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Research Article

Biotreatment of Un-/Pre-treatment Simulated Produced Water (SPW) Using *Chlorella vulgaris*

[1]Hameed Opeyemi Ottun, *[1]Daniel Ayodele Idowu, [1]Babajide Joseph Adisa, [2]Fortune Itoje Ebiala, [3]Yusuf Yusuf Arowosaye, [4]Cornelia Gordian Inoh, [5]Benjamin Madji Larweh

About Article

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About Author
[1] Department of Chemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
[2] Department of Microbiology, University of Benin, Benin, Edo State, Nigeria
[3] Department of Environmental Engineering, Kyungpook National University, Daegu, South Korea
[4] Department of Environmental Health, Metaspec Consult Limited, Port Harcourt, River State, Nigeria
[5] Department of Biological Sciences, Akenten Appiah-Menka University of Skills Training and Entrepreneurial Development, Kumasi, Ghana

Contact @ Daniel Ayodele Idowu
idowudaniel284@gmail.com

ABSTRACT

There have been increasing environmental concerns surrounding industrial wastewater especially from oil and gas industries, which call for the need for sustainable and effective treatment strategies. This study aims to investigate the biotreatment of *Chlorella vulgaris* for simulated produced water (SPW), looking at both untreated and pretreated water. This research used a laboratory bioreactor system where *Chlorella vulgaris* was nurtured and then introduced into SPW and 3,5-dimethylphenol (DMP) based produced water. Fenton oxidation was performed on the pretreatment of SPW to assess the effects with algal treatment. The parameters like pH, chemical oxygen demand (COD), volatile organic acids (VOAs), and UV-Visible spectrometer were observed for five weeks to evaluate treatment efficiency. The results obtained showed that *Chlorella vulgaris* developed substantial growth and adaptability in various treatments with notable changes occurring in colour, pH, and biomass. COD levels were significantly reduced across all treated samples, particularly in TSPW (884 to 315.5 mg/L) and SPW (2224.5 to 199.25 mg/L), which demonstrated strong pollutant removal capacity. The levels in VOA dropped to non-detectable limits in the TSPW CV sample, and it showed a major reduction in other treatments. UV spectrometry revealed changes in absorbance patterns at 272 nm and 687 nm, indicating organic matter transformation and algal activity. In conclusion, *Chlorella vulgaris* revealed it can be effectively used in the biotreatment of simulated produced water with commendable results obtained.

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1. INTRODUCTION

Produced water, which is a byproduct of many industrial processes such as oil and gas effluents, brings a significant environmental challenge due to its complex composition and possesses contaminants that can affect natural water bodies (Smith *et al.*, 2018). An increase in the volume of produced water has increased drastically owing to a more increased demand for energy (Huang *et al.*, 2019). The release of this produced water into the environment presents a challenge for industries involved in oil and gas extraction. Various methods of produced water treatment that include the physical, chemical and biological ways often exhibit limitations in form of efficiency, cost, and environmental sustainability (Sathish *et al.*, 2020). In present times, there is an urgent need to explore methods that can effectively treat produced water which can also offer the potential for water recovery.

While aiming for sustainable industrial practices, the industries releasing this produced water has become a major concern which must be looked into (Chen *et al.*, 2020). The produced water that is often generated from various industrial processes like oil and gas extraction poses a significant threat to our environment. The high salinity from it can disrupt the local ecosystem when they are improperly discharged. Also, it often carries a heavy load of contaminants like hydrocarbons, heavy metals, and other pollutants which further causes its potential for ecological harm (Mojiri & Bashir, 2012). Noting the importance of mitigating the adverse effects of produced water, the innovative scientific experts have been at the forefront of developing sustainable and environmentally friendly solutions. Treatment methods based on microalgae have recently attracted a lot of interest in treating municipal, industrial, and agro-industrial wastewater. Through the removal of contaminants, microalgae like *Chlorella vulgaris* reduce the danger of eutrophication (Alazaiza *et al.*, 2023; Dayana *et al.*, 2021). The use of microalgae like *Chlorella vulgaris* have become one of the leading discoveries within specialized bioreactor systems. Therefore, this study aim is to assess the effectiveness of using *Chlorella vulgaris* in a bioreactor system for treating produced water.

