



A novel full recycling process through two-stage anaerobic treatment of distillery wastewater for bioethanol production from cassava

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ARTICLE INFO

Article history:

Received 29 January 2010

Received in revised form 11 March 2010

Accepted 11 March 2010

Available online 18 March 2010

Keywords:

Mathematical model
Ethanol fermentation
Anaerobic treatment
Recycling process
Cassava

ABSTRACT

In the present study, a novel full recycling process for bioethanol production was investigated, where three mathematical models were established to simulate the accumulation of major soluble inhibitory substances, including organic compounds, total ions, volatile fatty acids (VFAs) and colorants. These inhibitory substances in the reused water reached a relative steady state after 3–7 batches of anaerobic treatment and recycling process, which coincided with the results of mathematical models. There were no negative effects of these inhibitory substances on ethanol fermentation and the final ethanol yield, fermentation time, starch utilization ratio were very close to that of the conventional process using tap water. However, approximately 7.54% (w/w) of water was lost during each circulation, which was replenished in subsequent circulations, to assure consistent fermentation broth volume. This novel process was confirmed to have a stable operation over 13 recycles. It is concluded the stable states of the inhibitory substances in the reused water can assure this recycling process will run successfully.

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1. Introduction

Bioethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the worldwide utilization of fossil fuels [1]. Various materials, such as wheat, corn, molasses, grain, cassava and plant fibres, can be utilized to produce fuel ethanol, but at present, the technology of bioethanol production using lignocellulosic biomass as raw materials is immature. Using food crops such as wheat, corn and grains to produce bioethanol is unacceptable. Cassava, a non-food material with virtues of large distribution, relative cheap, high yield and starchy content [2], would be one of the best choices to produce bioethanol at present time.

However, the large quantity of wastewater produced from ethanol fermentation has been an intractable problem in larger scale bioethanol production. For example, 3.3 t of cassava will produce 1 t of bioethanol, while the production of 1 t of bioethanol results in 9–15 t of distillery wastewater with high concentration of organic materials and low pH [3]. Consequently, the disposal costs of wastewater results in a large financial burden to the cassava based ethanol industry, and the environmental problems cannot be neglected. Therefore, in order to develop a cassava based ethanol

industry, it is urgent to devise a new strategy for treatment of the distillery wastewater.

Current treatment of distillery wastewater is mainly through traditional methods, including anaerobic and aerobic digestion to reduce the COD of the wastewater, which makes it meet the discharge criterion of most countries. However, such kinds of treatment are resource-wasting as well as environment-polluting. In the past few years, some new methods, such as coagulation [4], evaporation [5], and electrolysis [6], have been proposed for the treatment of distillery wastewater. Moreover, the distillery wastewater, if properly treated, can be reused in subsequent fermentation processes, and distillery wastewater recycling is known as one of the best available methods. While several cleaner technologies, including ultra-filtration [7–9], micro-filtration [10], bio-flocculation [11], have been proposed in ethanol production by recycling the distillery wastewater, a disadvantage of these technologies is the relatively high operation cost.

In this study, a novel full recycling process of ethanol production through two-stage anaerobic treatment was proposed to treat the distillery wastewater. In detail, the wastewater produced from ethanol fermentation was treated in a two-stage anaerobic treatment process, where a large amount of biogas was produced. The anaerobic digestion liquid was recycled to the ethanol fermentation, thus a full recycling process was established. In this process, cassava was used as raw materials to produce bioethanol and the anaerobic digestion step with low operation cost removed most of

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the impurities in wastewater, while producing a large amount of biogas formation, where the biogas can be used to produce electricity and the wastewater reused in bioethanol production. Such an operation can result in zero wastewater discharge and little energy consumption. With the aids of other technologies used in fermentative ethanol industry, such as cogeneration, multi-effect distillation, as well as very high gravity ethanol fermentation technology [12], the ideal fuel ethanol fermentation has great potential.

In recent years, many studies carried out on laboratory scale for the improvement of bioethanol production processes have been simulated in a preliminary way in order to evaluate their performance in terms of mass balances, where some mathematical models were proposed to simulate the process of ethanol production [13–15]. Similarly, in this work, several mathematical models were also established to simulate the accumulation of the inhibitory substances in the recycling system. The resulting research patterns were rarely found in present publications, especially in models of a coupled fermentation system. After comparing the simulated results, the real accumulation patterns of these substances were also investigated in practice.

