Journal of Hazardous Materials 172 (2009) 1252–1257

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Biological flocculation treatment on distillery wastewater and recirculation of wastewater

Wen Zhang, Rongchun Xiong, Gang Wei *

School of Materials Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, PR China

ARTICLE INFO

Article history:
Received 4 February 2009
Received in revised form 23 June 2009
Accepted 30 July 2009
Available online 11 August 2009

Keywords:
Ethanol
Distillery wastewater
Biological flocculation
Inhibitory
Recirculation

ABSTRACT

In the present study, a wastewater treatment system for the ethanol fermentation industry was developed by recycling distillery wastewater. The waste was able to be recycled for the next fermentation after being treated with bio-flocculation process. The bio-flocculation process contains three steps: screening, treatment with polyaspartic acid and filtration. When the filtrate from this process was recycled, the average ethanol production yield was very close to that in the conventional process using tap water. In contrast, the recycle of wastewater without flocculation and with chemical flocculation showed negative effects on ethanol yield as recycling was repeated. This new process was confirmed to have stable operation over ten recycles. Hazardous materials influencing distillery wastewater recycles on fermentation were also considered. It was found that the content of suspended solids (SS), volatile acid and Fe ions inhibited fermentation and resulted in a decreased ethanol yield. Bio-flocculation was shown to be an effective way to diminish the content of inhibitory compounds drastically when the waste was recirculated.

1. Introduction

As a clean and renewable source of energy in the future, ethanol is a promising alternative to fossil fuels. Especially lignocelluloses as raw materials for ethanol production have gained interest during these years. Production of bio-ethanol will help to cope with the over consumption of fossil fuels and further work for the reduction of carbon dioxide emissions. However, ethanol manufacture from different raw materials generates large volumes of high strength wastewater that is of serious environmental concern [1]. The production and characteristics of wastewater are highly variable and dependent on feedstocks and various aspects of the ethanol production process [1,2]. But these wastes are always with high concentration of organic materials (CODcr > 30,000 mg/l) and low pH (3.5–4.5).

Several methods have been proposed for the treatment of distillery wastewater such as anaerobic fermentation [3] including Up-flow anaerobic sludge blanket (UASB) technology [4] and Sequencing Batch Reactor Activated Sludge Process (SBR) method [5], membrane filtration process [6], adsorption process [7], electrolysis [8], evaporation process [9], coagulation [10], aerobic treatment [11,12], fermentation hydrogen production process [13], enzymatic treatment [14], catalytic wet oxidation process [15], and Dry Distilled Grain Soluble (DDGS) process [16]. The biological treatment of distillery wastewater (aerobic or anaerobic) always can result in 70–90% COD reduction [1,17]. Meanwhile the physicochemical methods are employed generally after the primary anaerobic treatment in order to further reduce the COD and color [1,2,17].

In China, the most widely used raw materials for ethanol production are grain. The commonly used treatment method of the wastewater from grain materials is anaerobic/aerobic biochemical process which needs a strict pretreatment. In contrast, the method DDGS which has been used in large-scale ethanol plants of China seems to be a good choice. The distillery wastewater was firstly separated into two parts—solid and liquid. The liquid fraction was further processed through an evaporator and concentrated. Then it was mixed with the solid fraction and finally concentrated to produce DDGS. DDGS can become a very popular feedstuff which is easy in storage and transport. DDGS process is very efficient for the treatment of ethanol distillery wastewater which contains high concentration of organic matters. However, the major drawbacks of this process are high energy consumption and significant difference in treatment efficiency with other raw materials (lignocellulose materials) used for the ethanol fermentation. This process is suitable for the treatment of wastewater from grain materials but not for the treatment of wastewater from other materials.

In the production of ethanol, large quantities of water are demanded in various process steps. Distillery waste recycle is one of the best methods and has bright future in practice. Although there

* Corresponding author. Tel.: +86 10 64434907; fax: +86 10 64416221.
E-mail address: rongchunxiong@163.com (G. Wei).

