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Gravity-driven membrane system for secondary wastewater effluent treatment:**Filtration performance and fouling characterization**

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Abstract

Gravity-driven membrane (GDM) filtration is one of the promising membrane bioreactor (MBR) configurations. It operates at an ultra-low pressure by gravity, requiring a minimal energy. The objective of this study was to understand the performance of GDM filtration system and characterize the biofouling formation on a flat sheet membrane. This submerged GDM reactor was operated at constant gravitational pressure in treating of two different concentrations of secondary wastewater effluent. Morphology of biofilm layer was acquired by an *in-situ* and on-line optical coherence tomography (OCT) scanning in a fixed position at regular intervals. The thickness and roughness calculated from OCT images were related to the variation of flux, fouling resistance and permeate quality. At the end of experiment, fouling was quantified by total organic carbon (TOC) and adenosine tri-phosphate (ATP) method. Confocal laser scanning microscopy (CLSM) was also applied for biofouling morphology observation. The biofouling formed on membrane surface was mostly removed by physical cleaning

confirmed by contact angle measurement before and after cleaning. This demonstrated that fouling on the membrane under ultra-low pressure operation was highly reversible. The superiority and sustainability of GDM in both flux maintaining and long-term operation with production of high quality effluent was demonstrated.

Keywords: Biofouling; fouling; gravity-driven membrane filtration; optical coherence tomography; secondary wastewater effluent

1. Introduction

Membrane bioreactor (MBR) technology, a combination of biological treatment with membrane filtration, has got an enhanced application in wastewater treatment and reclamation over the past two decades [1]. With several advantages such as higher quality of effluent and lower footprint, the global market of MBR was expanded with a compound annual growth rate (CAGR) of 11.2% through 2005 to 2015, and further it is expected to be \$777.7 million by 2019 [2, 3]. Among different process configurations, the submerged (or immersed) MBR is the mostly used one, which allows less energy consumption and more compact installation, and thus has possessed 98% of global MBR market [3].

Nevertheless, compared to biological treatment processes, the conventional MBR is to some extent constrained in higher capital equipment and operating costs such as cleaning and fouling control. Thus, researches on novel MBR configuration and reactor designs have been conducted to promote MBR technology into more feasible and practical implementation [4].

Gravity-driven membrane (GDM) filtration system is one of the beneficial submerged MBR developed configurations. It has a wide spectrum for water treatment, including diluted wastewater and surface water with variable contaminant levels and mainly shows attractive potential in water reuse or seawater pre-treatment [5]. The GDM filtration was firstly tested by the Swiss Federal Institute of Aquatic

Science and Technology (Eawag) [5-7]. The system is operated under ultra-low pressure (40-60 mbar) with less maintenance compared to traditional membrane filtration systems such as ultrafiltration (UF) [8, 9]. In a lab-scale test, the flux stabilization in the range of 2 to 20 L/m² h was reached without the need of backwashing [10, 11]. The viability of GDM filtration has been proved considering the scarcity of energy and stricter regulations in wastewater treatment. The increasing capacity and broaden application of MBR also calls for centralized robust systems and facilities, which are often insufficient in developing and transition countries, causing lack of adequate and microbiologically safe drinking water in these areas. Solutions to safe water supply in these regions have been proposed in decentralized forms such as ground water wells and point-of use (POU) systems [9]. Feasibility of these solutions has been verified in a slice of specific areas, but still failed in worldwide application in terms of either heavier burden on construction or unreliability of water quantity and quality. Thus GDM, as an energy-saving approach, can play an important part in both advanced wastewater and household tap water treatment.

The inevitable membrane fouling is a typical problem in all membrane filtration applications. Membrane fouling is mainly resulted from both pore blocking and cake layer formation, especially in UF and microfiltration (MF) systems, where the pore blocking is caused by colloids and soluble organics such as extracellular polymeric substances (EPS), and cake layer is caused by large particles like sludge flocs [12].

