Dynamic membrane-assisted fermentation of food wastes for enhancing lactic acid production

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ABSTRACT

A dynamic membrane (DM) module was inserted into a fermentation reactor to separate soluble products from the fermented mixture to increase lactic acid (LA) production from food wastes under acidogenic conditions (uncontrolled pH, pH 4 and 5). With a high total suspended solid content (20–40 g/L) in the fermenter, a stable DM could be maintained through regular backwashing. By effectively intercepting suspended solids and lactic acid bacteria (LAB), the fermenter was able to increase microbial activity and largely promote LA yield. Hydrolysis and acidogenesis rates increased with pH, and the highest LA yield (as high as 0.57 g/g-TS) was obtained at pH 4. The microbial community analysis showed that the relative abundance of Lactobacillus increased to 96.4% at pH 4, but decreased to 43.3% at pH 5. In addition, the DM could be easily recovered by intercepting larger particles in less than 2 h after each cycle of periodic backwashing.

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1. Introduction

Lactic acid (LA) is widely used in food, pharmaceutical, and cosmetic industries and has been highlighted as a raw material for polyactic acid (PLA) (Lasprilla et al., 2012; Dusselier et al., 2013; Dreschke et al., 2015). The continuous increase in demand for LA has led to rapid growth rate of the global market (Dusselier et al., 2013; Dreschke et al., 2015). Chemical synthesis and fermentation are the commonly used LA production methods, but the fermentation process is more attractive because it uses renewable materials and produces optically pure LA (John et al., 2009). Additionally, due to its large quantity and high organic content, food waste has become one of the main solid wastes in cities (Tang et al., 2016; Wu et al., 2015). Although LA fermentation from food waste has been reported in a number of studies (Tang et al., 2016; Ye et al., 2008), further investigations are required to improve the LA yield.

The conventional batch or continuous LA fermenters are widely used due to their high LA yield and stable operation (Wu et al., 2015; Liang et al., 2014). However, they have several drawbacks: first, they are not suitable for high concentrations of substrate because of their negative effect on the growth of lactic acid bacteria (LAB) (John et al., 2009; Tang et al., 2016); second, the feedback inhibition caused by accumulated free acids may restrict the bacterial activity and decrease the LA yield (Gao et al., 2011); third, substrates cannot be completely utilized and may retain at high levels...
in the effluent (Liu et al., 2016); and finally, LAB in the fermenter cannot accumulate to increase the population or accelerate the LA productivity. To improve the LA yield, fermentation conditions such as pH, organic loading rate (OLR), temperature, and types of inocula should be optimized (Tang et al., 2016; Wu et al., 2015; Liang et al., 2014). Continuous removal of LA with adsorption and ion exchange to counteract the negative effects caused by the accumulated end-products has been investigated in previous studies (Pal et al., 2009; Gao et al., 2011; Cui et al., 2016). Although these methods could improve the LA yield to some extent, they are usually costly and the down-stream processes may become complicated (Gao et al., 2011).

The utilization of membranes has appeared to be an option for solving the aforementioned problems because it can effectively separate the LA product from the fermenter to relieve the negative effect on further LA fermentation, intercept the particulate substrates to promote LA yield, and retain LAB to increase LA productivity (Pal et al., 2009; Zhao et al., 2010). Many efforts have been made to integrate membrane separation with LA fermentation (Wee et al., 2006; Mimitsuka et al., 2014). However, the high capital cost and short lifetime of the membrane unit often become the bottleneck problem to restrict low-cost production of LA. Therefore, it is required to establish a new fermentation system with low-cost membrane material.

Recently, the dynamic membrane bioreactors (DMBR), either operated aerobically (Wang et al., 2015; Ersahin et al., 2013) or anaerobically (Ersahin et al., 2016; Alibardi et al., 2014) have been studied as alternatives of conventional MBR, and showed their advantages of low-cost membrane module, high permeate fluxes (Ersahin et al., 2012; Chu et al., 2014; Hu et al., 2016). By intercepting suspended particles (e.g. sludge flocs and microbial cells) on a support material (e.g. nylon mesh and stainless steel mesh), a cake layer was formed, by which the solid particles were retained and only the soluble matter could pass through the membrane (Ersahin et al., 2012; Hu et al., 2016). Alibardi et al. (2016) succeeded in establishing an anaerobic DMBR with a large pore size mesh (200 μm) for wastewater treatment and achieved high organics removal under low transmembrane pressure (TMP). Recently, Liu et al. (2016) integrated an anaerobic digester into DMBR to produce volatile fatty acids (VFAs) and found that the system could enrich the functional bacteria, enhance enzymatic activities, and further improve the VFAs yield.

