The effects of Ca(II) on the structure of the cake layer in submerged membrane bioreactors (SMBRs) were investigated in this study. Three parallel laboratory-scale SMBRs were operated with synthetic municipal wastewater with three Ca(II) levels (82, 208 and 410 mg l$^{-1}$). As the Ca(II) concentration increased, the sludge floc size increased and the molecular weight of the soluble microbial products (SMP) in the bulk liquid decreased. These observations were attributed to the neutralization and bridging function of Ca(II). Furthermore, Ca(II) addition did not change the thickness of the cake layer, but inhibited the deposition of other elements, such as Al, Si, Mg, and Fe. As a result of Ca(II) addition, the cake layer became less compact and more porous. The interspaces among the flocs in the cake layer helped to reduce the membrane fouling potential.

**Keywords:** submerged membrane bioreactor; Ca(II); membrane fouling; cake layer structure; sludge flocs

**Introduction**

In recent years, considerable attention has been paid to the development of submerged membrane bioreactors (SMBRs) for municipal and industrial wastewater treatment. SMBRs have several advantages for wastewater treatment, including high-quality effluent, high loading rate, and low sludge production (Hermanowicz et al. 2006; Meng et al. 2009; Zhou et al. 2014a, 2014b). However, membrane fouling, which leads to declining membrane performance and increasing operating costs, has limited the application of SMBR in practice (Huang et al. 2001; Zhou et al. 2014b).

Cake layer formation plays the dominant role in membrane fouling (Meng et al. 2007; Xia et al. 2012; Jiang et al. 2013; Zhou et al. 2014a). The cake layer originates in the bulk liquid in the SMBR, where sludge flocs (including microorganisms), soluble microbial products (SMP) and inorganic compounds are mixed (Gao et al. 2012). Various physical and chemical methods have been used to reduce cake layer formation, eg backwashing, gas sparging, intermittent suction, and membrane fouling reducing (Hwang et al. 2007; Jiang et al. 2013). The cake layer is the dominant factor in membrane fouling, the effect of Ca(II) on the cake layer structure is still unclear. Consequently, although the effects of Ca(II) on membrane fouling in SMBr have been extensively investigated (Arabi & Nakhla 2008; Arabi & Nakhla 2009), the mechanism by which Ca(II) mitigates membrane fouling remains elusive.

The purpose of this study was to investigate the effects of Ca(II) on cake layer structure and to better understand the mechanism of the reduction in membrane fouling via addition of Ca(II). Two laboratory-scale SMBRs were operated under two Ca(II) concentrations, 208 and 410 mg l$^{-1}$, along with a control SMBR with a Ca(II) concentration of 82 mg l$^{-1}$. The medium Ca(II) concentration (208 mg l$^{-1}$) was chosen to represent municipal wastewater with high calcium carbonate hardness (Yang et al. 2010). The higher...
Ca(II) concentration (410 mg l\(^{-1}\)) was representative of the high concentration of Ca(II) in some industrial wastewaters, such as the wastewater from paper recycling manufacturing, bone manufacturing and citric acid manufacturing (Arabi & Nakha 2008). Cake layer structures (the major components of the cake layer and cake layer morphology) were analyzed through the membrane fouling process. Additionally, energy-diffusive X-ray analyzer (EDX) was used to identify the structural composition of the cake layer with the addition of Ca(II).

Materials and methods

Set-up and operation

Three parallel laboratory-scale SMBRs (Figure S1; Supplementary material is available via a multimedia link on the online article webpage), with an effective volume of 3.01 (20 cm × 4 cm × 25 cm of length × width × height), were employed in this study. A hollow fiber membrane module (Li-tree Company, Suzhou, China) was mounted in each SMBR. The module had a nominal pore size of 0.4 μm and an effective filtration area of 10 dm\(^2\). The inoculating sludge was drawn from the return activated sludge stream in the Quyang Wastewater Treatment Plant (Shanghai, China). Continuous aeration, at a flow rate of 0.6 m\(^3\) h\(^{-1}\) (1 atm; 25°C), was provided through an axial perforated tube below the membrane modules and the induction of a cross-flow velocity along the membrane surface to mitigate membrane fouling. Each SMBR was operated under the intermittent filtration mode (10 min suction followed by 2 min relaxation). When the transmembrane pressure (TMP) reached 40 kPa, the membrane module was removed for physical cleaning (washing with tap water) and chemical cleaning (1% NaOCl and immersion in 10% citric acid for 6 h) to recover the membrane permeability.

