

Elucidation of biofouling mechanisms on membranes in a semi-batch reverse osmosis process for surface water treatment

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ABSTRACT

With declining surface water quality and rising demand for water reuse, reverse osmosis (RO) has gained attention as a water treatment technology. Semi-batch reverse osmosis (SBRO) can achieve higher recovery than conventional RO, yet its application to surface water remains vulnerable to biofouling due to organic matter and microbial constituents in the feed. This study elucidates the biofouling mechanisms associated with SBRO during surface water treatment. Pretreatment evaluation indicated that soluble extracellular polymeric substances (EPS) remained largely unchanged, whereas adenosine triphosphate (ATP) decreased. Although pretreatment reduced ATP, water flux declined by 5.20% in RO and 38.69% in SBRO over three weeks, with more pronounced mixed fouling involving biofouling and silicate-related inorganic deposition observed in SBRO. During SBRO concentration, ATP increased without marked changes in soluble EPS, while microbial diversity and phylogenetic breadth decreased relative to RO. In parallel, Verrucomicrobiota became enriched under osmotic stress, and predicted functional profiles were dominated by biosynthesis and energy metabolism. These findings suggest that biofouling progression in SBRO was more closely associated with microbial metabolic activity than with EPS accumulation, and that ATP may serve as an early indicator of biofouling development. Accordingly, pretreatment strategies should target suppression of microbial energy activity in surface water, while biological or adsorptive polishing may further limit microbial energy production by reducing residual assimilable organic carbon.

1. Introduction

Global surface water quality has deteriorated progressively because of industrial effluent discharge, agricultural runoff, and climate-driven hydrological variability [1]. As contamination profiles become increasingly complex, surface waters now contain not only suspended solids and conventional organic pollutants but also emerging contaminants, including trace pharmaceuticals and heavy metals [2,3]. The coexistence of these contaminants raises significant concerns regarding human health and environmental safety, while their simultaneous removal remains difficult within conventional water treatment processes [4]. Consequently, the need for treatment systems capable of comprehensive contaminant control continues to expand [5].

Reverse osmosis (RO) has been widely recognized as an advanced technology for improving the performance of surface water treatment [6,7]. RO effectively removes various pollutants, including total dissolved solids (TDS), producing higher-quality permeate than conventional advanced treatment processes [2,8]. Despite these advantages, conventional RO operation suffers from limited recovery, which constrains overall water availability [9]. To address this limitation, modified RO configurations, such as semi-batch reverse osmosis (SBRO), hybrid semi-batch/batch RO (HSBRO), and batch reverse osmosis (BRO) technologies [10–12], have been developed to increase water recovery by recirculating concentrate to the RO feed [13]. Despite the high recovery and energy efficiency of BRO, its practical application remains relatively limited due to repeated pressurization and depressurization

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Table 1
Pretreated feed water quality characteristics.

Parameter	Unit	Value	Note
Electrical conductivity	[$\mu\text{S}/\text{cm}$]	242 ± 12	N/A
Temperature	[$^{\circ}\text{C}$]	20.2 ± 0.9	N/A
Ca^{2+}	[mg/L]	27.4	The ion profile was measured after adjusting the feed conductivity to $250 \mu\text{S}/\text{cm}$ with deionized water.
Mg^{2+}	[mg/L]	4.8	
SO_4^{2-}	[mg/L]	31.7	
Si	[mg/L]	4.5	
TOC	[mg/L]	14.2	

cycles, complex valve control, and difficulties maintaining continuous operation [14,15]. Instead, SBRO has been more widely adopted because of the practical challenges associated with process control [16,17].

Biofouling is a major limitation in surface water RO systems because it deteriorates membrane performance [18]. Surface water contains organic matter and diverse microorganisms, which promote microbial attachment and biofilm formation on membrane surfaces [19]. During this process, microorganisms secrete extracellular polymeric substances (EPS), mainly composed of proteins and polysaccharides, which form the structural matrix of biofilms [20]. The accumulation of EPS increases biofilm thickness and structural stability [21], thereby intensifying membrane fouling and mass transfer resistance [22,23]. Consequently, biofouling progresses rapidly and cannot be completely removed through conventional physical or chemical cleaning due to its irreversible nature [3,24]. These characteristics result in increased transmembrane pressure, reduced water flux, elevated energy consumption, and shortened membrane lifespan [25].

While effective mitigation of biofouling in SBRO systems requires a comprehensive understanding of its formation and progression mechanisms, biofouling behavior in SBRO systems remains insufficiently understood. The effects of different pretreatment types, including a $0.45 \mu\text{m}$ microfilter, sand filter (SF), and cartridge filter (CF), remain insufficiently clarified and may even enhance microbial activity or stimulate EPS release [26,27]. Also, differences in biofouling behavior between conventional RO and SBRO remain unresolved. Biofouling intensifies under high-recovery operation, where higher concentration promotes rapid accumulation of microorganisms and EPS [28]. Furthermore, the influence of biological indicators on biofouling during concentration has not been clearly elucidated. High-salinity environments with multivalent ions can further promote EPS aggregation and shifts in the microbial community [29,30]. In parallel, analyzing microbial community structure and functional potential remains necessary for a comprehensive understanding of fouling behavior. With this information, biofouling

mitigation strategies in SBRO can be developed.

This study is conducted to elucidate the mechanisms of biofouling in SBRO for surface water treatment. First, the changes in key biological indicators are evaluated through pretreatment experiments to identify pretreatment strategies beneficial for SBRO fouling control. The flux behavior under RO and SBRO operation is then compared, along with an investigation into the development of fouling on the membrane surface. To clarify biofouling development, biological indicators are examined during concentration, and microbial abundance and diversity are characterized. Microbial community structure and functional potential are further analyzed to provide ecological insight into microbial responses under SBRO conditions. This study represents the first attempt to investigate the mechanisms underlying biofouling in SBRO systems using real surface water based on biological indicators, membrane characterization, and microbial community analyses. Implications for the SBRO process are provided to mitigate biofouling for surface water treatment.

2. Materials and methods

2.1. RO and SBRO operation and analysis

2.1.1. Feed source

The feed water was collected from a lake at Wenzhou-Kean University (Zhejiang, China) at coordinates (27.922510, 120.651670). This source is a typical surface water, characterized by an electrical conductivity of $242 \pm 12 \mu\text{S}/\text{cm}$ and a temperature of $20.2 \pm 0.9 ^{\circ}\text{C}$ during sampling (Table 1). The feed characteristics, including multivalent ions, Si, and total organic carbon (TOC), were also further analyzed. As the water originates from mountain runoff and ongoing biological processes, organic matter and detectable biological activity remain present in the raw water. The feed was pretreated to remove suspended solids and biological components before being introduced into the RO and SBRO systems, thereby ensuring conditions for subsequent membrane operation.