The membrane fouling is of great interest in GDM research. A previous study assumed that at early stage, the pore blocking dominates the flux drop, while then the stable flux in GDM filtration was attributed by the cake layer formation on the membrane surface in expanded operation time [13]. Besides, while operated under dead-end mode with quite low TMP, the mechanism of membrane fouling in GDM can be different from that in normal MBR, which is usually equipped with pumps for extra

pressure [10, 11]. Moreover, studies about fouling mechanism in GDM will provide the fundamental information for optimizing the GDM system and achieving its practical application.

The effluent (or permeate) quality is also used for evaluation of filtration systems. The composition of effluent shows indirectly the retained substances by the filtration process by comparing with influent and reflects the filtration performance. In this regard, liquid chromatography-organic carbon detection (LC-OCD) has been used as an effective tool to analyze colloidal and soluble organics in solutions [14, 15]. Furthermore, biofouling characterization can be carried out by quantification of attached biomass in terms of adenosine triphosphate (ATP) and organic foulants in terms of total organic carbon (TOC) [16-18]. Fouling can be evaluated by measuring membrane surface properties such as hydrophobicity and hydrophilicity, which can be determined by contact angle. The contact angle measurement can also assess the fouling reversibility before and after cleaning [19].

Direct observation of biofouling structure on membranes is straightforward. In this way, various observation techniques have been widely reported. Confocal laser scanning microscopy (CLSM) yields images to display the morphology and composition of biofouling, showing different constituents such as proteins and polysaccharides by fluorescent staining [20]. However, sample preparation such as drying and staining also mechanically changes the morphology and components distribution of fouling layer [21, 22]. For this reason, some on-line imaging techniques have also been proposed. Among them, optical coherence tomography (OCT) has been used in biofilm monitoring in many studies, which surpasses others as an on-line, *in-situ* and non-destructive observation technique by maintaining the original appearance of object [23-24]. OCT was used to assess the impact of the biomass deposition on the flux decrease at early stage [25]. In spacer filled channel the image analysis of OCT scans enabled the spatial distribution the biomass [26-27]. The innovation facilitates the acquirement of authentic information of biofilm for quantitative analysis in the thickness, roughness and porosity of fouling layers

[10]. The *in-situ* observation also allows repeated and continuous investigation, providing the dynamic development of biofouling for further studies of fouling mechanisms [23,25-27].

Recent researches on GDM system are mostly limited to applied aspects of different processes such as seawater pretreatment and surface water treatment. For example, Peter-Varbanets *et al.* studied the flux stabilization in a side-stream GDM system. They found that the stable flux was attribute to the deposition and formation of non-dissolved material and irremovable fouling, as well as the structure changes of fouling caused by biological activities [5, 6]. They also tested the intermittent operation in treating river water by ultra-low pressure ultrafiltration, and confirmed its role in leading to higher fluxes [7]. Akhondi *et al.* explored the influence of different temperature and hydrostatic pressure on flux variation and fouling structure in a side-stream GDM ultrafiltration system for seawater pretreatment. The results showed the improved temperature and gravity pressure both contributed to higher flux in an extended operating time, where cake layer has predominated fouling on membranes. Besides, the general structural change of fouling had also been described and was suggested to be an explanation of permeate flux [13]. Wu *et al.* also studied the seawater pretreatment by GDM but in a submerged system. In this research, the stable flux was related to different system configurations, membrane types and prefiltration, likewise the fouling mechanisms. These studies provide information about the flux and fouling performance from different aspects, but only a few mentioned about wastewater treatment. Hence, more detailed and on-line information are required for better understanding of filtration and fouling mechanisms in GDM system.

The objective of this study was, therefore, to evaluate the filtration performance and fouling aspects in a submerged GDM system using UF membrane under ultra-low pressure. The flux variation and permeate quality was measured at regular intervals to assess the filtration performance. By using OCT, the on-line fouling development was monitored and related with the performance, as well as the formation rate, in

terms of thickness and cake layer resistance, could be obtained. The fouling characterization was also carried out at the end of operation.

