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

Jialing Tang[a,b,c], Xiaochang C. Wang[a,c,d*], Yisong Hu[a,c], Huu Hao Ngo[a,e], Yuyou Li[f]

[a] School of Environmental and Municipal Engineering, Xi'an University of Architecture and Technology, Xi'an 710055, China

[b] Key Lab of Northwest Water Resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an 710055, China

[c] International Science & Technology Cooperation Center for Urban Alternative Water Resources Development, Xi'an 710055, China

[d] Engineering Technology Research Center for Wastewater Treatment and Reuse, Shaanxi Province, China

[e] Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia

[f] Department of Civil and Environmental Engineering, Tohoku University, Sendai 9808579, Japan

* Corresponding author: xcwang@xauat.edu.cn; Tel/ Fax: +86-29-82201586

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.

Keywords: lactic acid; acidogenic fermentation; dynamic membrane (DM); pH; food waste

1. Introduction

Lactic acid (LA) is widely used in food, pharmaceutical, and cosmetic industries and has recently been highlighted as a raw material for polylactic 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.

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 m^2 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 $L/m^2 \cdot h$,

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).

Fig. 1.

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

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). Carbohydrates were measured by the phenol-sulfuric method with glucose

used as a standard. The elements in the food waste substrate were assayed by an elemental

analyzer according to Li et al. (2015). The bacterial enzymes in the fermentation broth were

detected based on the methods described by Li et al. (2015).

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) H_3PO_4 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 5C18-AR-II was used as the column

and 0.05 M phosphoric acid buffer liquid (50 mM NaH_2PO_4:50 mM H_3PO_4=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 taken out from the

fermenter and a piece of membrane 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 K_2HPO_4, 2 g diammonium citrate, 5 g CH_3COONa, 0.58 g $MgSO_4·7H_2O$, 0.25 g $MnSO_4·4H_2O$, 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, COD_{VFA} and $COD_{lactic\ acid}$ following Zhang (2016).

$$\text{Hydrolysis }(\text{COD}) = \frac{\text{SCOD-SCOD}_0}{\text{TCOD}_{in}} \qquad (1)$$

$$\text{Acidogenesis }(\text{COD}) = \frac{\text{COD}_{VFA} + \text{COD}_{lactic\ acid}}{\text{SCOD}} \qquad (2)$$

where $SCOD_0$ 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 15.9 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 in the effluent corresponding to a removal efficiency of 95% 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).

Fig. 2.

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 in the effluent 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 CO_2, 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.

Fig. 3.

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 by the DM prolonged the particle retention time and increased the

concentrations of the particulate organics in the reactor to benefit hydrolysis. The increase of pH

to 4 and 5 further promoted the hydrolysis to 46.9% and 50.4%, respectively. Higher pH tended to

bring about stronger hydrolysis.

Higher pH also resulted in higher acidogenesis as can be seen from Table 2 as 52.7%, 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 2.

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.

Table 3.

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/m^2·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/m^2·h (corresponding to a membrane resistance of 4.4×10^{10} 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/m^2·h (corresponding to a membrane resistance of 1.3×10^{11} 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/m^2·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/m^2·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/m^2·h, and then it fluctuated between 7 and 10 L/m^2·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/m^2·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.

Fig. 4.

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). 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.

Fig. 5.

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 slurry and effluent. When the reactor was

operated without the DM, approximately 23×10^{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 (40×10^8 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 12×10^9 cfu/L in the fermentation broth under the uncontrolled pH condition, while LAB in the effluent were one order lower as 8×10^8 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 72×10^9 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).

Table 4.

3.3.2 Enzyme activity enhancement by the DM filtration

Variations in α-Glucosidase activity are shown in Table 5. With the aid of DM, the enzyme activity increased with pH. As α-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.

Table 5.

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/m^2·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/m^2·h together with TSS as

1640 mg/L. The flux further decreased to 4.95 L/m^2·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 μm) 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/m^2·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/m^2·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.

Fig. 6.

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., 2010; Liu et al., 2016). Particles with a mean size of 14.0 μm were mainly present in the effluent after 1 min of backwashing (Fig. S4, Supporting Information). Some particles larger than 50 μm 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 μm and the mean size was 5.7 μm 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 μm with a mean size of 4.8 μm 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 μm. After a mature cake layer was 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.