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doi:10.1016/j.jhazmat.2009.07.150

Table 1
Characteristics of distillery wastewater accompanied by their standard deviation (numbers in parentheses correspond to the number of measurements used for the determination of the mean values and standard deviations).

Characteristics	Distillery wastewater
PH	3.8(5)
Total solids (g/l)	20.4 ± 2.5(10)
Sugar%(w/w)	1.4 ± 0.2(5)
Protein%(w/w)	2.4 ± 0.1(5)
COD_{cr} (g/l)	38.4 ± 2.3(5)
BOD_5 (g/l)	21.5 ± 1.2(5)
EC (ms/cm)	11.34 ± 0.71(5)

are a lot of studies on wastewater recirculation [6,9] but some of the processes require electricity derived from fossil fuel combustion so that they are energy intensive. At the same time, the requirements of the equipments on the other processes are special so that they are expensive. With this impetus, a new technology for distillery wastewater recycle emerges as the times require.

As is known, bio-flocculants are harmless to the environment and humans, indicating their potential to replace the existing chemical flocculants. Various bio-flocculants have been used in wastewater treatment [18,19]. The goal of this research was to apply a biological flocculation technology to the ethanol wastewater treatment process, which could eliminate the pollution problem caused by wastewater from different raw materials. The used bio-flocculant polyaspartic acid (PASP) which was firstly found to exist in the body of marine organism is recognized as a kind of green wastewater treatment agents in the world [20].

2. Materials and methods

2.1. Materials

The distillery wastewater, liquid saccharifying enzyme, *Saccharomyces cerevisiae* and corn residue (corn bran, by-products from starch processing) obtained from a local grain distillery company, were used in this investigation. The characteristics of the raw wastewater are given in Table 1. The *S. cerevisiae* was stored in the tube culture at 4 °C. It was inoculated into the liquid culture medium after activation. The PASP (molecular formula: $C_4H_6NO_3(C_4H_5NO_3)_m C_4H_6NO_4$; molecular weight: 1000–5000; pH (1% aqueous solution) = 10–11) used as bio-flocculant in the present study was prepared by Beijing University of Chemical Technology [21]. All other chemicals of analytical reagent grade were purchased from Beijing Chemical Factory (Beijing, China). All the biochemical reagents were bought from Beijing Biological Technology Factory (Beijing, China).

2.2. Ethanol production and distillery wastewater reuse

Experiments were performed in several glass flasks sealed with cotton stoppers to simulate the conventional ethanol fermentation process. Furthermore a lab-scale wastewater recycling system was devised.

The *S. cerevisiae* after activation was inoculated in the liquid culture medium under aseptic condition and carried out under 32 °C at 120 rpm for 48 h to form the seed liquid (suspended cells liquid). The optimal liquid culture medium composition in shake-flask scale were determined as followings: glucose 20 g/l, $(NH_4)_2SO_4$ 5 g/l, urea 2.5 g/l, peptone 5 g/l, KH_2PO_4 2 g/l, $MgSO_4{\cdot}7H_2O$ 1 g/l, yeast extract 5 g/l.

The corn residue (30 g) was weighted and mixed with 125 ml water (tap water or treated wastewater) in the 250 ml flasks. Then the mixture was heat-treated (70 °C, 30 min), boiled (125 °C, 1 h) and saccharified (60 °C, 60 min). Prior to fermentation, the pH was adjusted to 4.5 with dilute sulfuric acid. The fermentation condition was: culture temperature 30 °C, rotation speed 100 rpm, inoculation volume 12% and fermentation time 90 h. After fermentation, mixture was fully fed to distillation flask (500 ml) to separate ethanol and distillery wastewater. When the steam temperature reached 98 °C, the distillation was finished. This distillation obtained rough distillate.

