**Discrepant membrane fouling of partial nitrification and anammox
membrane bioreactor operated at the same nitrogen loading rate**

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Abstract

In this study, two times more serious membrane fouling was found in anammox membrane bioreactor, compared to partial nitrification membrane bioreactor (PN-MBR) operated at the same nitrogen loading rate. By protein, polysaccharide, amino acids and functional groups analysis, it was found that the discrepancy in membrane fouling was virtually due to the difference in microbial products of nitrifiers and anammox bacteria. Protein and polysaccharide were main foulants on membrane surface; meanwhile their content and ratio in the EPS, supernatant and membrane surface were significantly different in PN-MBR and anammox-MBR. The anammox metabolism products contained much more hydrophobic organics, hydrophobic amino acids, and hydrophobic functional groups than nitrifiers. A mass of anammox bacteria as well as hydrophobic metabolism products deposited on the hydrophobic membrane surface and formed serious fouling. In further, hydrophilic modification is more urgently needed to mitigate membrane fouling when running anammox-MBR, than PN-MBR.

Key words: Partial nitrification; Anammox; MBR; Membrane fouling; Microbial Products

1. Introduction

The partial nitrification (PN) –anaerobic ammonium oxidation (anammox) process is regarded as a new, efficient solution to remove ammonium from wastewater without organic carbon consumption (Liang et al., 2014). In this process, ammonium oxidizing bacteria (AOB) oxidize half of the ammonium to nitrite, and then the remained ammonium and nitrite are converted to nitrogen gas by anammox bacteria (Van de Graaf et al., 1996). PN-Anammox process is a novel nitrogen removal process with inherent superiority of no need external carbon source, less required oxygen and reduced sludge production etc, compared to the conventional nitrification and denitrification process (Jetten et al., 2002).

The functional bacteria, AOB and anammox bacteria both have a low proliferation rate (Van de Graaf et al., 1996), which results in a long term period to start up reactors. Thus, the optimization of reactor configuration and operation conditions is essential. Recently, membrane bioreactors (MBRs) have shown great potential in running the PN–anammox process (Shen et al., 2014). MBR allows a complete separation of hydraulic retention time (HRT) and sludge retention time (SRT) by the use of membrane filtration (Zhang et al., 2015; Li et al., 2015), so it could achieve high biomass concentration and establish a proper environment for the cultivation of these bacteria (Xue et al., 2009).

Although MBR has lots of advantages, membrane fouling is a non-ignorable problem during its operation. Therefore, the membrane fouling of PN–MBR and anammox–MBR has become a current research focus (Zhang et al., 2009; Shen et al., 2014). For PN–MBR and anammox–MBR, it has been found that reactor operation period plays a crucial role in membrane fouling. They have very low transmembrane pressure (TMP) values at the beginning period of reactor operation owing to the low

61 sludge concentration. With time course of reactor operation, the increase of soluble
62 microbial products (SMP) lead to the elevated fouling rates; meanwhile, the ratio of
63 polysaccharide and protein in EPS of membrane surface increased following a more
64 serious membrane fouling. The crosslinked structure of polysaccharide formed a gel
65 layer on membrane surface more easily than protein to cause membrane fouling (Shen
66 et al., 2014).

67 Actually, different types of microorganisms own variation of metabolites, finally
68 resulted in different extracellular polymeric substances (EPS) and SMP (Gao et al.,
69 2014), which can be derived in the excreting process of bacteria during substrate
70 metabolisms, biomass growth, and biomass decay (Jarusutthirak and Amy, 2006). As
71 reported by Hou et al. (2015), the microbial products by AOB and anammox are
72 clearly different. Anammox bacteria are more hydrophobic than activated sludge,
73 nitrifying bacteria and denitrifying bacteria, and contain more hydrophobic functional
74 groups in extracellular polymers (Hou et al., 2015). Many reports have claimed that
75 EPS and SMP mainly composed of proteins and polysaccharides is the key
76 contributor to the high membrane fouling in MBR (Nguyen et al., 2014; Luo et al.,
77 2015). EPS has a high correlation with composition change of a cake layer, which
78 significantly affects membrane fouling conditions (Nguyen et al., 2014). Thus,
79 different types of microorganisms are supposed to cause different membrane fouling
80 performances.

