	Mass	pH	Alk	DO	TDS	TS	TSS	EC	BOD	COD	N	P	Cl	Ca	Zn	Fe	Cu
Mass:	1																
pH:	.951**	1															
Alk:	.980**	.939**	1														
DO:	.970**	.928**	.980**	1													
TDS:	-.985**	-.959**	-.966**	-.960**	1												
TS:	-.996**	-.959**	-.983**	-.981**	.990**	1											
TSS:	-.993**	-.949**	-.985**	-.983**	.974**	.996**	1										
EC:	-.992**	-.934**	-.970**	-.967**	.985**	.987**	.978**	1									
BOD:	-.984**	-.945**	-.997**	-.975**	.966**	.987**	.990**	.967**	1								
COD:	-.984**	-.947**	-.993**	-.995**	.976**	.990**	.989**	.980**	.988**	1							
N:	-.996**	-.961**	-.984**	-.973**	.992**	.994**	.986**	.993**	.982**	.989**	1						
P:	-.973**	-.960**	-.990**	-.973**	.963**	.984**	.987**	.952**	.994**	.984**	.974**	1					
Cl:	-.974**	-.958**	-.934**	-.922**	.980**	.969**	.953**	.979**	.934**	.945**	.978**	.929**	1				
Ca:	-.983**	-.930**	-.946**	-.942**	.979**	.976**	.965**	.990**	.943**	.959**	.984**	.930**	.989**	1			
Zn:	-.986**	-.915**	-.986**	-.972**	.962**	.979**	.980**	.989**	.981**	.983**	.984**	.962**	.951**	.969**	1		

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Fe:	-.985**	-.941**	-.991**	-.988**	.980**	.988**	.983**	.987**	.983**	.997**	.993**	.974**	.954**	.969**	.988**	1	
Cu:	-0.659	-0.527	-0.542	-0.543	0.633	0.618	0.603	.707*	0.542	0.560	0.634	0.497	.732*	.743*	0.664	0.592	1

**Correlation is significant at the 0.01 level (2-tailed)

Correlation analysis

The correlation between the microalgal biomass production and the physic-chemical variables of ROC was carried out. The Pearson's correlation revealed the positive association of the production of algal biomass with pH (0.951**), alkalinity (0.980**), DO (0.970**). Negative correlation was observed with TDS (-0.985**), TS (-0.996**), TSS (-0.993**), EC (-0.9993**), BOD (0.984**), COD (0.984**), N (-0.976**), P (-0.973**), Cl (-0.974**), Ca (-0.983**). The heavy metals also showed negative correlation with calculated values for Zn (-0.986**), Fe (-0.985**) and Cu (-0.659**). The correlation of the algal biomass with the evaluated physic-chemical parameters was significant at $p < 0.01$.