2. Materials and methods

2.1. Strains and enzymes

Angel alcohol instant active dry yeast (a commercial strain of *Saccharomyces cerevisiae* for ethanol production was obtained from Hubei Angel Yeast Co. Ltd., China) was used throughout this study. α -Amylase (20,000 IU/mL, Genencor Biotech Co. Ltd., Wuxi, China) and glucoamylase (100,000 IU/mL, Genencor Biotech Co. Ltd., Wuxi, China) were used for liquefaction and saccharification of cassava medium, respectively. Thermo- and mesophilic anaerobic sludge were provided by Jinjiang Chemical Technology Co. Ltd. and DSM Citric Acid Co. Ltd., Jiangsu, China, respectively.

2.2. Pretreatment of cassava medium

Ethanol fermentation medium was prepared using cassava chips provided by Tianguang Fuel Ethanol Co. Ltd., Henan Province, China. First, the cassava was milled and passed through a 1 mm screen, and the cassava flour (raw starch content about 68%, w/w) was then mixed with tap water or digestion liquid at a ratio of 1:3 (w/w). For liquefaction, α -amylase was added at a dosage of 10 IU/g cassava, followed by heating of the mixture to 100 °C and liquefaction for 60 min. The hydrolyzed solution was then cooled down to 60 °C and 130 IU/g cassava of glucoamylase was added in. Finally, 0.3% (w/v) of sterilized urea was added in the saccharified broth and the pH was adjusted to 4.2–4.4.

2.3. Culture conditions of ethanol fermentation

14 g dry yeast powder was dissolved and activated in 50 mL of hot water at 35–40 °C for 30 min prior to fermentation. The batch fermentations for ethanol production were performed in a 10-L fermentor (Baoping Bioengineering Equipment Co. Ltd., Shanghai, China) with 8 L of the cassava medium at 30 °C until the end of fermentation. The seed broth was inoculated into the fermentation medium at a level of 10% (v/v). The culture pH was not controlled during the fermentation.

2.4. Evaporation and dehydration

When ethanol fermentation was completed, the fermented broth was transferred into a stainless steel evaporator where the broth was vigorously mixed and heated to 98 °C for 15 min to effec-

tively evaporate the ethanol. The removed ethanol was collected and then treated by dehydration.

2.5. Thermo- and mesophilic anaerobic digestion of the wastewater

An anaerobic treatment process using two UASB reactors was constructed to treat the distillery wastewater. Each glass-made UASB reactor had a working volume of 10 L. One liter of thermo- and mesophilic anaerobic digestion sludge was added into each UASB reactor, respectively.

The pH of the stillage was adjusted to 7.0 with calcium hydroxide prior to being put into a thermophilic UASB reactor, where the temperature was maintained at 60 °C by circulation of heated water through a water jacket, and the liquid in the reactor was continuously circulated everyday for 2 h using a peristaltic pump. After being treated in the thermophilic UASB reactor, the digestion liquid was centrifuged and the supernatant was then put into a mesophilic UASB reactor, where the temperature was kept 35 °C constantly through water bath. Each day the liquid of this mesophilic reactor was circulated for 12 h using a peristaltic pump with an up-flow velocity of 3 m/s.

2.6. The full recycling process

The cassava flour was mixed with water prior to liquefaction and saccharification step, and the saccharified liquor was then used in ethanol fermentation. When the fermentation stopped, the broth was distilled. Following by distillation, the distillery wastewater was treated in the thermo- and mesophilic UASB reactors, successively. The digestion liquid flowing out of the mesophilic UASB reactor was then mixed with the raw materials for next batch of ethanol fermentation. The recycling process (Fig. 1) was repeatedly performed and part of the sludge in the thermo- and mesophilic UASB reactors was periodically discharged.

2.7. Analytical methods

Samples were centrifuged at 10,000 rpm for 10 min and the supernatant was then filtered through 0.45 μ m filters to determine sCOD and VFAs. Total and soluble COD were analyzed in accordance with the standard APHA methods [16]. The ethanol content (volume) (v/v) and VFAs (e.g. acetic, propionic and butyric acids) were determined by gas chromatography (GC1690, Kexiao Instruments Co. Ltd., China) equipped with a flame ionization detector and CPWAX52CB column (30 m \times 0.25 mm \times 0.25 μ m), where N₂ and valeric acid were used as the carrier gas and the internal standard, respectively.