2. Materials and methods

2.1 Set-up and operation of submerged GDM system

A customized ultra-low pressure GDM system was used in this study with two following experiments (**Fig. 1**). A flat sheet, polysulfone (PS) UF membrane with 20 kDa molecular weight cut-off (MWCO, PHILOS, Republic of Korea) was immersed upright in a polymethyl methacrylate (PMMA) filtration tank of 70 cm height. Effective membrane area was 0.0045 m^2 ($0.09\text{m} \times 0.05\text{m}$). The OCT probe was mounted on a motorized frame (Welmex) to monitor the membrane and biofouling formation, allowing the movement in x, y and z directions as accurate steps in micrometer scale.

During the experiment, the system was operated under dead-end mode at constant temperature (20 ± 1 °C). A synthetic secondary wastewater effluent was used as feed solution to grow the biofilm on the membrane according to Nopens *et al.* [29]. A 0.5 mL activated sludge collected from a lab aerobic activated sludge system was added into the tank at the beginning to enhance the formation of biofilm. Based on different dilution ratio, the chemical oxygen demand (COD) of feed solutions used in two experiments were 15 mg/L (E1) and 7.5 mg/L (E2), respectively. Total organic carbon (TOC) concentration of two feed waters were 4.9 ± 0.2 mg/L (E1) and 2.6 ± 0.1 mg/L (E2). The feed water was pumped into a level regular to keep a constant level of water head in the tank, which is 45 cm above the membrane. Thus the gravity pressure was constant at 45 mbar. The permeation water was collected from the bottom of the tank. The whole system was covered with aluminium foil to avoid the growth of algae. GDM operation was continued for 6 weeks and 7 weeks, respectively in E1 and E2. The feed solution was refreshed every week.

2.2 GDM performance evaluation

2.2.1 Flux variation

The weight of permeated water was measured everyday to get the daily flux regarding the effective membrane area of 0.0045 m². At the same time, on-line flow rate was checked using a flow meter.

$$J = \frac{dQ}{A \cdot dt} \quad (1)$$

where, J (L/m² h, LMH) is the permeate flux, Q (L) is the quantity of the permeation water filtered through the membrane, A (m²) is the effective surface area of the membrane, and t (h) is the time interval between two measurements.

In addition, the hydraulic resistance (m⁻¹, R) was calculate based on Darcy's law:

$$R_{total} = \frac{TMP}{\mu_{20^{\circ}C} J_{20^{\circ}C}} \quad (2)$$

where, TMP is the transmembrane pressure which is around 45 mbar constantly in this case, and $\mu_{20^{\circ}C}$ is the permeate viscosity at temperature 20 °C, $J_{20^{\circ}C}$ is the flux measured in the experiment.

2.2.2 Permeate quality

Permeate samples were collected at regular intervals and measured by LC-OCD for evaluation of the effluent quality and removal efficiency of dissolved organic carbon (DOC) compounds. Hydrophilic dissolved organic carbon (HPI-DOC), including biopolymers, humics, building blocks and low

molecular weight (LMW) acids and neutrals, were analyzed based on size exclusion. The samples were filtered through 0.45 μm syringe filters. The sample injection and retention time were 2,000 μL and 150 min, respectively. Samples were transported to a chromatographic column (Toyopearl TSK HW50S dual-column, TOSOH Bioscience GmbH, Stuttgart, German) with a phosphate buffer mobile phase of pH 6.4 at a flow rate of 1.5 mL/min. The acquired data was processed using the customized software (ChromCALC, DCO-LABOR, Karlsruhe, German) [15, 30].

2.3 Fouling assessment

2.3.1 Fouling formation and development

An optical coherence tomography (OCT) system was used in this study to investigate the fouling formation and development in micrometer scale. A central wavelength of 930 nm was provided by the system to penetrate the sample to create depth profile. The OCT probe was equipped with a 5X telecentric lens (Thorlabs LSM 03BB), allowing scanning an area of 4.0 x 1.8 mm. A defined position was scanned every day at a relatively fixed time for fouling monitoring.