81 Accordingly, there is a question raised that whether the difference in characteristics
82 of microbial products between nitrifiers and anammox bacteria affects membrane
83 fouling. Therein, the purpose of this study was to compare membrane fouling
84 properties of PN-MBR and anammox-MBR operated at the same nitrogen loading
85 rate and analyze the reasons for different fouling levels.

In this study, PN-MBR and anammox-MBR were operated at the same nitrogen loading rate. Significant discrepancies were verified in the aspect of membrane fouling levels. To go inside for the mechanism, excitation-emission matrices (EEM), protein and polysaccharide analyses for supernatant were explored to validate the difference in microbial products in the two reactors. Proteins, polysaccharides, amino acids and organics functional groups on membrane surface analyses were further explored to clarify the hydrophobic property of organic matter produced by nitrifiers and anammox bacteria and the different production of these metabolisms. Most importantly, a conclusion was drawn that the different metabolites produced by these two consortia significantly contributed the discrepant membrane fouling profile.

2. Materials and methods

2.1. Reactor design and operation

PN-MBR and anammox-MBR have the same size and cylindrical shape with an effective volume of 5.0 L (Fig. 1). The membrane was made of polyvinylidene fluoride (PVDF) with an effective surface area of 0.11 m² (Yue Qing Membrane Technology Co., Ltd, China). The hollow fiber membrane with a pore size of 0.03 μm was arranged in the center of the reactor. No modification was applied on for the PVDF membrane surface. The contact angle of this PVDF membrane and water was 79.4±1.0° identifying by contact angle analyzer (Dataphysics Co., Germany), similar to the reference reported (Ochoa, 2003; Yan et al., 2006). A baffle between the main body of reactor and the membrane surface was arranged in these reactors to avoid the direct contact of aeration device and membrane surface. Anammox-MBR was inoculated with anammox bacteria (Liu et al., 2015) , and PN-MBR was inoculated with nitrifiers sampled from an aerobic bioreactor.

These two reactors were fed with a synthetic medium. Solutions containing

(NH₄)₂SO₄, NaNO₂ and some trace elements were added to the anammox–MBR. The trace element composition has been described previously (Van de Graaf et al., 1996). PN–MBR had the same influent composition except that all nitrogen was introduced in the form of (NH₄)₂SO₄. Besides the initial few days for the start-up, the nitrogen loading rate was controlled at 300 mg N/L day for both of the reactors, which were run for 50 days with HRT of 24 h. The temperature and pH of both reactors were controlled at 7.4–8.3 and 37±0.5 °C. The anaerobic (Dissolved Oxygen < 0.2 μmol/L) and micro aerobic environments (Dissolved Oxygen 1 mg/L) were maintained correspond to anammox–MBR and PN–MBR. The stirring speed of both reactors was set at approximately 100 rpm. The effluent was filtered by the membrane module driven by a peristaltic pump.

2.2. General water quality parameters

Samples were taken every two days to monitor the influent and effluent quality. The concentrations of ammonium, nitrite and nitrate were measured according to standard colorimetric methods, as set out by the American Publish Health Association (APHA, 1995). The pH was determined with a pH meter (TP–110, Mettler, China). The Dissolved oxygen (DO) level was measured by a DO meter (DOS–328B Mettler, China). TMP was measured by a Vacuum Pressure Gauge (MD–S600, MEOKON, China) with a range of from -0.1MPa to 0.1 MPa.

