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Exploration of water-recycled cassava bioethanol production integrated with anaerobic digestion treatment

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Due to the limited success of discharge target-hitting treatment in coping with environmental pollution from the alcohol industry, our attention was directed towards the distillery spent (DS) wash recycle in a cleaner bioethanol production by integrating anaerobic digestion treatment with conventional fermentation. An anaerobic digestion effluent from an alcohol waste water treatment plant is applicable for single use in ethanol fermentation. With further experimental estimate, the recycle of DS treated by a sole thermophilic up flow anaerobic sludge blanket (UASB) treatment was adverse to ethanol fermentation, resulting in a gradual increase of the residual total sugar from 1.2% at batch 1 to 8.0% at batch 5 after 48 h. With a combination of the thermophilic and mesophilic UASB treatment, the thirteen-batch fermentation recycling its DS achieved ~10.5% of ethanol production and > 90% of starch utilization after 48 h, which was comparable to that using tap water. This revealed a potential of the anaerobic digestion treatment in water-saving and emission reduction for bioethanol industry.

Key words: Anaerobic digestion, cassava, cleaner bioethanol production, distillery spent wash, thermophilic and mesophilic up flow anaerobic sludge blanket, recycle and reuse.

INTRODUCTION

Cassava (*Manihot esculenta*), also called yuca or manioc, is a good starchy material in food and fermentation industry (Apea-Bah et al., 2009). In many southern provinces of China, the cassava based ethanol production exceeds 1 million tons per year (Liu and Liu, 2010). With Chinese government's strong demand on non-grain based bioethanol, cassava listed as the second leading resource of non-food biomass has attracted more and more attention

recently (Wu et al., 2009). With 1 L ethanol production, 8-15 L distillery spent wash (DS) is generally discharged. The huge waste water has presented a considerable disposal or treatment problem because of its high organic content (100 g COD L⁻¹), strong acidity (pH 3.8 - 4.5) and dark brown color. As huge water utilization in ethanol production is mainly responsible for the concomitant DS discharge, some researchers have put forward a new concept of recycling or reusing the DS (Kim et al., 1997; Olukanni et al., 2006; Ryan et al., 2008). They argue that the DS recycle should take a top priority of decreasing water consumption and DS discharge from alcohol fermentation. However, direct use of the DS has been found to be adverse to both fermentation time and alcohol yields, so some physical and chemical methods are often adopted to enhance the recycling performance of DS (Kim et al., 1997; Morin Couallier et al., 2006). These earlier literatures indicate that by some innovative

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Abbreviations: DS, Distillery spent; UASB, up flow anaerobic sludge blanket; ADDW, anaerobic digestion discharge water; BOD, biological oxygen demand; COD, chemical oxygen demand.

Table 1. Physico-chemical property of the ADDW sampled from a waste water treatment plant.

Parameter	Value
pH	3.5 – 3.7
Total suspended solids (g/L)	41.5 – 42.5
Total COD (g/L)	60.0 – 65.0
Soluble COD (g/L)	24.0 – 26.0
SS (g/L)	25.0 – 27.0
VFA (g/L)	0.05 – 0.07
Total N (g / L)	0.80 – 0.90
Total P (g / L)	0.20 – 0.40
Cellulose (%)	22 – 26
Hemicelluloses (%)	9 – 13
Lignin (%)	8 – 12

pretreatment technologies, the DS recycling way can be an alternative for the target hitting discharge (Morin Couallier et al., 2008; Sagne et al., 2009).

As an environmentally friendly and socio-economically acceptable method for waste treatment, anaerobic digestion is popular for DS treatment (Asia et al., 2006; Melamane et al., 2007; Al-Zboon and Al-Ananzeh, 2008; Mohana et al., 2009), owing to: (a) High temperature and high organic load concentration of DS (b) unbalanced chemical oxygen demand/nitrogen/phosphorus (COD/N/P) ratio of DS for aerobic treatments (c) high-energy requirement of the distillery process (Pant and Adholeya, 2007; Ward et al., 2008). What's more, the anaerobic digestion offers numerous significant advantages, that is, less energy input, low nutrient demand, minimal sludge formation and surplus biogas cogeneration (Mshandete and Parawira, 2009; Buyukgungor and Gurel, 2009). However, most time, discharge standards are often too stringent and beyond the reach of anaerobic digestion (Acharya et al., 2008). However, following it, some post-treatments including physico-chemical and aerobic process are still necessary (Pant and Adholeya, 2007). As a result, these processes are less cost-competitive.