The concentration of the residual total sugar was measured by Fehling reagents; conductivity and pH values were measured by a conductivity meter (DDS-11C, Shanghai Leichi Instrument Co. Ltd., China) and a digital pH meter (PHSJ-4A, Shanghai Kangyi Instrument Co. Ltd., China), respectively. Colority was determined by dilution method [17]. All the measurement experiments were run in triplicate and the relative standard deviation for all analysis and experimental results was less than 5.0%. Error bar is used on graphs to indicate one standard deviation range on one experimental measurement.

3. Results and discussion

3.1. Mathematical models of the soluble inhibitory substances in the full recycling system

Several soluble inhibitory substances in the recycling system accumulated to some extent as the recycle repeated. When the con-

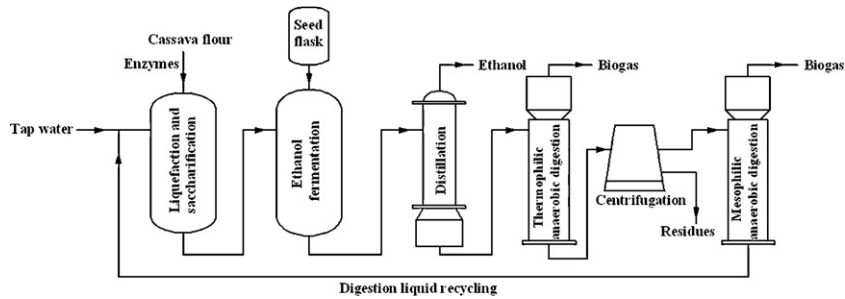


Fig. 1. The flow sheet of the full recycling technology.

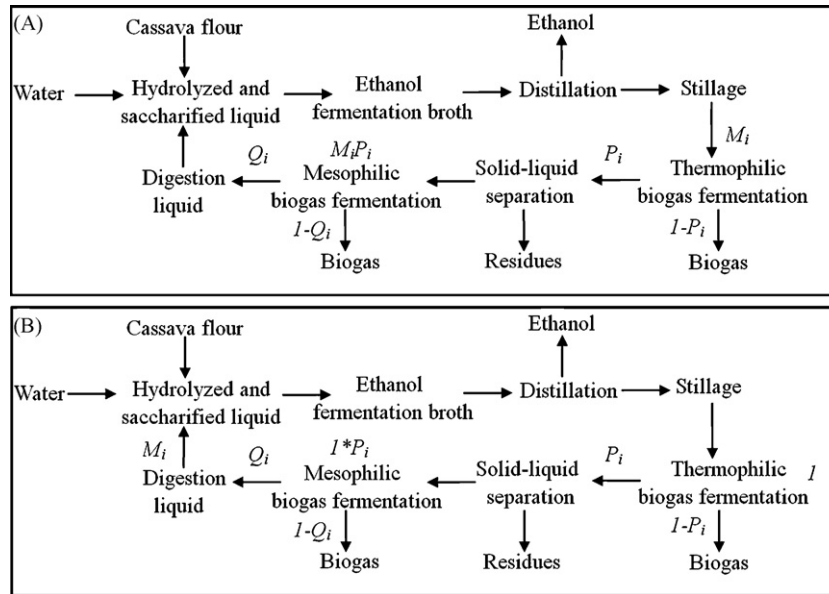


Fig. 2. Accumulation patterns of VFAs or organic compounds. Mark ' M_i ' indicates the stage where the accumulation of the i th inhibitory substance occurred and the relevant accumulation amount [M_i (g)] in each operation run, and P_i , Q_i refer to the remaining ratio (w/w) of the inhibitory substances after thermo- and mesophilic anaerobic treatment, respectively. (A) The inhibitory substances accumulated before anaerobic digestion (e.g. organic compounds); (B) the inhibitory substances accumulated after anaerobic digestion (e.g. VFAs).