2.1.2. Pretreatment

Pretreatment was conducted using three different approaches: a $0.45 \mu\text{m}$ microfilter, an SF, and a CF (or a sediment filter), applied separately. The $0.45 \mu\text{m}$ microfilter (MCE, Haining Delv New Material Technology, China) was pre-rinsed with deionized water before operation. The SF consisted of a column (0.08 m internal diameter, 1 m height), which was configured from top to bottom with a blank space, biological ceramicsite, activated carbon, quartz sand, and cobblestone at a height ratio of 1:3:2:3:1. The CF (SER-011PP, Soel, Republic of Korea) with a cylindrical depth-type filter was utilized. The primary function of pretreatment was to remove suspended solids and colloidal matter before RO operation.

2.1.3. MFI measurement

The modified fouling index (MFI) was determined using a dead-end

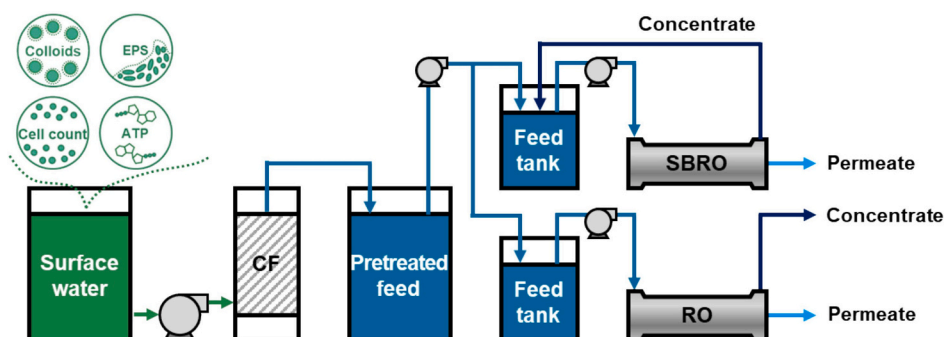


Fig. 1. A process diagram of the RO and SBRO experiments.

constant-pressure filtration protocol to quantify particulate and colloidal fouling potential. Filtration was conducted in a pressurized filtration cell equipped with a 0.45- μm microfilter as the fouling substrate. The system was operated at a transmembrane pressure of 2 bar, with a total filtrated volume of 0.5 L. The feed sample was introduced under constant pressure, and cumulative permeate volume was recorded as a function of filtration time. A new microfilter was used for each measurement to ensure reproducibility and to avoid interference from irreversible fouling.

2.1.4. RO and SBRO experiments

For the RO and SBRO experiments (Fig. 1), the feed was pretreated with CF after the selected pretreatment method was applied. Identical spiral-wound RO membranes (HP-2012-125, Huapai, China), each with a membrane area of 0.5 m^2 , were used in a single module for each experiment. Both systems were operated under constant pressure conditions at 6 bar. The feed flow rate was maintained at 64.9 ± 1.7 L/h, while the module recovery was $50.3 \pm 1.3\%$ and $48.8 \pm 1.3\%$ for RO and SBRO, respectively, on the initial day of operation. A relatively high recovery was observed, given the low feed TDS and the use of a single spiral-wound RO module. The crossflow velocity was maintained at 0.03–0.04 m/s, which is suitable for small laboratory-scale spiral-wound modules operated under low feed TDS conditions in biofouling studies [31,32].

For SBRO, the fresh feed flow rate was controlled identically to the permeate flow rate, while the concentrate was recirculated to the feed tank to maintain a constant volume of 1 L. In each run, the SBRO operation was conducted until the concentration factor reached 8. RO and SBRO were operated for identical durations to ensure direct performance comparability. However, the operation time was reduced after several runs due to the lower permeate flow rate, which accelerated concentration in SBRO owing to the reduced fresh feed flow rate [11]. After the operation, the membranes used in the RO and SBRO operations were stored in the modules containing fresh feed and concentrated feed, respectively, to accelerate fouling in SBRO. However, the RO and SBRO modules were sufficiently rinsed with the pretreated feed before each run to minimize residual effects from previous operation and storage, until stable electrical conductivity was observed in the permeate and concentrate streams. After three weeks of operation, further membrane surface analyses were performed to characterize the fouling, including scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR).

2.1.5. Water flux measurement

Because feed salinity remains constant during RO operation but increases progressively during SBRO, the osmotic pressure rises during SBRO. Under constant-pressure operation, this increase in osmotic pressure reduces the net driving pressure in SBRO, rendering direct flux comparison between RO and SBRO inappropriate. To enable a consistent comparison of water flux, the modules used for the RO and SBRO experiments were each operated in RO mode without circulation. Permeate volume collected over a 10-s interval was used to calculate the water flux. Three independent measurements were conducted at each operating stage, and the resulting average value was taken as the representative water flux for that condition. The initial water fluxes were 65.3 ± 0.3 L/ m^2 h and 63.4 ± 0.9 L/ m^2 h for RO and SBRO, respectively. A relatively high water flux was applied, given the use of a single spiral-wound RO module, and the elevated water flux accelerated fouling [33].

2.2. Biological indicators and microbial community analysis

2.2.1. ATP

Adenosine triphosphate (ATP) is directly associated with biological activity [34]. ATP content was determined using the Beyotime ATP Assay Kit (Cat# S0026, Beyotime, China). ATP detection working

solution was added to a Nest black 96-well plate and incubated for 3–5 min to reduce background signal. The test samples were added, and relative luminescence units (RLU) were measured using a Tecan Infinite Eplex Microplate Reader (Tecan Austria GmbH, Grödig, Austria). Sample ATP concentrations were calculated via a standard curve obtained ($R^2 \geq 0.99$) and converted to the measured ATP content by multiplying by a 2-fold dilution factor. Blank controls and 3 parallel samples (relative standard deviation $\leq 5\%$) were included in each assay to ensure assay accuracy.

2.2.2. EPS

The Ensonda Bio-Microbial EPS Enzyme-Linked Immunosorbent Assay Kit was used to quantify soluble EPS in the feed water, representing the dissolved and suspended EPS fraction in the bulk solution. Water samples and horseradish peroxidase were added to a detachable 96-well plate and incubated at 37 °C for 60 min to enhance the detection signal. The plate was then washed five times to remove non-specifically bound substances. Subsequently, the substrate and stop solution were added, and the optical density was measured using a Tecan Infinite Eplex microplate reader. Soluble EPS concentrations were calculated based on a standard calibration curve and corrected by a factor of five to account for sample pre-dilution. All measurements were performed in triplicate, with relative standard deviations maintained below 5% to ensure analytical accuracy.