Based on the above, to make the best use of the DS, our laboratory constructed a fermentation and ecological engineering strategy for bioethanol production. This follows an anaerobic digestion to treat and reuse the DS (Mao et al., 2006; Mao and Zhang, 2007). In the strategy, the anaerobic digestion treatment will not be responsible only for the recycling use of DS but also for a rich biogas cogeneration. This study was purposed to explore the recycling use of DS in cassava bioethanol production after anaerobic digestion treatment. In the experiment, some key variables of ethanol fermentation (that is, sugar consumption, starch utilization ratio and ethanol production) were determined when the DS treated by one- and two-stage anaerobic digestion was reused as dilution water of feedstock.

MATERIALS AND METHODS

Materials and setups

Cassava, supplied by He'nan Tianguan Group Co., Ltd, China, was dry milled into powder for an approximately 40-mesh size, dried to constant weight at 60°C and then stored for use. Anaerobic digestion discharge water (ADDW) was sampled from the waste water treatment plant of Jiangsu Huating Winery Ltd., Jiangsu Province, China, whose physico-chemical property is indicated in Table 1. The thermophilic anaerobic granular sludge (Taixing Jinjiang Chemical Industry Co., Ltd, Jiangsu Province, China) and mesophilic one (Disiman Citric Acid (Wuxi) Co., Ltd, Jiangsu Province, China) were used as the seed consortium, which were originally used in the waste water plant to meet the desired discharge standard. Two up flow anaerobic sludge blanket (UASB) bioreactors were maintained at $60 \pm 1^\circ\text{C}$ and $35 \pm 1^\circ\text{C}$ by a circulator bath, respectively. The former reacting region and settlement zone were 12 and 2 L, respectively, and the latter were 10 and 4 L, correspondingly (Table 1).

Distillery spent wash recycle in cassava ethanol fermentation with up flow anaerobic sludge blanket digestion

A recycling use of the DS in cassava ethanol fermentation with a single thermophilic or two-stage (thermophilic-mesophilic) UASB digestion is shown in Figure 1. The whole process was mainly composed of feedstock dilution, enzymatic saccharification, ethanol fermentation, distillation and thermophilic and (mesophilic) UASB digestion. To start up the UASB digestion treatment, the initial batch ethanol fermentation used tap water to dilute cassava feedstock. With the effluent reuse, some tap water was used to offset the water loss during batch fermentation. After ethanol distillation, the DS entered a reservoir (Reservoir 1) to conserve for the next continuous UASB digestion. For DS recycled ethanol fermentation with the single thermophilic UASB treatment, the slurry from the bioreactor settled down and separated into a settled residue and a supernate. The supernate, namely effluent 1, flowed into a reservoir (Reservoir 2) to conserve as dilution water. For DS recycled ethanol fermentation with two-stage UASB treatment, the supernate from the slurry after solid-liquid separation was fed into the second UASB bioreactor for mesophilic digestion. After the mesophilic UASB digestion treatment, its effluent (Effluent 2) was kept in a reservoir (Reservoir 3) to be use as dilution water (Figure 1).

Sweet mash preparation

In the batch run, a cassava powder of 2 kg was diluted at the solid-liquid ratio of 1:3 with the tap water, ADDW or effluent from anaerobic digestion. Then the broth was adjusted to pH 6.0 - 6.4 by 30% (w/v) diluted H_2SO_4 solution. After a short soak of 30 min, the broth was heated. At 50 - 60°C, it was added with a thermophilic amylase at 10 u g^{-1} dry feedstock, warming up was continued till up to 100°C and then maintained for 1 h. Then the mash was cooled down to 60°C, followed by calibration to pH 4.2 - 4.4. After that, glucoamylase was added into the slurry for saccharifying at 130 u g^{-1} dry feedstock. After 30 min of the saccharification, a cassava sweet mash was finally obtained.

Ethanol fermentation process

Pre-culturing of the yeast strain, *Saccharomyces cerevisiae*, was carried out in a 250 ml Erlenmeyer flask containing 100 ml sterilized medium (%) composed of glucose, 2; yeast extract, 0.85; NH_4Cl , 0.13; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 and CaCl_2 , 0.006. After a loop of seed

