Water Science and Engineering xxxx, xxx(xxx): xxx

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Rapid anaerobic bioreduction of 2-chlorophenol from water using biofilms supported on graphene oxide layers

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Received 14 November 2025; accepted 16 July 2026
Available online ■ ■ ■

Abstract

Chlorophenols are recognized as persistent environmental contaminants due to their toxicity and resistance to biodegradation. In particular, 2-chlorophenol (2-CP) is of concern because of its high aqueous solubility and tendency to accumulate in aquatic environments. This study proposed an anaerobic removal approach for 2-CP using a tubular ceramic-supported graphene oxide membrane (TCSGoM) bioreactor, which integrates separation and biological processes in a single operational unit. Anaerobic sludge was inoculated into the TCSGoM bioreactor to establish biofilm growth and was acclimated to 2-CP before evaluation under steady-state conditions with stepwise increases in 2-CP concentration. The system achieved 100% removal at feed concentrations up to 50 mg/L, with performance gradually declining at higher concentrations, reaching 24% removal at the tested maximum concentration of 80 mg/L. The results indicate that, during the start-up phase, initial 2-CP removal is primarily driven by adsorption onto the graphene oxide layer, while sustained removal over time is attributed to biofilm-mediated processes under quasi-steady-state operation. The dominant microbial populations were identified as *Comamonas* and *Cupriavidus*, both known for their capacity to degrade chlorinated phenolic compounds. Although high removal efficiencies were achieved, transformation products were not analyzed if any was present, which limits the confirmation of the degradation pathway and the extent of mineralization. The proposed system significantly reduces the acclimation period required for conventional anaerobic reactors and has the potential to reduce or even eliminate the need for post-treatment steps, demonstrating that it is an efficient and sustainable alternative for 2-CP removal compared to conventional methods.
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Keywords: Anaerobic process; 2-chlorophenol; Tubular membrane bioreactor; Graphene oxide; Wastewater treatment

1. Introduction

Chlorophenols are synthetic organic compounds that have received attention due to their persistence in the environment and widespread industrial use. These compounds consist of a phenolic ring substituted with one or more chlorine atoms and are employed in the production of pesticides, herbicides, disinfectants, and wood preservatives and in the pulp and paper bleaching process (Olaniran and Igbinosa, 2011; Jensen, 1996; Yadav et al., 2023). Increasing chlorination enhances their antimicrobial and pesticidal properties but reduces their biodegradability, thereby classifying them as priority contaminants (Yadav et al., 2023; Van Aken et al., 2019). Contamination by chlorophenols is common in soil and aquatic systems, mainly due to industrial discharges, improper waste management, and by-products from water chlorination (Olaniran and Igbinosa, 2011; Igbinosa et al., 2013).

The environmental and health impacts of chlorophenols are significant. They are toxic to aquatic and terrestrial organisms, and both acute and chronic exposure can induce physiological and biochemical alterations in humans and wildlife (Igbinosa et al., 2013; Wang et al., 2020b). Animal studies have associated exposure with hepatoxicity, nephrotoxicity, and endocrine disruption (Ingerman et al., 2001; Sarkar et al., 2022). Moreover, under high-temperature conditions, chlorophenols can generate dioxins, thereby exacerbating their environmental

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Peer review under responsibility of Hohai University.

https://doi.org/10.1016/j.wse.2026.08.003
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impacts (Beigh, 2024). These toxic and persistent characteristics highlight the need for effective and sustainable treatment methods for chlorophenol-containing wastewater.

Among these compounds, 2-chlorophenol (2-CP) is of particular environmental concern due to its chemical stability and solubility, which contribute to its persistence in aquatic ecosystems. It consists of a phenolic ring with a chlorine atom at the ortho position and exhibits high water solubility (approximately 28 g/L at 20°C) (Czaplicka, 2004; Singh and Chandra, 2019). The strong C—Cl bond in its structure renders it highly resistant to chemical and biological degradation, leading to its accumulation in the environment and posing risks to both aquatic life and human health (Michałowicz and Duda, 2007; Rao, 2003).

Conventional treatment methods, such as chemical oxidation, adsorption, and incineration, are often costly, energy-intensive, and prone to generating secondary pollutants (Buenaobra et al., 2025; Wang et al., 2023; Tazik et al., 2023). For instance, adsorption processes require adsorbent regeneration or disposal, oxidation may produce hazardous by-products, and incineration generates toxic emissions such as dioxins. In contrast, biological degradation provides a more sustainable and efficient alternative, using microorganisms to transform 2-CP into less toxic or harmless compounds.

Previous studies have demonstrated the feasibility of 2-CP biodegradation through bacterial and fungal systems (Yadav et al., 2023). Bacterial strains such as *Stenotrophomonas maltophilia* KB2 and *Pseudomonas putida* ATCC 49451 have exhibited significant degradation capacities, particularly when immobilized or supplemented with co-substrates that enhance aerobic degradation (Nowak et al., 2016; Li and Loh, 2005). In contrast, fungi such as *Coriolus versicolor* and *Funalia trogii* have achieved high removal efficiencies through enzymatic or biosorption processes (Kadhim et al., 1999; Bayramoglu et al., 2009). Studies on related compounds, such as the degradation of 2,4-dichlorophenol by *Pseudomonas putida* SM1443, have demonstrated the potential of microbial systems for dechlorination and detoxification of phenolic contaminants (Quan et al., 2003). Overall, these findings confirm that microbial biodegradation is a viable approach for chlorophenol removal, especially when combined with strategies that enhance microbial stability and activity.

However, biological systems often present practical limitations, such as reliance on structurally related co-substrates, long acclimation periods, complex immobilization or enzyme extraction protocols, and extended degradation periods. Addressing these limitations requires new bioreactor configurations capable of promoting rapid biofilm establishment and stable operation under anaerobic conditions.

Recent studies have proposed integrating membrane filtration and anaerobic biodegradation within a single system by supporting graphene oxide (GO) layers on ceramic filtration elements (Amin et al., 2022). The GO coating enhances surface conductivity, hydrophilicity, and roughness, thereby facilitating microbial adhesion and electron transfer during reductive bioprocesses. Both flat and tubular configurations have been evaluated, with GO-coated ceramic membranes achieving removal efficiencies above 90% for various azo dyes due to their improved surface and conductive properties (Amin et al., 2023). In such systems, pollutant removal involves initial adsorption processes during start-up, followed by biofilm-mediated biodegradation during steady-state operation, depending on system conditions and microbial activity.

Based on these promising results, this study investigated the potential of a tubular ceramic-supported graphene oxide membrane (TCSGoM) for the anaerobic biodegradation of 2-CP. This configuration integrates filtration and biodegradation in a single unit, aiming to achieve efficient pollutant removal under fully anaerobic conditions across a wide range of operational parameters.

2. Materials and methods

2.1. Preparation of TCSGoM

2.1.1. Characteristics of ceramic support

An ultrafiltration tubular ceramic membrane (ZrO_2—TiO_2, with a molecular weight cut-off of 50 kDa, an inner diameter of 6 mm, and a length of 250 mm; TAMI industries) was used as a support for depositing the GO layer.