Lab-scale ethanol production processes were carried out according to Fig. 1. As a first step, the vinasses were passed through a filter screen (50 mesh) to remove coarse solids (used as feedstock after drying) and then treated by biological flocculation. The wastewater supplemented with 1.0 g/l polyaspartic acid was stirred at 150 rpm for 2 min and then kept for 20 min. Under normal temperature, the dosage of flocculant was determined as follows: the flocculant was added to a certain amount of wastewater (stirring rate: 150 rpm) until there was a lot of precipitation generated and the color of the water was changed (from brown to reddish brown). Then the amount was recorded to determine the concentration of flocculant. Finally the solution was filtered by filter paper or filter cloth (pore size = 0.12 mm). The filtrate was mixed with the raw materials for the next fermentation. The wastewater was reused and recycled.

The process of chemical flocculation mentioned later was similar with biological flocculation. The vinasses were firstly passed through a filter screen (50 mesh) to remove coarse solids (used as feedstock after drying) and then treated by chemical flocculant. The chemical flocculant used was polyaluminium chloride–polyacrylamide (PAC–PAM). Then the wastewater supplemented with 1.0 g/l PAC–PAM was stirred at 150 rpm for 2 min and then kept for 20 min. After flocculation, the wastewater was passed through filter paper for reuse.

The ethanol yield (ml) in the experiments was calculated as

$$EV\,(\mathrm{mL}) = ED \times V_{\mathrm{distilled\ fluid}} \qquad (1)$$

in which ED was the ethanol content (volume) (v/v) present in the distilled fluid, $V_{\mathrm{distilled\ fluid}}$ was the volume of the distilled fluid.

The ethanol concentration (v/v) was calculated as the percent (v/v) of ethanol present in the body fraction per volume of fermentation broth.

$$EC\,(v/v) = \frac{EV}{V_{\mathrm{fermentation\ broth}}} \times 100\% \qquad (2)$$

The ethanol output rate (v/w) was calculated as

$$EP\,(v/w) = \frac{EV}{M_{\mathrm{corn\ residue}}} \times 100\% \qquad (3)$$

in which $M_{\mathrm{corn\ residue}}$ was the weight of the raw materials. Numbers in parentheses correspond to the number of measurements used for the determination of the mean values and standard deviations.

2.3. Analysis method

The reducing sugars were analyzed by the DNS method [22]. The ethanol content (volume) (v/v) in the distilled fluid was determined by the colorimetric method [23]. Water-soluble protein content in different batches of distillery wastewater was measured by Biuret method using bovine serum albumin [24]. A calibration curve was made with pure protein at different concentrations and the absorbance of the samples was read at 540 nm. The concentration of Ca^{2+}, Mg^{2+}, and Fe^{3+} was measured by the HANNA C200 WATER ANALYZER (Italy) throughout the studies. Total aldehyde (as acetaldehyde) was determined chemically using iodimetry [25]. The total acid content was measured by titration method with sodium hydroxide solution. Phenol red was used as indicator or automatic potentiometric titration was used and the end point of pH was 8. The total volatile acid (as acetic acid) was measured

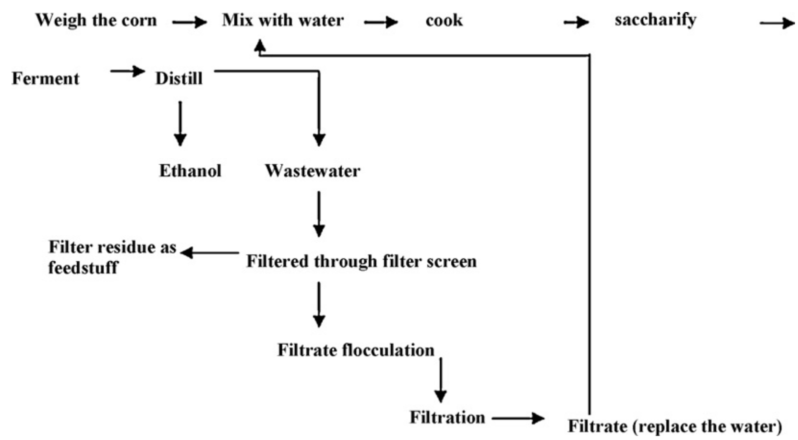

Fig. 1. Flow sheet of the lab-scale distillery wastewater recycling system.