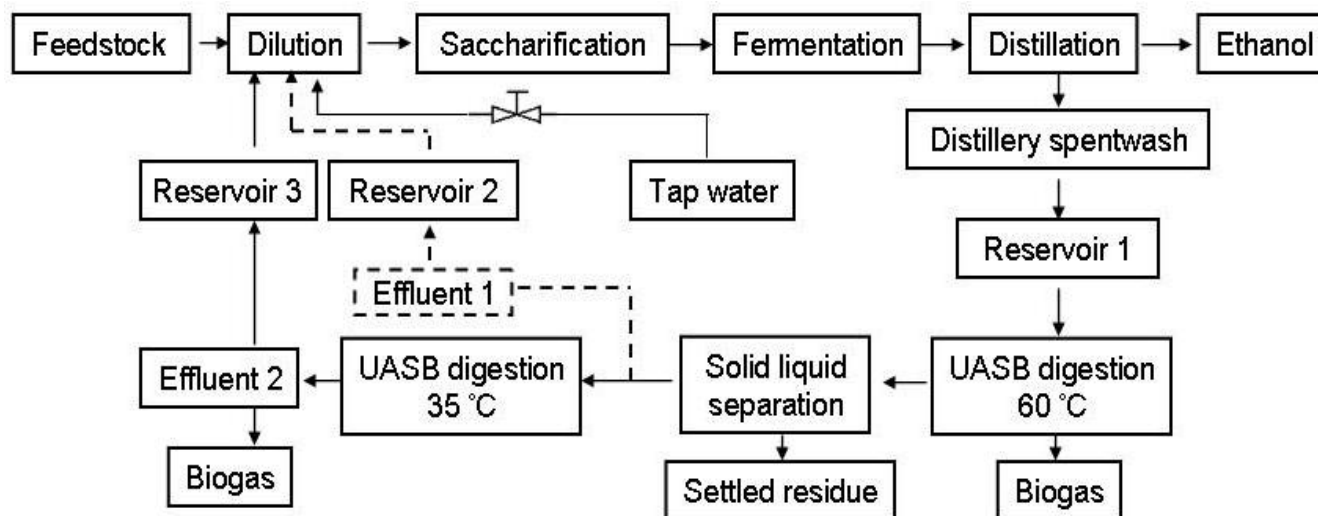


Figure 1. Scheme of the DS recycles in cassava bioethanol fermentation with UASB digestion. Dashed portion denotes that the effluent from a single thermophilic UASB digestion was used to dilute the feedstock.

from the slant culture was inoculated into the culture, the pre-cultivation was initiated in a rotary shaker at 28°C and 100 rpm for 12 h. Then, 10% (v / v) of liquid inoculums was inoculated in the sweet mash complemented with urea at 3 g L⁻¹. The fermentation experiment of using the ADDW and effluent from (one- or two-stage) anaerobic digestion treatment was done in a 250ml Erlenmeyer flask and 10 L fermentor, respectively. The fermentation finished when the residual sugar was detected to be below 1.0%. After the ethanol distillery, the hot DS (app. 6 L) was discharged into a reservoir and cooled to 60°C for the UASB digestion, whose physicochemical property is shown in Table 2.

Up flow anaerobic sludge blanket treatments of the distillery spent wash

Thermophilic and mesophilic UASB bioreactors were loaded with activated sludge of 4 and 3 L, respectively, with the rest of their effective volumes complemented with tap water, which both circulated by peristaltic pump overnight for acclimation. For the single thermophilic UASB digestion, the DS (COD > 100 g/L) from ethanol fermentation was cooled and directly pumped at 2 L day⁻¹ into the tank from the bottom with no dilution or residue removal, followed by the effluent discharge at 2 L day⁻¹. After the high-temperature effluent was out, it was centrifuged at 4,000 rpm for 30 min. Then the supernatant (app. 1.5 L day⁻¹ each batch) was kept at 5°C to use as an alternative for tap water to dilute the cassava feedstock for ethanol fermentation.

For the two-stage (thermophilic-mesophilic) UASB digestion, the DS was added with FeCl₂ at 2.5 g L⁻¹ and then was fed into the first stage, just like the single thermophilic UASB digestion. After the high-temperature effluent was out, it was centrifuged at 4,000 rpm for 30 min. Then the supernatant (app. 1.5 L day⁻¹ each batch) fed into the mesophilic UASB bioreactor at 35°C after the effluent was discharged with the same volume every day. The effluent from mesophilic digestion was kept at 5°C to be use directly as the dilution water alternative for tap water.

Analytical methods

The fermentation broth was centrifuged at 13,000 rpm for 10 min.

The supernatant was then determined for total sugar by a phenol-sulfuric acid method (Mecozzi, 2005). Reducing sugars were analyzed by Fehling titration method. Growth (cell concentration) was measured by OD measurements at 600 nm after the supernatant was diluted to ~ 5% of sugar concentration. For the fermentation using ADDW, the weight loss of the Erlenmeyer flask due to the amount of CO₂ evolved was measured at intervals (Teramoto et al., 1993). Ethanol was analyzed by gas chromatography (Agilent 6890A, USA). Solid phase: cross-linked polyethylene glycol, carrier gas: nitrogen, 180°C isothermol capillary column, injection temperature 200°C, flame ionization detector temperature 250°C, Agilent Chem Station Data Analysis System) and n-butanol was used as an internal standard. Each sample was performed in duplicate, with the average value reported. The standard deviation was less than 4.0 %.