2.1.2. Synthesis of GO

GO powder was synthesized using the modified Hummers' method according to the procedure described by Giménez-Pérez et al. (2016). Briefly, 2.5 g of graphite powder (with particle sizes below 20 μm) and 2 g of $NaNO_3$ were added to 70 mL of H_2SO_4 (with purity of 95%—97%) and stirred until homogeneous. The reaction mixture was cooled in an ice bath at 0°C—4°C, and 10 g of $KMnO_4$ was slowly added. The mixture was then maintained at 50°C under constant stirring overnight. After the initial oxidation stage, additional 10 g of $KMnO_4$ was added, followed by 70 mL of ultrapure water. The mixture was stirred for 24 h at room temperature. Subsequently, it was transferred to 400 mL of ice water, and 3 mL of 30% H_2O_2 was slowly added with stirring at room temperature. The resulting GO suspension was purified by dispersion in 500 mL of an aqueous solution (3% H_2SO_4 and 0.5% H_2O_2) for 72 h. The solid material was collected by centrifugation at 3 000 r/min for 30 min, rinsed at least six times with ultrapure water, and exfoliated in ultrapure water using an ultrasonic bath (at 40 kHz and 150 W) at 30°C for 2 h. The GO powder was obtained after drying at 40°C for 24 h, followed by drying at 60°C for 48 h and grinding with a mortar and a pestle.

2.1.3. Preparation of GO dispersion and membrane deposition

A GO dispersion (1 mg/mL) was prepared by dissolving the GO powder in ultrapure water and sonicating for 45 min in an ultrasonic bath. An 8-mL aliquot of the dispersion was deposited onto the tubular ceramic support (TCS) using in—out vacuum-assisted filtration. The membrane was placed in its filtration cell, and filtration was performed for 30 min until the GO layer was deposited over the support surface. The coated membrane was subsequently dried at 80°C for 24 h and

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then at 100°C for 72 h to consolidate the GO layer. A schematic representation of the TCSGoM synthesis process is shown in Fig. 1.

2.2. Experimental set-up for anaerobic biodegradation and analysis of 2-CP

2.2.1. Preparation of feed solution

The synthetic wastewater feed solution was prepared in a 1-L glass bottle (feed tank) containing 2-CP (98%; Sigma−Aldrich) as the model compound and sodium acetate (SA) (reference No. 11019−1; Sigma−Aldrich) as the co-substrate, serving as a carbon source and electron donor for the microorganisms. A 2-CP-to-SA mass ratio of 1:4 was maintained throughout all experiments. During the initial microbial acclimation phase (first 10 d), the feed solution contained 5 mg/L of 2-CP and 20 mg/L of SA. After achieving 100% removal at this initial concentration, the 2-CP concentration was progressively increased in steps of 5−10 mg/L (5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 50 mg/L, 60 mg/L, 70 mg/L, and 80 mg/L), with the SA concentration adjusted accordingly to maintain the 1:4 mass ratio. Each concentration was maintained for necessary days until a steady removal percentage was consistently achieved, before advancing to the next concentration level. 2-CP and SA were dissolved in ultrapure water, and the composition was supplemented with 1 mL/L (per liter of feed) of each of six basal media (BM) to provide essential microorganism nutrients.

2.2.2. Basal media composition

The basal media compositions are detailed in Table 1. All chemicals used for basal media preparation were of analytical grade and were dissolved in ultrapure water.

2.2.3. Bioreactor configuration and inoculation

TCSGoM was placed in the filtration module and operated as a compact bioreactor. 4 d prior to the experimental run, the bioreactor was inoculated with 7 mL of anaerobic sludge (from a mesophilic anaerobic digester at the Municipal Wastewater Treatment Plant Reus in Spain) to establish a biofilm. This inoculum volume corresponded to approximately 99% of the bioreactor internal volume (7.07 mL), essentially filling the entire tubular membrane. The inoculated bioreactor was immediately sealed and incubated at 37°C to allow microbial adaptation to the initial 2-CP concentration. The anaerobic sludge was characterized by a total solids (TS) concentration of 1.64 g/L and a volatile solids (VS) concentration of 0.57 g/L.

Table 1
Chemical composition of basal media used for microorganism nutrient supplementation.

Basal medium	Component	Concentration (mg/L)
BM-1 (trace metals)	$MnSO_4 \cdot H_2O$	0.155
	$CuSO_4 \cdot 5H_2O$	0.285
	$ZnSO_4 \cdot 7H_2O$	0.460
	$CoSO_4 \cdot 7H_2O$	0.307
	$(NH_4)_6Mo_7O_{24}$	0.285
BM-2 (phosphate buffer)	K_2HPO_4	21.750
	$Na_2HPO_4 \cdot 2H_2O$	33.400
	KH_2PO_4	8.500
BM-3 (iron source)	$Fe_2(SO_4)_3$	21.470
BM-4 (calcium source)	$Ca(NO_3)_2$	19.930
BM-5 (magnesium source)	$MgSO_4 \cdot 7H_2O$	15.200
BM-6 (nitrogen source)	$(NH_4)_2SO_4$	235.700

2.2.4. Operational conditions

Biodegradation experiments were conducted under the following conditions: temperature was set at 37°C (maintained in an incubator), with an operational flux of 0.2 L/($m^2 \cdot h$) and a transmembrane pressure (TMP) in the range of 5−10 kPa (gage). The feed change strategy was maintained by complete feed replacement at each concentration increment. The bioreactor was continuously operated from the start of each experiment, and nitrogen gas (with purity greater than 99.99%) was continuously flowed to the feed tank to maintain anaerobic conditions and control the flux through the tubular membrane. The nitrogen flow was adjusted to maintain the desired TMP of 5−10 kPa (gage) and ensure complete deoxygenation of the feed solution. The feed tank was stored in a refrigerator at 1°C to minimize undesired microbial growth in the feed solution and extend feed stability over the duration of the experimental runs.

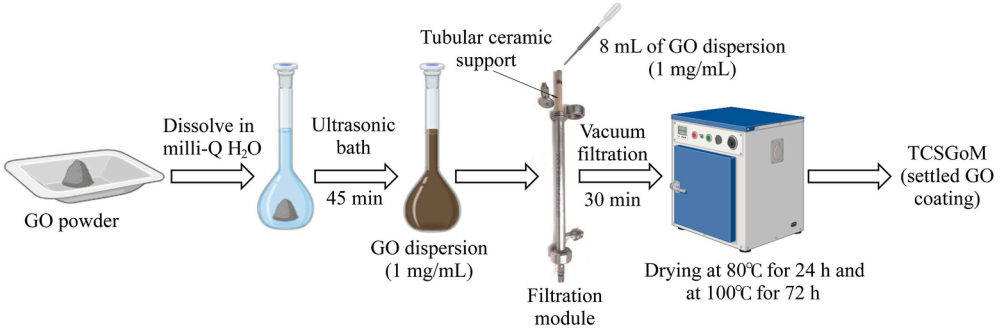


Fig. 1. Schematic representation of TCSGoM synthesis process.