The reactor's mixture liquid (10 mL) was withdrawn from the reactor once a week and centrifuged for 10 min at 3000 rpm to obtain the supernatant. Dissolved organic matter (DOM) was obtained by filtering the supernatant by a cellulose acetate membrane filter with a pore size of 0.45 μm (Tianjin Dongtang Technology Co., Ltd, China). EPS were extracted based on cation ion exchange resin method (Oslash et al., 1996). The protein and polysaccharide in supernatant, DOM and EPS could be

determined by a phenol-sulfuric acid method (Dubois et al., 1956) and Lowry method (Lowry et al., 1951), respectively, and all the analyses were performed for three times to get the average value. In addition, EEM analysis was applied to depict the organic compounds in the supernatant. EEM spectrograph (F7000, Hitachi Limited, Japan) was applied here. The EEM spectra were collected by scanning emission wavelengths from 300 nm to 700 nm. The excitation wavelength was increased from 200 nm to 600 nm at 10 nm increments. The scanning speed was set at 1200 nm/min.

2.3. Membrane surface analysis

All the samples for membrane surface analysis were probed at the terminal operation day (day 50) of PN-MBR and anammox-MBR.

2.3.1. SEM analysis

Scanning electron microscopy (SEM) was used to observe the characteristics of the membrane surface, which was performed with an SEM-energy dispersive X-ray spectrometry (EDS) analyzer (Nova_Nano SEM430, USA). All samples were fixed overnight with 2.5% glutaraldehyde solution, washed three times with 0.1 M phosphate buffer and then dehydrated with 50%, 70%, 80%, 95%, and 100% ethanol solution for 15 min each. Both virgin and fouled membrane sheets were dried and sputter coated in gold. EDS was used to analyze inorganic elements content.

2.3.2. ATR-FTIR Analysis

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) could elucidate the functional groups present on the membrane surface, which was measured by ATR-FTIR equipment (Tensor 27, Bruke, Germany) from 400 cm⁻¹ to 4000 cm⁻¹. The foulants loosely accumulated on the membrane surface were washed with physiological saline for ATR-FTIR analysis, and the samples were lyophilized for 24 h before being measured.

2.3.3. XPS Analysis

X-ray photoelectron spectroscopy (XPS) measurements were performed on an Imaging Photoelectron Spectrometer (Axis UltraKratos Analytical Ltd., Japan). The membrane samples (10 mg) were got from the fouled membrane surface of MBR and then lyophilized for 24 h before XPS analysis. In this study, a dual anode (aluminum/magnesium target) X-ray source and a power of approximately 225 W (operating voltage of 15 kV, emission current 15 mA for high-resolution C 1s XPS spectra) were applied.

2.3.4. Amino acid composition analysis

The EPS extracted by cation ion exchange resin method (Oslash et al., 1996) was subjected to the amino acid composition analysis using an automatic amino acid analyzer (Hitachi Co., Japan). The details of this analysis can be found elsewhere (Hou et al., 2015). 10 mg EPS were hydrolyzed in 30 mL 6 mol/L HCl at room temperature for 10 min. Then the hydrolysate was filtrated by 0.22 μ m cellulose acetate membranes and analyzed in an injection volume of 50 μ m. Sodium citrate system was used for standard analysis.

2.3.5 Protein and polysaccharide composition analysis

The foulants accumulated on the membrane surface were washed with physiological saline, and then EPS were extracted based on cation ion exchange resin method (Oslash et al., 1996). The protein and polysaccharide in EPS could be determined by a phenol-sulfuric acid method (Dubois et al., 1956) and Lowry method (Lowry et al., 1951), respectively as information above in section 2.2.

3. Results

3.1. Reactor performance during the operation time

The nitrogen removal performance of the two reactors is shown in Fig. 2. During

the operation of the reactor, the ammonium and nitrite concentration in effluent of anammox-MBR was always 1.0–2.2 mM and 0.7–2.3 mM, respectively. The total nitrogen removal rate of anammox-MBR reached 288 mg / (L day). With the improved performance of the reactor, the pH of the effluent increased from 7.2–7.6 in influent to 7.6–8.0 in effluent, consistent with previous reports (Strous et al., 1999).