The application of DMBR in wastewater treatment and anaerobic digestion processes has indicated the applicability of low-cost membranes to assist waste disposal and resource recovery. However, little has been known about the feasibility of LA fermentation assisted by dynamic membrane (DM). It thus became the objective of this study to establish a DM fermenter for enhancing LA production from food wastes under acidogenic conditions. Attention was paid to the performance and LA yield of the DM fermenter under varied pH conditions, as well as the stability of the DM layer in long-term operation.

2. Methods and materials

2.1. Food waste substrate

The fresh food waste was collected from a university canteen in Xi’an, China. It mainly consists of rice, vegetables, and meat. The pretreatment procedures followed the ones described in our previous study (Tang et al., 2016). Briefly, food waste was homogenized with an electrical blender after animal bones and clamsHELLS were separated and grease was removed. The resulting slurry was sieved (1 mm) and stored in a refrigerator (4 °C). Before adding the slurry into the reactors, the TS content of the fresh food waste slurry was adjusted to approximately 3% with tap water. The characteristics of the food waste slurry are shown in Table 1.

2.2. DM-assisted fermenter

The lab-scale DM assisted fermenter with a working volume of 25 L is shown in Fig. 1. A nylon mesh with an equivalent aperture of 50 μm and an effective filtration area of 0.04 m² was used as a support material for DM formation (Fig. S1, Supporting information). Two agitators (200 rpm) were installed beside the membrane module to continuously mix the fermentation broth and scrub the membrane to relieve the membrane fouling. The effluent was withdrawn continuously under a hydraulic head of merely 10 cm between the bioreactor and the effluent port. With the attachment of the particles on the mesh support, a cake layer was formed and acted as a DM filter. As the filtration continued, the DM layer might become compacted, resulting in a decrease in the membrane flux. When the membrane flux dropped to the prescribed value of 2.0 L/m²·h, backwashing was conducted using the effluent (200 mL) to remove the foulants on the mesh support module and recover the membrane flux. The pH of the fermentation broth was continuously recorded with an on-line pH meter and automatically adjusted by adding NaOH or HCl (5 M) to the prescribed value except for the pH uncontrolled condition. A water bath was equipped to maintain the temperature of the broth at 37 °C. All devices were controlled by a program logical controller (PLC).

2.3. Operation of the fermenter

At the beginning of the experiment, the reactor was filled with 25-L food waste substrates (described in Section 2.1) and initiated. The indigenous microorganisms in fresh food waste were used as the starters as discussed in our previous study (Tang et al., 2016). During the first eleven days, the reactor was operated as a continuous stirring tank reactor (CSTR) with the hydraulic retention time (HRT) of seven days as a start-up stage at uncontrolled pH (pH = un). When the products in the effluent became stable, a nylon mesh support module was inserted into the reactor to start the DM assisted fermentation process. To investigate the effect of pH on LA fermentation and membrane performance, the fermenter was operated in three stages with different pH values, namely, uncontrolled pH (Stage 1) followed by pH 4 (Stage 2) and pH 5 (Stage 3). Hydraulic retention time (HRT) of the reactor varied between 7 and 10 d depending on the membrane flux; the solid retention time (SRT) was controlled at 30 d during the entire fermentation period by daily discharge of the broth from the reactor.

2.4. Analytical methods

2.4.1. Chemical analysis

Immediately after collecting the mixture and effluent from the fermenter, chemical analyses were conducted regarding the total chemical oxygen demand (TCOD), total nitrogen, total