Discussion

The membrane filtration process utilised in Reverse Osmosis technology for the purification of drinking water produces a concentrate that contains a higher concentration of dissolved organic and inorganic substances compared to the original water source (Umar et al., 2015). When released into the environment without proper treatment, this concentrate, known as reverse osmosis concentrate (ROC), can lead to health and environmental issues. This is because it contains toxic organic and inorganic substances. Untreated disposal of ROC into water bodies is a significant cause of eutrophication, a pressing environmental concern. Therefore, it is crucial to ensure the appropriate pretreatment of ROC in order to safeguard the environment and promote sustainable development (Zhong et al., 2019). Utilising waste water reclamation alongside biomass production can be a highly effective strategy for achieving a circular economy solution. The nutrients that are typically disposed of in effluents can be recovered through the production of biomass. In addition, a decrease in greenhouse gas emission was accomplished as microalgae utilise CO2 as a carbon source during photosynthesis and transform it into biomass (Sayre, 2010). In this study, microalgae *C. vulgaris* were used to treat ROC. The ROC was gathered from five distinct mineral water plants located in and around Kumbakonam. The physicochemical properties of the ROC were assessed, including pH, BOD, COD, dissolved oxygen, total solids, total suspended solids, electrical conductivity, total dissolved solids, and alkalinity. In addition, the evaluation also included the analysis of micro and trace elements like calcium, phosphate, nitrate, chloride, iron, chromium, mercury, zinc, copper, lead, and cadmium. No traces of lead or cadmium were found in the ROC. Utilising ROC for the growth of microalgae results in significantly higher production of algae biomass compared to using wastewater. The increased biomass production by ROC was attributed to the concentrated form of secondary effluent (Wu et al., 2014). In a study conducted by Wang et al. (2016), a biomass concentration of 318.7 mg/L was achieved using a batch process over a period of 16 days. However, the presence of toxic chemicals can pose a challenge to the growth of microalgae, even though ROC contains essential nutrients that are beneficial for their growth (Zhang et al., 2017). In addition, the level of ROC has a significant impact on the development of microalgae. The highest amount of microalgae biomass was obtained when using a ROC concentration of 25-30% (Matos et al., 2017). Therefore, in the current study, a 30% ROC was used for the cultivation of microalgae. The pH of the untreated ROC was measured to be 6.85. Following 20 days of microalgae cultivation, the ROC observed a rise in pH to 7.74, as indicated in the table. A study by Ikehata et al. (2018) reported that *C. vulgaris* showed growth in an alkaline pH of 8.2 in ROC. In a study conducted by Chang et al. (2021), it was found that the pH of ROC increased to 7.8 after the treatment period, which aligns with the findings of the present study. The increase in pH of ROC (growth media) suggests that cell growth is occurring as a result of photosynthetic activity and the breakdown of organic acids in ROC (Cho et al., 2014). The pH level of 8.1, as observed in the study conducted by Matos et al. in 2017, aligns with the findings of our present study regarding the growth of *C. vulgaris*. Therefore, the development of microalgae relies on maintaining a stable pH in the growth medium, which is crucial for increasing the biomass concentration (Akerstrom et al., 2014). It appears that the *C. vulgaris* experienced a rapid growth phase where nutrient uptake reached its peak. Another study by Mohensi et al. (2020) reported a similar finding regarding nitrate uptake by *C. vulgaris* using batch treatment. By the 20th day of treatment, the phosphate content of ROC had been significantly reduced to 0.79 mg/L from its initial concentration of 2.43 mg/L. In a study conducted by Mohensi et al. (2020), it was noted that the phosphate content decreased to less than 1.9 mg/L after 10 days of microalgae treatment of ROC. This finding aligns with the results of the present study. Microalgae have a tendency to consume a significant amount of