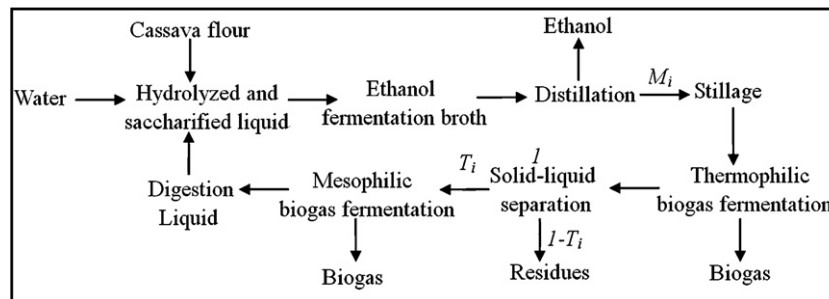


Fig. 3. Accumulation patterns of colorants or ions. Mark ' M_i ' indicates the stage where the accumulation of the i th inhibitory substance occurred and the relevant accumulation amount [M_i (g)] in each operation run, and T_i refers to the permeating ratio (w/w) of the inhibitory substances during centrifugation operation.

centration of these inhibitory substances accumulated to a high level, the growth of the microbes was inhibited, thus resulting in a failure of the recycling. In most cases, organic and inorganic substances contained in the distillery wastes came from the raw materials or fermentation by-products, which could not be utilized by yeast cells. Some proteins, fermentation by-products, metal ions and excreted toxic metabolites still remained in the wastes and inhibited cell growth and ethanol production [8]. However, these inhibitory substances in this recycling system were partly degraded by thermo- and mesophilic anaerobic treatment or partly discharged by the centrifugation operation.

The accumulation rules of the inhibitory substances, such as VFAs (including acetic, propionic and butyric acids), organic compounds (indicated by COD and sCOD), total ions (indicated by conductivity) and colorants (indicated by colority) were investigated in this recycling system. According to the different removal styles and accumulation places of these inhibitory substances, two situations were proposed as follows:

- (1) Some inhibitory substances were partly degraded in anaerobic digestion (such as VFAs and organic compounds).

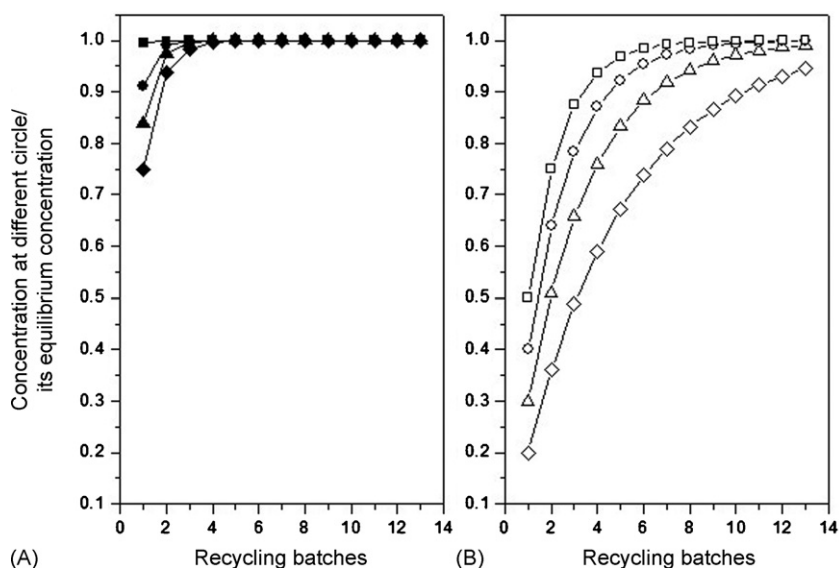


Fig. 4. Ratio of the concentration of the *i*th inhibitory substance at different repeated fed-batch cycles versus its equilibrium concentration under different remaining ratio P_i and Q_i or permeating ratio T_i (constant M_i and V)—the simulation results using the models summarized in Table 1 (■, $P_i = Q_i = 20\%$; ●, $P_i = Q_i = 30\%$; ▲, $P_i = Q_i = 40\%$; ◆, $P_i = Q_i = 50\%$; □, $T_i = 50\%$; ○, $T_i = 60\%$; △, $T_i = 70\%$; ◇, $T_i = 80\%$). (A) The inhibitory substances were partly degraded by the anaerobic microbes (e.g. organic compounds and VFAs); (B) the inhibitory substances were partly removed by the centrifugation operation (e.g. colorants and ions).

(2) Other inhibitory substances were removed by centrifugation (such as colorants and ions).

As shown in Figs. 2 and 3, the accumulation patterns of the inhibitory substances are illustrated in the recycling system, where different kinds of inhibitory substances were represented by COD, VFAs, colority and conductivity accumulated in different ways. ' M_i ' indicates the stage where the accumulation of the *i*th inhibitory substance occurred and the relevant accumulation amount [M_i (g)] in each operation run, and P_i , Q_i refer to the remaining ratio (w/w) of the inhibitory substances after thermo- and mesophilic anaerobic treatment, respectively, while T_i refers to the permeating ratio (w/w) of the inhibitory substances during centrifugation operation.