RESULTS AND DISCUSSION

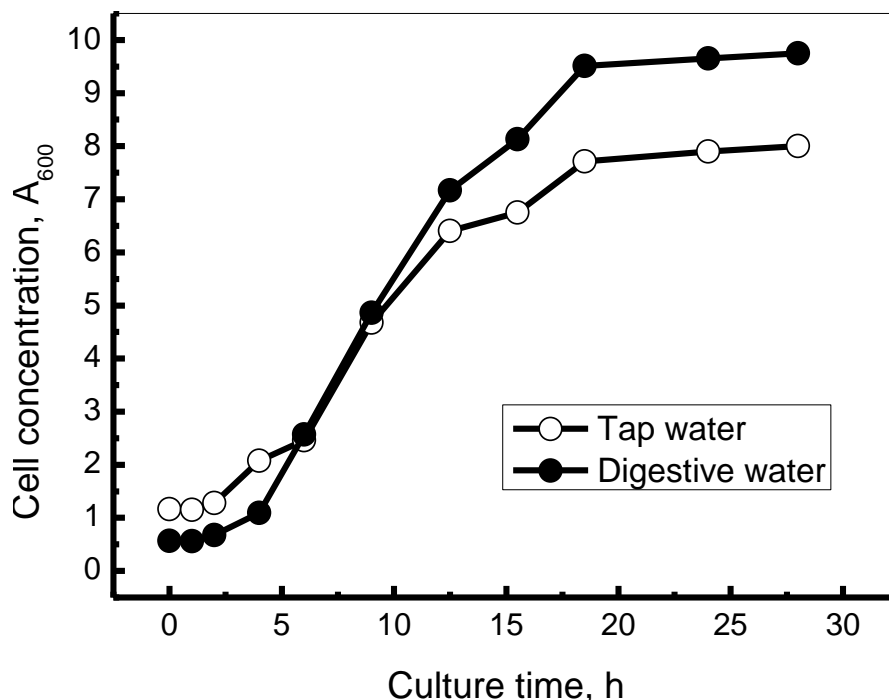
Utilization of ADDW in cassava ethanol fermentation

In order to evaluate the reuse of DS after anaerobic digestion treatment, an ADDW was taken directly as dilution water to determine its effect on ethanol fermentation and as such some key variables as microbial biomass, sugar consumption and ethanol production were characterized, as seen in Figure 2. During the whole fermentation using tap water and ADDW (Figure 2a), the cell biomass both increased rapidly, indicating a good growth of yeast. However, the fermentation using ADDW had a much higher microbial biomass than that with tap water, even if the former had a small inoculums size. At the stationary phase, the former A₆₀₀ value reached 9.75, far higher than that (8.00) of the latter. This indicated that the ADDW seemed to have a positive role on yeast growth.

As seen in Figure 2b, the total sugar and reducing sugar were both consumed rapidly. The total sugar of ethanol fermentations using tap water and ADDW reduced

Table 2. Physico-chemical characteristic of the influent DS from batch ethanol fermentation.

Parameter	Value	Parameter (g / L)	Value
pH	5.04	Crude fat	2.6
Total suspended solids (g / L)	60.5	Cellulose	18.2
Total COD (g / L)	119.0	Hemicelluloses	10.9
Soluble COD (g / L)	47.0	Lignin	2.4
Gross protein (mg / L)	63	Ash content	8.1

**Figure 2a.** Effect of the ADDW on yeast growth of ethanol fermentation. The ADDW was sampled from the anaerobic digestion process of a wastewater treatment plant that treated the DS to meet the discharge standard; the ethanol fermentation was developed at 30°C in a 250 ml Erlenmeyer flask, with a working volume of 150 ml.

from 20.3 and 20.9 to 3.8 and 2.0%, respectively. Meanwhile, their reducing sugars also decreased from 5.9 and 8.0 to 2.1 and 0.3%, correspondingly, though there both appeared significant increases at a prior fermentation stage. Interestingly, with the ADDW as dilution water, the fermentation presented a much faster degradation of total sugar and reducing sugar than that using tap water, regardless of a slight more initial content of the former. Also, the maximum reducing sugar content (9.7%) of the former was lower than the latter's (10.7%). These results showed that the ADDW used as dilution water seemed to expedite the sugar consumption during ethanol fermentation. On the other hand, Figure 2b also describes the profile of ethanol production. Compared with that (7.7%) using tap water as dilution water, the fermentation with ADDW gave a higher weight loss of 9.0% after 60 h fermentation, indicating that the ADDW used as dilution

water favored the ethanol production (Figure 2a).