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Average</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>–</td>
<td>4.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Total solid content (TS)</td>
<td>% of wet weight</td>
<td>3.0</td>
<td>0.5</td>
</tr>
<tr>
<td>VS/TS</td>
<td>%</td>
<td>90.4</td>
<td>10.8</td>
</tr>
<tr>
<td>Total COD (TCOD)</td>
<td>g/L</td>
<td>33.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Soluble COD (SCOD)</td>
<td>g/L</td>
<td>12.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Total carbohydrate</td>
<td>g/L</td>
<td>18.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Total protein</td>
<td>g/L</td>
<td>3.6</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Note: S.D. represents standard deviation.
carbohydrate, and protein immediately. After being centrifuged at 6000 rpm for 10 min, the supernatant was obtained and then filtered through 0.45-μm filters. The filtrate was used to measure the total organic carbon (TOC), soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), soluble carbohydrate, proteins, and lactic acid. SCOD, TCOD, and total suspended solid (TSS) were measured according to the standard methods (APHA, 1998). Protein was determined by the Lowry-Folin method with bovine serum albumin (BSA) used as a standard (Lowry et al., 1951). Protein was detected based on the methods described by Li et al. (2015). The bacterial enzymes in the fermentation broth were assayed by an elemental analyzer according to Li et al., 2016). Briefly, the filtrate was collected in a 1.5-mL gas chromatograph (GC) vial and 3% (v/v) H3PO4 was added to adjust the pH to approximately 4.0. A gas chromatograph (GC2014, Shimadzu, Japan) with a flame ionization detector (FID) equipped with a 30 m × 0.32 mm × 0.25 μm CPWAX52CB column was utilized to analyze the composition of VFAs. Nitrogen was used as the carrier gas, with a flux of 50 mL/min. The injection port and the detector were maintained at 200 °C and 220 °C, respectively. The oven of the GC was programmed to stay at the starting temperature of 100 °C for 2 min. The temperature was then increased at a rate of 10 °C/min to 200 °C, and maintained at 200 °C for 2 min. The sample injection volume was 0.5 μL.

2.4.2. Volatile fatty acids (VFAs)

The analysis of VFAs was based on our previous study (Tang et al., 2016). Briefly, the filtrate was collected in a 1.5-mL gas chromatograph (GC) vial and 3% (v/v) H3PO4 was added to adjust the pH to approximately 4.0. A gas chromatograph (GC2014, Shimadzu, Japan) with a flame ionization detector (FID) equipped with a 30 m × 0.32 mm × 0.25 μm CPWAX52CB column was utilized to analyze the composition of VFAs. Nitrogen was used as the carrier gas, with a flux of 50 mL/min. The injection port and the detector were maintained at 200 °C and 220 °C, respectively. The oven of the GC was programmed to stay at the starting temperature of 100 °C for 2 min. The temperature was then increased at a rate of 10 °C/min to 200 °C, and maintained at 200 °C for 2 min. The sample injection volume was 0.5 μL.

2.4.3. Lactic acid

The concentration of lactate was measured using a liquid chromatograph (LC-10AD, Shimadzu, Japan) equipped with an ultraviolet detector. COSMOSIL SC18-AR-IL was used as the column and 0.05 M phosphoric acid buffer liquid (50 mM NaH2PO4; 50 mM H3PO4 = 9:1, pH = 3) was used as the carrier liquid. The analysis was performed at a detector temperature of 40 °C, flow velocity of 1.0 mL/min, and UV at 210 nm.

2.4.4. Scanning electron microscopy (SEM)

To observe the membrane morphology, the nylon mesh module was cut from the middle of the nylon mesh support module and pretreated according to Hu et al. (2016). The samples were fixed with 2.0% glutaraldehyde for 8 h, dehydrated with ethanol, coated with platinum, and observed using SEM (Quanta 600 FEG, FEI Corporation, USA).

2.4.5. Particle size distribution (PSD)

To investigate the DM formation processes, the variations in the PSD of the fermentation mixture and membrane effluent were analyzed using a laser granularity distribution analyzer (LS 230/SVM+, Beckman Coulter Corporation, USA) with a detection range of 0.4–2000 μm. Each sample was tested for at least five times.

2.4.6. Lactic acid bacteria counts

The viable lactic acid bacteria (LAB) in the fermentation slurry and effluent were detected using the De Man-Rogosa-Sharpe (MRS) agar according to Ye et al. (2008). The mixture of 14 g agar, 20 g glucose, 20 g peptone, 10 g beef extract, 5 g yeast extract, 2 g K2HPO4, 2 g diammonium citrate, 5 g CH3COONa, 0.58 g MgSO4·7H2O, 0.25 g MnSO4·4H2O, and 1 mL Tween-80 was diluted in 1 L pure water. The pH was adjusted to 6.5 ± 0.2. The solution was sterilized at 121 °C for 15 min and cooled down to approximately 50 °C. The samples obtained from the reactor and the effluent were serially diluted with pure water. Then, 1 mL diluted sample was added into the plate, the agar solution was dropped into the plate, and mixed with the sample completely. The colony-forming units (cfu) were determined by incubating the MRS agar at 36 °C for 48 h in an incubator. Each sample was tested in triplicate and the cell counts of LAB were averaged.