During this recycling process, the organic compounds and VFAs were mainly degraded by the anaerobic microbes, while their removal ratios in centrifugation operation were relatively minor. Therefore, the removal ratios of these substances were not considered as a factor in this study. In contrast, the colorants and ions were only slightly degraded by anaerobic microbes, but they were partly removed by the centrifugation operation, so the degradation effect of the anaerobic treatment was not considered to be a significant factor in this pattern.

The mass balance of the *i*th inhibitory substance in the system was based on the assumption of that, M_i (g), P_i (%), Q_i (%) and T_i (%) in each operation cycle are constant, where P_i , Q_i and T_i can be expressed as follows:

$$P_i = \frac{\text{Amount of } i\text{th inhibitory substance in thermophilic digestion liquid (g)}}{\text{Total amount of the } i\text{th inhibitory substance (g)}}$$

$$Q_i = \frac{\text{Amount of } i\text{th inhibitory substance in mesophilic digestion liquid (g)}}{\text{Total amount of the } i\text{th inhibitory substance after treated by thermophilic digestion (g)}}$$

$$T_i = \frac{\text{Amount of } i\text{th inhibitory substance in supernatant after centrifugation (g)}}{\text{Total amount of the } i\text{th inhibitory substance (g)}}$$

The above assumption is true for the repeated continuous fermentation cases as the initial and the final states do not change significantly cycle by cycle. The mass balance model describing the change and accumulation pattern of the *i*th inhibitory substance in each operation cycle is formulated in Table 1, where V represents the final working volume of each continuous run.

Due to P_i , Q_i and $T_i < 1$, the concentration of the *i*th inhibitory substance tends to reach an equilibrium level, and the accumulated and discharged amounts of the *i*th inhibitory substance tend to coincide with each other, when the repeated fed-batch cycle number n is approaching infinite (∞). As shown in Table 1, the equilibrium concentration depended on the value of M_i , P_i , Q_i and T_i , where the equilibrium concentration was high when the values of these parameters were large. According to the patterns in Table 1, the accumulation rules of these inhibitory substances were simulated as follows (Fig. 4).

In general, during the anaerobic treatment process, the COD removal ratio is 80–90% [18,19], which means the remaining ratio P_i and Q_i was very low. For example, if $P_i = Q_i = 30\%$, it would take about 2 cycles to reach 99% of the equilibrium concentration (Fig. 4A). In contrast, the colorants and ions were only partly removed by the centrifugation operation and could not be degraded by anaerobic microbes, so their permeating ratios were relatively higher than the remaining ratios of COD and VFAs in the system. For example, if $T_i = 50\%$, it would take about 6 cycles to reach 99% of the equilibrium concentration (Fig. 4B). As shown in Fig. 4, because M_i and V_i were constant in the models, the recycling batches were less when approaching equilibrium states if P_i and Q_i or T_i was very small. In a

Table 1
The change and accumulation patterns of the i th inhibitory substance at different operation cycles.

Recycle number	Concentration of the inhibitory substance in the end of each run (g/L)	Amount accumulated in the end of each run (g)	Amount degraded in the end of each run (g)
(1) Inhibitory substances were partly degraded in anaerobic digestion			
(a) Inhibitory substances accumulated before anaerobic digestion (e.g. organic compounds)			
1	$M_i P_i Q_i / V$	M_i	$M_i(1 - P_i Q_i)$
2	$M_i P_i Q_i(1 + P_i Q_i) / V$	M_i	$M_i(1 - P_i^2 Q_i^2)$
3	$M_i P_i Q_i(1 + P_i Q_i + P_i^2 Q_i^2) / V$	M_i	$M_i(1 - P_i^3 Q_i^3)$
...
n	$M_i P_i Q_i(1 - P_i^n Q_i^n) / V(1 - P_i Q_i)$	M_i	$M_i(1 - P_i^n Q_i^n)$
$n \rightarrow \infty$	$M_i P_i Q_i / V(1 - P_i Q_i)$	M_i	M_i
(b) Inhibitory substances accumulated after anaerobic digestion (e.g. VFAs)			
1	M_i / V	M_i	0
2	$M_i(1 + P_i Q_i) / V$	M_i	$M_i(1 - P_i Q_i)$
3	$M_i(1 + P_i Q_i + P_i^2 Q_i^2) / V$	M_i	$M_i(1 - P_i^2 Q_i^2)$
...
n	$M_i(1 - P_i^n Q_i^n) / V(1 - P_i Q_i)$	M_i	$M_i(1 - P_i^{n-1} Q_i^{n-1})$
$n \rightarrow \infty$	$M_i / V(1 - P_i Q_i)$	M_i	M_i
(2) Inhibitory substances were partly removed by centrifugation (e.g. colorants and ions)			
1	$M_i T_i / V$	M_i	$M_i(1 - T_i)$
2	$M_i T_i(1 + T_i) / V$	M_i	$M_i(1 - T_i^2)$
3	$M_i T_i(1 + T_i + T_i^2) / V$	M_i	$M_i(1 - T_i^3)$
...
n	$M_i T_i(1 - T_i^n) / V(1 - T_i)$	M_i	$M_i(1 - T_i^n)$
$n \rightarrow \infty$	$M_i T_i / V(1 - T_i)$	M_i	M_i