2.2.3. Cell count

Cell counts in lake water were determined using the Beyotime LIVE/DEAD Bacterial Staining Kit (Cat# C2030S, Beyotime, China) containing *N,N*-dimethylaniline (DMAO) and propidium iodide (PI). Appropriate volumes of DMAO and PI stock solutions were mixed with detection buffer at a 1:1:8 ratio to prepare the bacterial viability staining working solution. An aliquot of the lake water sample was transferred into a 96-well plate, followed by the addition of 1 μL of the staining working solution to each well. The plate was gently mixed and incubated at 37 °C in the dark for 15 min. After incubation, relative fluorescence units were measured using a Tecan Infinite Eplex microplate reader. DMAO exhibited green fluorescence with excitation and emission wavelengths of 503 and 530 nm, respectively, whereas PI exhibited red fluorescence with excitation and emission wavelengths of 535 and 617 nm, respectively.

2.2.4. 16S rRNA gene amplicon sequencing

Microbial community profiling was conducted by Hangzhou Yanqu Information Technology Co., Ltd. (Hangzhou, China) to characterize the microbial communities associated with RO and SBRO operation. Total genomic deoxyribonucleic acid (DNA) was extracted using the OMEGA Soil DNA Kit (D5625–01, Omega Bio-Tek, Norcross, Georgia, USA), and DNA quality and concentration were evaluated by agarose gel electrophoresis and UV spectrophotometry. Polymerase chain reaction (PCR) products were purified, quantified, and pooled prior to library preparation using the Illumina TruSeq Nano DNA LT Library Prep Kit. Sequencing was performed on the Illumina NovaSeq platform using a 2 $\times$ 250 bp paired-end sequencing approach. Microbial functional prediction was conducted using PICRUSt2 based on amplicon sequence variant (ASV) representative sequences and abundance profiles. The predicted functional features were subsequently mapped to the MetaCyc pathway database to generate pathway abundance profiles.

3. Results and discussion

3.1. Effect of pretreatment for foulant removal

Variations in pretreatment strategies directly influence the stability and performance of RO membranes by controlling biofouling-related constituents. Because SBRO is applied under high-recovery conditions that intensify fouling stress, pretreatment becomes particularly crucial

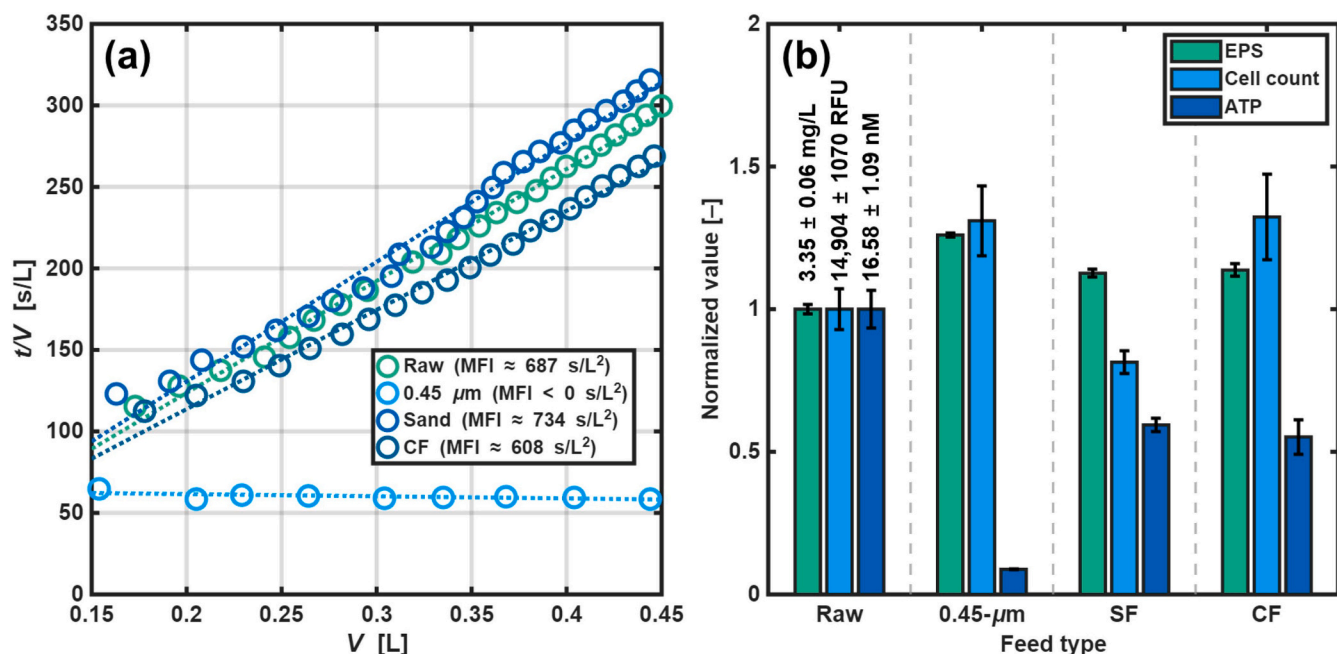


Fig. 2. (a) MFI and (b) normalized biological indicators of raw water and water treated by different pretreatment methods. In (b), the actual values for the raw water are indicated, and the subsequent values are normalized based on the raw water conditions.

for controlling fouling. Accordingly, the effects of different pretreatment methods for RO membrane feed were examined with respect to the control of colloids and biofouling-related biological indicators.

While the 0.45- μ m microfilter effectively controlled colloidal matter, several biofouling-related biological indicators increased after filtration. MFI analysis indicates that pretreatment methods impose distinct colloidal loading conditions on downstream membranes (Fig. 2a). Despite the MFI of the raw water being 687 s/L², the MFI of SF increased to 734 s/L², with possible particle release [35]. While CF reduced the MFI modestly to 608 s/L², the 0.45- μ m microfilter lowered the MFI to near zero, achieving highly effective colloid removal despite practical

cost and maintenance constraints. However, enhanced colloid removal did not correspond to reduced biological indicators (Fig. 2b). The 0.45- μ m microfilter increased soluble EPS and cell counts by 1.26-fold and 1.13-fold, respectively, while markedly suppressing ATP. SF, commonly regarded in practice as beneficial for algal bloom control, increased soluble EPS to 1.13-fold, accompanied by concurrent declines in cell counts and ATP to 0.81-fold and 0.59-fold, respectively. The CF exhibited soluble EPS and cell count trends comparable to the 0.45- μ m microfilter, with only a minor reduction in ATP to 0.55-fold. Overall, all pretreatment methods led to elevated soluble EPS levels after treatment.