Two sets of experiments (SMBR I with 208 Ca mg l\(^{-1}\) and SMBR II with 410 Ca mg l\(^{-1}\)) were conducted to investigate the effects of Ca(II) on cake layer structure. Additionally, a control experiment (SMBR control) was performed with a typical Ca(II) concentration (82 mg l\(^{-1}\)).

Table 1. Operating conditions of the three SMBRs.

| Parameter                            | SMBR control | SMBR I     | SMBR II     |
|--------------------------------------|--------------|------------|-------------|
| Influent Ca(II) concentration (mg l\(^{-1}\)) | 82           | 208        | 410         |
| HRT (h)                              | 8            | 8          | 8           |
| SRT (days)                           | 30           | 30         | 30          |
| Dissolved oxygen (mg l\(^{-1}\))     | 7.14         | 7.07       | 7.10        |
| pH                                   | 7.10         | 7.02       | 7.01        |
| MLSS (g l\(^{-1}\))                 | 3.02         | 3.21       | 3.48        |

Before the experiments, each newly inoculated SMBR was initially operated for 60 days with the corresponding Ca(II) concentration to achieve steady state (based on effluent water quality) for the acclimatization of activated sludge. Then the membrane module was replaced with a new unit and each inoculated SMBR was operated for the experiments. As described in Figure S1, SMBRs were fed with synthetic municipal wastewater. The operational conditions and effluent quality are shown in Tables 1 and S1.

Filtration resistance analysis

The hydraulic resistance was calculated using Equation (1):

\[ R = R_m + R_p + R_c = \frac{\Delta P}{\mu J} \]  

(1)

where \( R_m \) is the constant resistance of the clean membrane (m\(^{-1}\)), \( R_p \) is the resistance due to pore blocking (m\(^{-1}\)), \( R_c \) is the fouling layer resistance (m\(^{-1}\)), \( \Delta P \) is TMP (kPa), \( J \) is permeate flux (l m\(^{-2}\) h\(^{-1}\)), and \( \mu \) is the viscosity of the mixed liquor (Pa s). The method used to calculate the resistances of the membrane was as follows: (1) \( R_m \): the flux and TMP of the new membrane module were analyzed in deionized water before the operation; (2) \( R \): after the operation, the flux and TMP of the membrane module were measured; (3) \( R_m + R_p \): after removing the cake attached to the membrane surface by physical cleaning, the flux and TMP of the membrane module were determined in deionized water; (4) \( R_c \): \( R \) was subtracted from \( R_m + R_p \) to get \( R_c \). From these values, each of \( R \), \( R_m \) and \( R_p + R_m \) could be obtained using Equation (1).

SMP extraction and measurement

Forty ml of mixed liquor were centrifuged (MILTIFUGE X1R, Thermo Electron Corporation, Waltham, MA, USA) at 6,000 g for 5 min. Then the supernatant was filtered through a 0.45-μm filter (SCAA-101, ANPEL, Shanghai, China). The content of the filtrate was considered as SMP (Jiang et al. 2013). The molecular weight (MW) distribution of SMP was determined with a gel filtration chromatography (GFC) analyzer, consisting of a TSK G4000SW type gel column (TOSOH Corporation, Shunan-shi, Japan) and a liquid chromatography spectrometer (LC-10ATVP, SHIMADZU, Kyoto-fu, Japan).

Analyses of other parameters

A focused beam reflectance measurement (EyeTech particle size and shape analyzer, Ankersmid, Nijverdal, The Netherlands) was used to analyze the particle size distribution (PSD) of the sludge flocs. Chemical oxygen demand (COD), total nitrogen (TN), ammonia (NH\(_4\)-N), and...
total phosphorus (TP) and mixed liquor suspended solids (MLSS) were measured according to standard methods (APHA 1998). The dissolved oxygen (DO) concentration and pH were measured via a DO-and-pH meter (HQ4d, HACH, USA).

When TMP reached 40 kPa, a piece of membrane fiber was taken from the membrane module. Scanning electron microscopy (SEM) (XL30, Philips, Amsterdam, The Netherlands) coupled with an EDX (Oxford Isis, Oxford, UK) was used to examine the cake layer and determine its chemical components.