The images were optimized by Fiji (NIH, USA) to remove the background noise for higher identification, and then were analyzed by a customized MATLAB code to measure the thickness of fouling layer in pixel unit. Then, according to the original information of pixel size from the OCT scanning, the real average thickness ($\bar{Z}$, μm) of biofilm was corrected and calculated using Eq. (3) [10, 13].

$$\bar{Z} = \frac{1}{n} \sum_{i=1}^n Z_i \quad (3)$$

Roughness (absolute and relative) was acquired using the following equations,

Absolute roughness coefficient (R_a , μm),

$$R_a = \frac{1}{n} \sum_{i=1}^n |Z_i - \bar{Z}| \quad (4)$$

Relative roughness coefficient (R'_a),

$$R'_a = \frac{1}{n} \sum_{i=1}^n \left(\frac{|Z_i - \bar{Z}|}{\bar{Z}} \right) \quad (5)$$

where, n is the number of measurements and Z_i is the biofouling layer thickness (μm) in specific position.

The cake layer porosity was calculated based on Eq. (6) according to Carman-Kozeny equation [10, 31].

$$R_{\text{cake layer}} = \frac{75(1-\varepsilon)^2}{2\varepsilon^3 r_p^2} \bar{Z} \quad (6)$$

where, ε is the cake layer porosity, r_p (m) is the characteristic radius of the particles forming the cake layer which is 28 nm according to the measurement of particle (in supernatant) size using zeta potential analyzer (Zeta Plus, Malvern, United Kingdom). Average thickness ($\bar{Z}$) is calculated using Eq. (3). Based on previous researches, the cake layer resistance possesses the most part of the total resistance. As published by Derlon *et al.*, after a long term running, a GDM system operated under different predation conditions all showed significant biofilm resistance, corresponding to 95% or even higher of the total resistance [10]. Thus, here, the cake layer resistance ($R_{\text{cake layer}}$) was assumed to be the total resistance, which has been acquired by Eq. (2).

2.3.2 Foulant extraction

After finishing the GDM operation, the membrane module with biofilm accumulation was taken out of the filtration cell and the fouling characterization was carried out immediately. The membrane pieces were cut and transported into 50mL tubes containing 30 mL of 1X phosphate buffer solution (PBS). The foulants were extracted by vortex followed by ultra-sonication in water bath 3 times to fully detach the biofilm layer from the membrane and distribute homogeneously in the solution. The extracted foulant samples were then used in different fouling analyses.

2.3.3 Organic fouling

The organic foulants were measured by total organic carbon (TOC) method using a TOC analyzer (TOC-V-CSH, Shimadzu, Japan), and was calibrated by a fresh membrane with specific area. The non-purgeable organic carbon (NPOC) method was used.

2.3.4 Biological fouling

The attached active biomass of biofilm layer was detected by measuring the adenosine triphosphate (ATP) concentration in extracted fouling samples. ATP was measured using ATP Analyzer (Celsis, USA), which employs the luciferin–luciferase bioluminescence reaction method [32, 33].

2.3.5 Fouling distribution

CLSM was used to investigate the biofilm internal structures and characterize the component distributions. Two fouled membrane pieces were used as CLSM samples. One is for investigating the cell viability in the biofilm, while the other is for polysaccharide and nucleic acid (total cell) detection.

The membrane samples were rinsed with DI water. A 3 $\mu\text{L/mL}$ SYTO®9 and propidium iodide (PI) mixed reagent (LIVE/DEAD® BacLight™, Invitrogen, USA) was used for live and dead cells investigation. DAPI (4',6-diamidino-2-phenylindole, Thermo Fisher Scientific, USA) was used for staining of total cell as it can penetrate the intact cell membrane of bacteria and bind strongly with DNA. The biopolymer mainly existed as β -polysaccharide. It was identified by 0.2 mg/mL concanavalin A (ConA, Alexa Flour® 633, Invitrogen, USA). Samples were all soaked and stained by reagents at room temperature (20 ± 1 °C), incubated in a dark room for 30 min. The ConA and DAPI staining were done separately. DI water was used to wash the samples three times after each staining. The stained samples were then fixed on the glass slides for later CLSM investigation. To maximum maintain the original morphology and acquire the components of the biofilm, an upright Zeiss LSM 710 confocal microscope (Germany) was used to capture the images. A 10X objective was equipped and the detected positions were selected randomly on the sample surface with an area of $900 \mu\text{m} \times 900 \mu\text{m}$. The acquired images were processed and analyzed by Imaris (Bitplain, USA).