Overall, fouling control depends on selecting a pretreatment that

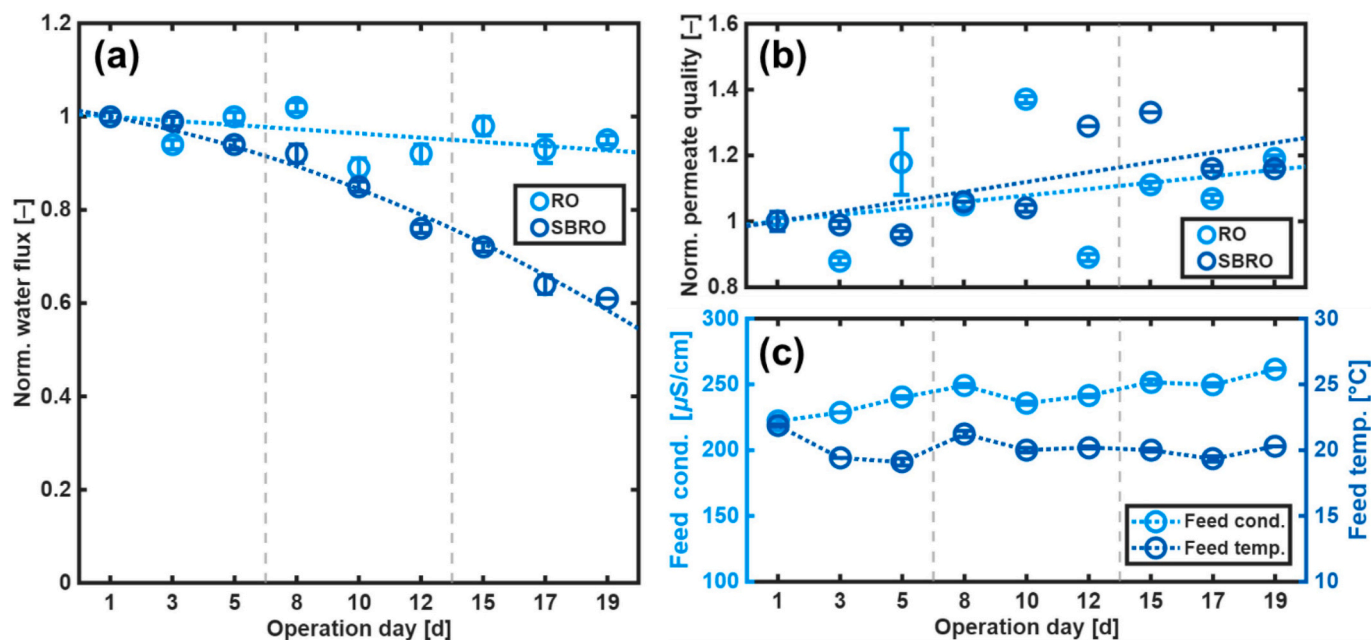


Fig. 3. (a) Water flux and (b) permeate conductivity in RO and SBRO with a single RO element. Feed temperature and conductivity during the experiments are indicated in (c). Initial water fluxes were 65.3 ± 0.3 L/m² h and 63.4 ± 0.9 L/m² h for RO and SBRO, respectively, in (a), whereas the initial permeate electrical conductivities were 6.8 ± 0.1 μ S/cm and 7.3 ± 0.2 μ S/cm, respectively, in (b). The subsequent values are normalized based on the initial values.

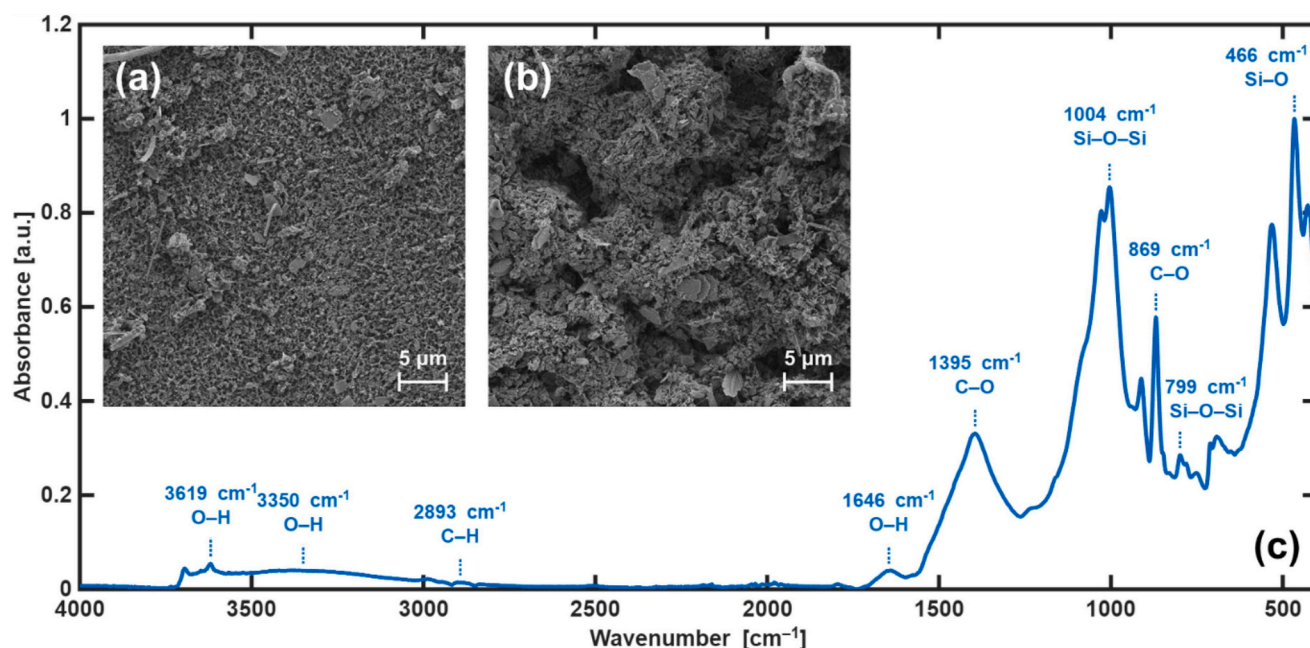


Fig. 4. SEM images of the membrane surfaces from (a) RO and (b) SBRO, together with (c) FTIR analysis of the foulant layer composition on the membrane operated under SBRO conditions.

balances colloidal removal with regulation of biological activity. Because individual pretreatment methods differ in their effectiveness across physical and biological indicators, stable operation may benefit from integrated or optimized pretreatment schemes that consider both functional performance and economic feasibility. Due to the effective control of colloidal and biological matter and its high water production rate, CF was selected as the pretreatment process in this study.