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2.2.5. Membrane cleaning protocol

Following each experiment (i.e., after completion of all concentration levels), the membrane system was cleaned by backward flushing with ultrapure water for 2 min at a TMP of 400 kPa. A schematic representation of the laboratory-scale experimental setup in this study is shown in Fig. 2.

2.2.6. Analytical methods and data treatment

2-CP removal was evaluated by daily concentration measurements using high-performance liquid chromatography (HPLC; Agilent 1220 Infinity Series) equipped with a diode array detector (DAD). The analytical method was adapted from Ghani et al. (2023). The HPLC system comprised a column with dimensions of 4.0 mm × 250 mm and a particle size of 5 μm (Hypersil ODS, Agilent Technologies) operated at 30°C, with a mobile phase consisting of ultrapure water (pH of 3, adjusted with phosphoric acid) and acetonitrile (with a volume ratio of 45:55) in isocratic elution. The injection volume was 50 μL, and the detection wavelength was set at 254 nm. The limit of detection (LOD) and the limit of quantification (LOQ) for 2-CP were 0.5 mg/L and 1.0 mg/L, respectively, based on the lowest reliably quantified concentrations in the calibration curve, which was linear over the range of 0.5−200 mg/L (with a determination coefficient (R^2) of 0.999). Calibration curves were prepared periodically using standard 2-CP solutions.

The 2-CP removal efficiency (E) was calculated using Eq. (1):

$$E = \frac{A_0 - A}{A_0} \times 100\% \tag{1}$$

where A_0 is the peak area corresponding to the feed solution, and A is the peak area corresponding to the samples periodically taken during the treatment. A_0 and A were averaged from two analytical replicates per sample.

2.3. Characterization of TCSGoM

2.3.1. Structural and chemical characterization

The morphology and thickness of TCSGoM were analyzed using field-emission scanning electron microscopy with focused ion beam (FESEM-FIB). The cross-sectional images of the GO-coated membrane were used to estimate an average GO layer thickness of approximately 0.530 μm, based on three measurements taken at different positions along the membrane. The surface and cross-sectional elemental compositions of the ceramic support and the GO-coated membrane were analyzed by environmental scanning electron microscopy with energy-dispersive X-ray spectroscopy (ESEM-EDX), confirming the presence and uniform distribution of carbon-rich GO on the ceramic surface.

The chemical structure of GO used for membrane preparation was characterized by Raman spectroscopy (with an excitation wavelength of 514 nm and laser power below 2 mW), Fourier transform infrared (FT-IR) spectroscopy (over a scan range of 600−4 000 cm^{-1}), and X-ray diffraction (XRD) (with a 2θ range of 5°−80°, where θ is the diffraction angle). Raman spectra exhibited the typical D- and G-bands of GO, while FT-IR and XRD analyses confirmed the presence of oxygenated functional groups and the characteristic diffraction pattern of oxidized graphene, indicating that GO possessed the expected chemical structure and crystallinity for the deposited layer.

2.3.2. Filtration performance characterization

The filtration performance of the membrane was evaluated through pure water flux measurements and hydraulic resistance calculations. Pure water flux experiments were conducted at room temperature under a TMP range of 10−80 kPa, using ultrapure water as the feed. Flux measurements were performed for TCS without the GO coating and TCSGoM. The reported values represent the average of three repeated measurements for each condition. The permeate flux was calculated using Eq. (2):

$$J = \frac{V}{tA_m} \tag{2}$$

where J is the permeate flux (L/(m^2·h)), V is the volume of permeate collected in a specific time period (L), t is the filtration time (h), and A_m is the effective membrane area (m^2). The hydraulic resistance was obtained from Eq. (3):

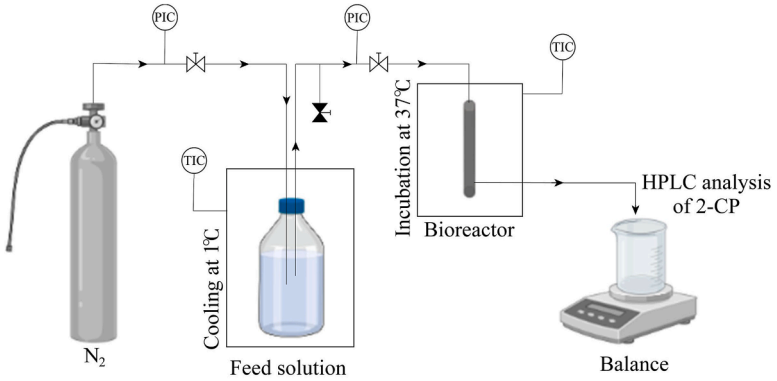


Fig. 2. Scheme of experimental system for biodegradation of 2-CP using TCSGoM (with PIC denoting pressure indictor and controller and TIC representing temperature indicator and controller).

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$$H_R = \frac{\Delta P}{\mu J} \quad (3)$$

where H_R is the hydraulic resistance (m^{-1}), ΔP is the TMP (kPa), and μ is the dynamic viscosity of water (Pa·s) at the experimental temperature. For clarity, the operational pressure range of 10−80 kPa used in the bioreactor corresponds to ΔP = 10−80 kPa. The characterization of the GO layer thickness and hydraulic resistance provides a link between the membrane structure and the observed performance in the bioreactor, as discussed in Section 3.

2.4. Microbial analysis

Microbial diversity was assessed in two individual samples: one from the initial anaerobic sludge (inoculum) and another collected from the bioreactor at the end of the entire experiment, following operation at the maximum 2-CP concentration of 80 mg/L. Total DNA was extracted using the DNeasy PowerSoil kit (Qiagen), with modifications to the standard protocol. The bead-beating step was extended to 15 min to improve cell lysis efficiency.

For microbial profiling, the ITS2 region and V3−V4 regions of the 16 S rRNA gene were amplified via polymerase chain reaction (PCR). Amplification of the 16 S rRNA gene targeted bacterial diversity, while the ITS2 region was analyzed to assess the presence of potential fungi in the anaerobic sludge and bioreactor sample. This dual approach enabled comprehensive characterization of the microbial community, including both bacteria and fungi. The amplified products were tagged with Illumina adapters and unique barcodes prior to sequencing on the NextSeq 2000 platform (Illumina; paired-end 2 × 300 bp reads).

Raw sequences were quality-filtered and processed using the Quantitative Insights Into Microbial Ecology (QIIME) 2 (version 2020.8), with default Divisive Amplicon Denoising Algorithm 2 (DADA2) parameters applied for denoising and generation of amplicon sequence variants (ASVs). Taxonomic assignment for 16 S rRNA sequences was performed using the Greengenes 2 database (version 2022.10), which enabled more precise taxonomic resolutions. Alpha diversity was evaluated using Shannon and Chao 1 indices to estimate species richness, based on rarefied data at a sequencing depth of 25 826 reads per sample. No formal statistical comparison was performed between the two samples, as this study aimed to examine qualitative shifts in microbial community composition under progressive 2-CP exposure.