Based on all the above experiment, it was evident that the ADDW from anaerobic digestion of DS was not only applicable as an alternative for tap water to dilute the feedstock, but also had a positive role on the ethanol fermentation. The result agreed with some reports that the ADDW still had a notable biological oxygen demand (BOD)/COD content, considerable nutrients (K, S, N and P) and some micronutrients (Ca, S, Cu, Mn and Zn) (Mohana et al., 2009). When the ADDW was used as the dilution water, these rich nutritive substances probably contributed to a good yeast growth and ethanol production. Besides, the data implied that by anaerobic digestion, it was possible to reuse the DS from ethanol fermentation as dilution water. Therefore, the ensuing work was carried out to recycle the DS in cassava bio-ethanol production by integrating some UASB digestion

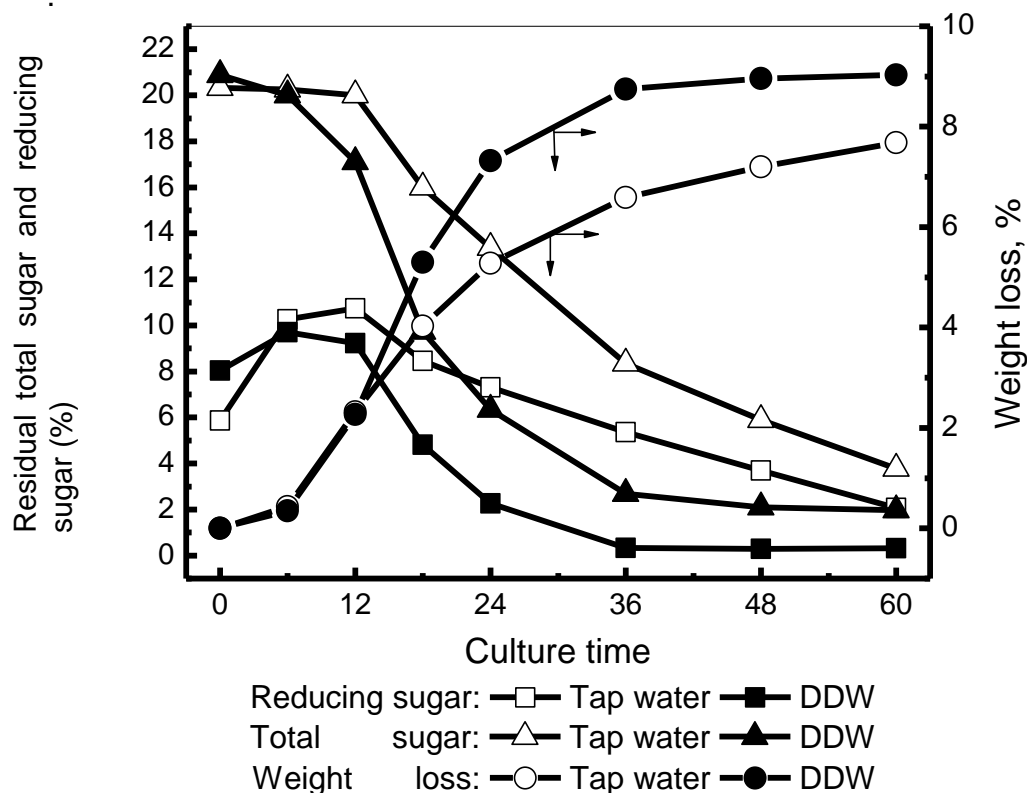


Figure 2b. Effect of ADDW on sugar consumption and ethanol production.

treatment such as cassava ethanol fermentation recycling DS by a single thermophilic UASB digestion.

Based on the above findings, the experiment was tried to reuse the DS as an alternative for tap water to dilute the cassava feedstock for ethanol fermentation after the DS was treated in a single thermophilic UASB bioreactor. As such, some key variables such as total sugar consumption and ethanol production were detected to make an elementary judgment on the ethanol fermentation, as shown in Figures 3a and b.

After ethanol fermentation using tap water as dilution water, its DS was treated by a thermophilic UASB digestion and the settled effluent was reused as dilution water for the next fermentation, so was the latter recycled. Total sugar consumption of batch ethanol fermentation is shown in Figure 3a. For the fermentation using tap water, the sugar content decreased to 0.9% after 48 h fermentation. Compared with it, the residual total sugar content of batch fermentation recycling the DS increased gradually, rising from 1.2% at the first batch to 8.0% at the fifth batch. The result indicated that the digested DS seemed adverse to ethanol fermentation. In order to reduce the above over high content (> 1.0%) of residual total sugar, the next experiment was developed, as shown in Figure 3b. When compared to tap water, the batch ethanol fermentation using digested DS all achieved an equivalent ethanol production, but the batch fermentation time pro-

longed strikingly, increasing from 55 h at batch 1 to 105 h at batch 5.