Acknowledgments

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Table 1. Characteristics of the food waste slurry.

Table 2. The extent of hydrolysis, acidogenesis, and LA yield during the operation period.

Table 3 Comparison of the LA yield with other types of reactors.

Table 4. Viable LAB in the fermentation broth and effluent.

Table 5 Variations of α-Glucosidase activity during the fermentation

Fig. 1. Schematic of the DM-assisted fermenter

Fig. 2. TSS (a), COD (b), and carbohydrates (c) in the reactor and in the effluent during the

operation.

Fig. 3. Variations of lactate and acetate in the effluent during the fermentation.

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

Fig. 5. Microbial communities in raw food waste and fermentation slurry at different pH.

Fig. 6. The cake layer formation process after backwashing.

Table 1. Characteristics of the food waste slurry.

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

Note: S.D. represents standard deviation.

Table 2. The extent of hydrolysis, acidogenesis and LA yield during the operation period.

		Extent of hydrolysis	Extent of acidogenesis	LA yield	LA/SCOD
		%	%	g/g-TS	%
Start-up stage	pH=un	9.3±0.6	47.4±10.3	0.20±0.01	37.7±9.3
DM-assisted	pH=un	26.2±12.1	52.7±9.3	0.28±0.02	43.8±7.8
fermenter	pH=4	46.9±9.5	73.6±17.0	0.57±0.08	65.1±15.9
	pH=5	50.4±7.3	77.4±10.3	0.44±0.10	54.3±12.3

Table 3 Comparison of the LA yield with other types of reactors.

Reactor	Volume (L)	Substrates	pH	Temperature (°C)	Highest LA concentration (g/L)	LA yield* (g/g-TS)	Reference
Batch test	1.3	food waste	5.5	41	42	0.29**	RedCorn et al., 2016
Fedbatch reactor	1.5	cellulosic biosludges	4r.9	45	42	0.38	Romaní et al., 2008
Lab-scale semi-continuous fermentation experiment	10	food waste	4-6	37	37.6	0.21-0.44	Tang et al., 2016
Pilot-scale semi-continuous fermentation experiment	90	food waste and sludge	7	-	12.05	0.66***	Li et al., 2015
DM-assisted fermenter	25	food waste	4	37	17.2	0.57	This study

* LA yield was obtained under the optimal fermentation condition

** g/g-VS

*** calculated according to the data, g/g-TSS

Table 4. Viable LAB in the fermentation broth and effluent.

Unit: cfu/L

	Substrate	Start-up stage	DM-assisted fermenter		
pH	-	un	un	4	5
Fermentation broth	$40\pm12\times10^8$	$23\pm13\times10^8$	$12\pm2\times10^9$	$57\pm21\times10^9$	$72\pm15\times10^9$
Effluent	-	$25\pm8\times10^8$	$8\pm2\times10^8$	$17\pm2\times10^8$	$28\pm9\times10^8$