3. Results and discussion

3.1. GO and membrane characterization

3.1.1. GO powder characterization

Three characterization techniques, including Raman spectroscopy, FT-IR spectroscopy, and XRD, were used to verify the synthesis of GO. Each technique provides information on the structure, defects, and functional groups present in the material.

The Raman spectrum (Fig. 3) confirmed GO synthesis with characteristic D- (approximately 1 353 cm^{-1}), G- (approximately 1 600 cm^{-1}), and 2D-bands (approximately 2 840 cm^{-1}). The D-band represents the breathing modes of aromatic rings associated with structural defects and disorder in the carbon lattice, and its intensity indicates the presence of oxygenated functional groups. The G-band corresponds to sp^2 carbon atom vibrations. The calculated I_D/I_G (where I_D and I_G are the intensities of the D- and G-bands, respectively) of 0.97 indicated a significant defect density typical of GO nanosheets (Alam et al., 2017; Aimon et al., 2024).

FT-IR spectroscopy (Fig. 4) confirmed the presence of oxygen-containing functional groups characteristic of GO. The key absorption bands included O−H stretching vibrations at 3 268 cm^{-1}, C=O stretching vibrations of carboxyl and/or carbonyl groups at 1 714 cm^{-1} and 1 616 cm^{-1}, and C−O stretching vibrations at 1 218 cm^{-1} and 1 041 cm^{-1} (Rattana et al., 2012).

The structural changes induced by oxidation were analysed using XRD. As shown in Fig. 5, the XRD pattern exhibited a characteristic GO peak at 2θ = 11.2°, corresponding to an interlayer spacing of 7.96 Å and the (001) crystal plane, in contrast to graphite, which showed a characteristic peak at 2θ = 26.4°, corresponding to a d-spacing of 3.37 Å and the (002) plane (Alam et al., 2017). This approximately 2.4-fold expansion confirmed the oxidation-induced structural modification and interlayer separation.

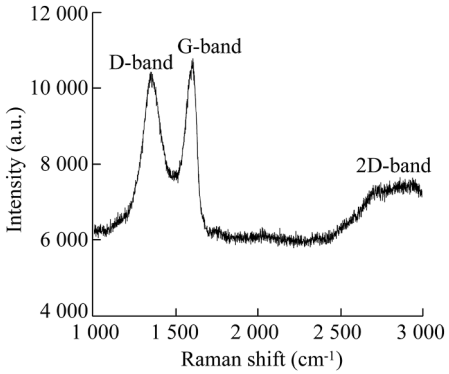


Fig. 3. Raman spectrum of synthesized GO.

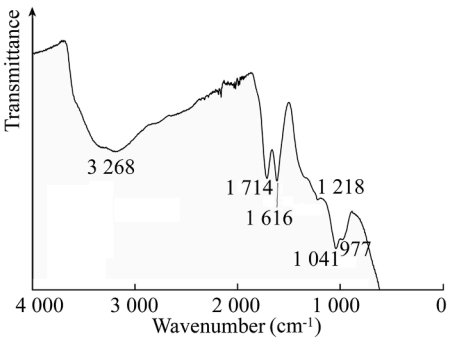


Fig. 4. FT-IR spectra of synthesized GO.

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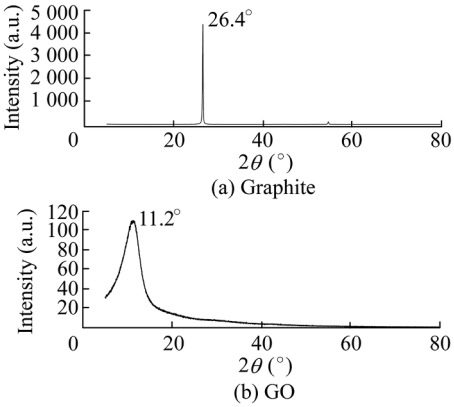


Fig. 5. XRD patterns of graphite and synthesized GO.

3.1.2. Characterization of GO-coated ceramic membrane

After deposition of the GO layer onto the tubular ceramic filtration membrane, field-emission scanning electron microscopy (FESEM) analysis was performed to evaluate its morphology and thickness. Fig. 6(a) and (b) reveals a continuous and uniform GO layer with an average thickness of 0.530 μm (averaged over three cross-sections) and a surface pore diameter of 0.108 μm (averaged over five pores), consistent with the ultrafiltration (UF) range suitable for bacteria retention.

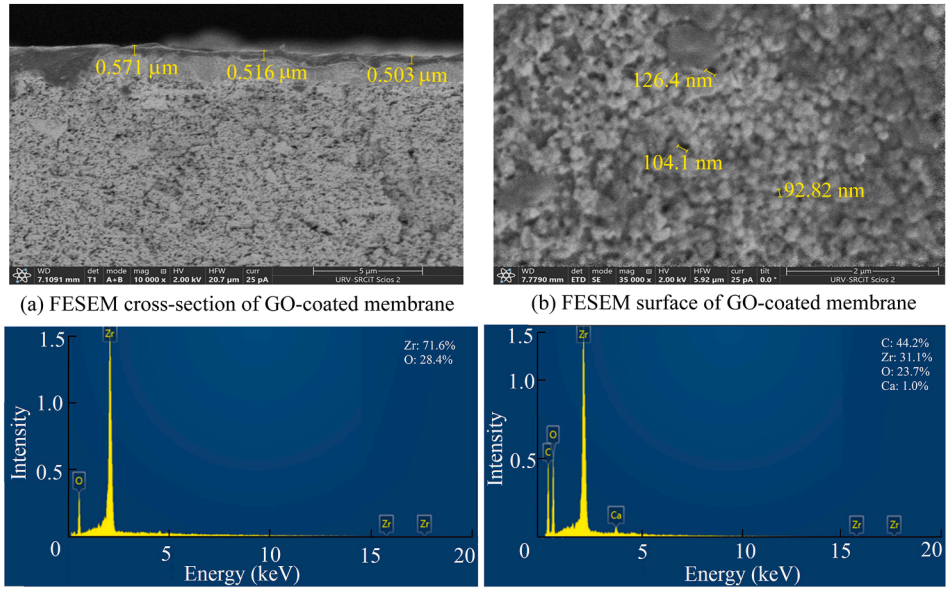
Energy-dispersive X-ray spectroscopy (EDX) analysis confirmed the successful deposition of the coating. The uncoated ceramic membrane (Fig. 6(c)) revealed Zr (71.6%) and O (28.4%), confirming a ZrO_2 composition consistent with the composition reported by the TCS manufacturer. The GO-coated membrane (TCSGoM) (Fig. 6(d)) displayed C (44.2%), alongside Zr and O, demonstrating the formation of an effective GO layer on the ceramic membrane surface during the vacuum filtration deposition process.

The uniform carbon-rich coating, hydrophilic oxygen groups, and UF-range porosity of the TCSGoM support facilitate bacterial attachment, biofilm development, and microorganism retention, which are essential to anaerobic bioreactor performance, as discussed in detail in Section 3.3.