For PN-MBR, the performance of the reactor is unstable at the very beginning. After 10 days, half of the $\text{NH}_4^+\text{-N}$ in influent was converted to $\text{NO}_2^-\text{-N}$, with the $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ ratio in the effluent was nearly 1:1.32. It just met the requirement of the successful startup of partial nitrification process. $\text{NO}_2^-\text{-N}$ accumulation could be effectively achieved, and $\text{NO}_2^-\text{-N}$ was the main ammonium oxidation product. The nitrification process was a typical acid production process, with increased performance of the partial nitrification reactor; the pH decreased slightly in the effluent compared to that of the influent.

3.2. Membrane fouling analysis

Although the nitrogen loading rate was the same in these two reactors with a successful startup, the membrane fouling profiles were totally different (Fig. 3). Anammox-MBR exhibited much more serious fouling (24 kPa on day 50) than that of PN-MBR (12 kPa on day 50). To understand the reasons, microbial products in the supernatant of PN-MBR and anammox-MBR, as well as the membrane surface deposition of both reactors were investigated.

3.3. Characteristics of protein and polysaccharide in PN-MBR and anammox-MBR

From Fig.4, we can see that the protein and polysaccharide concentrations in the supernatant and DOM of the PN-MBR were higher than those in the anammox-MBR. For PN-MBR, the protein and polysaccharide concentrations (7th week) in the supernatant were 7.6 mg L⁻¹ and 18.3 mg L⁻¹, whereas the values in the DOM were

211 7.2 mg L⁻¹ and 16.6 mg L⁻¹, respectively. In contrast, for anammox-MBR, the protein
212 and polysaccharide concentrations were much smaller, irrespective of the supernatant
213 and DOM, with values of 5.7 mg L⁻¹ and 11.6 mg L⁻¹, 4.8 mg L⁻¹ and 11.0 mg L⁻¹ (Fig.
214 4), respectively.

215 The distribution profile of protein and polysaccharide in EPS of membrane surface
216 is also measured. Compared to PN-MBR, the content of protein and polysaccharide in
217 EPS of membrane surface of anammox-MBR was higher than that of PN-MBR. The
218 protein and polysaccharide concentration in EPS of membrane surface in
219 anammox-MBR were 1.35 g m⁻² and 0.60 g m⁻², respectively, and the corresponding
220 values in PN-MBR were 0.39 g m⁻² and 0.38 g m⁻². Compared with the correspondent
221 contents in the supernatant and DOM, it's obvious that the content of protein and
222 polysaccharide in EPS of membrane surface of anammox-MBR were higher than
223 those in PN-MBR, meanwhile the protein were higher than polysaccharide on the
224 membrane surface. It was different from the phenomenon that polysaccharide were
225 higher than protein in the supernatant. Due to the PVDF membrane surface is
226 hydrophobic, we could learned that the EPS of anammox bacteria are more
227 hydrophobic than that of nitritiers, while protein is more hydrophobic than
228 polysaccharide (Bos et al., 1999; Oss et al., 1986). Therefore nitritiers EPS are
229 considered to be more hydrophilic than that of anammox bacteria.

230 3.4. Membrane surface morphology

231 Morphologies of the deposition on the membrane surface were observed by SEM.
232 The photos (Fig. S1) showed that a high degree of compactness in the cake layer was
233 achieved in anammox-MBR, whereas the cake layer in PN-MBR was relatively loose,
234 which may be associated with the occurrence of more serious membrane fouling in
235 anammox-MBR than that in PN-MBR.

3.5. Characteristics of organic compounds in PN-MBR and anammox-MBR

For the EEM analysis, the apparent excitation wavelength at 280–310 nm with emission wavelength at 350 nm (peak A) is likely to be attributed to water Raman (Singh et al., 2010). Both reactors have this peak (Fig. 5). Particularly, the membrane surface disposition of anammox-MBR has an excitation wavelength at 290–310 nm with an emission wavelength at 370–410 nm (peak B, the humic substances) and an excitation wavelength at 225–237 nm with an emission wavelength at 340–381 nm (peak C, the tryptophan). However, the PN-MBR has an excitation wavelength at 300–370 nm with an emission wavelength at 400–500 nm (peak B, the humic-like substances), and an excitation wavelength at 280–310 nm with an emission wavelength at 380–455 nm (peak C, the fulvic acid) (Stedmon and Markager, 2005; Li et al., 2011). EEM could evaluate organic compounds released by anammox bacteria and nitrite bacteria, the significant difference in the peak locations between the supernatant of anammox-MBR and PN-MBR is thought to be a crucial proof to present the difference in characteristics of microbial products in these reactors, and all these different kinds of microbial metabolites may result in the changes in composition or proportion of EPS and SMP, which might be the cause of different membrane fouling behaviors.