2.5. Calculation

The following equations are used for calculating the hydrolysis and acidification ratios based on SCOD, CODVFA and CODlactic acid following Tang et al. (2016).

Hydrolysis(COD) = \frac{SCOD - SCOD_0}{TCOD_{in}} \tag{1}

Acidogenesis(COD) = \frac{COD_{VFA} + COD_{lactic acid}}{SCOD} \tag{2}

where SCOD0 and TCOD_{in} are the soluble and total COD of the influent, respectively. SCOD is the soluble COD of the effluent.

3. Results and discussion

3.1. Performance of the DM in LA fermentation

3.1.1. DM performance for solid interception

The variations of total suspended solids (TSS), COD, and carbohydrates in fermentation slurry and effluent were analyzed to evaluate the performance of the fermenter (Fig. 2a–c). With the effective interception of particulates by the DM, TSS in the reactor gradually increased from 32.3 g/L to approximately 39.1 g/L (Fig. 2a). With the discharge of the fermentation mixture from the reactor to maintain a constant SRT, TSS in the fermentation slurry was stabilized at 40 g/L. Although the TSS content in the reactor was high, a very low level (less than 2 g/L) was observed because of the effective interception function provided by DM. Additionally, the constant TSS content in the effluent further manifested the stable operation of the DM in the long-term applications.

Due to the retention of particulate organics by the DM, TCOD of the fermentation slurry in reactor gradually increased from 32.3 g/L to 61.4 g/L after 10 d at uncontrolled pH (Fig. 2b) and maintained stable about 60 g/L. With the formation of the DM on the membrane module, particulate organics, especially those with larger...
sizes than the pores of the DM, could not pass through and thus retained by the membrane (Hu et al., 2016). As a result, the TCOD gradually increased to 73.6 ± 5.2 g/L and 90.1 ± 4.9 g/L, respectively, at pH 4 and 5.

Regardless of the increase in TCOD of the fermentation slurry in the reactor, TCOD of the effluent was relatively stable during the entire fermentation period and was much lower than that in the reactor, mainly due to the high retention rate of the DM. Moreover, the SCOD and TCOD concentrations in the effluent were almost identical, indicating that few particulate organics remained in the effluent because of the perfect particle interception by the DM. At uncontrolled pH, SCOD in the effluent increased from 14.9 g/L to 22.8 g/L, which might be attributed to the retention of particulate organics and higher solubilisation rate enhanced by the membrane (Liu et al., 2016). When pH was adjusted to 4 and 5, the effluent SCOD gradually increased to 25–30 g/L, indicating a stronger solubilisation, which was consistent with the previous studies (Tang et al., 2016; Wu et al., 2015). Moreover, the relatively stable COD in the effluent demonstrated that the fermenter could stably work during the long-term operation.

At uncontrolled pH (Fig. 2c), the total carbohydrate showed an increasing profile possibly due to the following two reasons: the interception of the carbohydrates, especially the particulate carbohydrates by the DM, and the relatively low carbohydrate degradation rate at the uncontrolled but low pH (3.3 ± 0.2). There were little differences between the soluble and total carbohydrates because most particulate carbohydrates were intercepted by the DM. Soluble carbohydrates were resulted from a net balance between competing rates of release and degradation, and its increase in the effluent at uncontrolled pH demonstrated a higher production rate than the degradation rate, possibly caused by the enhancement of enzyme activity and accumulation of hydrolytic bacteria in the reactor (Liu et al., 2016). However, when pH was increased to 4 and 5, the soluble carbohydrate concentration gradually decreased, and the total carbohydrate concentration in the reactor became about 7 g/L which was much lower than that in the influent (18.4 g/L), indicating a higher degradation rate possibly due to the higher bacterial activity and larger populations of LAB in the reactor (Dalie et al., 2010; Tang et al., 2016; Liu et al., 2016).

3.1.2. Variation of LA in the DM filtrate

Fig. 3 shows the variation of LA concentration in the effluent at different pH in comparison with acetate. At the start-up stage of the operation, an initial LA increase was observed from 3 g/L to about 6 g/L but almost kept unchanged afterwards. The low LA production was mainly due to the low pH (pH 3.3 ± 0.2) of the fermentation broth. It was reported that the free LA and low pH conditions would act as feedback inhibitors to the growth of LAB and activity of enzymes, resulting in lower LA production (Abdel-Rahman et al., 2011; Dalie et al., 2010; Wee et al., 2006). The carbohydrate concentrations were maintained at 5.0 g/L after 5 d (Fig. 2c) and LA in the effluent showed no increase.