3.2. Flux decline and membrane fouling

Membrane fouling development leads to flux decline and increased energy consumption. Experiments compared RO and SBRO performance over three weeks, with the membrane module operated on fresh feed for RO and concentrated feed for SBRO. Water flux was monitored and compared, followed by an analysis of the fouling layer's structure and chemical composition. The discussion progresses from macroscopic performance trends to microstructural and compositional evidence, enabling a mechanistic interpretation of the higher flux decline observed in SBRO compared to conventional RO.

Fouling formation led to a more pronounced decline in performance for SBRO than for RO. While RO maintained a relatively gradual reduction of 5.20% over three weeks, SBRO exhibited a continuously declining water flux of 38.69% (Fig. 3a). Because water flux was measured under identical RO-mode conditions for both membranes (Section 2.1.5), the measured values represent the relative fouling state of each membrane. The more pronounced decline observed for SBRO was potentially attributable to extended membrane exposure to high-salinity conditions during both operation and storage. In SBRO, the cumulative concentrations of biomass, dissolved organics, and inorganic species would increase osmotic pressure and diminish the effective driving force at the fixed operating pressure. In parallel, the growth and compaction of the fouling layer increase hydraulic resistance, resulting in a sustained decline in water flux. While permeate conductivity remained low yet exhibited a mild upward tendency, a pattern consistent with progressive enhancement of concentration polarization (CP) and local transport resistance as operation proceeds (Fig. 3b). Feed conductivity and feed temperature fluctuated within a limited range without a pronounced monotonic drift, which constrains explanations based solely on changing feed conditions (Fig. 3c).

SEM and FTIR analyses were used to characterize the morphology and chemical composition of the fouling layer, suggesting mixed fouling involving biofouling and silicate-related inorganic constituents. Under RO operation, the membrane surface remained comparatively uniform with sparsely distributed deposits (Fig. 4a). In contrast, SBRO operation exhibited rough, heterogeneous aggregates with pronounced clustering (Fig. 4b). The FTIR spectrum indicates the coexistence of organic biofouling substances and silicate-related inorganic constituents during SBRO operation, consistent with the annotated peaks (Fig. 4c). Bands near 1395 and 869 cm^{-1} correspond to carboxylate-related and C-O-related functional groups associated with organic biofouling constituents. In contrast, bands at lower wavenumbers, near 1004, 799, and 466 cm^{-1} , indicate silicate-related inorganic constituents embedded within the organic fouling matrix, with further enrichment under SBRO conditions. Given the relatively low Si concentration (Table 1), the detected silicate-related constituents are unlikely to represent significant inorganic scaling and were instead considered part of the mixed fouling layer. These silicate-related inorganic constituents could stabilize and accelerate fouling accumulation within the biofouling matrix [36,37]. Therefore, the concentrated feed in SBRO promoted mixed fouling involving both biofouling and silicate-related inorganic constituents.

SBRO operation promotes cumulative concentration effects that intensify osmotic pressure penalties and accelerate the development of a compact fouling layer. This structure increases transport resistance and sustains the decline in water flux. The combined SEM-FTIR interpretation reinforces the causal linkage between SBRO operational characteristics and fouling-layer evolution. A mechanistic understanding requires clear identification of the biological indicators associated with SBRO biofouling and careful interpretation of the associated microbial activity.

3.3. Biological indicators and microbial diversity

In the SBRO operation, progressive feed concentration alters the behavior of biological indicators. Although enrichment would conventionally imply proportional increases in these indicators, their responses are not determined solely by physicochemical mass balance but are instead regulated by microbial activity [38]. This distinction necessitates systematic evaluation of how concentration-driven changes in

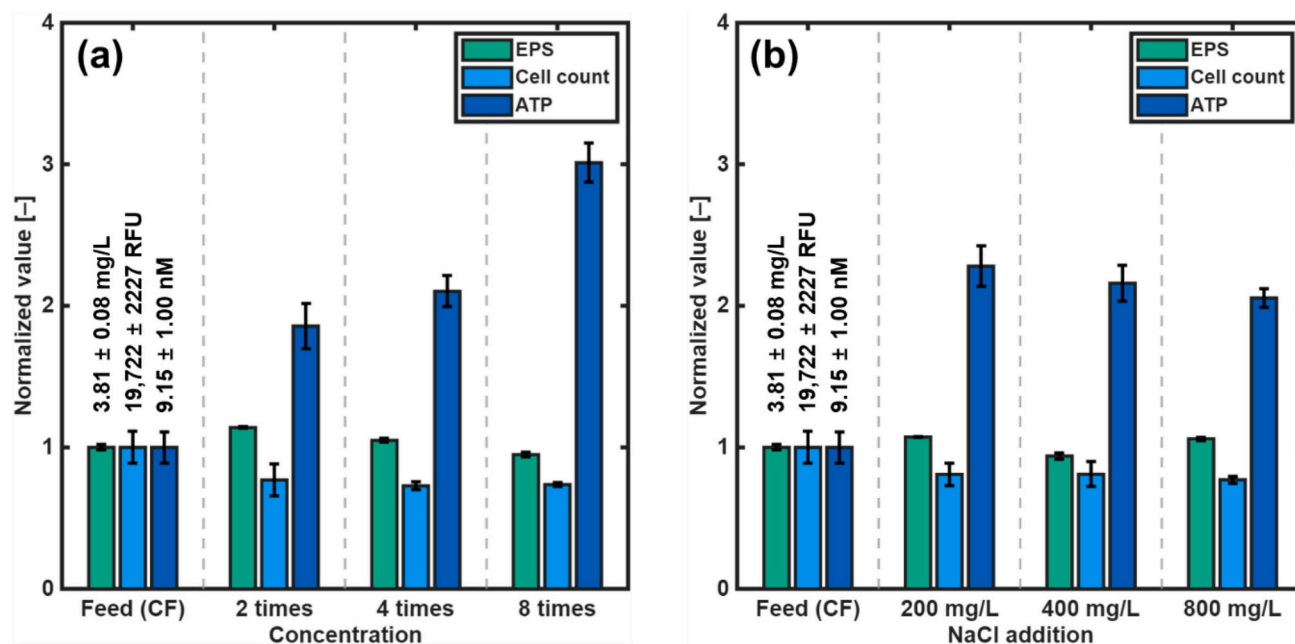


Fig. 5. Changes in biological indicators of the feed under (a) different concentration factors in SBRO and (b) different NaCl concentrations added. The actual values for the initial feed are provided, and subsequent values are normalized accordingly.