Table 5 Variations of α-Glucosidase activity during the fermentation

Unit: U/L

	Start-up stage	DM-assisted fermenter		
pH	un	un	4	5
Fermentation broth	25.2±2.2	49.7±8.1	89.8±8.3	104.7±10.3
Effluent	25.2±2.2	19.7±5.6	23.8±2.9	41.5±7.8
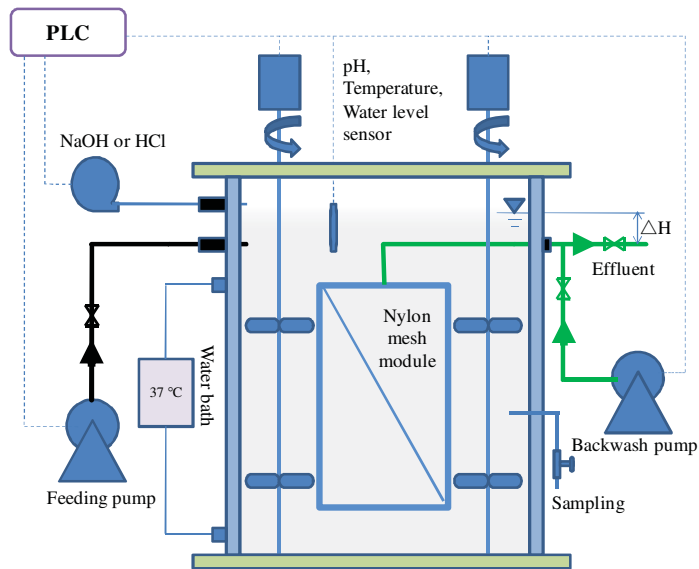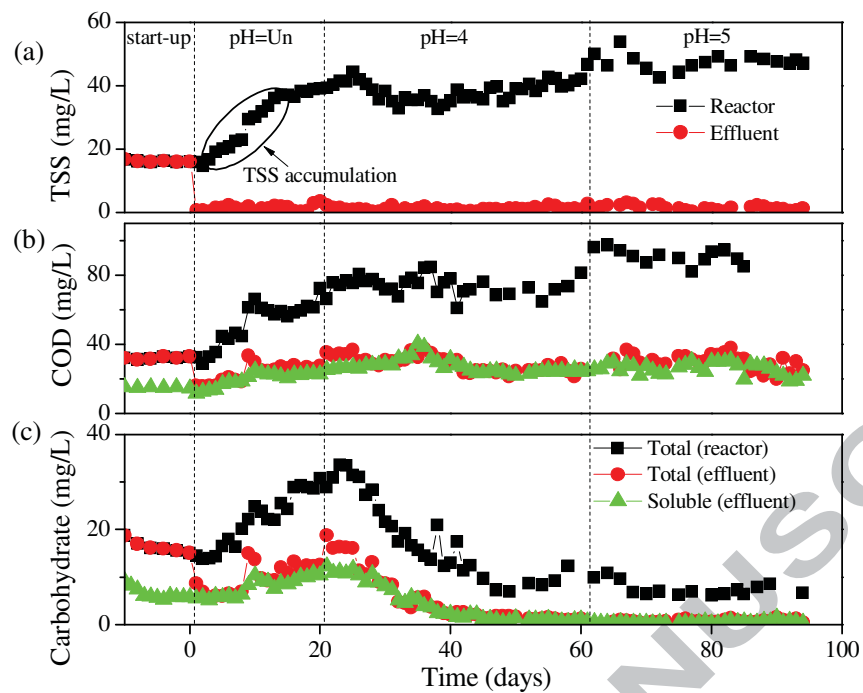
Fig. 1. Schematic of the DM-assisted fermenter

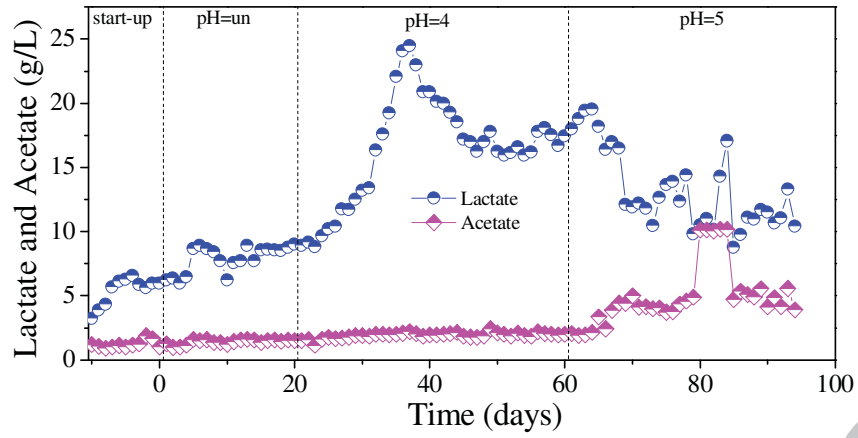**Fig. 2.** TSS (a), COD (b), and carbohydrates (c) in the reactor and in the effluent during the operation.