2. LITERATURE REVIEW

Chlorella vulgaris possesses remarkable properties that make them well suited for tackling the challenges brought about by produced water from the oil and gas industries. These organisms are known to be highly efficient in removing contaminants from water which is due to their ability to absorb and dissolve a wide range of pollutants, including hydrocarbons and heavy metals. *Chlorella vulgaris* can survive in high salinity water, making them an ideal choice for treating the rich salinity produced water (Abunada *et al.*, 2020). Bioreactor systems provide a controlled environment where microalgae can flourish and effectively perform their remediation tasks. These systems come in various dimensions including open pond systems, closed photobioreactors and raceway ponds with its own advantages and limitations (Jalilian *et al.*, 2020). The choice of using bioreactor system depends on factors like the scale of the operation, the specific characteristics of the produced water, and economic importance (Kunjapur & Eldridge, 2010).

However, research on source-separated produced water treatment with microalgae-based systems is very limited and has focused solely on anaerobically pre-treated produced water (Moges *et al.*, 2020; Fernandes *et al.*, 2017). There is an increasing interest in the use of anaerobic digestion for treatment of source-separated produced water (Moges *et al.*, 2018) and, therefore, research on microalgae-based systems in relation to produced water is focused on treating nutrient rich effluents from anaerobic digestion (Moges *et al.*, 2020). The usage of microalgae in produced water treatment has significant advantages in comparison with other methods of removing biogenic substances. Namely: effective and simultaneous removal of nitrogen and phosphorus without reagents management facilities, oxygen formation. During the life of microalgae absorb carbon dioxide. Another advantage of microalgae is the integration of produced treatment processes and carbon dioxide biofixation, especially in industrialized regions (Molazadeh *et al.*, 2019). It should be readily accessible, cheap, sustainable, and easily regenerated, while also demonstrating high selectivity for the target substances (Idowu *et al.*, 2025). The results of research show that the usage of microalgae of the species *Chlorella vulgaris* to get the effect of purification of domestic produced from nitrogen and phosphorus compounds by more than 95 % (Mayhead *et al.*, 2018). The high efficiency of using *Chlorella vulgaris* to remove nutrients from industrial wastewater in both aerobic and anaerobic conditions (Amenorfenyo *et al.*, 2019; Szwarc *et al.*, 2020; Khalekuzzaman *et al.*, 2019) and for purification landfill filtrates with high concentrations of ammonium nitrogen and chemical oxygen demand (COD) were proven (Pereira *et al.*, 2016).

3. METHODOLOGY

3.1. Sample collection

Simulated produced water (SPW), chemically treated SPW (TSPW), and 3,5-dimethylphenol (DMP) were prepared in the TETFUND NRF 2020 Chemistry laboratory, and were collected. DMP is the main component of simulated produced water. Digital pH meter was calibrated using buffer solution of pH 4,7,10 and pH value of the samples were adjusted to pH 7-8 using 0.1 M NaOH and 0.1 M H_2SO_4. Other parameters like COD, VOA and UV analysis were carried out before treated with C. vulgaris. Freshwater algae *chlorella vulgaris* was collected from Veterinary Microbiology Laboratory, FUNAAB. The 7-days old algal cells were collected and inoculated into 2 L Erlenmeyer flasks filled with 1 L of BBM solution under natural lights and 25 ℃ for 9 days.

3.2. Bioreactor set-up

The 50 mL volume bottle containing *chlorella vulgaris* strain and simulated produced water was used in the ratio of 1:10 i.e 2.5 mL of the algae and 25 mL of simulated produced water covered with cotton wool. All the experiments were carried out using 50 mL bottles in duplicates. *Chlorella vulgaris* from the stock solution was centrifuged (SL8 centrifuge, Thermo Scientific) at 7500 rpm for 10 minutes. The supernatant was discarded, and algal biomass was resuspended with a minimum amount of BBM media. The optimum amount of algae for treatment was obtained in the ratio 1:10.