Results
Membrane fouling

As Figure 1 shows, the TMP of the SMBR control increased faster than that of SMBR I and SMBR II, indicating that Ca(II) addition delayed membrane fouling. TMP variations in SMBR I, SMBR II and the SMBR control were classified as a two-phase fouling phenomenon, i.e., a slow increase in TMP (Phase I) followed by a rapid increase (Phase II). The gradients of TMP were different in the three SMBRs during Phases I and II.

To further investigate the effects of Ca(II) on cake layer structure, various membrane fouling resistances against membrane permeability during the operation were experimentally determined and are shown in Table 2. The intrinsic membrane resistance was ~ 0.13 × 10^{12} m^{-1} in each SMBR. As shown in Table 2, the total resistance (R) and pore blocking resistance (R_p) were reduced with the addition of Ca(II), and the R_p of SMBR was insignificant.

For each SMBR, R_m, R_p and R_c were also plotted as a percentage relative to the total resistance (Figure S2). Cake layer resistance (R_c) played the dominant role in membrane fouling. With Ca(II) concentration increase, the lower percentage of R_p and the higher percentage of R_c indicated that Ca(II) addition not only delayed membrane fouling (including pore blocking and cake layer fouling), but also promoted the dominance of the cake layer in membrane fouling. The result of membrane fouling indicated that Ca(II) addition induced the variation in cake layer structure.

Variations in the major components of the cake layer

PSD of sludge flocs

The volume-weighted PSD of sludge flocs is presented in Figure S3 and Table 3. The sludge flocs in the SMBR control had a mean size of 160.17 μm with a narrow size distribution profile ranging from 44.15 to 245.53 μm. Conversely, the sludge flocs in SMBR I and SMBR II had broader ranges of size distribution: 84.00–349.99 μm and 39.50–896.83 μm, respectively.

MW distributions of SMP

Figure S4 shows the MW distributions of SMP in the three SMBRs. The SMBR control and SMBR I had a similar main peak (about 25 kDa) in MW chromatograms of SMP, but the main peak of SMP in SMBR II was much lower.

Elements

The elements C, O, Al, Mg, Fe and Ca were determined by SEM–XRD (see Table 4). The relative content (weight, %) of C increased as the Ca(II) concentration increased. Organics (mainly sludge flocs, including microorganisms and SMP) were continuously deposited on the membrane surface and dominated in the cake layer with the addition of Ca(II). Additionally, Table 4 shows that Ca(II) accumulated in the cake layer as the Ca(II) concentration increased and the addition of Ca(II) reduced the percentage of other elements (i.e., Al, Si, Mg, and Fe) in the cake layer.

Morphology of the cake layer

The membrane surface of each SMBR was covered with a cake layer (Figure 2). The cake layer seemed to be dense and nonporous in the SMBR control, but the membrane surface in SMBR II was covered with a loose and porous cake layer. As Figure 3 shows, Ca was well distributed along the cross-section of the cake layer in each SMBR. The thickness of the cake layer
Table 2. Membrane fouling resistances of the three SMBRs (10^{12} m^{-1}) (n = 6).

|        | R     | R_m  | R_p  | R_c  |
|--------|-------|------|------|------|
| SMBR control | 3.57±0.51 | 0.140±0.010 | 0.080±0.003 | 3.35±0.57 |
| SMBR I | 2.88±0.72 | 0.126±0.035 | 0.014±0.003 | 2.74±0.48 |
| SMBR II | 1.8±0.24  | 0.136±0.11  | 0.004±0.001  | 1.66±0.32 |

Table 3. Particle size distributions (μm) of sludge flocs in the three SMBRs (n = 10).

| Parameters          | Volume weighted, D[4,3] |
|---------------------|-------------------------|
|                     | Mean | D10   | D50   | D90   |
| SMBR control        | 160.17±20.41 | 44.15±12.56 | 170.15±15.14 | 245.53±24.81 |
| SMBR I              | 214.89±14.46 | 84.00±10.43 | 195.99±8.53 | 349.99±38.53 |
| SMBR II             | 341.83±25.13 | 39.56±8.41  | 204.09±10.85| 896.83±93.14 |

Table 4. Compositional element analysis of the cake layer (weight %) (n = 8).