Understanding the distribution of various types of hydrophilic or hydrophobic amino acids on the membrane surface is helpful to investigate the hydrophobic property of these two types of sludge (Hou et al., 2015). Content of different amino acids on the membrane surface was shown in Fig.6. The relative yields of hydrophilic amino acids Serine (SER), Glutamic Acid (GLU), Glycine (GLY), Lysine (LYS), and Arginine (ARG) in PN-MBR was higher than those of anammox-MBR; at the same time, hydrophilic amino acids Aspartic Acid (ASP) and Histidine (HIS) and

261 hydrophobic amino acids Methionin (MET), Isoleucine (IIE), and Tyrosine (TYR)
262 were lower than those of anammox–MBR. The sum of hydrophobic amino acid
263 content on the surface of anammox–MBR was slightly higher than those of PN–MBR,
264 possibly because the anammox bacteria secreted more hydrophobic products than
265 nitrifiers.

266 In order to further confirm the discrepancy in the hydrophobic property of these
267 two types of bacterial consortia, FTIR and XPS analyses for identifying the
268 compounds group were also carried out. In the FTIR spectrum (Table 2), the existence
269 of peaks at 1641 cm^{-1} and 1549 cm^{-1} of the two investigated samples suggests the
270 deposition of protein on the membrane surface (Cho et al., 1998). The peak at 1040 cm^{-1}
271 represents pollutants containing polysaccharides (Howe et al., 2002). The main
272 differences of these two samples from PN–MBR and anammox–MBR lie in: (1) The
273 peak at 1549 cm^{-1} (amide II) existed only in anammox–MBR; (2) Contents of groups
274 representing amide I (1641 cm^{-1}) were higher in PN–MBR; (3) The peak at 2562 cm^{-1}
275 appeared only in PN–MBR. These differences indicated that the organic matter
276 released by anammox sludge and nitrifying sludge were totally different. The original
277 FTIR spectra (Fig. S2) could be found in Supplementary information.

278 XPS analysis could help to determine the elemental content deposited on the
279 membrane surface. High-resolution C 1s XPS spectra of the two membrane surface
280 dispositions are shown in Table 3. Comparing the two MBRs, the membrane surface
281 disposition in the PN–MBR contained more hydrophilic groups, such as C–O, C–OH,
282 and C–O–C (31.87%) than anammox–MBR (24.97%) at the peak of 286.3 eV, as well
283 as higher C=O (12.32%) of PN–MBR than anammox–MBR (10.2%) at the peak of
284 288.1 eV. This result once again confirmed the discrepant microbial products of
285 nitrifiers and anammox bacteria, and the more hydrophilic properties of nitrifiers than

286 anammox bacteria. The original XPS-spectra could be found in Supplementary
287 information (Fig. S3).

288 **4. Discussion**

289 Partial nitrification and anammox are new emerging nitrogen removal technology
290 to treat ammonium rich wastewater. As the slow growth rate of the functional bacteria,
291 MBR is an efficient reactor for operating these processes. Although the optimal
292 operation parameters and operation performance have been well discussed before
293 (Dongen et al., 2001; Jetten et al., 2001), the discrepant membrane fouling has not
294 been paid attention.

295 10 mL mixture liquid that harvested every week to test polysaccharide and protein
296 in the supernatant and DOM was very small compared to the reactor working volume
297 (5 L). In addition, amount of sludge was deposited on the membrane surface.
298 Therefore, it could be regarded as no sludge discharging during the reactor operation.