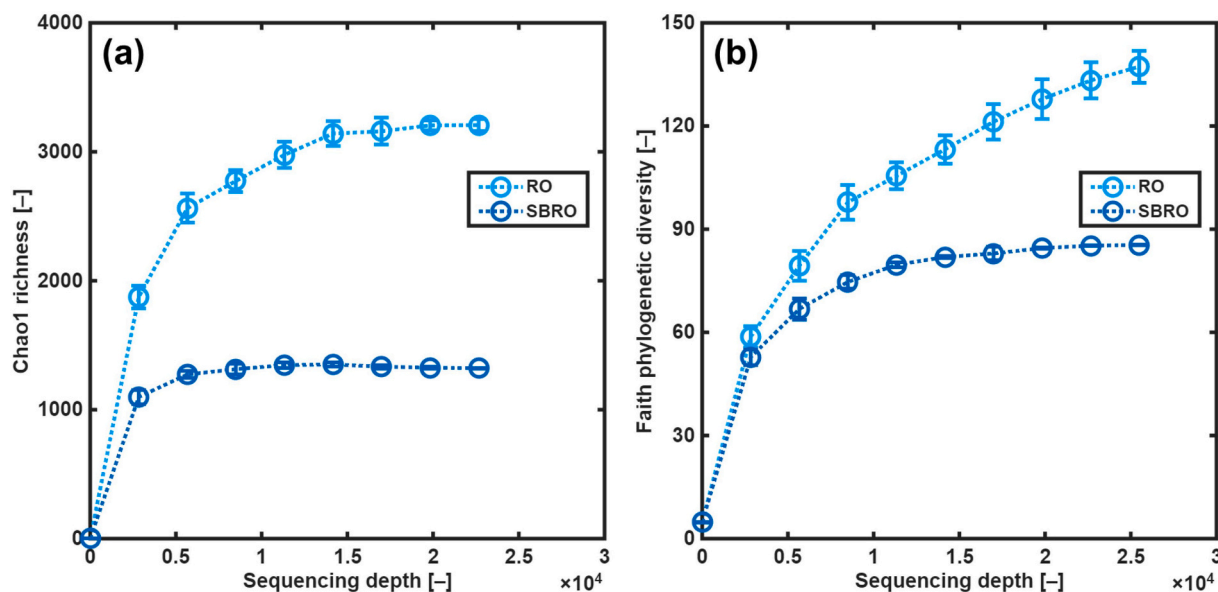


Fig. 6. Temporal evolution of microbial diversity in RO and SBRO systems with (a) Chao1 richness index and (b) Faith's phylogenetic diversity.

microbial physiology influence biofouling development [39]. Under SBRO conditions, sustained exposure to higher feed concentrations may further differentiate surface-associated microbial communities from those observed during conventional RO operation [40]. Examination of these shifts provides insight into the relationship between concentration stress and fouling behavior [41]. Interpreting biological indicators in parallel enables a mechanistic linkage between microbial stress responses and fouling potential.

ATP in the feed increased sharply with increasing SBRO concentration factor (Fig. 5a). The ATP values rose progressively, reaching approximately 1.8 at twofold concentration, about 2.1 at fourfold, and nearly 3.0 at eightfold relative to the feed baseline. In contrast, soluble EPS in the feed remained near unity, with only a slight increase at a twofold concentration, and cell counts declined below baseline and

remained suppressed across all concentration ratios. A similar response was observed following the addition of NaCl to the feed (Fig. 5b). ATP reached a maximum of approximately 2.3 at moderate salinity (200 mg/L) and then declined to about 2.1 at 400 mg/L and 2.0 at 800 mg/L. In contrast to the SBRO concentration, nutrient availability remained unchanged, while only salinity varied. Under these conditions, this trend suggests that ATP elevation does not result solely from concentration enrichment but instead reflects an osmotic stress response [42]. These patterns indicate a salt-stress reaction in which SBRO enhances intracellular energy metabolism in surviving cells, while cell abundance declines and soluble EPS remain weakly responsive to increasing osmotic stress. Although membrane-attached EPS was not analyzed, soluble EPS has also been reported to contribute to membrane biofouling [43,44]. In light of this, the relatively stable soluble EPS concentration