2.4 Cake layer reversibility test

The CAM 200 contact angle goniometer (KSV instruments Ltd, Finland) was used to examine the reversibility of the fouling membrane by comparing the contact angle of three different types of membranes, i.e. fresh, fouled and cleaned membrane. This enabled to compare contact angle values of fouled membrane and cleaned one with virgin one. The syringe of the goniometer was filled with DI water and fixed by a syringe clamp upon the sample stage. The fresh and dry membrane sample was removed from a pristine UF membrane module. The fouled membrane samples were cut and washed by DI water and dried with pressurized air. All of the samples were modified into $20 \times 10 \text{mm}$ dimensions

and fixed on slides. The principle of the goniometer is based on sessile drop method. The images of the water drop formed on the sample surface can be recorded and used to calculate the contact angle then.

3. Results and Discussion

3.1 GDM filtration performance

3.1.1 Flux reduction and stabilization

The GDM reactor was operated under a constant gravitational pressure of 45 mbar (4.5 kPa) supported by the water head. Two different experiments using two different feed solutions (E1: COD 15mg/L and E2: COD 7.5 mg/L) were continued for 6 weeks and 7 weeks, respectively. The weight of collected permeation water (or effluent) was measured every day during the whole operation, and the flux was calculated as described in earlier.

Fig. 2a presents the flux decline of two experiments and four distinct phases based on flux decline pattern were observed. The flux change trends in both two experiments (E1 for the first experiment and E2 for the second experiment) were the same. The flux dropped significantly during the first 6 days (phase I: 1st-6th day) from around 30 to 6 LMH and became stable after 30 days of operation (phase IV) at 2 LMH and 4 LMH till the end of the E1 and E2, respectively. The flux variation exists the similar pattern observed in a previous GDM system study reported by Akhondi *et al.* [13]. **Fig. 2b** displays the variation of total resistance under constant TMP of two experiments. The fouling resistances were calculated according to the flux and displayed with a converse trend as flux. It is also found to be four phases, indicating the resistance development was closely related to flux decline.

The flux stabilization has been observed in most cases of GDM system in spite of different operating conditions. However, previous researches on flux level demonstrated that the degree of flux stabilization could be quite different and depend on the operating temperature, hydrostatic pressure and influent

properties etc. [13]. Given that the gravity pressure was maintained at 45 mbar as well as a constant ambient temperature and other operations, the different level of stabilized flux (E1: 2 LMH and E2: 4 LMH) could be explained by the different COD concentrations of feed solution. In the GDM filtration study by Perter-Varbanets *et al.* [5], feed water with varying TOC concentrations collected from river, lake and diluted wastewater were applied for flux test under the same hydrostatic pressure. They found that the diluted wastewater with the highest TOC of 12.5 mg/L showed the lowest flux level, stabilizing at around 7 LMH, while the river water with TOC of only 2.5 mg/L reached the highest flux level up to 10 LMH. In their study, larger pore size of membrane (100kDa) than the membrane (20kDa) used in the present study was applied. It made different flux and fouling behaviour even though the similar concentration of feed water was used (TOC of E2: 2.6 ± 0.1 mg/L). The same trend was observed in the present study. In the filtration systems operated under stable TMP, the feed water with higher TOC valued led to higher fouling formation on the membranes, resulting in the achievement of lower flux level over the long-term operation (**Fig. 2b**).

3.1.2 Permeate quality in GDM system

The effluent (or permeate) was collected at regular intervals and the effluent quality was measured by LC-OCD, which is quantifying dissolved organic carbon (DOC) based on molecular size.