3.2. Pure water permeability

The pure water permeability (PWP), membrane flux, and hydraulic resistance of the uncoated TCS and GO-coated membrane (TCSGoM) were determined via filtration tests conducted at room temperature under steady-state conditions, with three replicate measurements per pressure point. Tests were performed using ultrapure water as the feed at TMP ranging from 20 kPa to 80 kPa. In this study, PWP refers to the experimentally determined permeance (i.e., $J/\Delta P$).

Pure water flux increased linearly with the applied TMP for both membranes (Fig. 7), confirming Darcy-type flow behavior. The PWP values, obtained from the linear regression slopes, were 1.976 L/(m^2·h·kPa) for TCS and 0.167 L/(m^2·h·kPa) for TCSGoM, representing a near 12-fold reduction due to GO coating. This substantial reduction in permeance reflects increased resistance to water flow, quantitatively confirmed by the hydraulic resistance (H_R) values calculated from Eq. (3). H_R increased from 2.04×10^{12} m^{-1} for TCS to 2.38×10^{13} m^{-1} for TCSGoM, consistent with the additional resistance imparted by the denser GO layer. Reduced permeance facilitates biofilm formation and contaminant retention in the TCSGoM bioreactor, as discussed in Section 3.3. As a



(a) FESEM cross-section of GO-coated membrane
(b) FESEM surface of GO-coated membrane
(c) EDX spectrum of uncoated membrane
(d) EDX spectrum of GO-coated membrane

Fig. 6. FESEM and EDX analyses of membranes.

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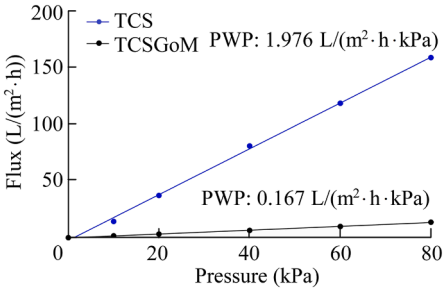


Fig. 7. Variations in membrane flux and PWP as a function of pressure.

reference for system productivity, the maximum fluxes at 80 kPa were 158.5 L/(m^2·h) for TCS and 13.6 L/(m^2·h) for TCSGoM. These results align with those of Amin et al. (2023), who reported a PWP value of 0.228 L/(m^2·h·kPa), a maximum flux of 18.2 L/(m^2·h), and an H_R value of 1.78 × 10^{13} m^{-1} for GO-coated tubular ceramic membranes under comparable conditions.

3.3. Effects of GO coating and biofilm formation on 2-CP removal

To evaluate the 2-CP removal efficiency in different membrane systems, tests were conducted using a 2-CP feed solution with an inlet concentration of 10 mg/L. The flux was maintained at 0.2 L/(m^2·h) under dead-end filtration mode. Four systems were tested: (1) ceramic membrane without GO coating (TCS), (2) ceramic membrane without GO coating but with anaerobic sludge (B-TCS), (3) ceramic membrane with GO coating but without anaerobic sludge (TCSGoM), and (4) ceramic membrane with GO coating and with anaerobic sludge (B-TCSGoM). All data correspond to a single experimental run. Samples were collected under steady-state conditions and analyzed in duplicate by HPLC, with mean values reported.

As shown in Fig. 8, the removal of 2-CP was primarily influenced by the presence of the GO layer and the microbial community. In all cases, a notable decrease in 2-CP concentration was observed during the first 24 h, with removal efficiencies of 42.1% for TCS and 86.9% for TCSGoM. After this initial period, removal declined until reaching steady values of 4.7% for TCS and 17.6% for TCSGoM. For B-TCS, removal increased from 14.1% after 24 h to 46.0% after 48 h, followed by a gradual decline to a steady value of 2.8%. In contrast, B-TCSGoM exhibited a different trend: 2-CP removal reached 87.0% after 24 h and continued to increase steadily until complete removal (100%) was achieved, remaining stable over time.

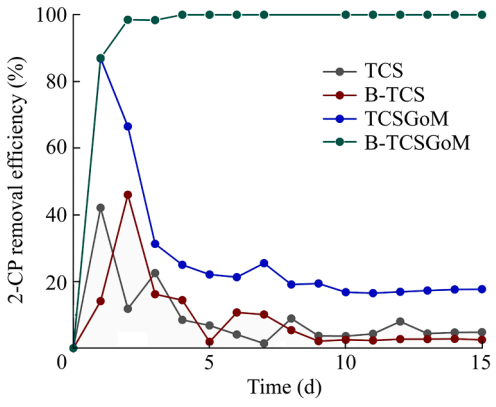


Fig. 8. Anaerobic removal of 2-CP in TCS and TCSGoM reactors and B-TCS and B-TCSGoM bioreactors (with flux of 0.2 L/(m^2·h), initial 2-CP concentration of 10 mg/L, temperature of 37°C, and data corresponding to a single run).

Overall, these results indicate that 2-CP is initially adsorbed onto the membrane surface, resulting in apparent removal that is not attributable to biodegradation. Once the adsorption sites become saturated, the retention capacity diminishes, explaining the observed decline in removal efficiency unless an active biofilm develops on the surface. TCS exhibited a limited adsorption peak and nearly negligible 2-CP removal, owing to the low adsorption capacity of ZrO_2 and the absence of microorganisms. Similarly, B-TCS followed a comparable pattern, suggesting that the inoculated microorganisms were unable to establish a stable biofilm directly on the unmodified ceramic surface.

In TCSGoM, the removal efficiency stabilized at approximately 17.6% for 15 d, nearly four times that observed for TCS and B-TCS. This behavior indicates that the GO layer enhanced the adsorption capacity and partial retention of 2-CP. It should be noted that this stabilization reflects physical retention and adsorption, rather than active biodegradation. The GO layer, with its oxygenated functional groups, exhibited lower porosity and strong adsorption properties, thereby improving the initial capture and retention of 2-CP.

On the other hand, the high and sustained removal efficiency observed for B-TCSGoM suggests that the GO layer served as a suitable support for biofilm growth, enabling efficient removal of 2-CP. In comparison, the B-TCS membrane exhibited considerably lower removal, indicating that without GO modification, stable biofilm formation was limited. This may be attributed to the larger pore size of the ceramic support relative to bacterial dimensions, which could hinder cell attachment and biofilm stabilization, although further surface characterization would be necessary to confirm this hypothesis.

In summary, only the B-TCSGoM anaerobic bioreactor achieved complete and stable removal of 2-CP. The GO layer deposited on the ceramic support plays a threefold role: (1) enhancing adsorption, (2) providing a conductive and hydrophilic surface favorable for biofilm development, and (3) promoting electron transfer between bacteria and the pollutant. The oxygenated functional groups in GO (hydroxyls, epoxides, and carboxyls) facilitate electrostatic and hydrophobic interactions with 2-CP, improving its proximity and availability for bacterial degradation (Soltani and Lee, 2016; Khalid et al., 2020). Moreover, GO provides an ideal surface for anaerobic biofilm formation due to its hydrophilic and conductive properties, promoting the adhesion and proliferation of microorganisms (Amin et al., 2021; Casabella-Font et al., 2023). GO-based materials have been reported to

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enhance the stability of anaerobic biofilms by reducing biomass leaching and improving cell retention, thereby increasing the degradation efficiency of chlorinated organic compounds (Wu et al., 2023). Finally, GO can act as a conductive bridge, potentially accelerating electron transfer between microbial cells and 2-CP during anaerobic processes. This role is associated with its biological reduction to reduced GO (Casabella-Font et al., 2024) and its function as an electron shuttle in microbial systems (Li et al., 2016; Igarashi et al., 2020). This mechanism may facilitate reductive dechlorination, as reported in previous studies (Yang et al., 2021). However, as transformation products were not identified in this study, the conversion of 2-CP into phenol and its subsequent mineralization pathways to CH_4 and CO_2 should be considered as plausible hypotheses rather than confirmed mechanisms.