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phosphate and store it in vacuoles as polysaccharides, as observed in a study conducted by Powell et al. (2011). According to Miura (2013), the presence of salinity in the medium does not affect the uptake of phosphate and nitrate. Studies conducted by Chan et al., (2014) and Yao et al., (2013) have shown that microalgae, specifically *C. vulgaris*, demonstrated high efficacy in eliminating heavy metals from ROC. The current study revealed the presence of heavy metals like zinc, copper, chromium, and mercury in the ROC. The effectiveness of *C. vulgaris* in copper removal from the ROC was remarkable, with complete elimination of copper achieved within just 48 hours of treatment. Through observation, it was found that the initial Ca^{2+} content of ROC was 282.60 mg/L. After 20 days of treatment, *C. vulgaris* consumed 63.95% of the Ca^{2}. Wang et al., (2016) reported a low Ca^{2+} content (164.1 mg/L) and removal efficiency (58.7%) by *C. vulgaris* treated ROC. It is possible that the variation in Ca^{2+} uptake is a result of the disparity in initial concentration. In addition, the pH levels of the ROC could potentially impact the absorption of Ca^{2+}. According to a study by Wang et al. (2016), adjusting the pH of the growth medium to 10 enhances the efficiency of calcium removal. In addition, the pH level of the growth medium can have an impact on the way microalgae absorb heavy metals (Suresh Kumar et al., 2015). The chloride concentration of 1632 mg/L was found to be lower compared to the chloride concentration (5480.1 mg/L) (Chang et al., 2021). There was a significant increase in chloride levels from 1632 mg/L to 945 mg/L during the rapid growth phase of microalgae. Studies have shown that the growth of microalgae, *C.vulgaris*, remains unaffected by changes in chloride concentration, even when grown in low salinity conditions (Shen et al., 2019; Chang et al., 2021). After 20 days of treatment, *C. vulgaris* was able to reduce the initial concentration of TDS from 3568.60 mg/L to 2780 mg/L. During the exponential growth phase of microalgae, researchers observed a notable decrease in the concentration of TDS. Another study reported a similar TDS content in the ROC, measuring at 3410 mg/L. However, it has been reported that *C. sorokiniana* is highly effective in eliminating 100%. The dissolved oxygen concentration of the raw ROC measured 8.14 mg/L. Microalgae had a significant impact on the dissolved oxygen (DO) content of the ROC. After 20 days of treatment, the DO concentration increased to 9.44 mg/L. In a previous study conducted by Mohseni et al. (2020), it was noted that there was a comparable rise in the dissolved oxygen concentration (9.1 mg/L) when *C. vulgaris* treatment was applied to ROC. In a study conducted by Matos et al. (2017), they found that the dissolved oxygen (DO) concentration in ROC ranged from 10.1 to 12.5 mg/L. Interestingly, our present study observed slightly lower DO content in ROC compared to their findings. Above a concentration of 0.032 mg/L, chlorella species experience significant toxicity due to the presence of copper. However, the copper concentration in ROC obtained from the water purification plant was measured to be 0.02 mg/L. Therefore, the copper concentration was found to be significantly below the toxic level, and it did not have any noticeable impact on the growth of *C. vulgaris* in terms of biomass production. In addition, the study found that copper was completely reclaimed within 48 hours of treatment, indicating that the microalgae effectively absorbed the copper. A higher electrical conductivity (EC) of ROC was observed (11.34 5.6 mS cm^{-1}) compared to the EC (5.6 mS cm^{-1}) reported by Matos et al., (2017). Indications of microalgae growth can be observed through the increase in EC of the growth medium, as demonstrated by Mostafa et al. (2012) and Chen and Oswald (1998).

Prior to treatment with *C.vulgaris*, the GC-MS analysis of ROC revealed the presence of 25 distinct compounds. The most prominent compound, occupying 44.87% of the area, was identified as Diethylmalonic acid, 3,4-difluorobenzyl heptyl ester. However, through the process of microalgae treatment, a different compound was discovered and identified as 1,4-Benzenedicarboxylic acid, dimethyl ester. This particular compound is known for its anti-hemorrhagic properties. The FTIR analysis of ROC after microalgae treatment revealed the presence of various organic compounds, including aromatic primary amine, carboxylate, aryl thioester, methyl, and alkenyl compounds. These findings support the identification of 1,4-Benzenedicarboxylic acid, dimethyl ester as determined by GC-MS analysis.

The FTIR spectrum of treated wastewater showed a significant reduction in polysulfides and complete removal of aliphatic bromo compounds. This was evident from the absence of peaks at 671.91 cm−1, 657.00 cm−1, and 609.00 cm−1 in the treated wastewater. In addition, there was a noticeable decrease in peaks at 506.99 cm−1, 516.78 cm−1, 538.48 cm−1, and 546.10 cm−1 in the treated samples, indicating the removal of aliphatic iodo compounds. The algal biomass productivity in ROC showed a rise in algal biomass concentration over the course of cultivation days. The calculated linear regression equation is $y = 0.0438x + 0.0086$, and it has an R^2 value of 0.974. The correlation study showed positive correlation with pH, alkalinity and dissolved oxygen. The other parameters evaluated in the study were negatively correlated with algal biomass production.

Conclusion

The following conclusions are made from the present study investigation. The treatment of ROC with *C.*

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vulgaris showed an increase in the pH, alkalinity and dissolved oxygen content. The solids including total solids, dissolved solids and suspended solids were considerably reduced with the treatment of microalgae. The nutrients such as chloride, nitrate, phosphate and calcium were found to be decreased. Similarly, the microalgae, *C. vulgaris* showed effective removal of heavy metals from the ROC. Hence, the study suggests that utilization of microalgae, *C. vulgaris* in the treatment of wastewater, particularly ROC could be an effective alternative in the removal of xenobiotics.

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Conflict of Interest

The authors state that they do not have any competing interests.

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