As shown in **Table 1**, the concentration of different organic fractions generally shows a tendency of decline. At phase IV (day 48 of the second experiment), DOC in the permeate was decreased to 835 $\mu\text{g/L}$ compared to 1326 $\mu\text{g/L}$ at the beginning (day 2), reflecting a higher organic removal due to either size exclusion or effect of adsorption onto biofilm on the membrane. The hydrophilic DOC contributes as the main part of the total DOC. Among the HPI-DOC components, the biopolymer is composed of substances in high molecular weight including polysaccharides and proteins, and can be mostly rejected

by the UF membrane used during the experiment. But at day 48, the concentration of biopolymers only decreased slightly from 74 $\mu\text{g/L}$ to 64 $\mu\text{g/L}$. As the protein was completely rejected by the filtration in the end (0 $\mu\text{g/L}$ in day 48), the insignificant decline is attributed to the polysaccharides, which is almost the same in different stage. The explanation can be the linear structures of polysaccharides composed of long chains of monosaccharide, thus much easier passed through the pore tunnels of the membrane [34]. Furthermore, building blocks and humic substances (or humics) with molecular weight cut-off around 1 kDa, are smaller than the membrane. The size of low molecular weight (LMW) substances are less than 350 Da [30], so in theory, these substances could not be rejected by the membrane. However, the concentration of humics and LMW neutrals also lowered over the operation time from 155 $\mu\text{g/L}$ and 883 $\mu\text{g/L}$ to 55 $\mu\text{g/L}$ and 456 $\mu\text{g/L}$, respectively, which supports an assumption that the fouling layer at phase IV can work as a second filtration layer under ultra-low pressure [35].

3.2 Fouling formation and development

OCT was used in this study for *in-situ* and on-line fouling monitoring in the GDM. The daily scanning was carried out to investigate the development and evolution of fouling layer on membrane surface at a fixed position. The acquired images were then processed and the fouling morphology was quantified in terms of thickness, roughness and porosity.

3.2.1 Fouling investigation using OCT

The obtained morphology of fouling layer from OCT scanning shows an evolution of fouling from thin, loose and porous structure to thick, dense and homogeneous structure in both experiments (**Fig. 3**). Generally, the morphology change was well matched the flux and resistance change, which can also be divided into four phases. Hence, the result of E2 is reported in this paper.

In phase I (**Fig. 3a**), the biofilm layer started with a thin and fluffy structure, and then a smooth and dense layer was detected in the bottom part of the biofilm by the end of phase I (day 6), where the flux has dropped to a significant low level (**Fig. 2a**). From day 7, when phase II (**Fig. 3b**) started and a short period of stable flux was observed, the fouling layer were subjected to the deposition of materials in different size such as bacteria, colloids, particulate organics and inorganic substances [36]. Accordingly, the upper layer formed in phase I was condensed and the biofilm altered into more compact but irregular morphology after around 10 days of operation. With the accumulation of biomass on the membrane, the fouling layer grew into a relatively homogeneous structure, resulting in thickness decreasing compared to the beginning of this stage.

In time of phase III (**Fig. 3c**), with the continuous filtration, the observed thickness of biofilm increased steadily. Meanwhile, more organic substances such as biopolymers were retained in the fouling layer, facilitating the growth of bacterial and then in return, there were more biopolymer excretion. These gel-like materials filled the vacancy and squeeze the pores. So the fouling layer became denser and more homogeneous, revealing the enhanced fouling resistance and decreased flux.

After 30 days' operation, the formed biofilm layer has been fairly stable and homogeneous in phase IV (**Fig. 3d**), which is different from some previous GDM system studies, where heterogeneous structures of biofilm layers were observed by CLSM scanning and maybe on account of the destructive operation in sample preparation as well as the inoculation and predation of eukaryotes [10, 13]. A recent research in diverse membrane modules applied in GDM filtration cells also supported the uniform fouling architecture formed in the final stable stage [37], providing an explanation of the stable flux during this phase. In addition, it is noticed that the thickness of fouling decreased slightly and gradually during this stage. This is probably due to the detachment of aging biofilm and the condensation by accumulated biomass. Overall, the subsequent change in the fouling structure indicated that a dynamic evolution of

the biofilm morphology occurred during GDM filtration, and the morphology showed strong correlation with flux and resistance change.