After DM module was installed into the reactor, the LA in the effluent apparently increased to a higher concentration about 8.5 g/L, showing the function of DM to promote LA production. The DM could continuously separate LA from the fermentation broth, avoid the accumulation of free LA in the broth to cause inhibition to microorganisms and bacterial enzymes. More biomass and longer particle retention time also contribute to the promotion of LA yield (Gao et al., 2011; Liu et al., 2016). Meanwhile, the
carbohydrates also gradually increased to a higher level at 10.4 g/L (Fig. S2, Supporting Information), providing a larger amount of substrates to the microorganisms and promoting the LA production. The increase in soluble carbohydrate levels indicated a stronger production rate, which was due to the fact that some hydrolytic microorganisms were intercepted by the membrane filtration or the enzymatic activities were enhanced in the fermenter (Liu et al., 2016). The increase in biomass and longer particle retention time in the reactor also supported the hydrolysis.

When pH was increased to 4, sharp increase in LA concentration was observed, which may be mainly benefited from the relief of feedback inhibition. As a result of pH adjustment, the soluble and particulate substrates (e.g. carbohydrates) accumulated in the reactor were hydrolyzed and transformed into LA by lactic acid bacteria. The effluent LA peaked as about 25 g/L on Day 37 and turned stable (about 16.5 g/L) after Day 40. Meanwhile, soluble carbohydrate levels gradually decreased to approximately 1 g/L (Fig. 2c), indicating a high degradation rate of carbohydrates. The effectiveness of pH control at 4 to enhance LA production was also reported in previous studies (Tang et al., 2016; Wu et al., 2015; Itoh et al., 2012) in which the high enzyme activity maintained by LAB was stressed.

As pH was increased to 5, carbohydrates further decreased to lower levels of 0.3–0.8 g/L, indicating a higher carbohydrates degradation rate. However, it is noticeable that the effluent LA gradually decreased while an increase of acetate was observed. It has been reported that the main product of homofermentation is lactic acid, while heterofermentation results in equimolar amounts of CO2, lactate, and acetate or ethanol (Kandler, 1983). As the molar ratio of lactate-to-acetate in the effluent was 1.0–2.5, it could thus be deduced that the heterofermentation and homofermentation coexisted in this condition, which was consistent with the results of Wu et al. (2015). Another reason may be the shift of microbial communities as will be discussed later in Section 3.2.

Table 2 compares the extent of hydrolysis, acidogenesis and LA yield in the start-up stage and with DM under different pH values. Under uncontrolled pH, the extent of hydrolysis increased from 9.3% in the start-up period to 26.2% with the DM module because more bacteria were intercepted by the DM and thus enhanced hydrolysis (Liu et al., 2016). Additionally, the interception provided more bacteria were intercepted by the membrane filtration or under uncontrolled pH, LA yield increased from 52.7% to 73.6% and 77.4% at uncontrolled pH, pH 4 and pH 5, respectively. The main reason, as was discussed in the former sections, was that pH adjustment could effectively relieve the inhibition from free acid and promote bacterial activity.

With the aid of DM and under uncontrolled pH, LA yield increased from 0.2 g/g-TS to 0.28 g/g-TS while the LA/SCOD increased from 37.7% to 43.8%, indicating a higher transformation of substrates into LA. When pH was adjusted to 4, the LA yield sharply increased to 0.57 g/g-TS together with a higher LA/SCOD (65.1%), implying that most of the substrates were effectively transformed into LA. On the contrary, when pH was adjusted to higher level (pH 5), the LA yield decreased to 0.44 g/g-TS, because a portion of the substrates were degraded into other products, such as acetate and propionate.

Table 3 compares the LA yield of this study with other types of reactors. Although the highest LA concentrations among different reactors differed much due to the different settings of substrate loading rate in the documented studies, the LA yield in this study was obviously higher than many of the other reactors, especially those using food waste as the sole substrate. The extended degradation time of soluble and particulate organic matter should be the main factors responsible for the high LA yield in this study. Additionally, the accumulation of bacteria that could effectively degrade refractory organic matter and hydrolyze particulate organics might be another key contributor. All these might have enhanced LA fermentation and resulted in higher LA yield.