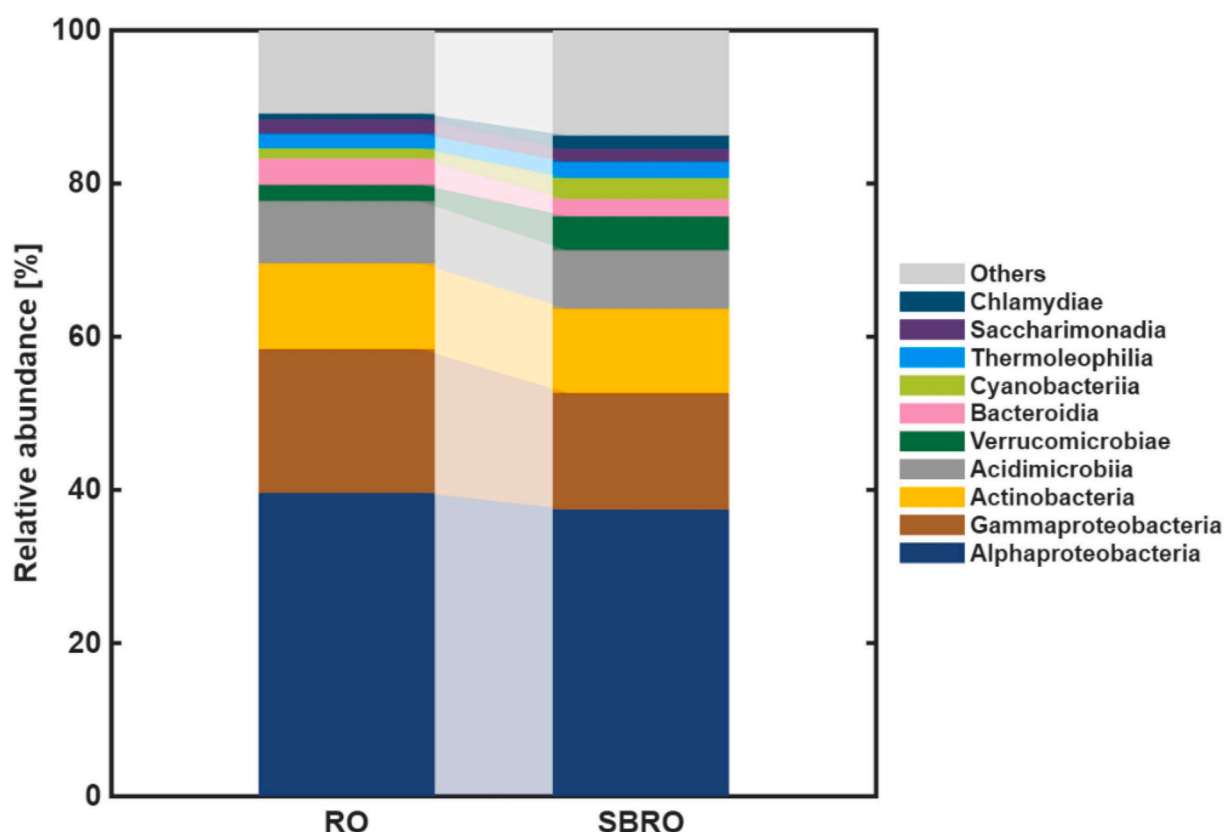


Fig. 7. Phylum-level taxonomic composition of microbial communities on the RO membrane operated under SBRO conditions.

suggests that soluble EPS alone did not reflect the observed biofouling behavior under the tested conditions.

Microbial community diversity on the membrane decreased during SBRO operation compared with RO operation due to osmotic stress and operational instability. Chao1 richness reflects taxonomic richness; it increased with sequencing depth and then plateaued, confirming adequate sampling [45]. Across the whole depth range, SBRO consistently exhibited lower Chao1 values than RO, indicating reduced taxonomic richness under concentrated operation (Fig. 6a). Changing feed conditions from low TDS to high TDS with a concentration factor of 8 in SBRO would be unfavorable to a subset of microorganisms adapted to RO operation [46]. Similarly, Faith phylogenetic diversity, which captures phylogenetic breadth, remained lower in SBRO than in RO (Fig. 6b). When considered together with biological indicator responses, SBRO sustains a less diverse microbial community due to its feed-concentration characteristics.

Given the reduction in cell counts and the shift in microbial diversity, osmotic stress-tolerant microorganisms were preferentially retained under SBRO conditions. This selective environment was associated with increased ATP despite reduced microbial abundance. Consequently, elevated ATP levels were closely associated with biofouling progression rather than with proportional increases in biomass or soluble EPS. However, the possibility that increased microbial metabolic activity was further reinforced by the progressively changing membrane environment associated with flux decline and CP cannot be excluded.

3.4. Microbial community structure and functional analysis

While microbial community diversity was reduced under SBRO conditions, taxonomic composition and functional potential remain necessary to interpret the ecological selection imposed by SBRO conditions. Accordingly, phylum-level relative abundance was analyzed to identify dominant lineages and structural shifts between RO and SBRO.

Also, MetaCyc-based predicted functional pathways were analyzed across major functional categories to provide ecological insight into the metabolic potential associated with the observed community composition. Given the exploratory nature of the sequencing analysis, these results were interpreted to identify overall ecological shifts rather than statistically definitive differences in taxonomic abundance.

The ratio of osmotic stress-tolerant microorganisms increased during SBRO operation. At the phylum level, both RO and SBRO communities were dominated by Proteobacteria and Actinobacteria (Fig. 7). However, the relative abundance shifted from Proteobacteria toward Verrucomicrobiota and several minor phyla under SBRO conditions. Proteobacteria are commonly detected as dominant bacterial groups in surface water and wastewater [47]. They are associated with rapid growth under relatively stable and nutrient-rich conditions, such as those maintained during conventional RO operation [48]. In contrast, Verrucomicrobiota are frequently associated with oligotrophic adaptation, utilization of complex carbon sources, and tolerance to osmotic and physicochemical stress [49]. The intermittent concentration cycles and elevated salinity inherent to SBRO progressively intensify ionic strength and CP near the membrane surface, generating a selective environment for stress-tolerant microorganisms. Despite elevated nutrient concentrations, fast-growing copiotrophic microorganisms, such as Proteobacteria, were less favored under these conditions, whereas metabolically versatile and osmotic-stress-tolerant taxa became enriched [50]. The enrichment of minor taxa such as Cyanobacteriia further suggests ecological adaptation toward microorganisms capable of surviving under concentrated conditions within the biofilm matrix [51]. These results indicate that SBRO operation altered the ecological characteristics of the membrane-associated microbial community, enhancing osmotic stress tolerance and environmental resilience.

Predicted functional profiles were dominated by biosynthesis and central carbon/energy metabolism during SBRO operation (Fig. 8). Biosynthesis-related functions were primarily represented by amino acid