3.3 SPW pretreatment before biological treatment

A Fenton oxidation experiment was carried out using 300 mL wastewater based on the optimum conditions determined from preliminary electrolytic experiments in the 500 mL flask. The Fenton process consists of four stages: pH adjustment, oxidation, neutralization and sedimentation. Firstly, the pH of the treated wastewater was adjusted to 3.0 with sulphuric acid. Immediately, a known volume of H_2O_2 was added. All these operations were carried with 800 rpm agitation speed to provide rapid mixing of reagents. In the case of experiments at acidic conditions, Fe^{2+} was oxidized by H_2O_2 to ferric ion by dissolved oxygen. After the desired duration of oxidation, calcium oxide was added to adjust the pH to 8.0 – 9.0. The solution was allowed to settle for 1 h, the supernatant collected and its COD, VOA and UV were detected.

3.4. Analysis of samples

3.4.1. Procedure for carrying out COD

COD was carried out using spectroquant colorimeter move 100 with measuring range: 300-3500 mg/L COD or O_2 16-mm cell containing COD standard reagents of sulphuric acid, mercury (II) sulphate. The bottom sediment in the reaction cell test were suspended into two cells by swirling and 2.0 mL of each of the samples (DMP, SPW, TSPW) were pipetted into each reaction cells and closed tightly with screw caps and mixed each of them vigorously, 2.0 mL of distilled water was pipetted into another reaction cell (blank cell) and closed tightly with the screw cap and mixed vigorously.

Each of the reaction cells with samples and blank cell were heated in the thermoreactor TR320 for digestion at 148 ℃ for 2 h. The reaction cells were then removed from the thermometer TR320 and placed in a test tube rack for cooling. The reaction cells were swirled after 10mins of cooling and replaced back to the test tube rack for cooling to room temperature and COD was determined.

3.4.2. pH and physical change

The pH was monitored using glass electrode pH meter for 5 weeks and the physical change was monitored as well over the period of weeks.

3.4.3. Procedure for carrying out VOA

VOA was carried out using spectroquant colorimeter move 100 with volatile Organic Acid Standards: ethylene glycol, NaOH, H_2SO_4 (OA-1 to OA-5), 0.75 mL of reagent OA-1 were pipetted into cleaned round cells consecutive then 0.50 mL of reagent OA-2 were pipetted into the same round cells then 0.5 mL of pretreated samples were added into each solution and closed tightly and mixed.

The cells were heated in a thermo-reactor TR320 for 15 mins, then cooled to a room temperature under running water, after cooling 1.0 mL of reagent OA-3 were added into each round cells containing the solution of reagents OA-1, OA-2 and sample, followed by 1.0 mL of reagent 0A-4 and finally 1.0 mL of reagent of OA-5 were added. They were left to stand for 1min (reaction time), then the samples were measured in the photometer.

4. RESULTS AND DISCUSSION

4.1. pH Analysis

The pH of the treatment system was continuously monitored throughout the experiments. Results in week 0, showed that DMP 1 & 2 were within the set control (7) except SPW 1 & 2 with level of pH (9). First week, DMP 2, SPW 1 & 2 except DMP 1 (8, 9, 9 respectively) were all above the set control (7). Third week showed that all parameters including DMP 1&2 and SPW 1 & 2 (9.50, 9.97, 9.45, 9.44 respectively) were above the control (9.04). Fourth week showed all were within the control (9) except for SPW control (7). Fifth week showed that all were within the set control (7) except SPW 1 & 2 which were above but within the SPW control (8).