3.2.2 Fouling quantification based on OCT image analysis

To further quantify the biofilm on membrane surface, image analysis was carried out to calculate the average biofilm thickness, absolute and relative roughness as well as porosity as presented in **Fig. 4**. The relative roughness coefficient (**Fig. 4c**) shows how homogeneous and uniform the biofilm is. In addition, the porosity was estimated by assuming the cake layer resistance as the fouling resistance (**Fig. 4d**). However, considering the relatively thin layer of biofilm formed during the first several days, such descriptor is more convective after a long period of filtration when the biofilm has significantly deposited and formed.

Overall, different from flux and resistance, non-linear trend was found over the observation period in all four descriptors of both experiments, but with general consistency to the morphology changing captured by OCT in **Fig. 3**. **Fig. 4a** showed that the thickness increased linearly in the first few days in phase I, where additional fouling resistance presented and flux dropped significantly. Afterwards, as shown in **Fig. 3b**, with foulants aggregating and biofilm compacting, the thickness decreased in the end of phase II after a short fluctuating period. The relative roughness coefficients in both phase I and phase II provided a higher value (**Fig. 4c**), indicated that some parts of the biofilm were locally thinner or thicker than general conditions of other parts, matching well with the heterogeneous morphology captured by OCT in these two phases (**Fig. 3a** and **3b**). Thus the temporary flux stabilization can be explained by the dynamic equilibrium between heterogeneity and thickness. The fouling layer in this phase was subjected to the increased thickness of deposited biomass. However, at the same time, the biological process was more active and the fouling structure was more heterogeneous. Thus the increased resistance can be

offset, contributing to a stable flux. In phase III, the thickness slowly increased when the coefficients kept decreasing. It is noteworthy that the thickness in phase II was higher than the beginning part of phase III, which in theory, means higher resistance and lower flux. According to the OCT images (**Fig. 3b** and **3c**) as well as the coefficients, however, the fouling structure in phase II was more heterogeneous than that in phase III, leading to less filtration resistance and thus higher flux. This also indicates that the thickness is not inversely proportional to the flux exactly and is not the only demanding factor to flux changing. After entering phase IV, the biofilm compacted into constant morphology and the values of thickness were varied in a quite small range. The coefficient also stabilized at quite low level, indicating a stable and homogeneous biofilm structure, showing consistency to the OCT investigations (**Fig. 3d**). During the four phases of operating, the porosity of biofilm was fluctuated in a slight range around 0.60-0.75. Such moderate porosity facilitates the biofilm to filtrate water and confirms again the role of the cake layer as a secondary filtration. Moreover, it was found that the thickness in E1 was higher than E2 in the final stage, and the relatively higher porosity of E2 compared to E1 confirming the influence of the different concentrations of feed water. The variation of thickness, roughness and porosity strongly supports as well a dynamic change of fouling layer during GDM filtration.

3.3 Fouling characterization

After finishing the experiment, foulant was extracted immediately from the fouled membrane. The fouling was characterized in terms of TOC and ATP. In addition, several pieces of cut membrane were used for CLSM investigation. **Table 2** shows the TOC and ATP values measured in two different experiments (E1 and E2). The amounts of organic foulants deposited on the membranes (132 ± 17 and 135 ± 16 μg of TOC/ cm^2 , respectively) were very similar in E1 and E2, implying that feed water concentration did not significantly influence the organic fouling. On the other hand, biomass

concentrations developed on the membrane in E1 (5.46 ± 1.15 ng of ATP/cm²) was slightly higher than that in E2 (4.09 ± 0.12 ng of ATP/cm²) indicating that biofouling was more influenced by higher concentration of feed solution to some extent.

Fig. S1 displays the morphology of fouling layer observed by CLSM. Specific biofouling constituents (i.e., total cell amount, live and dead cell amount as well as polysaccharides) were also found in the images by different fluorescent stains. The findings obtained from CLSM observation are as follow: (1) Mostly found biomass are live cells (**Fig. S1a**) which indicates that the biomass in biofilm on the membrane in GDM are very active, which is consistent to reported study [13]; and (2) polysaccharides are in quite low concentration, which is consistent to the LC-OCD result, where the concentration of polysaccharide in the permeate is high, confirming the low removal efficiency of polysaccharide in this experiment.