by steam distillation [26]. The pH was measured in a pH meter (PHS-3B, Shanghai Precision & Scientific Instrument Co. Ltd., China). Measurements of COD, and SS were performed according to the standard methods [27] (APHA, 1998). BOD_5 was measured by the HANNA BOD Trak (Italy) and EC was measured by Conductivity Meter (DDSJ-308A, Shanghai Precision& Scientific Instrument Co. Ltd., China). The morphology of yeast was observed by the microscope (XSP-3X, Shandong Photoelectric Instrument Co. Ltd., China). Numerous yeasts could be observed under low magnification of the microscope. High-magnification microscope can be used for the observation of individual yeast cell.

3. Results and discussion

3.1. The recirculation of distillery wastewater with common treatments

As shown in Table 1, the distillery wastewater contains proteins and sugars which can provide nitrogen and carbon for the growth of microorganisms. So the wastewater reuse for the ethanol fermentation process should be feasible, and it is necessary to study on the wastewater recirculation technology. It is possible to eliminate the wastewater pollution instead of the conventional wastewater treatment steps using the biological treatment processes such as anaerobic digestion and activated sludge steps widely being operated in industry.

In this study, the recirculation of distillery wastewater was evaluated (Fig. 1). The tap water; distillery wastewater after the primary filtration (through a filter screen) and distillery wastewater after chemical flocculation treatment were reused for ethanol production. Table 2 showed the results of ethanol fermentation with tap water, without and with flocculation treatment for the reuse of distillery wastes, respectively. The number of recycles was determined by the ethanol production rates. With a high ethanol yield the number of recycles was increased. The wastewater from the last fermentation was mixed with the raw materials as brewing water for the next fermentation.

Ethanol concentration (v/v) at 90 h using tap water was 8.16% (Table 2). The yield was high. The process of yeast fermentation was observed by microscope. The large yeast cells could be recognized easily without staining. The yeast cells were generally round, oval, cylindrical or citriform. Each yeast cell had its own form of a certain size, and did not agglomerate.

Without flocculation treatment, final ethanol concentrations (v/v) were 6% at 1st recycle and 4.8% at 2nd recycle (Table 2). The yield was lower than the ethanol yield with tap water (8.16%). The direct recycling without flocculation treatments gives a severe negative effect on ethanol fermentation and yeast cell growth (observed by the microscope). The negative effect would become greater as the number of recycles increases, and to achieve the same ethanol yield the run requires much more time for fermentation. The cause may be the relative high concentration of soluble solids and some specific toxic substances.

With chemical flocculation treatment for the reuse of distillery wastes, final ethanol concentrations (v/v) were 6.41% at 1st recycle and 7.2% at 2nd recycle (Table 2). The yield was lower than the ethanol yield with tap water (8.16%). But ethanol fermentation was better than the ethanol yield with direct recycling of stillage. The solids content of the wastes was greatly reduced, but the toxic substances were not removed using this common chemical flocculation treatment. The filter residue may be toxic from such inorganic-organic compound flocculation and cannot be used as feedstuff. This may result in secondary pollution. This post-treatment of filter residue might be problematic for the ethanol industry.

3.2. The recirculation of distillery wastewater with biological flocculation treatment

After treating the wastewater with common recycling process, the effects of recycled filtrate from biological flocculation on ethanol fermentation were investigated. This process was carried out according to Fig. 1 too.