Similar phenomenon has also been observed by other researchers. Teramoto et al. (1993) found, when reusing the concentrated *shochu* distillery waste in semi continuous ethanol fermentation, the 3rd - 8th batch ethanol fermentation needed approximately for 5 days while the contrast took less than 3 days. Kim et al. (1997) reported that, without and with membrane filtration of DS, the total fermentation time was prolonged from 60 to 90 - 100 h and to 70 - 80 h, respectively, but the average ethanol production yield (8.8%) was similar to that in the conventional process (9.0%). They speculated that the accumulation of some low molecular weight organics and salts were probably responsible for the slow sugar consumption. Further, Morin Couallier et al. (2008) pointed out why the DS could not be recycled, finding that at least eight toxic volatile compounds existed in the recycled distillery condensate. Our further experiment also demonstrated that some fermentation inhibitive substances survived the single thermophilic UASB digestion process and accumulated progressively in batch ethanol fermentation, thus resulting in the fermentation failure of DS recycle (Mumtaz et al., 2008; Zhang et al., 2010).

Besides, it was implied that the single thermophilic UASB digestion was unable to achieve the goal of DS recycle in ethanol fermentation. It was indispensable to

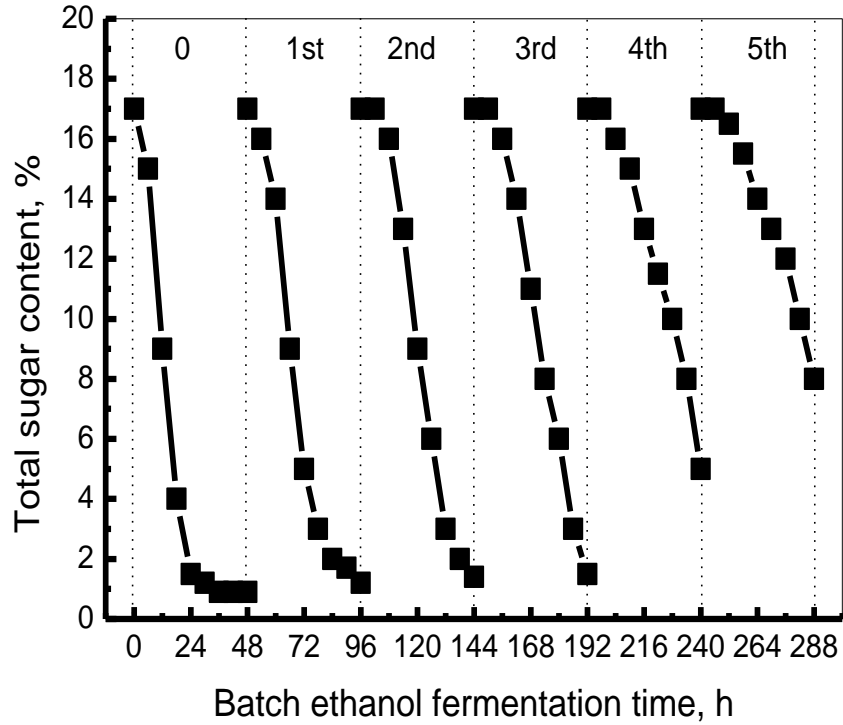


Figure 3a. Total sugar profile of batch ethanol fermentation reusing the treated DS. The fermentation was implemented in a 10 L fermentor, whose static fermentation comprised of three stages, namely a prior fermentation at 28°C for 6 – 8 h, a main fermentation at 32°C for 18 – 16 h and a late fermentation at 30 °C for 24 or 26 h.