3.1.3. Variation of the dynamic membrane flux

Membrane flux of the fermenter is shown in Fig. 4. Although fluctuations could be seen in each operation cycle (about 6 days on average), the working flux was mostly maintained at 2–4 L/m2·h during the entire operation period, indicating the high stability of the DM system. At the start of the operation (clean nylon mesh), the instant flux was as high as 11.4 L/m2·h (corresponding to a membrane resistance of 4.4 × 1010 m–1), while after operation for 12 h, as a result of cake layer formation on the nylon mesh, the flux sharply decreased to 3.9 L/m2·h (corresponding to a membrane resistance of 1.3 × 1011 m–1). As the filtration continued, the cake layer on the nylon mesh became compacted, and gradual decrease of membrane flux was observed down to 2.4 L/m2·h on day 6. At this time, the first backwashing was conducted and the DM was put into the second work cycle. The backwashing resulted in the recovery of membrane flux to 10.3 L/m2·h, slightly lower than the clean nylon mesh.

As shown in Fig. 4, continuous decrease in the initial flux after each backwashing was seen in the first five cycles to about 7 L/m2·h, and then it fluctuated between 7 and 10 L/m2·h. The decrease in the initial flux after backwashing might be caused by the following factors: (1) the backwashing could not completely remove the cake layer on the membrane mesh, leading to an increase in membrane resistance; (2) the pore blocking or the existence of physically irremovable fouling occurred during the operation (Hu et al., 2016); (3) the increase of SS in the fermentation broth caused a higher potential of membrane fouling; (4) the intercepted fine particles and foulants (e.g., carbohydrate and protein) in the broth easily caused membrane fouling (Hu et al., 2013; Zhang et al., 2011). However, the decrease and fluctuation in the initial flux after each backwashing did not affect much the working flux in the whole operation period. Even after 3 months of continuous operation, a flux higher than 2 L/m2·h could still be maintained and the frequency of backwashing did not change much. This proved the stability of the DM system for continuous operation.

3.1.4. Effects of backwashing on DM maintenance

As discussed above, the membrane flux could be effectively recovered by periodic backwashing. To investigate the effects of backwashing, SEM was used to observe the membrane surface before and after backwashing (Fig. S3, Supporting Information).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Extent of hydrolysis</th>
<th>Extent of acidogenesis</th>
<th>LA yield</th>
<th>LA/SCOD</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>g/g-TS</td>
<td>%</td>
</tr>
<tr>
<td>Start-up stage</td>
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<tr>
<td>pH = un</td>
<td>9.3 ± 0.6</td>
<td>47.4 ± 10.3</td>
<td>0.20 ± 0.01</td>
<td>37.7 ± 9.3</td>
</tr>
<tr>
<td>DM-assisted fermenter</td>
<td>pH = un</td>
<td>26.2 ± 12.1</td>
<td>52.7 ± 9.3</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>pH = 4</td>
<td>46.9 ± 9.5</td>
<td>73.6 ± 17.0</td>
<td>0.57 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>pH = 5</td>
<td>50.4 ± 7.3</td>
<td>77.4 ± 10.3</td>
<td>0.44 ± 0.10</td>
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</table>
Comparison of the LA yield with other types of reactors.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Volume (L)</th>
<th>Substrates</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Highest LA concentration (g/L)</th>
<th>LA yield (g/g-TS)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch test</td>
<td>1.3</td>
<td>Food waste</td>
<td>5.5</td>
<td>41</td>
<td>42</td>
<td>0.29*</td>
<td>RedCorn and Engelberth (2016)</td>
</tr>
<tr>
<td>Fedbatch reactor</td>
<td>1.5</td>
<td>Cellulosic biosludges</td>
<td>4.9</td>
<td>45</td>
<td>42</td>
<td>0.38</td>
<td>Romani et al. (2008)</td>
</tr>
<tr>
<td>Lab-scale semi-continuous fermentation experiment</td>
<td>10</td>
<td>Food waste</td>
<td>4–6</td>
<td>37</td>
<td>37.6</td>
<td>0.21–0.44</td>
<td>Tang et al. (2016)</td>
</tr>
<tr>
<td>Pilot-scale semi-continuous fermentation experiment</td>
<td>90</td>
<td>Food waste and sludge</td>
<td>7</td>
<td>–</td>
<td>12.05</td>
<td>0.66**</td>
<td>Li et al. (2015)</td>
</tr>
<tr>
<td>DM-assisted fermenter</td>
<td>25</td>
<td>Food waste</td>
<td>4</td>
<td>37</td>
<td>17.2</td>
<td>0.57</td>
<td>This study</td>
</tr>
</tbody>
</table>

* LA yield was obtained under the optimal fermentation condition. 
** g/g-VS. 
*** Calculated according to the data, g/g-TSS.