With biological flocculation treatment for the reuse of distillery wastes, final ethanol concentrations of the repeated fermentation were as follows; 9.12% (lst), 9.12% (2nd), 9.12% (3rd) etc. . . . (Fig. 2). The average ethanol yield was slightly higher than if using fresh tap water (8.16%). One interesting observation, however, is that final ethanol concentration (v/v) does not change significantly as the number of recycles increases (Fig. 2). Through the flocculation the SS of the wastewater had been greatly reduced to avoid influence on repeated fermentation. Polyaspartic acid was effective to separate hazardous impurities (metal ions, acid, aldehyde and so on) which inhibited ethanol fermentation. With bio-flocculation, the sludge is harmless and can be used as a kind of feedstuff or agricultural fertilizers. This process was non-toxic, non-pollution and truly green.

3.3. Hazardous materials influencing distillery wastewater recycles on fermentation

Organic and inorganic materials contained in the distillery wastes may influence ethanol fermentation rate. These substances come from the raw materials which cells cannot utilize and from fermentation by-products. Some proteins, fermentation by-

Table 2
Fermentation with recycled distillery waste. (a) Ethanol fermentation without flocculation treatment for the reuse of distillery wastes; (b) ethanol fermentation with chemical flocculation treatment for the reuse of distillery wastes (numbers in parentheses correspond to the number of measurements used for the determination of the mean values and standard deviations).

Tap water (ml)	Wastewater (ml)	Corn residue (g)	Ethanol concentration (%)	Ethanol output rate (%)
125	–	30.0 ± 0	8.16 ± 0.12(4)	34.0 ± 0.5(4)
–	125(a)	30.0 ± 0	6.0 ± 0.5(4)	25.0 ± 2(4)
–	125(a)	30.0 ± 0	4.8 ± 0.3(4)	20.0 ± 1.3(4)
–	125(b)	30.0 ± 0	6.41 ± 0.82(4)	27.0 ± 3.4(4)
–	125(b)	30.0 ± 0	7.2 ± 0.2(4)	30.0 ± 0.8(4)

products, metal ions and excreted toxic metabolic products would still remain in the wastes and could inhibit cell growth and ethanol fermentation. The concentrations of various compounds in the distillery wastewater after different treatments (primary filtration, biological flocculation treatment and chemical flocculation) were analyzed compared to those with the tap water.

COD, carbohydrates, aldehydes, volatile acid and non-volatile acids, inorganic ions (such as iron, magnesium and calcium), water-soluble proteins, as well as the suspended substance (including insoluble proteins) of the using water for fermentation were measured.

Bio-flocculation mainly got a reduction of SS, volatile acid, total acid and the amount of iron ions (Table 3). The biological flocculation process for distillery wastewater recycle on fermentation could have stable operation over ten recycles because cumulative adverse effects on ethanol yield were removed by the process. A simultaneous increase in the concentration of chemical compounds occurred, some of them causing inhibition of microbial metabolism and reducing cell growth and product yield. The following sections summarize the factors influencing distillery wastewater recycling on fermentation.

The SS was greatly reduced by the flocculation and filtration processes no matter what flocculants were used (Table 3). The SS of the filtrate was not increased as the recycles were repeated. The result suggested that the concentration of suspended substance inhibited fermentation and resulted in a decreased yield. The SS gave a severe negative effect on the respiratory metabolism of yeast cell and ethanol fermentation.

Furfural and hydroxymethylfurfural delay the fermentation during their assimilation or degradation, causing a period of cell death [28]. The majority of aldehyde compounds (by-products of cooking) were removed by the distillation process and their concentrations in the recycled water were very low. During low passages of recycling, the concentration of aldehyde compounds