long run, the concentration of these inhibitory substances reached stable states after several times recycling.

3.2. The accumulation of COD and sCOD in the full recycling system

In this study, after ethanol fermentation, the COD and sCOD in the distillery wastewater reached 108,000 and 60,000 mg/L, respectively. However, after thermo- and mesophilic anaerobic treatment, the organic compounds were drastically degraded, thus resulting in a rapid decrease of COD and sCOD. Because the degradation ability of the anaerobic microbes in this system was very high and over 80% in most situations, the COD and sCOD in the digestion liquid after 3 batches recycling remained constant in a very low level (Fig. 5). It was also found that 2000 mg/L COD had no negative effect on ethanol fermentation in this system.

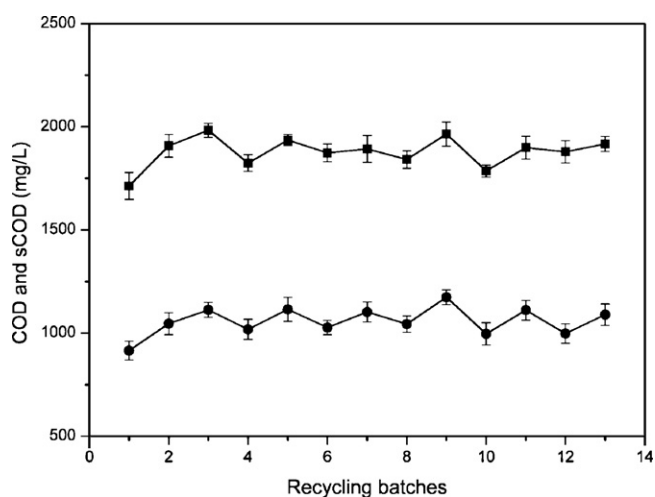


Fig. 5. The change curves of COD and sCOD in the reused liquid at different recycling batches (■, COD; ●, sCOD).

3.3. The accumulation of VFAs in the full recycling system

In the present study, the VFAs had negative impact on ethanol fermentation if their concentrations were high enough to surpass the endurable ability of yeast. Because many substances, such as fattiness, glucide and amino acids in the stillage existed after ethanol fermentation, a large amount of propionic acid was produced when these substances were treated by anaerobic microbes [20]. According to Hanaki et al., among these VFAs, propionic acid was the most toxic acid to methanogens and its inhibitory concentration was below 1000 mg/L [21]. Similarly, it was also found that 1000 mg/L propionic acid drastically inhibited ethanol fermentation in our previous studies (data not shown). In addition, the acetic acid is known as the precursor of methane in biogas fermentation and there would be little of it existing in the reused liquid. It was found that lower acetic acid concentration (358 mg/L) was responsible for inhibiting the growth of the yeast cells [11].