299 Under conditions of influent nitrogen loading rate of 300 mg/L day, both reactors
300 exhibited good performance for partial nitrification and anammox reaction. However,
301 the membrane fouling profiles were totally different. In the very beginning (day 0-
302 day 10), the reactor operation was non-steady. During this period, the TMP was
303 nearly to zero for both reactors. After this original phase, the reactors were operated
304 under steady state. For the TMP monitoring, we mainly compare the data on day 50,
305 at which time the reactors have been steady for more than one month. The TMP of
306 anammox-MBR (24 kPa) was double higher than that of PN-MBR (12 kPa) at the
307 terminal day (day 50), which revealed that the membrane fouling of anammox-MBR
308 was much more serious. This study is mainly focus on the emergence of discrepant
309 membrane fouling and the inherent cause for this discrepancy, between partial
310 nitrification process and anammox process. After 7 weeks (nearly 2 month) operation,

it could be obviously seen the discrepant TMP of these two process (24 kPa and 12 kPa). Therefore, longer operation was not applied. For the longer period, the discrepant membrane cleaning should be done and the discrepant restoration of membrane permeability for these two processes should be discussed. This is another topic, which remained to do in the further.

The PN–MBR and anammox–MBR were operated in parallel at the same nitrogen loading rate. They have the same reactor configuration and operation parameters except the oxygen supplement and nitrogen supplement form, as anammox–MBR should be totally anaerobically operated and PN–MBR was micro–aerobically operated. Thus, one may ask whether the different aeration demand in these two reactors significantly affected the membrane fouling profile. In order to avoid this interference factor and mainly focus on the difference originated from bacteria characteristics, we arranged a baffle between the main body of reactor and the membrane surface in these reactors (see Materials and methods and Fig. 1). To avoid potential effects of scouring force produced by aeration on the membrane surface layer cake. In this way, this study could exclude the mechanical effects and mainly focus on the biological roles, namely different membrane fouling significantly associated with discrepant microbial product of anammox consortia and nitrifiers.

Previously, Duan et al. (2015) once compared the membrane fouling between an integrated fixed-film activated sludge membrane bioreactor (IFAS–MBR) and MBR. The result suggested that the membrane fouling in MBR was more serious than that in IFAS–MBR. It is worth noting that the protein and polysaccharide concentrations in MBR were apparently higher than that in IFAS–MBR, which supported the assumption: SMP and EPS are the main components causing membrane fouling, and they could transform between each other (Meng et al., 2009). Polysaccharides and

336 protein, as main ingredients of SMP and EPS, have been known to be significant
337 contributors to membrane fouling (Aslam et al., 2015). Therefore, the important and
338 essential issues should be considered to explain the difference in membrane fouling is
339 to identify the difference in microbial products by anammox bacteria and nitrifiers.

340 In this study, it has been well verified that microbial products excreted by these two
341 kinds of consortia was different, which affected a lot on the membrane fouling. For
342 the case of supernatant samples (10 mL) in the reactors probed every week, EEM
343 analysis confirmed the different secretions of these microorganisms especially for the
344 component of humic and fulvic acid. We also noticed that the mass ratio of protein
345 and polysaccharide in the supernatant was clearly discrepant between the two reactors
346 during the operation. The protein and polysaccharide concentrations in the
347 supernatant and DOM of the PN-MBR were obviously higher than those in
348 anammox-MBR, though membrane fouling developed more severely in
349 anammox-MBR. This discrepancy may not be amazing. For example, it has been
350 found that fouling potential of certain polysaccharides based on unit mass
351 significantly differed depending upon the types of polysaccharides (Kimura et al.,
352 2012). From the SEM figure, we can see clearly the bacteria shape (Spherical) on the
353 PN-MBR membrane surface, we can also see some pores here. Whereas, the cake
354 layer formed on the anammox-MBR membrane surface was so thick that we could not
355 get the clear bacteria pattern. During the preparing of SEM samples, we first used
356 Glutaraldehyde to fix biofilm structure before dehydrating (see Methods part),
357 therefore the pretreatment process could not affect the morphologic observation of the
358 layer cake.