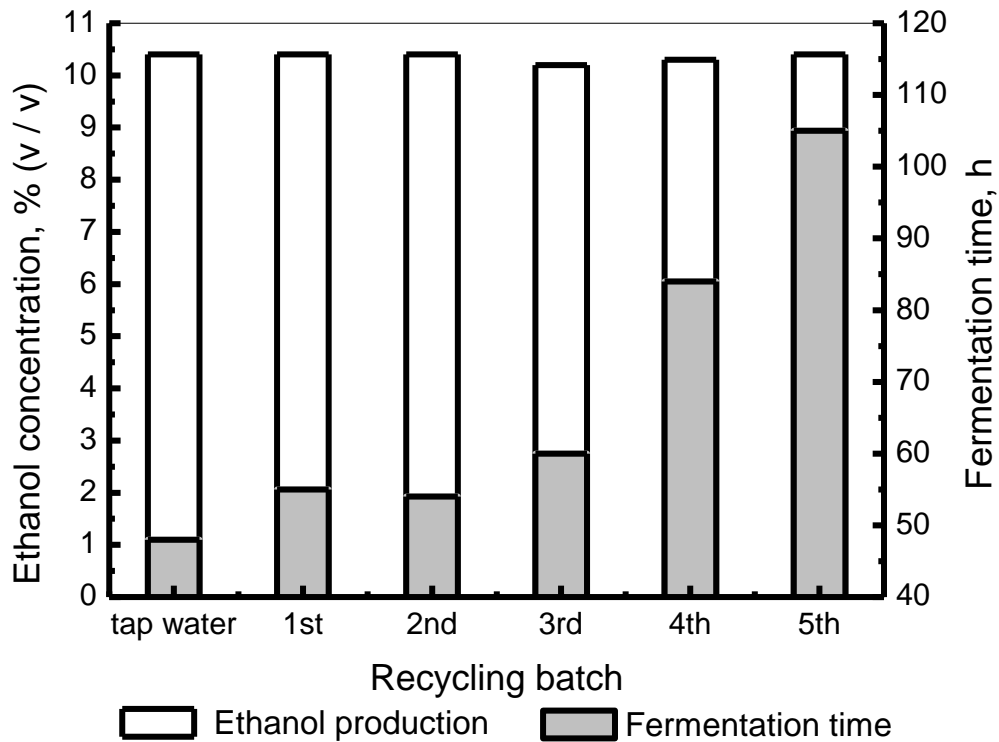


Figure 3b. Performance of batch ethanol fermentation recycling the DS treated by a single thermophilic UASB digestion.

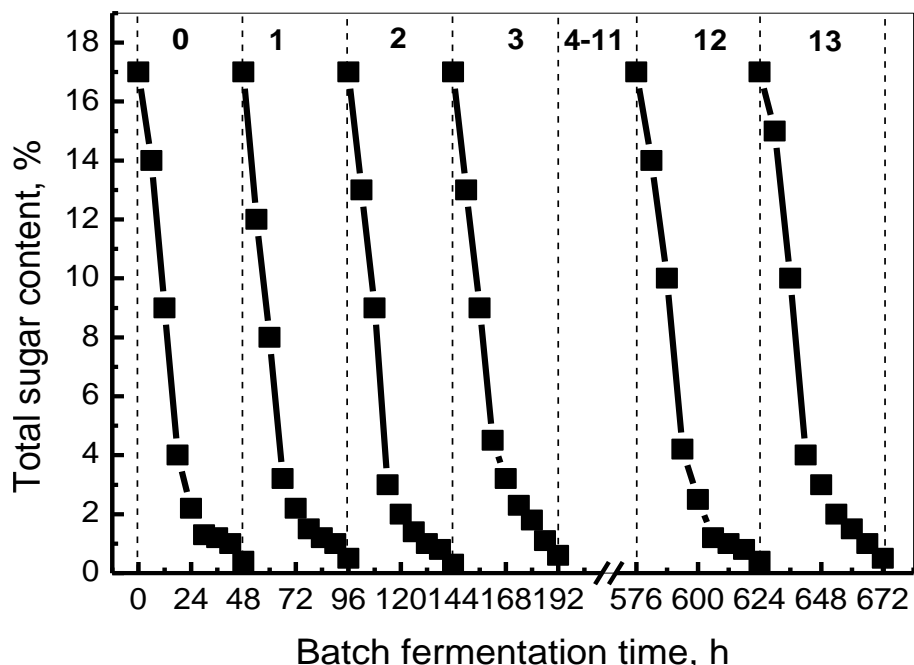


Figure 4a. Total sugar consumption of thirteen batch ethanol fermentation with the DS recycle. The fermentation was also done in the 10 L fermentor, just the same to the above in Figure 3.

engineer rationally an anaerobic digestion system to coordinate the nutrient accumulation and inhibitive elimination for the DS recycled ethanol fermentation. Considering individual advantage of thermophilic and mesophilic UASB digestions (Singh and Prerna, 2009; Mohana et al., 2009), the ensuing experimental work was carried out to examine the DS recycle in ethanol fermentation by a two-stage UASB digestion.

Cassava bioethanol production recycling the distillery spent wash by a two-stage digestion

To assess ethanol fermentation using the DS after thermophilic-mesophilic UASB digestion as dilution water, thirteen batch fermentation experiments were done to investigate the sugar consumption, starch utilization and ethanol production of each batch in the DS recycled process (Figure 4). As seen in Figures 4a and b, for the fermentation using tap water, the residual total sugar content reduced to 0.5% after 48 h and its starch utilization and ethanol production reached 90 and 10.9%, respectively. When the DS treated by two stage digestion was used as dilution water, thirteen-batch ethanol fermentation all had fast sugar consumption. The residual total sugar content stayed at 0.5% and the starch utilization achieved above 90% after 48 h, while their ethanol production maintained basically stable at 10.5%. The data showed that for the dilution water of ethanol fermentation, there was no marked difference between the tap

water and biotreated DS, though some slight waves of the starch utilization and ethanol production occurred. It was demonstrated that the treated DS was applicable as a substitute for tap water to dilute the cassava feedstock. More importantly, the two-stage UASB digestion enabled the ethanol fermentation to recycle its DS for use, which supplied bioethanol industry with a promising future in emission reduction and water-saving (Figures 4a and b).