The accumulation rules of these VFAs in the reused liquid were illustrated in Fig. 6. As the recycling batches increased, the concentrations of VFAs also increased, however, after the 7th recycle-

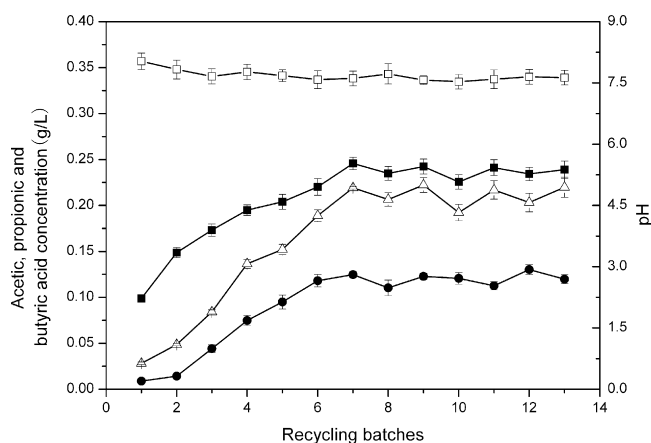


Fig. 6. The change curves of VFAs and pH in the reused liquid at different recycling batches (□, pH; ■, acetic acid; △, butyric acid; ●, propionic acid).

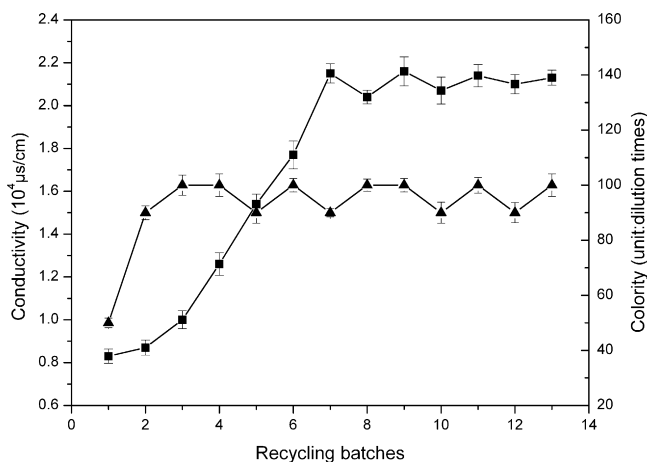


Fig. 7. The change curves of conductivity and colority in the reused liquid at different recycling batches (■, conductivity; ▲, colority).

clung, their concentrations were kept nearly constant. These results suggested that the majority of VFAs could be degraded by anaerobic treatment process. It was found that low concentration of butyric acid (less than 225 mg/L) had no negative effect on ethanol fermentation under this experimental condition. The ultimate concentration of acetic and propionic acid were almost stable and below their inhibitory concentrations (1000 and 358 mg/L mentioned above). Meanwhile, although small amounts of the VFAs were accumulated during this process, the pH of the reused liquid was also found to be almost stable. These results strongly demonstrated that the VFAs in this system reached balanced states and have no negative effect on the recycling process.

3.4. The accumulation of conductivity and colority in the full recycling system

The conductivity and colority of the digestion liquid were also investigated in this recycling system. As shown in Fig. 7, the conductivity and colority were both increased as the recycles repeated. However, with the recycling batches increased, the conductivity and colority of the digestion liquid ultimately balanced and reached constant states after the 7th and 3rd recycles, respectively.

In present study, conductivity represents the strength of ions which include SO_4^{2-} , PO_4^{3-} , Fe^{3+} , Ca^{2+} , etc. The increase of conductivity means the concentration of total ions in the digestion liquid was increased, which might be due to the continuous transformation of the ions from the raw material and the added nutrient salts. In addition, the distillery wastewater has a large amount of dark brown colour in this study. This colour was hardly removed by the conventional treatments and even increased during anaerobic treatments, due to repolymerization of compounds [22]. However, as the recycling process proceeded, part of the ions and colorants were discharged with the residues from the recycling system at the centrifugation step. As a result, the total ions and colorants in the digestion liquid did not permanently accumulate and ultimately reached constant states.

3.5. Water balance analysis in the full recycling system

In the process of ethanol fermentation, the cassava flour was mixed with tap water (1:3, w/w). The contents of the raw starch, water and non-starchy components in the cassava flour were 68%, 13% and 17% (w/w), respectively. Usually, 3.3 t of cassava can produce 1 t of bioethanol with the concentration of 99% (w/v). The water was mainly lost in two operation steps, i.e. dehydration and