The fluctuations in pH levels observed during the experiment can be attributed to the photosynthetic activity of *Chlorella vulgaris*. As the microalgae photosynthesize and consume carbon dioxide, the pH increases. Conversely, at night or in the absence of light, pH levels decreases due to respiration. In DMP & SPW samples, the fluctuating trends observed in the DMP and SPW samples reflects the dynamic nature of produced water and its treatment by *Chlorella vulgaris*. The flunctuations suggest that the microalgae's metabolic activities, including photosynthesis and respiration, affect pH levels. The slightly constant trend in SPW 1 and SPW 2 samples, with a slight decrease in the fourth and fifth weeks, indicate that *Chlorella vulgaris* may have reached a stable state with these types of produced water. This stability might be linked to a more efficient treatment process due to the microalgae's adaptation to the specific conditions of SPW 1 and SPW 2.

The inconsistent trend shown in TSPW 1 in (Table 2) possibly showed that the responses observed by the *Chlorella vulgaris* is due to variations in the produced water. The value of 8 observed can be an adaptation stage where the *Chlorella vulgaris* adjust to the produced water treatment. The decrease to 7 observed in the third week can be as a result showing the period of stress or decreased metabolic activity by the *Chlorella vulgaris*. The increase to 8.2 observed in the fourth week showed a recovery stage or an adaptation to the changing produced water treatment. The decrease back to 7 observed in the fifth week can be indications of ongoing irregularities in treatment efficiency. The slight irregular trend in TSPW 2 in (Table 2) with a peak in the third and fourth weeks, shows a more stable response of *Chlorella vulgaris* to the produced water conditions. The consistent increase from 7 to 9 in the fourth week followed by a decrease to 8 in the fifth week indicate a phase of active growth and possible pollutant removal.

Table 1. pH of samples reflecting the metabolic activities of *Chlorella vulgaris* in SPW and DMP.

Week	Control	DMP 1	DM 2	SPW 1	SPW 2	DMP Control	SPW Control
0	7	6	6	9	9	-	-
1	7	7	8	9	9	7.9	7.5
2	9.04	9.50	9.97	9.45	9.44	7.99	9.13
3	9	9	9	9	9	7	9
4	7	7	7	8	8	7	8

Table 2. pH of the treatment system reflecting the metabolic activities of *Chlorella vulgaris* in TSPW1 and TSPW2.

Week	TSPW 1	TSPW 2
O	8	7
1	8	8
2	7	8
3	8.20	9.0
4	7.0	8.0

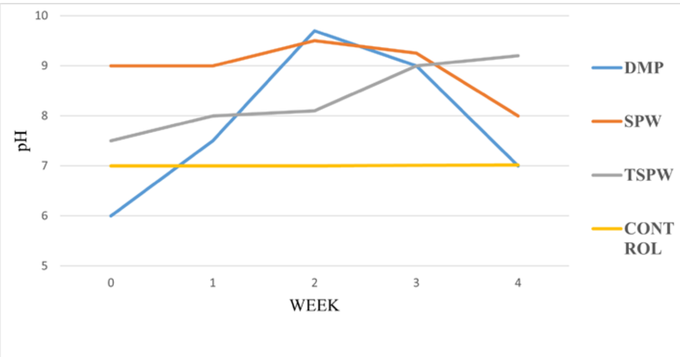

Figure 1. Average pH of samples reflecting the metabolic activities of *Chlorella vulgaris* in SPW, DMP and TSPW

4.2. Physical appearance

Visual observations of the treated produced water were recorded. Results in week 0, showed that only DMP 1 was within the set control (Light green), while DMP 2, SPW 1 & 2 had variation in their colour. First week, only DMP 1 was within the set control (Light green) while DMP 2, SPW 1 & 2 had variation in their colour. Second to fourth week showed that DMP 1 & 2 were within the set control (Light green) while SPW 1 & 2 had (Yellowish brown) colour. Growth condition from week 0 to third week showed that *Chlorella vulgaris* was alive in DMP, SPW, and TSPW. Fourth week showed that *Chlorella vulgaris* was gradually dying in DMP, dead in SPW and alive in TSPW. Fifth week showed that *Chlorella vulgaris* had died in DMP and SPW, but remained alive in TSPW.