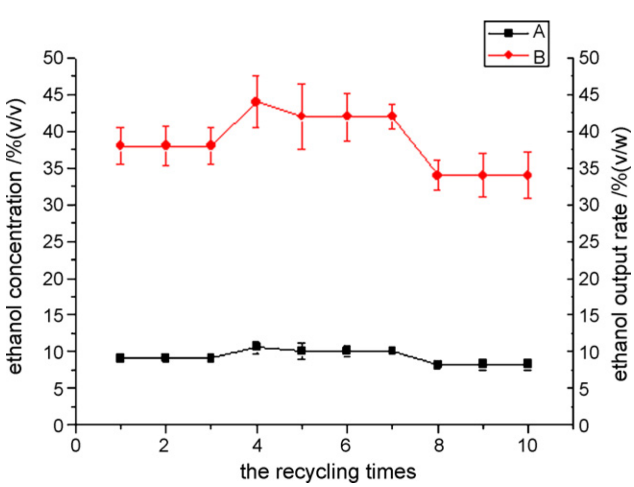

Fig. 2. Fermentation with recycled filtrate after biological flocculation treatment. (A) Ethanol concentration; (B) ethanol output rate.

increased but, after the 4th recycle, the concentration somewhat decreased or was kept nearly constant. These results suggest that aldehyde compounds are partially removed by bio-flocculation and filtration process (Table 3). It was found that low concentration of aldehyde compounds ($c < 500$ mg/l) had no negative effect on the fermentation under this experimental condition.

Acetic acid is the most studied inhibitor by many researchers (see Table 3). At acidic pH, acetic acid can diffuse into cell cytoplasm resulting in uncoupled energy production and impaired transport of various nutrients with increased ATP requirements [28]. The acetic acid inhibition on yeast growth is lager than furfural [29]. The acetic acid concentration of the filtrate after chemical flocculation treatment was almost the same as the wastewater without any treatment. The inhibitory action depends on the concentration. The accumulation of volatile acid in the recycled wastewater was more toxic. The acetic acid was fully removed by biological flocculation treatment. The pH of treated wastewater ranged from 9 to 10. The recycled filtrate after biological flocculation treatment did not inhibit fermentation and result in a decreased yield. It was found that a small acetic acid concentration ($c = 358$ mg/l) was responsible for inhibiting the growth of the yeast cells.

The total acid concentration of the distillery wastewater ($c = 831.6$ mg/l) and that of the filtrate after chemical flocculation treatment ($c = 812.8$ mg/l) were both high. The accumulation of total acid in the recycled wastewater was toxic. The acid was fully removed by biological flocculation treatment. The inorganic acids (sulfuric acid etc) with inhibitory effect were less obvious than the acetic acid.

The incomplete fermented sugar which still remained in the wastewater can be reused to avoid the resource waste. The concentration of residual reducing sugars was low when the ethanol yield was high. It was found that the concentration of residual reducing sugars had little influence on fermentation.

Excessive concentrations of metal ions usually affect yeast cell growth and ethanol production rate. Fe ions usually have an inhibitory effect on yeast cells growth. The Fe^{3+} concentration of the distillery wastewater and the filtrate after chemical flocculation treatment was high. The accumulation of Fe^{3+} in the recycled wastewater was more toxic. The Fe^{3+} was partly removed by biological flocculation treatment. During low passages of recycling, the concentration of Fe^{3+} was low and kept nearly constant but after the 7th recycle, the concentration somewhat increased because of the biomass accumulation (Table 3). It was found the low concentration of Fe^{3+} ($c < 1.0$ mg/l) in the wastewater had no negative effect on the fermentation under ten times recycles using biological flocculation treatment.

Inorganic salts are essential substances to microorganism vital activity. Mg^{2+} is an important accessory factor of many enzymes in the yeast metabolism. But an increase in osmotic pressure might influence cell growth or fermentation rate. In our process the Ca^{2+} ($C_{Ca} < 650$ mg/l) does not significantly affect fermentation (Table 3). Mg^{2+} concentration observed were lower than the critical concentration ($C_{Mg} = 25{,}000$ mg/l [30]) that would inhibit cell growth and ethanol production. Ca^{2+} and Mg^{2+} did not affect the fermentation significantly.