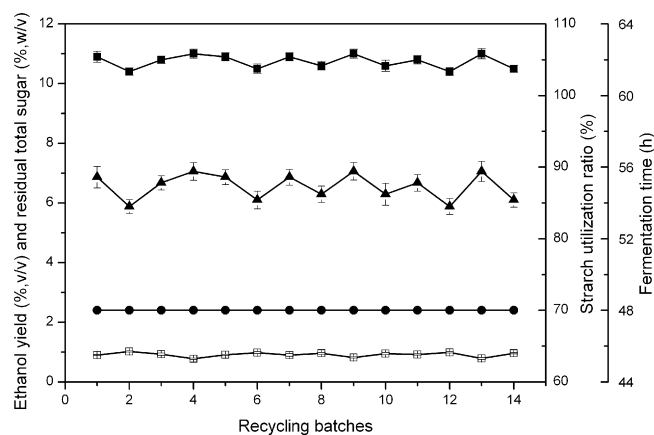


Fig. 8. The ethanol yield, starch utilization ratio, fermentation time and residual total sugar of the fermentation broth at different recycling batches (■, ethanol yield; ▲, starch utilization ratio; ●, fermentation time; □, residual total sugar).

centrifugation step, while little water was lost in other operation processes, so it can be ignored in this work.

According to the flow sheet of bioethanol production (Fig. 1), the ethanol content could reach 93% (w/v) after distillation in this study. This ethanol solution was then dehydrated and ethanol with a concentration of 99% (w/v) was achieved. During this dehydration process, 6% (w/v) water was lost. On the other hand, a large amount of residues formed by the non-starchy components of cassava, comprising 55% (w/w) of water, were produced during the centrifugation step. So the water in the recycling system was analyzed as follows.

Every 3.3 t cassava will produce 1 t fuel ethanol with the concentration of 99% (w/v), so in the dehydration step, the water loss volume of every produced 1 t 99% (w/v) ethanol was:

$$1 \times (0.99 - 0.93) = 0.06 \text{ t}; \quad (1)$$

3.3 t cassava producing dry residues:

$$3.3 \times 0.17 = 0.561 \text{ t}; \quad (2)$$

From (2) we get that the water volume contained in the wet residues was:

$$\left(\frac{0.561}{1 - 0.55} \right) \times 0.55 = 0.686 \text{ t}; \quad (3)$$

From (1) and (3) we obtain that the water loss ratio of each recycling batch was:

$$\left(\frac{0.686 + 0.06}{3.3 \times 3} \right) \times 100\% = 7.54\% \text{ (w/w)}. \quad (4)$$

So in order to keep the water balance in this recycling system, 7.54% (w/w) tap water was added in each subsequent ethanol fermentation process. Due to the constant operation condition, the water loss ratio in later recycling operations remained the same as the first run. Therefore, the recycling process would proceed consistently if 7.54% (w/w) tap water was added in this system when last recycling operation was completed.

3.6. Effects of the full recycling process on ethanol fermentation

In this work, the ethanol fermentation was performed 14 times, where tap water was used in the first time, the recycled digestion liquid after two-stage anaerobic treatment was then reused in the later ethanol fermentation 13 times. According to Fig. 8, a conclusion was reached that, although the digestion liquid was reused for 13 times, the ethanol yield, starch utilization ratio and residual total sugar were almost stable and the fermentation time was kept constant compared with the first fermentation. These results

demonstrated that this full recycling process of ethanol production after two-stage anaerobic treatment had no negative effect on the ethanol fermentation and it was feasible in practice.

4. Conclusion

A novel cleaner technology is proposed to treat the distillery wastewater in the ethanol production industry. By introducing a two-stage anaerobic treatment process, the wastewater can be totally recycled to the ethanol fermentation step, and the ethanol yield, fermentation time, starch utilization ratio and residual total sugar will be similar with that in the conventional process in which tap water was used for raw material mixing. This novel full recycling technology was confirmed to have stable operation over 13 recycles. In the experiment, the concentration of the inhibitory substances including organic compounds, VFAs, ions and colorants, reached a relative steady state after 3–7 batches recycling and these substances were found to have no negative effect on ethanol fermentation. Meanwhile, 7.54% (w/w) water will be lost during each recycling process, with the same amount of tap water added in next recycling process. Such operation assured that the water keeps balance in the whole process and will not influence the ethanol fermentation. Furthermore, three mathematical models have been established in this work to simulate the accumulation of the inhibitory substances. Through analyzing these models, the recycling batches of this process can be obtained when these substances reached equilibrium states at different situations. Compared with real recycling operations, it was found that these models can be used as a general instrument to evaluate the stability of this recycling system.

Acknowledgements

This work is financially supported by the National High Technology Research and Development Program of China (863 Program) (No. 2008AA10Z338). We also thank Mr. Jim Wright for his kind assistances during the paper preparation.

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