A colour change observed towards green shows increased chlorophyll production suggesting the active growth of *Chlorella vulgaris*. The texture changes such as flocculation points to the coagulation of *Chlorella vulgaris* biomass. In DMP 1 and DMP 2 observed in (Table 3) shows light green colour as being observed that the presence of DMP does not impact the appearance of the solution. The colour change in DMP 2 from green to light green shows a possible adaptation of *Chlorella vulgaris* to the initial exposure. In SPW 1 and SPW 2 observed in (Table 3) shows more notable changes in colour. The changes observed from deep green to greenish yellow and from yellowish brown also indicate that *Chlorella vulgaris* was responding to the presence of produced water contaminates possibly by altering its pigment production or cellular composition. The colour changes observed can be linked to the *Chlorella vulgaris* ability to adapt and remove certain components in the produced water making it an essential aspect of the produced water treatment.

Table 3. Visual observations of the treated waste

Week	Control	DMP 1	DMP 2	SPW 1	SPW 2
0	Light	Light	Green	Deep	Deep
	green in	green in		green	Green
	colour	colour			
1	Light	Light	Green	Greenish	Greenish
	green	green		yellow	Yellow
2	Light	Light	Light	Yellowis	Yellowi
	green	green	green	brown	h brown
3	Light	Light	Light	Yellowish	Yellowish
	green	green	green	brown	brown
4	Light	Light	Light	Yellowish	Yellowish
	green	green	green	brown	brown

Table 4. Growth condition of *chlorella vulgaris* over 5 weeks

Week	DMP	SPW	TSPW
0	Alive	Alive	Alive
1	Alive	Alive	Alive
2	Alive	Alive	Alive
3	gradually dying	Dead	Alive
4	Dead	Dead	Alive

4.3. Chemical Oxygen Demand (COD) Analysis

The COD of the treated produced water was determined using standardized methods and recorded. Results below showed that the highest was recorded in SPW (2224.5 mg/L) while the lowest COD was recorded in DMP CV (192.75 mg/L).

The SPW sample which represent simulated produced water exhibited the highest COD value among all the samples. This observation indicates that SPW contain a substantial amount of organic and inorganic matter which is a typical produced water from industrial processes. The high COD value in SPW highlight the contaminate load that need effective treatment making it a suitable choice. In DMP and TSPW, while lower in COD compared to SPW demonstrate the presence of significant organic and inorganic matter. DMP CV, SPW CV and TSPW CV where *Chlorella vulgaris* was introduced into the produced water simulants showed lower COD values compared to the ones without *Chlorella vulgaris.*

Table 5. Result showing COD values

Samples	**COD mg/L**
DMP CV	192.75
SPW CV	199.25
TSPW CV	315.5
TSPW	884
DMP	1720.5
SPW	2224.5

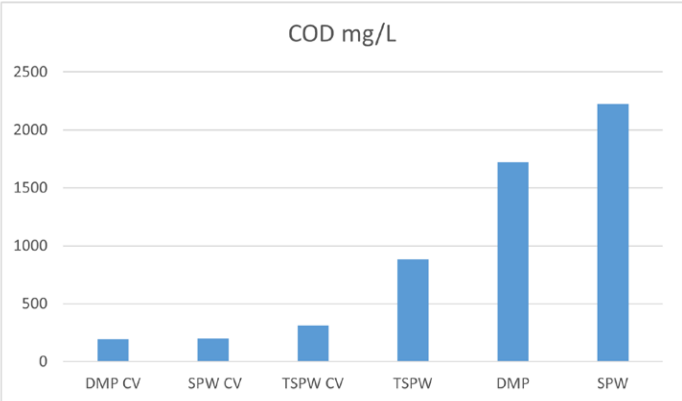

Figure 2. Reduction in COD levels observed demonstrating the potential for pollutant removal

4.4. Volatile Organic Acid (VOA) Analysis

This was employed to identify and quantify volatile organic acids present in the treated produced water. Results showed that highest VOA was recorded in TSPW (731 mg/L) while the lowest was recorded in DMP CV & TSPW CV (0 mg/L).