Table 3
Variation of COD, carbohydrates, aldehydes, volatile acid and total acids, reducing sugars, inorganic ions (such as iron, magnesium and calcium), water-soluble proteins, and SS in the brewing (recycling) water as well as the ethanol output rate during recycling step comparing to the tap water (numbers in parentheses correspond to the number of measurements used for the determination of the mean values and standard deviations).

The brewing water	Concentrations										
	SS (mg/l)	Total aldehyde (as acetaldehyde)	Total volatile acid (as acetic acid) (mg/l)	Total acid (as acetic acid) (mg/1)	Reducing sugars (mg/1)	Fe^{3+} (mg/l)	Ca^{2+} (mg/l)	Mg^{2+} (mg/D	Water-soluble proteins (g/l)	COD (g/l)	Ethanol concentration (v/v)%
Tap water	242 ± 0.8(4)	6.7 ± 0.9(4)	–	–	–	0.03 ± 0(4)	48.1 ± 3.6(4)	20.4 ± 2.8(4)	–	0.0035	8.16 ± 0.12(4)
Wastewater1	3875 ± 82(4)	20.0 ± 1.0(4)	379.8 ± 20.1(4)	525.0 ± 20.5(4)	1754 ± 21(4)	0.53 ± 0.01(4)	128.3 ± 10.2(4)	48.6 ± 13.5(4)	1.8 ± 0.1(4)	30.8 ± 1.7(4)	6.0 ± 0.5(4)
Wastewater2	3985 ± 154(4)	50.0 ± 14.2(4)	372.6 ± 22.2(4)	831.6 ± 36.1(4)	1961 ± 18(4)	0.62 ± 0.07(4)	76.15 ± 8.2(4)	70.5 ± 147(4)	1.6 ± 0.2(4)	32.0 ± 0.8(4)	4.8 ± 0.3(4)
PAC–PAM1	32.1 ± 2.5(4)	20.0 ± 2.1(4)	376.1 ± 25.7(4)	512.4 ± 24.5(4)	838 ± 20(4)	1.22 ± 0.12(4)	24.1 ± 2.5(4)	29.2 ± 2.4(4)	0.89 ± 0.01(4)	23.8 ± 1.4(4)	6.41 ± 0.82(4)
PAC–PAM2	342 ± 3.2(4)	50.0 ± 15.1(4)	358.4 ± 21.1(4)	812.8 ± 23.4(4)	1242 ± 29(4)	1.81 ± 0.2(4)	80.2 ± 110.4(4)	16.2 ± 1.4(4)	0.73 ± 0.05(4)	22.6 ± 1.5(4)	7.2 ± 0.2(4)
PASP1	28.8 ± 2.1(4)	20.0 ± 0.9(4)	–	–	779.5 ± 17.2(4)	0.29 ± 0.01(4)	184.4 ± 18.3(4)	68.1 ± 2.8(4)	1.41 ± 0.11(4)	23.5 ± 0.3(4)	9.12 ± 0.6(4)
PAKP2	16.2 ± 1.2(4)	140.1 ± 7.4(4)	–	–	654.6 ± 23.4(4)	0.3 ± 0.02(4)	432.9 ± 25.7(4)	24.3 ± 1.7(4)	1.32 ± 0.09(4)	19.0 ± 0.7(4)	9.12 ± 0.6(4)
PASP3	38.9 ± 4.2(4)	220.2 ± 15(4)	–	–	523.4 ± 19.6(4)	0.3 ± 0(4)	513.0 ± 22.4(4)	14.6 ± 3.9(4)	1.86 ± 0.14(4)	26.1 ± 1.4(4)	9.12 ± 0.6(4)
PASP4	29.2 ± 3.5(4)	380.4 ± 25(4)	–	–	521.2 ± 27.2(4)	0.3 ± 0(4)	573.1 ± 35.4(4)	24.1 ± 3.7(4)	1.06 ± 0.08(4)	22.8 ± 2.1(4)	10.56 ± 0 8(4)
PASP5	36.5 ± 2.9(4)	180.2 ± 19(4)	–	–	492 ± 24(4)	0.3 ± 0(4)	522.2 ± 34.1(4)	19.7 ± 3.4(4)	1.3 ± 0.1(4)	26.5 ± 2.7(4)	10.08 ± 1.1(4)
PASP6	34.0 ± 4.5(4)	150.2 ± 17.0(4)	–	–	387.1 ± 15(4)	0.27 ± 0.02(4)	391.7 ± 15.2(4)	21.9 ± 3.8(4)	1.27 ± 0.07(4)	19.0 ± 2.0(4)	10.08 ± 0.7(4)
PASP7	36.2 ± 3.9(4)	90.1 ± 9.8(4)	–	–	485.8 ± 17.2(4)	0.538 ± 0.04(4)	514.1 ± 31.1(4)	17.3 ± 1.0(4)	1.36 ± 0.13(4)	24.6 ± 1.4(4)	10.08 ± 0.4(4)
PAKP8	34.9 ± 2.5(4)	130.1 ± 8(4)	–	–	442 ± 21(4)	0.825 ± 0.07(4)	648.7 ± 38.7(4)	36.5 ± 10.4(4)	1.28 ± 0.13(4)	25.8 ± 1.2(4)	8.16 ± 0.5(4)
PASP9	29.2 ± 1.9(4)	60.1 ± 7.1(4)	–	–	614.6 ± 22(4)	0.78 ± 0.04(4)	563.1 ± 28.7(4)	48.6 ± 2.1(4)	1.63 ± 0.15(4)	26.0 ± 1.0(4)	8.16 ± 0.7(4)
PASP10	33.1 ± 3.7(4)	100.1 ± 11(4)	–	–	867.3 ± 19.9(4)	0.08 ± 0.02(4)	612.0 ± 22.7(4)	48.6 ± 13.0(4)	1.86 ± 0.08(4)	27.3 ± 1.7(4)	8.16 ± 0.8(4)