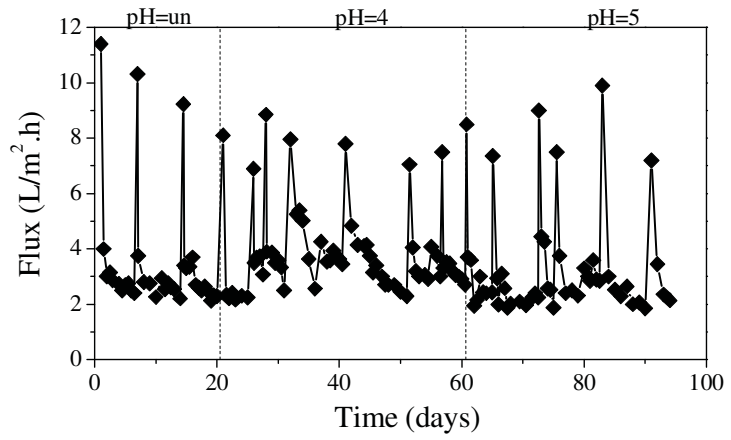**Fig. 3.** Variations of lactate and acetate in the effluent during the fermentation.

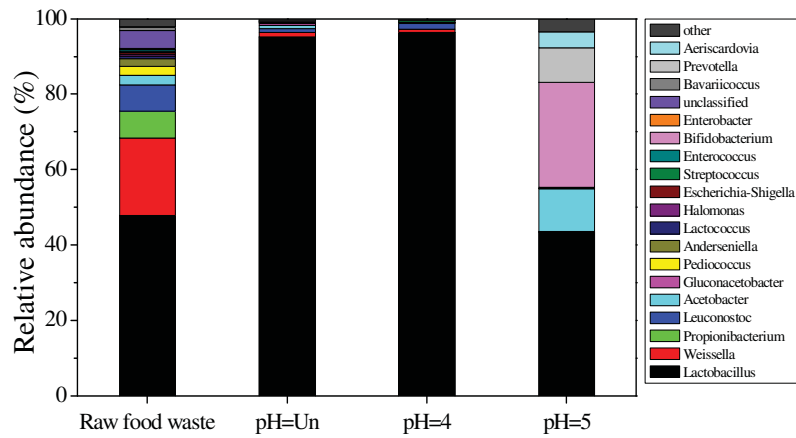**Fig. 4.** Variation of membrane flux during the operation period.

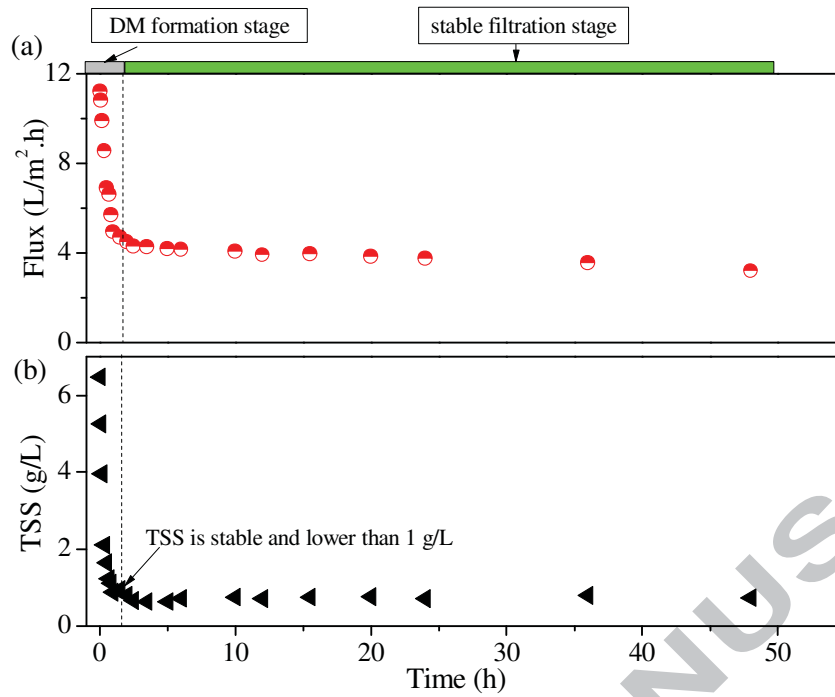**Fig. 5.** Microbial communities in raw food waste and fermentation slurry at different pH.

Fig. 6. The cake layer formation process after backwashing.

Highlights

- Fermenter was stably operated under acidic conditions for LA production.

- DM could significantly intercept particles and enhance LA yield.

- High abundance of *Lactobacillus* dominated at uncontrolled pH and pH 4.

- Backwashing could effectively maintain the DM permeability.