The combined mechanisms of adsorption, biofilm support, and electron transfer render GO-modified ceramic membranes a promising strategy for 2-CP removal, which integrates multiple intensification approaches within a single unit.

A limitation of this study is the absence of transformation product identification. Consequently, although the observed shift from initial adsorption to sustained removal suggests a potential contribution of biofilm-mediated biodegradation, neither complete biodegradation nor specific degradation pathways can be directly confirmed. Future work should incorporate advanced analytical techniques (e.g., liquid chromatography−mass spectrometry (LC−MS) and gas chromatography−mass spectrometry (GC−MS)) to identify intermediate compounds and better elucidate the fate of 2-CP.

Furthermore, it should be noted that all experiments were conducted as single runs. This is attributable to the complexity of the membrane bioreactor system, including biofilm development and extended operation required to evaluate performance under gradually increasing contaminant concentrations. Therefore, the results should be interpreted as indicative of system behavior under the tested conditions rather than as statistically validated trends. However, previous studies involving similar biodegradation schemes applied to other compounds have confirmed their reproducibility, even when starting from a different sludge seed (Amin et al., 2023).

3.4. Performance of 2-CP removal in B-TCSGoM

3.4.1. Effect of feed 2-CP concentration

Fig. 9 shows the evolution of 2-CP removal over 88 d using the B-TCSGoM system under anaerobic conditions. To evaluate the impact of pollutant load on biodegradation efficiency, the feed 2-CP concentration was incrementally increased upon achieving stable removal performance. This approach provided insights into the adaptive capacity of the biofilm and the stability of the process under fluctuating contaminant concentrations.

All data correspond to a single experimental run. Samples collected at each time point were analyzed in duplicate by HPLC, with mean values reported. Due to the extended operation required to evaluate system performance under

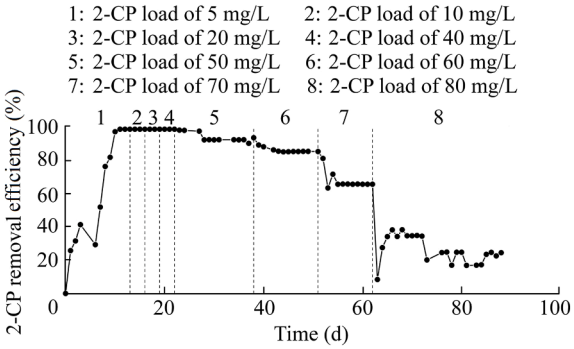


Fig. 9. Impact of increasing 2-CP concentrations on 2-CP removal (with flux of 0.2 L/(m^2·h), temperature of 37°C, and data corresponding to a single run).

progressively increasing 2-CP concentrations, full experimental replication was not feasible within the scope of this study.

The experiment began at an initial feed 2-CP concentration of 5 mg/L based on previous evidence that higher initial concentrations (greater than 40 mg/L) can hinder biofilm formation and cause system instability during the start-up. Thus, a low initial concentration facilitated the progressive acclimation of microorganisms to the target compound. During the first stage, 26.1% 2-CP removal was achieved after 24 h, with a progressive increase to 98.5% on day 10, reaching 100% on day 11, which remained for two additional days.

After complete removal was confirmed, the feed concentration was increased sequentially. At 2-CP concentrations of 10 mg/L, 20 mg/L, and 40 mg/L, the system maintained 100% removal for 3 d after each increase in 2-CP concentration. However, from day 22 onwards at 50 mg/L, the 2-CP removal efficiency gradually decreased and stabilized at 93.2% after 5 d. When the 2-CP concentration was increased to 60 mg/L on day 38, 2-CP removal stabilized at 86.4% after 4 d. At 70 mg/L on day 51, 2-CP removal decreased to 64.2% and then stabilized at 66.5% after 4 d. Finally, at 80 mg/L on day 62, the removal efficiency dropped sharply to 8.5% within 24 h. A limited recovery was observed, reaching 35.9% over 8 d, followed by a further decline. No stabilization occurred during the remaining 12 d of operation, suggesting that the biofilm lost its ability to effectively biodegrade 2-CP above this concentration.

These findings demonstrate that the biofilm developed on TCSGoM maintained excellent stability and removal performance at feed 2-CP concentrations up to 50 mg/L, with a gradual decline in performance at higher loads.

To better evaluate system performance, the removal rate per unit membrane area was calculated as a function of the 2-CP loading rate (Fig. 10). This analysis provides a clearer representation of the biodegradation capacity under increasing contaminant loads.

The removal rate increased from 0.98 mg/(m^2·h) at a 2-CP loading rate of 0.005 mg/h to a maximum of 10.4 mg/(m^2·h) at 0.057 mg/h. Beyond this point, the removal rate decreased slightly to 9.80 mg/(m^2·h) at 0.066 mg/h, followed by a decline to 4.08 mg/(m^2·h) at 0.075 mg/h. These results

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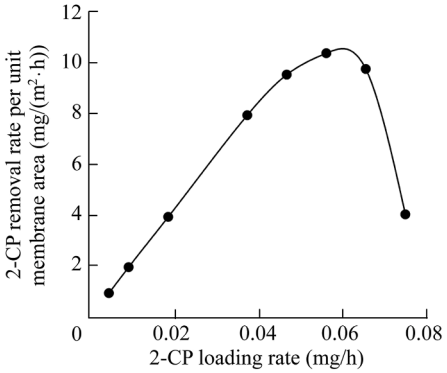


Fig. 10. 2-CP removal rate per unit membrane area as a function of 2-CP loading rate under increasing 2-CP initial concentrations (5−80 mg/L).

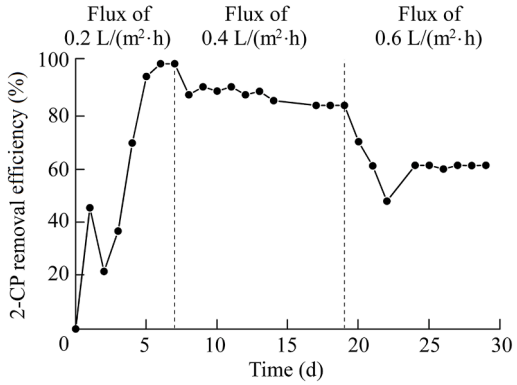


Fig. 11. Effect of flux on 2-CP removal (with 2-CP concentration of 40 mg/L, temperature of 37°C, and data corresponding to a single run).

indicate that the system maintained a stable removal capacity up to approximately 0.066 mg/h, corresponding to a feed 2-CP concentration of 70 mg/L. Above this level, biofilm activity became limited, likely due to substrate inhibition and toxic effects on the microbial community, consistent with established substrate inhibition models for chlorophenols (Patel and Kumar, 2016).