(–) The concentration was lower than 100 μg/l.

The water-soluble proteins generated from ethanol production can be reused to avoid the resource waste. But the concentration of water-soluble proteins was decreased a lot by chemical flocculation treatment that leaded to production loss. It was found that the concentration of water-soluble proteins had little influence on fermentation in this process.

The total organics in the wastewater from fermentation by-products and yeast cell debris produced during the distillation step can be measured as COD in content. COD was partly removed by flocculation process, but it was still high. It was found that COD had little influence on fermentation in this process.

The accumulation of Al^{3+} may inhibit the fermentation. The filter residue may be toxic from such inorganic-organic compound flocculation. The post-treatment of filter residue would bring trouble to the Ethanol Factory. But bio-flocculation was effective to separate hazardous impurities and was non-toxic, non-pollution and truly green.

4. Conclusions

A new clean technology was applied in the ethanol production industry to treat the distillery wastewater. By introducing a bio-flocculation process, the distillery waste could be recycled to the fermentation step. The average ethanol production yield was similar to that in the conventional process using tap water. In contrast, the recycle of wastewater without flocculation and with chemical flocculation showed negative effects on ethanol yield as recycling was repeated. This new bio-flocculation process was confirmed to have stable operation over ten recycles.

Hazardous materials influencing distillery wastewater recycles on fermentation were also considered. It was found that the content of SS, volatile acid and Fe ions inhibited fermentation and resulted in a decreased yield. The concentration of aldehydes, COD, water-soluble proteins and reducing sugars had no negative effect on the fermentation under this experimental condition. Bio-flocculation was shown to be an effective way to drastically diminish the build-up of inhibitory compounds when process streams are recirculated.

Acknowledgments

This work was financially supported by the High-Tech Research and Development Program (863 program) of China (2007AA05Z409).

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