359 For the case of membrane surface sediment, the totally different FTIR spectra well
360 verified the discrepant compositions of organic compounds on these two membrane

361 surfaces. For both of samples, FTIR identified that the protein and polysaccharides
362 were main foulants on the membrane surface. The further analysis of protein and
363 polysaccharide content in EPS of the membrane surface illustrated that protein was
364 more easily to be deposited on the membrane surfaces. This phenomenon was
365 explainable in our study. The membrane module used in this research was PVDF,
366 which is a hydrophobic material. Polysaccharide is more hydrophilic than protein
367 (Bos et al., 1999; Oss et al., 1986), so there is more protein on the membrane surface.
368 In order to penetrate into the protein characteristic deposited on the membrane surface,
369 amino acid content analysis was following performed. As expected, for
370 anammox–MBR, the content of hydrophobic amino acids, such as MET, IIE, and
371 TYR were much higher than that on the membrane surface of PN–MBR. Without a
372 doubt, microbial products excreted by anammox bacteria have higher proportion of
373 hydrophobic amino acids. The characteristics of amino acids affect the property of
374 proteins, and the ratio of hydrophobic amino acids influences the hydrophobicity of
375 proteins (Hou et al., 2015). Hydrophobic residues of amino acids play a key role in
376 protein folding and hydrophobic structure stabilities (Satish et al., 2016; Ueno et al.,
377 2016). This result provide the further strong proof about that the more hydrophobic
378 products excreted by anammox bacteria than nitrifiers.

379 Now we turn to the evidence of the higher hydrophilic characteristics of nitrifiers
380 given by XPS analysis for the organic compounds on the membrane surface. It
381 showed the higher hydrophilic groups of PN-MBR, than that of anammox–MBR. This
382 once again illustrated the assured more hydrophilic characteristics of nitrifiers than
383 anammox consortium. These phenomena showed that the different microbial products
384 induced the different hydrophilic and hydrophobic characteristics of nitrifiers and
385 anammox bacteria. This was a crucial cause for the discrepant membrane fouling of

386 PN-MBR and anammox-MBR. The SEM result revealed more bacteria deposited on
387 the membrane surface of anammox-MBR to form close grained cake. The
388 hydrophobic characteristics of anammox consortium make them more easily to
389 deposit on the hydrophobic membrane material, to cause more serious membrane
390 fouling in anammox-MBR.

391 In this study, it is verified that PVDF membrane was easily fouled in
392 anammox-MBR. In the further work, hydrophilic modification for PVDF membrane
393 such as surface modification and blending modification may be more urgently
394 necessary to be carried out to slow down the membrane fouling when operating
395 anammox-MBR.

396 This study show that even the two processes were operated at the same nitrogen
397 loading, the membrane fouling level was significantly different. The discrepancy in
398 membrane fouling was virtually due to the difference in microbial products of
399 nitrifiers and anammox bacteria (the more hydrophobic compounds released by
400 anammox bacteria contributed a lot). At last, it is claimed that hydrophilic
401 modification is more urgently needed to mitigate membrane fouling when running
402 anammox-MBR, than PN-MBR.

403 **5. Conclusion**

404 Membrane fouling on PVDF membrane surface more easily occurred in
405 anammox-MBR than PN-MBR. The difference in the characteristics of microbial
406 products were considered to be the crucial reason to cause the differences in
407 membrane fouling. Direct relationship between the concentrations of polysaccharides
408 and proteins in the supernatant and membrane fouling could not be established.
409 Anammox metabolism products contained more hydrophobic groups including C=O,
410 C-O, C-OH and C-O-C; more hydrophobic amino acids such as MET, IIE and TYR,

and therefore more easily to be attached to the hydrophobic membrane surface in anammox-MBR, compared to PN-MBR.