3.4 Reversibility of cake layer on the fouled membrane

According to previous studies, cake layer formation dominates the fouling on membranes and contribute to the stable flux in GDM system [13]. Cake layer was assessed by hydrophilicity reduction using contact angle measurement. As can be seen from **Table 3**, the contact angle of virgin membrane was $61.22 \pm 1.23^\circ$ and it was decreased significantly to $27.46 \pm 2.42^\circ$ after GDM operation. This indicated that fouling membrane became more hydrophilic and it is probably due to the hydrophilic organic deposition on the membrane. This was also observed in organic fouling fractionation by LC-OCD (see **Table 1**). The contact angle (or hydrophilicity) of the membrane after physical cleaning recovered again ($60.53 \pm 0.58^\circ$) to initial value of virgin membrane, revealing that the cake-fouling layer formed on the membrane in GDM was highly reversible.

4. Conclusions

In this study the filtration performance of a GDM system under ultra-low pressure was evaluated and the fouling development on a flat-sheet UF membrane was monitored. In addition, fouling characterization and reversibility were tested. The findings obtained from this study are as follow:

- During the GDM operation, the flux decreasing was closely associated with the biofouling development. After 30 days running, the flux was stabilized at a certain level range, which is not observed in other conventional MBR systems.
- The measurement of effluent quality indicated satisfying removal efficiency in the system. Especially, with developing of the fouling layer, permeate quality was improved.
- A dynamic evolution of the biomass deposited on the membrane was confirmed by OCT scanning. Acquisition of morphology descriptors (i.e. thickness, relative roughness, absolute roughness and porosity) matched well with flux and resistance variation.
- A fouling characterization was carried out and the contact angle of membranes in different conditions was measured. The recovered contact angle of the membrane after basic physical cleaning revealed the reversibility of cake layer and the potential of GDM for a long-term operation of secondary wastewater treatment.

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Table 3 Variations of contact angle of virgin membrane and fouled membrane before and after cleaning.

Table 1 Organic fractions of supernatant in the filtration tank and permeate samples (at phases I and IV) analyzed by LC-OCD.

Concentration of organic fractions ($\mu\text{g/L}$)	Supernatant	Permeate phase I	Permeate phase IV
DOC	2762	1326	835
Hydrophobic DOC	248	173	197
Hydrophilic DOC	2514	1153	638
Biopolymers	539	74	64
Polysaccharides	329	64	64
Proteins	210	10	0
Humic Substances	738	155	55
Building Blocks	157	41	63
LMW Neutrals	1080	883	456
LMW Acids	0	0	0

Table 2 Organic fouling and biological fouling concentration on membrane coupons measure by TOC and ATP (Effective membrane area was 0.0045m². Pressure and temperature were constant at 45mbar and 20 ± 1 °C, respectively).

	E1	E2
TOC concentration of feed water, mg/L	4.9±0.2	2.6±0.1
Average concentration of TOC, µg/cm ²	132±17 (n=9)	133±16 (n=12)
Average concentration of ATP, ng/cm ²	5.46±1.15 (n=6)	4.09±0.12 (n=6)

Table 3 Variations of contact angle of virgin membrane and fouled membrane before and after cleaning.

Samples	Average contact angles (°)
Virgin membrane	61.22±1.23
Fouling membrane	27.46±2.42
Physical-cleaning membrane	60.53±0.58

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Fig. 3 Evolution of fouling morphology by OCT scanning: **(a)** phase I, **(b)** phase II, **(c)** phase III and **(d)** phase IV.

Fig. 4 Biofilm descriptors calculated for each obtained routine scan from OCT: **(a)** average thickness, **(b)** absolute, **(c)** relative roughness and **(d)** porosity.

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(b)

(c)

(d)

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Highlights

- A gravity-driven membrane (GDM) system was tested for wastewater post-treatment.
- Fouling evolution was monitored by optical coherence tomography (OCT) in a real-time.
- Flux variation and effluent quality was investigated to associate with fouling.
- Fouling characterization and reversibility confirmed a sustainable operation of GDM.