| Element  | C     | O     | Al    | Mg    | Si    | Fe    | Ca     |
|----------|-------|-------|-------|-------|-------|-------|--------|
| SMBR Control | 44.80±3.14 | 49.89±8.58 | 0.47±0.01 | 0.64±0.08 | 1.40±0.28 | 1.45±0.18 | 1.35±0.30 |
| SMBR I   | 58.76±7.48 | 38.01±7.69 | 0.32±0.003 | 0.29±0.04 | 0.93±0.13 | 0.24±0.08 | 1.44±0.48 |
| SMBR II  | 71.76±9.14 | 24.37±2.48 | 0.28±0.03 | 0.25±0.02 | 0.69±0.18 | 0.25±0.03 | 2.42±0.21 |

Figure 2. SEM images of the cake layer in (a) the SMBR control, (b) SMBR I and (c) SMBR II.
in the three SMBRs was similar (~25 μm). These results indicate that addition of Ca(II) did not affect the thickness of the cake layer and the Ca distribution along the cross-section of the cake layer. According to the SEM results and EDX line analysis, addition of Ca(II) caused structure variation in the cake layer. The particle size of the sludge flocs attached to the membrane surface increased as the Ca(II) concentration increased. Intervals, which were formed within the sludge flocs, made the cake layer porous along with the Ca(II) concentration increase.

Discussion

Membrane fouling resulted in an increase in TMP, which served as a macroscopic indicator for variations in the cake layer structure. The TMP gradient, represented as dP/dt, is equivalent to the membrane fouling rate. There was a slow TMP rise in Phase I (Figure 1) due to the accumulation of organic macromolecules and the deposition of the bulk liquor (Meng et al. 2009). The SMBR was kept in a state of low energy consumption in Phase I. As shown in Figure 1, the operation time of Phase I was prolonged with an increase in Ca(II) concentration. This result was consistent with recent studies (Arabi & Nakhla 2008; Zhang et al. 2009), which showed that a Ca(II) concentration of 410 mg l⁻¹ reduced the accumulation and deposition of foulants, keeping the energy cost low. During Phase II, a sudden TMP increase occurred due to fouling that covered the membrane. As a result, the local flux exceeded the critical flux, leading to a decrease in membrane performance (Tian & Su 2012).

Meng et al. (2007) suggested that the substantial increase in the TMP was due to the formation of a cake layer on the membrane surface. Compared with the SMBR control and SMR I, the higher TMP gradient of SMBR II during Phase II showed that a Ca(II) concentration of 410 mg l⁻¹ slowed down cake layer formation, indicating that cake layer structure varied with addition of Ca(II).

The cake layer is comprised of variable and complex compounds. For instance, sludge flocs, SMP and the elements (i.e. Ca, Al, Si, Mg, Fe) are in the majority in the cake layer (Le-Clech et al. 2006; Meng et al. 2009). Their characteristics play a significant role in cake layer structure. The mean particle size of sludge flocs increased with the addition of Ca(II). Ca(II) played a significant role in neutralizing the negative colloid surface charge of carbohydrates, proteins and bacteria, leading to destabilization of the colloid structure and biopolymer formation (Zhang et al. 2009) as well as bridging the carbohydrates, proteins and bacteria (divalent cation bridges of Ca(II)) negatively charged functional groups of carbohydrates, proteins and bacteria, promoting the

![Ca distribution along a cross section of the cake layer in (a) the SMBR control, (b) SMBR-I and (c) SMBR-II. The SEM image predicts the XRD scanning location. The y-axis shows the intensity of Ca, which indicates the Ca content at this point. The content of Ca at each point shows a similar intensity, and some differences can be ignored due to low intensity. (a), (b) and (c) indicate that the Ca was well distributed along the cross section of the cake layer on the membrane in each MBR.](image-url)
aggregation and stabilization of biopolymer (Kim & Jang 2006); the other elements were also involved in Ca(II) bridging due to their connection with the same organic compound). Ca(II) also combined with SMP to form gels, which were the backbone of the floc structure, and enhanced floc deposition on the membrane surface (Bruus et al. 1992). Therefore, Ca(II) enhanced bioflocculation and enlarged the size of the sludge flocs. The large sludge flocs were continuously deposited onto the membrane surface as part of the cake layer due to pressure suction.