Compared with ADDW, the DS treated by two-stage UASB digestion during the whole reuse displayed no significant stimulative role on ethanol fermentation. This was possibly due to the different anaerobic microbial consortium and chemical composition of DS, which resulted finally in different nutrition of the DS effluent (Mohana et al., 2009). Also unlike the single thermophilic UASB digestion, the two-stage UASB digestion treatment was able to circumvent some adverse effects and thus realized the DS recycle in ethanol fermentation probably by eliminating some fermentation inhibitive substances. Further work had developed on why the two-stage UASB digestion treatment enabled the DS to recycle in ethanol fermentation. A relatively stable, low content of SO_4^{2-} , COD, N and P in the DS effluent was found mainly responsible for it, which would be reported elsewhere.

Conclusion

As an alternative for tap water to dilute the feedstock, the anaerobic digestion effluent presented some positive roles

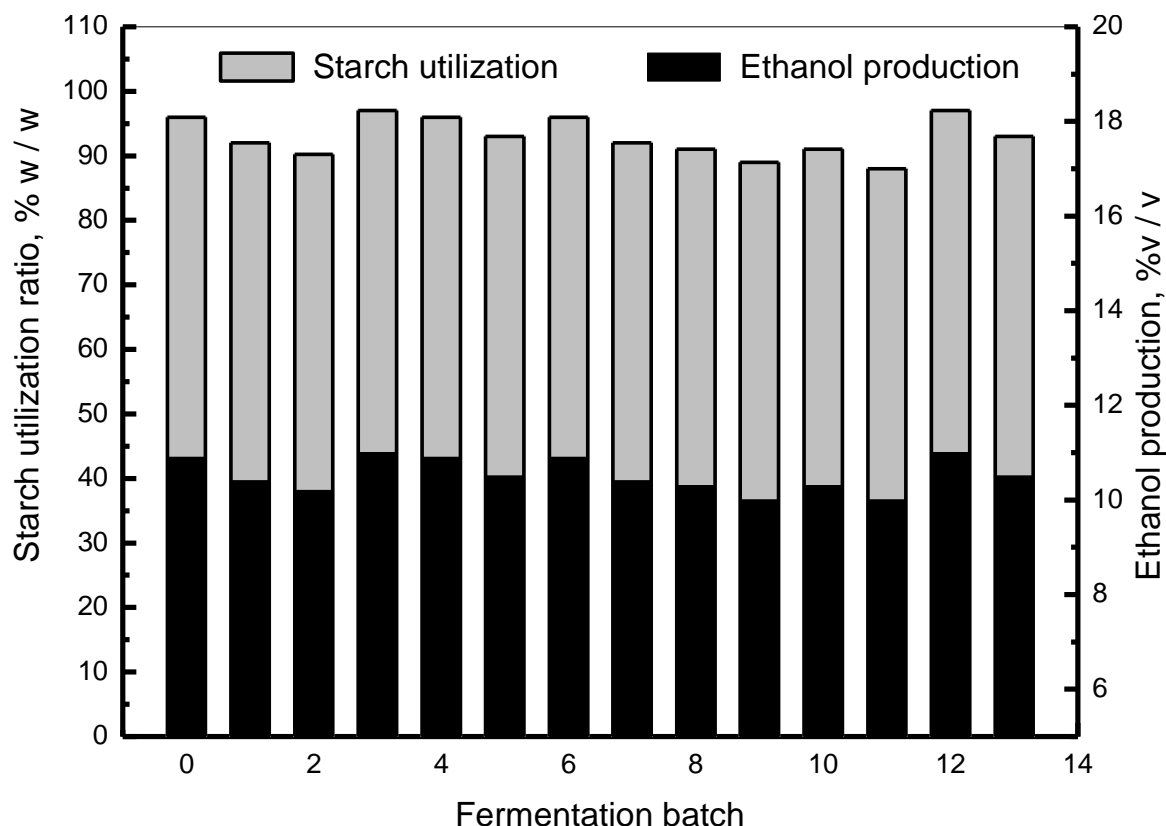


Figure 4b. Starch utilization ratio and ethanol production of batch ethanol fermentation with the DS recycle.

on ethanol fermentation. With a sole thermophilic UASB digestion treatment, the DS recycle was hard to reach. The recycled water was adverse to ethanol fermentation, resulting in a gradually long fermentation time from 55 h at batch 1 to 105 h at batch 5. The combination of thermophilic and mesophilic UASB treatments allowed ethanol fermentation to recycle the DS for over thirteen batches, reaching ~10.5% of the ethanol production and > 90% of the starch utilization after 48 h batch fermentation. For bioethanol industry, the anaerobic digestion treatment revealed a considerable potential in water-saving and emission reduction.

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