Fig. 4. Variation of membrane flux during the operation period.

For a new nylon mesh, the pores could be clearly seen on the membrane surface and the pore size was approximately 50 μm (Fig. S3a). After putting into operation, with the deposition of particles on the membrane, the pores on the mesh membrane were covered and a cake layer was formed (Fig. S3b). The cake layer acted as a filtration layer, which only allowed soluble matter and smaller particles to pass through.

After backwashing, the cake layer on the membrane surface was effectively removed and the pores on the nylon mesh could be clearly seen again (Fig. S3c), and the flux was recovered after backwashing. However, some foulants still existed in the pores or attached on the intersection of the mesh fibers, which could be considered as physically irremovable fouling (Hu et al., 2016). This can explain the reason for the decrease of the initial flux after backwashing discussed in the former section. For a DM system, the existence of physically irremovable fouling may not always be negative because the residual substances on the nylon mesh surface may also assist quicker formation of the DM layer for the stable operation in the next working cycle.

### 3.2. Microbial community in the fermenter under varied pH conditions

Microbial communities under different operating conditions were analyzed to further explain the differences in the LA yield. Fig. 5 shows the distinct microbial communities in different samples. In the raw food waste slurry, a relatively high microbial diversity was observed, in which Lactobacillus and Weissella accounted for 47.9% and 20.4% of the total population, respectively. In addition, other bacteria such as Propionibacterium (7.2%), Leuconostoc (6.9%), and Acetobacter (2.5%) were also detected in the slurry.

At uncontrolled pH and pH 4, Lactobacillus gradually accumulated in and dominated the fermentation broth, accounting for 95.3–96.4%, which was primarily due to the fact that in such low pH conditions, only LAB (e.g. *Lactobacillus*) could dominantly exist (Tang et al., 2016; Wu et al., 2015; Itoh et al., 2012). When pH was increased to 5, the abundance of *Lactobacillus* decreased to 43.3%, and other genera such as *Bifidobacterium* (27.9%), *Prevotella* (9.1%), and *Aeriscardovia* (4.3%) were also detected. It is distinctive that *Acetobacter* increased from 0.1% to 11.3%, which closely related to the higher content of acetic acid at pH 5.

Although *Lactobacillus* was abundant at uncontrolled pH, the LA yield was relatively low, primarily due to the restriction by low pH. However, when pH was adjusted to 5, the relative abundance of LA producers (*Lactobacillus*) decreased, accompanying the growth of other types of microorganisms so that the substrates could be transformed into other products, thus bringing about a decreased LA yield.

#### 3.3. Mechanism of LA fermentation enhancement

#### 3.3.1. Interception of LAB during the fermentation

Table 4 shows the viable LAB in the fermentation broth and effluent. When the reactor was operated without the DM, approximately $23 \times 10^{8}$ cfu/L of LAB were detected in the broth, and a similar number existed in the effluent, even lower than that in the substrate ($40 \times 10^{6}$ cfu/L). This might be due to the extremely deteriorative conditions (low pH) in the reactor and resulted in the low LA yield (Table 2). However, when the mesh membrane module was installed, the LAB in the broth gradually increased to $12 \times 10^{8}$ cfu/L in the fermentation broth under the uncontrolled pH condition, while LAB in the effluent were one order lower as $8 \times 10^{8}$ cfu/L, indicating that a large proportion of LAB were intercepted in the reactor by the DM. As the operation proceeded, the number of LAB in the broth continuously increased and reached
72 \times 10^8 \text{cfu/L} at pH 5. Although the population of LAB at pH 5 was larger than that at pH 4, the LA production was lower, probably due to the changes in metabolic pathways as mentioned in Section 3.1.2. In addition, the consumption of substrates by other types of microorganisms would also lead to low LA yields. The LAB in the effluent was always 10–30 times lower than that in the broth. The high retention rate by the DM and large populations of LAB in the broth promoted the LA production and yield (Zhang et al., 2014; Liu et al., 2016).

### 3.3.2. Enzyme activity enhancement by the DM filtration

Variations in \( \alpha \)-Glucosidase activity are shown in Table 5. With the aid of DM, the enzyme activity increased with pH. As \( \alpha \)-Glucosidase is a type of endoenzyme, the effective retention of microorganisms by the DM should be one of the main reasons for the enhanced enzymatic activities (Liu et al., 2016). In addition, the substrates intercepted by the DM could provide more nutrients to the microorganisms and increase the enzymatic activities of bacteria. The higher hydrolytic enzyme activity promoted the transformation of substrates and further enhanced the LA production.