A key advantage of this configuration is its ability to operate without prior microbial acclimation and its potential to minimize post-treatment requirements, thereby offering an integrated single-stage solution for 2-CP removal. Unlike other studies that require extended adaptation periods before achieving stable degradation, this approach enabled the direct inoculation of anaerobic sludge onto the TCSGoM membrane. Microorganisms colonized the GO surface within 4 d, and the gradual increase of 2-CP concentration facilitated natural adaptation, ensuring stable degradation across a wide range of contaminant loads. In contrast, Majumder and Gupta (2006) used a sequential anaerobic−aerobic reactor system to remove 2-CP and 2,4-dichlorophenol (2,4-DCP) and reported that the microorganisms required 190−215 d of acclimation to reach optimal biodegradation rates. Similarly, Geng et al. (2019) implemented an anaerobic fixed-bed bioreactor (AnFBR) and observed that their system required a 205-d acclimatization period for microorganisms to efficiently degrade 2-CP.

Furthermore, whereas other systems require secondary treatments to eliminate residual intermediates, the B-TCSGoM configuration integrates adsorption, biodegradation, and filtration in a single stage. Moussavi et al. (2014) demonstrated effective 2-CP removal using a baffled granular anoxic reactor, but it relied on a denitrification step to enhance performance. Similarly, Zhou et al. (2012) combined catalytic hydrodechlorination over Pd or C and biodegradation for 4-chlorophenol (4-CP) and 2,4-DCP, producing phenol intermediates that required an additional treatment. In contrast, the present configuration achieved high 2-CP removal in a single compact system, simplifying operation and improving process stability.

3.4.2. Effect of flux on 2-CP removal

A second experiment was conducted to evaluate the influence of flux on 2-CP removal. Based on previous findings, a feed concentration of 40 mg/L was selected as it enabled complete removal at a flux of 0.2 L/(m^2·h) in the first experiment. As shown in Fig. 11, complete 2-CP removal was achieved on day 7. The flux was then doubled to 0.4 L/(m^2·h), leading to a gradual decline in removal efficiency to 84.3%. On day 19, the flux was increased to 0.6 L/(m^2·h), and the removal efficiency dropped to 48.2%, stabilizing at 61.7% after day 29. These values represent decreases of 16% and 38.0%, respectively, relative to the baseline condition.

The observed decrease in 2-CP removal efficiency with increasing flux can be attributed to multiple interacting factors. At a low flux, the contact time between 2-CP molecules and the biofilm is sufficient for adsorption on the GO layer and subsequent biodegradation. As the flux increases, shorter contact times disrupt the adsorption−biodegradation equilibrium, reducing interactions between the contaminant and biofilm (Amin et al., 2021; Trivedi and Chhaya, 2022). In addition, a higher flux generates shear forces that may partially detach biofilm layers, thereby diminishing bioreactor performance (Picioreanu and Heijnen, 2001).

To complement these results, the removal rate per unit membrane area was again calculated and plotted as a function of the 2-CP loading rate (Fig. 12). The removal rate per unit membrane area increased from 8.02 mg/(m^2·h) at a load of 0.038 mg/h (i.e., a flux of 0.2 L/(m^2·h)) to 13.72 mg/(m^2·h)

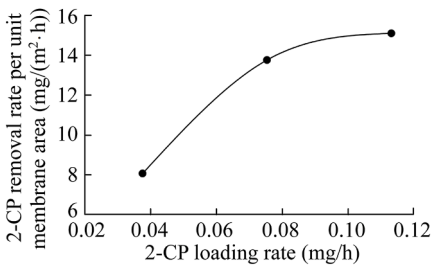


Fig. 12. 2-CP removal rate per unit membrane area as a function of 2-CP loading rate under increasing flux conditions.

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at 0.076 mg/h (0.4 L/(m^2·h)), reaching a maximum of 15.06 mg/(m^2·h) at 0.113 mg/h (0.6 L/(m^2·h)). Although this trend initially indicated an improvement in 2-CP removal due to enhanced mass transfer, the curve plateaued at higher 2-CP loads, revealing that the system was approaching its maximum biodegradation capacity. The initial increase is likely attributable to improved substrate transport to active sites, whereas the subsequent decline reflects shear-induced biofilm detachment and reduced contact time.

However, compared to the trends observed when the feed load was increased via higher inlet concentrations (Fig. 10), a shift of the peak removal rate towards a greater feed load was observed, along with higher maximum removal rates, approximately 11 mg/(m^2·h) in Fig. 10 and about 15 mg/(m^2·h) in Fig. 12. This indicates that, prior to reaching the maximum, the increased flux had a beneficial effect on the removal rate capacity of the system, likely due to enhanced mass transfer of 2-CP from the bulk liquid to the sites where biodegradation occurs.

To provide a clearer overview of the system's behavior under different operational conditions, Table 2 summarizes the key parameters, removal efficiencies, and limiting factors observed in both experimental series (concentration and flux).

3.5. Microbial analysis

Microbial diversity analysis based on 16 S rRNA gene sequencing enabled the identification and comparison of phylum composition in two samples: the anaerobic sludge used as inoculum and the biofilm that formed on B-TCGoM after the 2-CP biodegradation process. The results are presented in Fig. 13.

The microbial analysis of the anaerobic sludge revealed 19 distinct phyla. The most abundant phyla were Proteobacteria (19%), Bacteroidota (18%), Firmicutes A (12%), and Chloroflexota (6%), each comprising over 5% of the total microbial community. These phyla are known to play essential roles in anaerobic digestion processes, including hydrolysis, fermentation, and methanogenesis. Several minor phyla were also detected, confirming a highly diverse microbial ecosystem typical of anaerobic sludge from wastewater treatment plants. Similar community structures have been reported by Chen et al. (2023) and Wang et al. (2020a), who found that Proteobacteria, Bacteroidota, Firmicutes, and Chloroflexota dominate anaerobic sludge systems treating complex organic wastes.

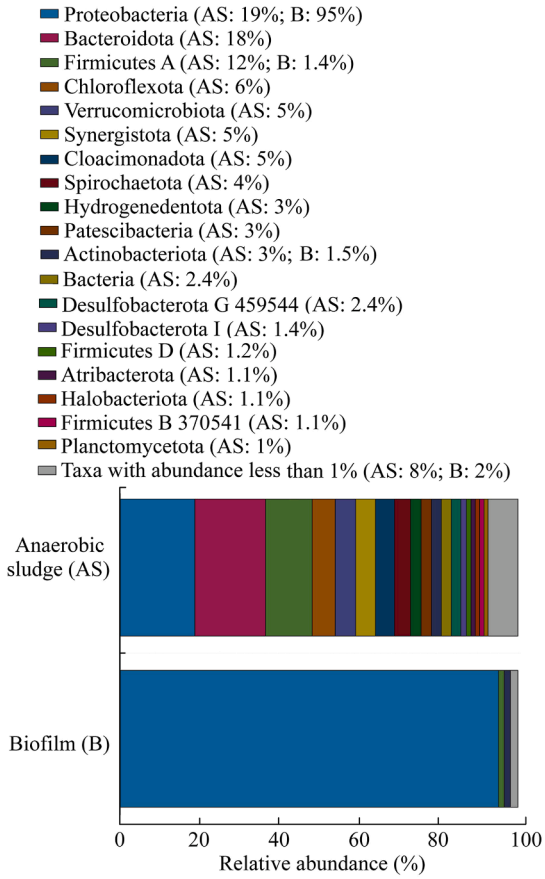


Fig. 13. Relative abundances of bacterial phyla in anaerobic sludge and biofilm.