A Ca(II) concentration of 410 mg l\(^{-1}\) reduced the MW of the SMP, and enhanced bioflocculation through combining low MW molecules for the formation of

![Figure 4](image-url)

**Figure 4.** The membrane fouling cake structure. (a) New membrane surface; (b) membrane surface with a Ca(II) concentration of 82 mg l\(^{-1}\); (c) membrane surface with a Ca(II) concentration of 208 mg l\(^{-1}\); (d) membrane surface with a Ca(II) concentration of 410 mg l\(^{-1}\).
sludge flocs (Kim & Jang 2006). In addition, the high-MW SMP in the SMBR control and SMBR I has been reported to be a major component of cake layer structure (Chang & Lee 1998; Yeo et al. 2006). Therefore, addition of Ca(II) reduced the MW of the SMP and further led to the postponement of membrane fouling. Additionally, as the backbone of cake layer structure, sludge flocs increased in size and deposited on the membrane surface together with SMP and Ca(II), further leading to the variation in cake layer structure (Arabi & Nakhla 2008; Zhang et al. 2009).

The elements, eg Ca, Mg, Al, Fe and Si, played a significant role in the formation of the cake layer (Meng et al. 2007). Table 4 indicates that addition of Ca(II) reduced the deposition of elements Al, Si, Mg and Fe onto the membrane surface. Elements such as Al, Si, Mg and Fe mainly contributed to the cake layer by combining with organic compounds or microorganisms as flocs. Ca(II) combined more strongly with organic compounds than with other elements, such as Al, Si, Mg and Fe, due to the flocculation of Ca(II). Therefore, addition of Ca reduced the content of other elements (ie Al, Si, Mg and Fe) in the cake layer structure.

Large sludge flocs formed through combination with the SMP, microorganisms and compounds of Al, Si, Mg, Fe, etc via the neutralization and bridging function of Ca(II). Most of these sludge flocs were continuously deposited on the membrane surface and dominated the cake layer structure. Additionally, the morphology and the thickness of the cake layer in three SMBRs were similar. Therefore, Ca(II) mainly altered the inner structure of the cake layer, as shown in Figure 4. In the cake layer with 82 mg l\(^{-1}\) of Ca(II) (Figure 4b), sludge flocs tightly bonded together and formed a dense cake layer on the membrane surface, which caused both higher cake layer resistance and total resistance. Small particles and colloids deposited on the membrane surface also reduced the porosity of the cake and increased the density of the cake layer. As in the cake layer with 208 mg l\(^{-1}\) of Ca(II) shown in Figure 4c, both the amount and the size of the sludge flocs increased due to neutralization and bridging of Ca(II). The colloids (mainly SMP and compounds of Al, Si, Mg, Fe, etc) were reduced and agglomerated together as large sludge flocs via the flocculation of Ca(II). Additionally, the rate of sludge floe deposition onto the membrane surface increased as the Ca(II) concentration increased, because Ca(II) aggravated the transport of particles towards the membrane surface (Zhu & Elimelech 1997). Consequently, there were some pores in the cake layer structure due to the interspace between the sludge flocs, and the cake layer resistance was reduced. With 410 mg l\(^{-1}\) of Ca(II) (Figure 4d) most of the colloids were bioflocculated, and the charge neutralized (surface charge neutralization) and formed into large sludge flocs due to Ca(II) flocculation (Arabi & Nakhla 2009; Zhang et al. 2009). Large sludge flocs increased the interspace between them in the cake layer, which reduced the total resistance. When the small particles were continuously deposited into the interspace between flocs in the cake layer, the cake layer was blocked, causing rapid membrane fouling (Figure 1). Therefore, Ca(II) bonded with small colloids (SMP and compounds of Al, Si, Mg, Fe, etc), which contributed to the increased size of the sludge flocs via its flocculation. Ca(II) changed the inner structure of the cake layer, but not the thickness. Large sludge flocs altered the cake layer from dense and unconsolidated to incompact and porous. The interspace between flocs in the cake layer led to the postponement of cake layer fouling.

Conclusions
Ca(II) influenced the cake layer structure in SMBR. In particular, Ca(II) varied the cake layer structure and mitigated membrane fouling potential through changing cake layer formation. The size of the sludge flocs increased and the MW of the SMP was reduced as the Ca(II) concentration increased, due to the neutralization and bridging function of Ca(II). Additionally, Ca(II) did not affect the thickness of the cake layer. Large sludge flocs led to the formation of a loose and porous cake layer. The reduction in total resistance and cake layer resistance was due to the increased porosity and interspacing of sludge flocs on the cake layer surface.

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