### 3.3.3. DM formation and substrate retention processes

To further explore the mechanisms of the particle interception process by the DM, a typical operation cycle was considered (Fig. 6). The DM in one operational cycle may involve three stages: DM formation and maturation stage, stable filtration stage and regeneration stage (Hu et al., 2016). It can be seen from Fig. 6a that the initial flux was 11.2 L/m²⋅h after backwashing, with a TSS content of 6480 mg/L in the effluent (Fig. 6b). Both the flux and TSS content of the effluent decreased with time; after 30 min, the flux sharply decreased to 6.9 L/m²⋅h together with TSS as 1640 mg/L. The flux further decreased to 4.95 L/m²⋅h after 1 h, accompanied with a very low content of TSS (875 mg/L) in the effluent.

Backwashing eliminated the cake layer on the mesh support and removed the particles from the membrane pores, increasing the flux. Subsequently, the particles smaller than the membrane pores (50 \( \mu \)m) passed through the membrane and entered into the effluent. Due to the attachment and deposition of larger particles on the mesh support, the cake layer became thicker, and the pore size became smaller. Only smaller particles could pass through the membrane and enter into the effluent and the TSS content of the effluent gradually decreased and the flux decreased at the same time.

After 2 h, TSS gradually decreased to 795 mg/L and stayed constant thereafter; meanwhile the flux (4.5 L/m²⋅h) became mostly stable with a slight decline, indicating the maturation of the DM layer. The DM then entered into the stable filtration stage. The DM formation was slower in this study because of the lower flux applied during the DM formation stage than that reported by Hu et al. (2016).

During the stable filtration stage, the cake layer became compacted and the flux gradually decreased with the attachment of particles on the membrane (Liang et al., 2013). After 2 d, the flux gradually decreased to 3.2 L/m²⋅h and a stable concentration of TSS (732 mg/L) was observed. The variations of flux and TSS in the effluent clearly described the DM formation and the particle interception process.

In principle, the DM is formed by retaining the particles on the mesh support and is responsible for preventing the particles from entering into the effluent (Hu et al., 2016; Zhang et al., 2011; Liu et al., 2016). Particles with a mean size of 14.0 \( \mu \)m were mainly present in the effluent after 1 min of backwashing (Fig. S4, Supporting Information). Some particles larger than 50 \( \mu \)m were also detected due to the uneven pore size of the nylon mesh support. As the filtration proceeded, the detected particle size became smaller than 40 \( \mu \)m and the mean size was 5.7 \( \mu \)m after 1 h. The smaller particle size was mainly attributed to the formation of intact DM on the mesh support, which resulted in smaller pores on the DM.

After 2 h, the detected particles in the effluent were smaller than 15 \( \mu \)m with a mean size of 4.8 \( \mu \)m and meanwhile, the TSS content was lower than 1000 mg/L. This further demonstrated a perfect DM formed on the mesh support. After 24 h, the mean particle size in the effluent was 2.6 \( \mu \)m. After a mature cake layer was

### Table 5

<table>
<thead>
<tr>
<th>pH</th>
<th>Start-up stage</th>
<th>DM-assisted fermenter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substrate</td>
<td>un</td>
</tr>
<tr>
<td>Fermentation broth</td>
<td>40 ± 12 \times 10^8</td>
<td>12 ± 2 \times 10^9</td>
</tr>
<tr>
<td>Effluent</td>
<td>23 ± 13 \times 10^8</td>
<td>8 ± 2 \times 10^9</td>
</tr>
</tbody>
</table>

### Fig. 6

The cake layer formation process after backwashing.
established, stable filtration was maintained, and stable TSS content was also obtained in the effluent in the long-term operation (Fig. 2a).

4. Conclusions

The DM fermenter could effectively intercept the particulate organics and LAB, enhance the enzyme activity, and significantly promote the LA yield during the long-term operation. The highest LA concentration and yield (0.57 g/g-TS) was obtained at pH 4. Based on the results of the high-throughput sequencing analysis, a high abundance of Lactobacillus (95.3–96.4%) was observed at uncontrolled pH and pH 4, but a lower abundance (43.3%) was detected at pH 5. DM could be formed in 2 h by intercepting particles on the mesh membrane. By periodic backwashing, DM could be effectively regenerated and long-term stable operation could be achieved.

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Appendix A. Supplementary data

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References