In addition, the anaerobic sludge sample tested positive for ITS2 amplification. Sequence analysis revealed the predominance of a single fungal taxonomic group, classified as an uncultured soil fungus. However, its relative abundance was low, and no fungal sequences were detected in the biofilm sample, indicating that the fungal contribution to 2-CP degradation was negligible.

In contrast, the biofilm sample exhibited a significantly distinct microbial profile, dominated by Proteobacteria (95%), indicating strong selective pressure exerted by 2-CP. This dominance suggests that only specific bacterial groups were capable of surviving, adapting, and contributing to 2-CP removal. To further investigate this shift, Fig. 14 shows the relative abundances of species in the biofilm. The biofilm

Table 2
Summary of 2-CP removal performance under various concentration and flux conditions.

Experiment	Parameter range	Maximum 2-CP removal efficiency (%)	Stable removal efficiency (%)	Peak removal rate (mg/(m^2·h))	Main limiting factor
Concentration effect (with flux of 0.2 L/(m^2·h))	5−80 mg/L	100 (at 2-CP concentration no greater than 50 mg/L)	66.5 (at 2-CP concentration of 70 mg/L)	10.4	Substrate inhibition and biotoxicity at 2-CP concentration greater than 70 mg/L
Flux effect (with 2-CP concentration of 40 mg/L)	0.2−0.6 L/(m^2·h)	100 (at flux no greater than 0.2 L/(m^2·h))	61.7 (at flux of 0.6 L/(m^2·h))	15.1	Reduced contact time and biofilm detachment at high flux

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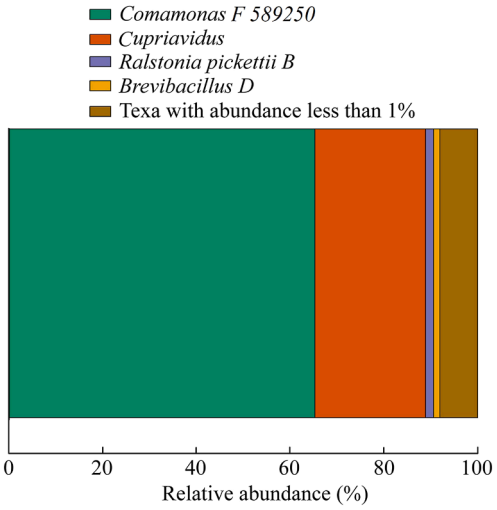


Fig. 14. Relative abundances of bacterial species in biofilm.

community was primarily composed of *Comamonas F 589250* (65%) and *Cupriavidus* (24%), suggesting that these genera played a key role in 2-CP removal. Both genera have previously been reported to participate in the degradation of aromatic compounds under aerobic or facultative conditions, supporting their potential involvement in the transformation of chlorophenolic compounds within the biofilm. Several studies have demonstrated the efficient degradation of chlorinated phenolic compounds by these genera. Yadav et al. (2023) reviewed microbial degradation of chlorophenols, highlighting *Comamonas* and *Cupriavidus* as key organisms involved in dechlorination and the subsequent breakdown of toxic phenolic contaminants. Similarly, Arora and Bae (2014) reported that *Comamonas testosteroni* degrades monochlorophenols via a meta-cleavage pathway involving phenol hydroxylase and dioxygenases, while Tobajas et al. (2010) demonstrated that *Cupriavidus necator* achieves complete 4-CP degradation through the hydroxyquinol pathway, emphasizing its adaptability to environments containing chlorinated compounds.

In contrast to the highly diverse inoculum, the dominance of *Comamonas* and *Cupriavidus* in the biofilm reflects strong adaptive selection that optimized biodegradation efficiency without requiring additional acclimation periods. This finding highlights a key advantage of the B-TCSGoM system: its ability to rapidly establish a specialized microbial community capable of efficiently degrading synthetic 2-CP wastewater under anaerobic conditions.

4. Conclusions

This study demonstrated that TCSGoM can serve as an effective biofilm support for the anaerobic removal of 2-CP, enabling simultaneous pollutant removal and effluent filtration in a single-stage system.

The GO coating plays a key role in enhancing biofilm formation and facilitating electron transfer, thereby improving microbial activity for 2-CP degradation. In contrast, negligible removal was observed in the absence of GO. GO has been reported to promote biofilm development, enhance contaminant adsorption, and facilitate electron transfer in biodegradation pathways for 2-CP.

The TCSGoM bioreactor achieved complete (100%) removal of 2-CP at influent concentrations up to 50 mg/L, after which performance gradually declined at higher concentrations. The system maintained stable operation for several months, even under feed fluctuations. When removal performance was expressed in terms of 2-CP load versus removal rate, the removal capacity increased with load up to approximately 0.07 mg/h, beyond which the removal rate either declined or plateaued, indicating that the biofilm had reached its maximum removal capacity.

Microbial community analysis revealed that the biofilm was dominated by *Comamonas F 589250* (65%) and *Cupriavidus* (24%), both of which are known for their ability to degrade chlorophenolic compounds. This dominance suggests the selective enrichment of microbial species best suited for 2-CP metabolism. In contrast to conventional anaerobic reactors, which require extended acclimation periods, this system facilitates rapid microbial adaptation, simplifying operation and reducing start-up time.

Furthermore, the TCSGoM configuration has the potential to reduce or even eliminate the need for post-treatment steps, providing a cost-effective and sustainable alternative for wastewater treatment. These findings highlight the potential of TCSGoM as a low-energy technology for 2-CP removal and encourage future research into its long-term biofilm stability and performance under real wastewater conditions.

Declaration of generative AI in scientific writing

During the preparation of this work, the authors used Scholar GPT (OpenAI) to enhance the clarity and fluency of the English language. After utilizing this tool, the authors reviewed and revised the content as necessary and take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by Ministerio de Ciencia, Innovación y Universidades de España and Agencia Española de Investigación (MICIU/AEI, Project PID 2021-126895OB-I100), the European Regional Development Fund (FEDER, Project PID 2021-126895OB-I100), and Universitat Rovira i Virgili (Contract 2021 PMF PIPF-22).

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Please cite this article as: Guevara, D et al., Rapid anaerobic bioreduction of 2-chlorophenol from water using biofilms supported on graphene oxide layers, Water Science and Engineering, https://doi.org/10.1016/j.wse.2026.08.003

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