DMP and DMP CV showed no detectable VOA values, which indicate the absence of volatile organic acids. This shows that either the nature of the produced water used in the experiment or the *Chlorella vulgaris* treatment process was effectively removed or neutralized VOAs. The TDMP exhibited a VOA value which indicate the presence of volatile organic acids. The SPW had a significantly high VOA value compared to SPW CV, which describes the untreated produced water having a high level of VOAs. The *Chlorella vulgaris* treatment in SPW CV slightly reduced the VOAs but they were still present. TSPW showed the highest VOA value among all samples, which indicate a high level of VOAs. TSPW CV displayed no detectable VOA values which indicated the absence of volatile organic acids.

Table 7. Result showing VOA values

Wastewater	**VOA (mg/L)**
DMP CV	0 (<50)
TDMP	84
SPW	628
SPW CV	42
TSPW	731
TSPW CV	0

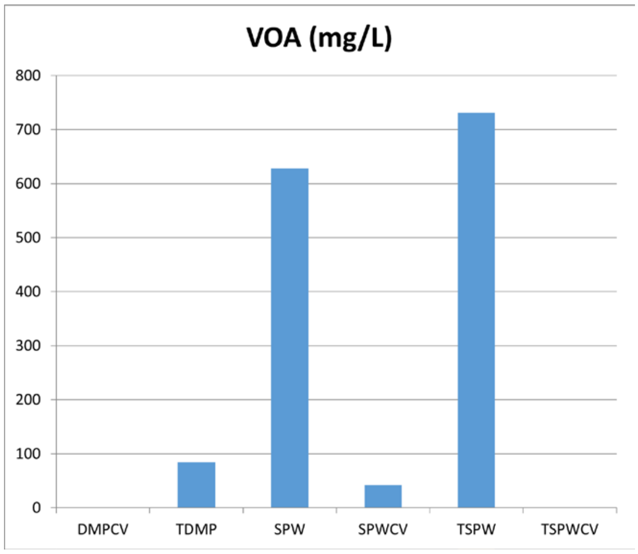

Figure 3. Reduction in VOA levels observed

4.5. UV Spectrometry analysis

The UV Spectrometry analysis is an important method for assessing the treatment process efficiency. Results showed the absorbance of light was measured at different wavelengths between 272 nm and 687 nm for five consecutive weeks. AVDMP CV, AV SPW CV, and DMP showed lower absorbance in the UV region (200-300 nm).

In AVDMP CV, the upward trend indicate an increasing variability of the samples and this could be an important factor, as this changes also reflected *Chlorella vulgaris* response to the produced water treatments. In AVSPW CV, the upward trend give an increasing variability in the spectral. In DMP, the irregularities shown over the five weeks gave different variations in the samples. In SPW, The general upward trend indicate an increase in the spectral of the samples. In AVTSPW CV, the upward trend until the third week followed by a slight decrease in the fourth week gave an increasing variability in the total spectral power weight of the samples over time. The decrease in variability in the last week indicate a stabilization of the process.