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510 Table 1 The abbreviation table

Abbreviation	Nomenclature
PN	Partial Nitrification
ANAMMOX	Anaerobic Ammonium Oxidation
AOB	Ammonium Oxidizing Bacteria
MBR	Membrane Bioreactor
HRT	Hydraulic Retention Time
SRT	Sludge Retention Time
SMP	Soluble Microbial Products
EPS	Extracellular Polymeric Substances
EEM	Excitation-Emission Matrices
PVDF	Polyvinylidene Fluoride
DO	Dissolved Oxygen
TMP	Transmembrane Pressure
DOM	Dissolved Organic Matter
SEM	Scanning Electron Microscopy
ATR–FTIR	Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy
XPS	X–Ray Photoelectron Spectroscopy
ASP	Aspartic Acid
THR	Threonine
SER	Serine

GLU	Glutamic Acid
GLY	Glycine
LYS	Lysine
HIS	Histidine
ARG	Arginine
ALA	Alanine
VAL	Valine
MET	Methionin
IIE	Isoleucine
LEU	Leucine
TYR	Tyrosine
PHE	Phenylalanine

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Table 2 The peak locations and corresponding constituents of the membrane surface deposition by FTIR analysis (a) anammox–MBR (b) partial nitrification–MBR

Waves (cm ⁻¹)	Vibration types	Corresponding groups	Relative transmittance*	
			a	b
3427	O–H stretching	Hydroxyl	1	1
2925	C–H stretching	Lipid-carbon chain	0.05	0.37
2562	S–H stretching	Protein	–	0.03
2366	N≡N stretching	Protein	0.03	0.01
1641	C=O stretching	Amide I (protein peptide bond)	0.42	0.64
1549	C–H stretching	Amide II (protein peptide bond)	0.13	–
	N–H bending			
1450	CH ₃ bending	Methyl	0.27	0.22
	CH ₂ stretching	Methylene		
1403	C=OO stretching	Carboxyl	0.16	0.29
1385	CH ₃ stretching	Methyl	0.23	0.35
1267	C–OH stretching	Polysaccharide	0.02	0.2
1120	C–O–C stretching	Polysaccharide	–	0.36
1040	C–H in-plane bending	Polysaccharide	0.13	0.41
<1000	Fingerprint region	Sulfur and phosphorus groups	–	–

* In order to compare the content of each functional group, we set the maximum peak in each group to be 1.0. Then the relative transmittances of other peaks are represented as the ratio to the maximum peak.

524 Table 3 Corresponding constituents of C atomic groups by high-resolution C 1s XPS

525 spectra analysis

Peak	Position	Assignment	C atomic content (%)	
			Anammox-MBR	Partial nitrification-MBR
1	284.8	C–C,C–H	59.67	52.58
2	286.3	C–O,C–OH, C–O–C	24.97	31.87
3	288.06	C=O	10.2	12.32
4	289.63	COO–	5.16	3.23

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540 **Figure Captions**

541 Fig.1 Membrane bioreactor configuration, 1-intake pipe, 2-pressure gauge,
542 3-dissection used for water bath heating, 4-sampling port, 5-intake for water bath, 6-
543 baffle, 7- membrane module, 8-stirring paddle, 9-ari-outlet, 10-aeration pipe, 11-gas
544 flowmeter, 12- outlet for water bath

545 Fig. 2 Ammonium, nitrite and nitrate in influent and effluent of (a) anammox–MBR
546 (b) partial nitrification–MBR

547 Fig. 3 Change of TMP during the reactor operation

548 Fig. 4 Protein and polysaccharide concentrations in the supernatant and DOM (a)
549 anammox–MBR (b) partial nitrification–MBR

550 Fig. 5 EEM analysis result of supernatant (a) anammox–MBR (b) partial
551 nitrification–MBR

552 Fig. 6 Content of different amino acids on the membrane surface
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Fig.1

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576 Fig.2

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Fig.3

Fig.4

Fig.5

Fig.6

Graphical Abstract

Membrane fouling is less severe in partial nitrification-MBR because nitrifiers are more hydrophilic tended.

Membrane fouling is more severe in Anammox-MBR due to high hydrophobicity of anammox consortia.