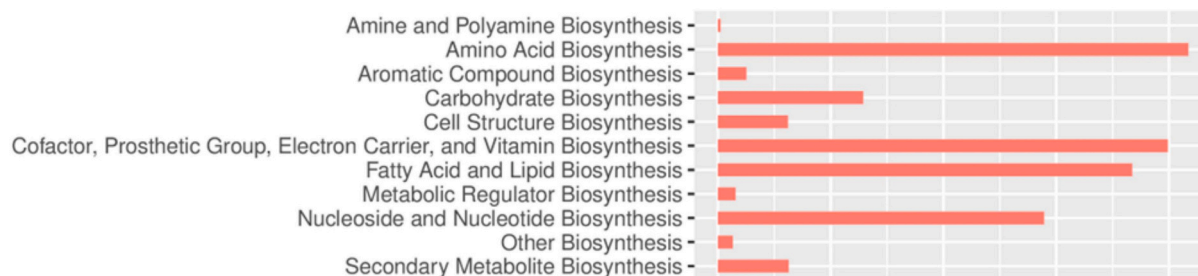
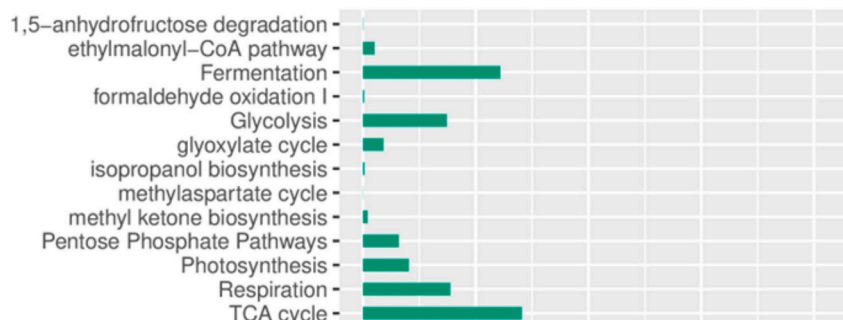
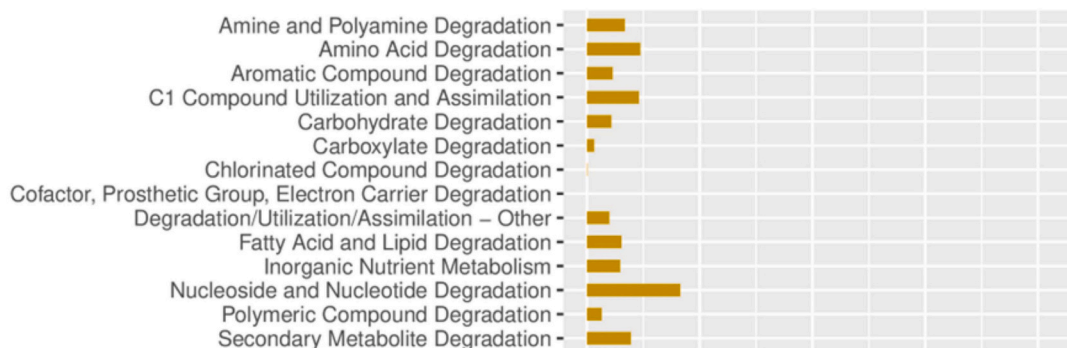
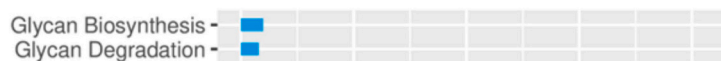
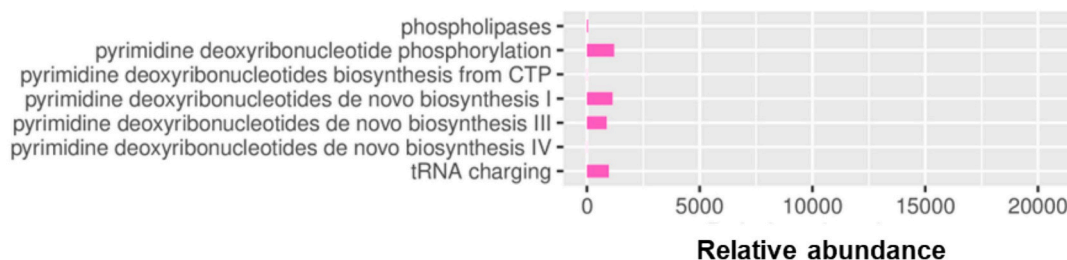
(a) Biosynthesis**(b) Central carbon and energy metabolism****(c) Degradation****(d) Cellular modification and detoxification processes****(e) Glycan pathways****(f) Metabolic clusters**

Fig. 8. MetaCyc-based functional pathway profile of microbial communities on the RO membrane under SBRO operation: (a) Biosynthesis, (b) central carbon and energy metabolism, (c) degradation, (d) cellular modification and detoxification processes, (e) glycan pathways, and (f) metabolic clusters.

biosynthesis, cofactor/prosthetic group/electron carrier/vitamin biosynthesis, fatty acid/lipid biosynthesis, and nucleoside/nucleotide biosynthesis (Fig. 8a). These results suggest enhanced biosynthesis of cellular constituents required for microbial cell membrane integrity, metabolic maintenance, and osmotic stress adaptation under concentrated SBRO conditions [52]. In particular, increased fatty acid and lipid biosynthesis reflects microbial cell membrane remodeling and stabilization in response to salinity and concentration stress [53]. Pathways associated with central carbon and energy metabolism also exhibited high relative abundance (Fig. 8b). The tricarboxylic acid (TCA) cycle, fermentation, glycolysis, and respiration contributed strongly to the functional profile, indicating sustained energy production and carbon flux to support microbial activity and maintenance under recirculating high-recovery operation [54]. In contrast, pathways associated with degradation, cellular modification/detoxification processes, glycan pathways, and metabolic clusters remained relatively low in abundance (Fig. 8c–8f).

Overall, the SBRO operation corresponded to a taxonomic reallocation in which the dominance structure was reshaped, with contributions from several non-dominant phyla becoming more pronounced. At the same time, the functional profile remained strongly oriented toward biosynthesis and central carbon/energy metabolism. The taxonomic and predicted functional analyses, supported by the observed ATP increase, provide complementary evidence that SBRO favored metabolically active microbial communities adapted to concentrated conditions.

4. Conclusions

With increasing contamination of surface waters and stricter water quality requirements, SBRO has attracted attention as an effective technology for high-recovery water production. Because surface water is prone to biofouling during high-recovery operations, identifying the formation and development of biofouling in SBRO is essential for guiding mitigation strategies. The key findings of the study are summarized below:

- Pretreatment regulated distinct foulant fractions. The 0.45- μm microfilter most effectively reduced colloids and ATP but suffered severe clogging under high biofoulant loads. SF increased MFI due to particle release, while reducing cell counts, whereas CF provided limited colloid control and lowered ATP levels in the feed. Soluble EPS increased under all pretreatment conditions.
- Prolonged exposure to highly concentrated feed during SBRO led to a pronounced decline in water flux, exceeding that observed with RO, indicating intensified mixed fouling involving biofouling and silicate-related inorganic deposition. Accordingly, effective mitigation requires targeted control of biological activity while considering the potential contribution of inorganic deposition.
- High SBRO concentration led to marked increases in ATP under osmotic stress, while soluble EPS changed little, and cell counts declined in the feed. Microbial diversity and phylogenetic breadth decreased relative to RO, indicating selective enrichment of halotolerant taxa with elevated metabolic activity.
- SBRO operation shifted microbial community composition, reducing Proteobacteria and enriching Verrucomicrobiota and other minor phyla. In addition, the predicted functional profile was dominated by biosynthesis and central carbon/energy metabolism pathways, suggesting microbial adaptation to concentrated conditions under SBRO operation.

Biofouling progression under SBRO was more closely associated with increased microbial metabolic activity than with changes in soluble EPS or cell abundance in the feed. Elevated ATP levels were consistently observed during the concentration process, suggesting their potential as an early indicator of biofouling risk. Thus, pretreatment strategies should aim to suppress microbial metabolic activity in surface water,

with approaches such as biological or adsorptive polishing (e.g., activated carbon) to limit per-cell energy production by reducing residual assimilable organic carbon. This mechanistic understanding enables more effective biofouling mitigation and supports high-recovery surface water treatment.

CRedit authorship contribution statement

Yumiao Song: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Weijian Jia:** Project administration, Investigation. **Heriberto Véléz:** Validation, Data curation. **Kiho Park:** Writing – review & editing, Validation, Data curation. **Jungbin Kim:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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