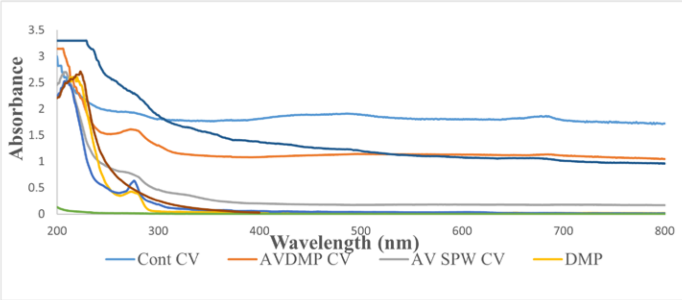

Figure 4. UV-Visible appearance at 272 nm and 687 nm for 5 weeks

4.6. Discussion

pH recorded in this study slightly correlates with (Alazaiza *et al*., 2023) who stated the initial pH varied between 7.38 and 7.78 using all wastewater mixing ratios (50%, 60%, 70%, 80%, and 90%). Between the 2nd and 7th day of aeration, the pH rose to 8.03 and 8.38 utilizing a wastewater mixing ration higher than 60%, while the highest pH value (8.64) was reported at day 14 of aeration using the 80% mixing ratio. Microalgae have been utilized to treat wastewater because of their ability to utilize both organic and inorganic carbon, nitrogen, and phosphorus while also accumulating biomass and reducing N, P, and chemical oxygen demand (COD) in the produced water (Ferro *et al*., 2018). COD obtained in this study was lower than (Rani *et al.* 2021) who observed synergistic connections between a microalgae strain and bacteria. According to the study, two days of incubation with 700 mg/L microalgae and 200 mg/L sludge resulted in a 67% elimination of COD. Since algae's photosynthesis produces a lot of oxygen without using any electricity and also absorbs nutrients, algae ponds are a desirable solution for wastewater treatment (WWT) to eliminate impurities. Microalgae are particularly appealing for bio-treatment because they are capable of photosynthesizing, transforming solar energy into useful biomasses, and incorporating nutrients that cause eutrophication (Nu and Buma, 2019). Studies related the removal of phosphorus, nitrogen, and ammonia by algae with the quantized fixation of CO2. For example, the treatment of industrial produced water using Chlorella sp. was reported by (Tarlan *et al.* 2002), They revealed that Chlorella sp. was able to treat 80% of absorbable organic xenobiotics, 58% of COD, and 84% of colour. Due to the photosynthesis of microalgae, oxygen is released, which is used by activated sludge for the decomposition of organic substances in the processes of biological wastewater treatment. This allows to reduce energy consumption for traditional produced water aeration (Muylaert *et al*., 2019; Wang *et al*., 2017). The use of microalgae is a way to dispose of valuable impurities in produced water. The obtained biomass use as animal feed, biofertilizer, production of biologically active substances in the pharmaceutical and cosmetic industries (Renuka *et al*., 2015; Wang *et al*., 2017).

5. CONCLUSION

The experiment conducted in this study yielded several reasonable results. This demonstrated its potential for pH regulation in produced water treatment processes. The physical observations of the treated produced water indicate changes in colour and texture observed which proved active growth and adaptation of *Chlorella vulgaris* in the treatment environment. The treated produced water exhibited a reduction in COD levels which indicated the potential of *Chlorella vulgaris* to remove organic and inorganic contaminants. This reduction described the promises of *Chlorella vulgaris* treatment for contaminant removal in produced water streams. The analysis of the treated produced water identified and quantified volatile organic acids. This research also provides insight into understanding the metabolic pathways of the Chlorella vulgaris and optimizing treatment conditions. In conclusion, *Chlorella vulgaris* showed a promising method for treating simulated produced water and the ability to regulate pH, display physical indicators of growth, reduced COD, and produced volatile organic acids that suggests its potential applications.

RECOMMENDATIONS

Though this study provided valuable research, there is still room for further investigation and improvement. Further research should still be conducted to assess the long-term performance of *Chlorella vulgaris* in produced water treatments. Experiments with variations in light intensity, temperature and nutrient supply to enhance growth and pollutant removal should be done. Also, the scope of this study should be expanded by experimenting with different types of produced water streams. Understanding how *Chlorella vulgaris* perform under various conditions and produced water compositions will also be valuable. The feasibility of scaling up *Chlorella vulgaris* treatment systems for industrial applications should be investigated while development of robust monitoring and control systems that can maintain favourable conditions for *Chlorella vulgaris* growth and treatment performance. Conducting thorough environmental impact assessments to evaluate the ecological implications of using *Chlorella vulgaris* in produced water treatment. Assess the potential benefits and risks associated with the introduction of *Chlorella vulgaris* into different ecosystems.

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