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A novel strategy for 1, 3-propanediol recovery from fermentation broth and control of product colority using scraped thin-film evaporation for desalination

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[5] Biological production of 1, 3-propanediol (1,3-PD) by microbial fermentation is promising since by-product glycerol produced in biodiesel production can be used as a carbon source. However, the salts present in the fermentation broth are negative to the downstream processing, especially for the product colority. In the present work, we first studied the effects of several salts on the increase of colority and analyzed the possible mechanism. Ammonium salt ($(NH_4)_2SO_4$) showed the most negative effect, which was probably due to the decrease of pH caused by the hydrolysis of ammonium salt thus facilitating the chromophoric reaction. A novel strategy was thus made [10] by adjusting the initial pH of the feeding liquid for distillation. It was found that high pH (alkali condition) indeed reduced the distillate colority but showed no negative effects on the recovery yields of the main product 1,3-PD and major by-product 2,3-butanediol (2,3-BD). Scraped thin-film evaporation was greatly effective for desalination and recovering 1,3-PD and 2,3-BD with high recovery yields. High pH was also found to be beneficial to reduce the concentration of the impurity, acetic acid, in the distillate, which was of great importance for producing qualified 1,3-PD for polymerization. The novel strategy is thus very promising for recovering 1, 3-PD from [15] fermentation broth, especially for the downstream processing at an industrial scale.

Introduction

1,3-propanediol(1,3-PD) is an important bulk chemical. It can be used as emulsifiers, cosmetics, lubricants, and for medicine production with annual demand growing very fast [1, 2]. [20] However, 1,3-PD is more famous as an important monomer for producing polytrimethylene terephthalate (PTT) [1], which has been known for many years because of its excellent physical properties [3]. PTT is reported to have several advantages versus PET and PBT, such as better elastic recovery and lower modulus [25] [3].

Commercially, 1,3-PD generally can be produced by chemical or biological synthesis [4-6]. However, the application of the chemical synthesis has been limited by the requirement of high temperature, high pressure and expensive catalyst, use of [30] non-renewable materials made from petroleum and the relative low yield. Thus, a cleaner, safer, more sustainable and economic way to produce 1,3-PD is necessary [7]. Nowadays, several microorganisms have been reported to have the ability to convert glycerol into 1,3-PD [7, 8]. Owning to the rapid development of [35] biodiesel industry, the price of glycerol has been decreased sharply since the glycerol is a main by-product of biodiesel production in a weight ratio of about 10%. It is reported that the price of crude glycerol (80%) drop down from 55 cent/kg in 2004 to 4.4 cents/kg in 2006 [9]. The cheap glycerol attracts more [40] attention for its bioconversion to 1,3-PD. However, there are still some problems restricting the large-scale biological production of 1,3-PD, one of which is the high cost of the downstream processing. The recovery of 1,3-PD from fermentation broth can make up 50%-70% of the total production cost when by-product

[45] glycerol is used as the raw material [9-11]. Technically, 1,3-PD is relatively difficult to recover and purify from fermentation broth because of the complexity of the broth which contains the main product 1,3-PD, major by-product 2,3-butanediol (2,3-BD), un-converted feedstock glycerol, minor by-products acetic acid, [50] succinic acid and lactic acid, etc. The low volatility and high hydrophilic characteristics of 1,3-PD in dilute aqueous solutions (1,3-PD concentration ranging from 30-130 g/L) also makes the downstream processing more complicated [8, 12-15].

Two of the major problems that restrict the process of 1,3-[55] PD recovery from fermentation broth are the presence of salts in the broth and the increase of product colority during purification. So far, a variety of separation methods have been introduced to separate the salts from the polyhydric alcohols, such as distillation/evaporation, electrodialysis, liquid-liquid extraction, [60] reactive extraction, aqueous two-phase extraction, chromatography, and membrane separation technology such as nanofiltration [9, 11, 15-25]. All of those separation methods have their advantages and disadvantages, but no single method is proved to be efficient and economically feasible [9]. For example, recent [65] works by Bastrzyk et al., [24, 25] have shown that nanofiltration was very effective for separation of post-fermentation glycerol solution. However, in our previous work (data not shown), we found that nanofiltration using organic membranes (DL-1812C and DK-1812, GE Co., USA) or 0.9 nm ceramic membrane (GFT, [70] Germany) indeed was effective to remove multivalent organic salts such as citrate, lactate and succinate, but not so effective for removal of acetate. Furthermore, the losses of 1,3-PD and 2,3-BD were also significant when nanofiltration was used for desalination. The presence of salts in the fermentation broth not [75] only increases the viscosity of the broth, but also have a negative effect on the colority of 1,3-PD product. Therefore, a more effective process has to be developed to effectively remove salts with high recover yields of 1,3-PD, 2,3-BD and glycerol, and obtain 1,3-PD product that meets the criteria for polymerization [80] of PTT.

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In a conventional recovery and purification of 1,3-PD from fermentation broth, the broth is usually filtrated by membrane to remove cell biomass and protein followed by a concentration/distillation process to remove a part of water. The
5 concentrated broth is then treated for desalination before going to rectification column for 1,3-PD purification (Fig.1). In the present work, the effects of several impurities including salts present in the fermentation broth on the colority of distillate and bottom liquid during distillation were first studied. Scraped thin-
10 film evaporation (STFE) were further used to remove salts from the concentrated broth. The objective of this work is thus to study the possible mechanism for the increase of colority during distillation, and further to make novel strategies to control the product colority.
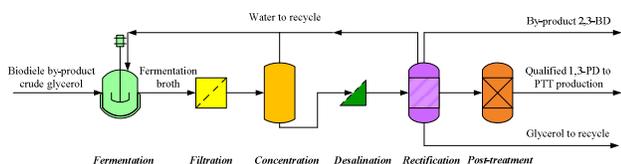

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Fig. 1 General flow scheme of fermentative production of 1,3-PD using biodiesel by-product crude glycerol as the carbon source

Materials and methods

The effect of salts on the colority

20 The fermentation broth was obtained according to our previous work [25]. Before vacuum concentration to remove a part of water, the fermentation was treated by ultrafiltration using a ceramic membrane with pore size of 5 nm and cut-off molecular weight of 8000-10000 (GFT, Germany). For investigating the effects of
25 salts on the colority, the experiments were conducted with a simulated concentrated fermentation broth (SCFB), which consists of 240.0g/L 1,3-PD, 50.0g/L 2,3-butanediol (2,3-BD), 7.00 g/L glycerol and the rest are deionized water. The natural pH of this solution was 6.08. The salts used in this experiment
30 including $(NH_4)_2SO_4$, K_2HPO_4, KH_2PO_4, $MgSO_4$, sodium succinate, sodium acetate and sodium lactate, were analytically pure and the same as those used or produced in the fermentative production of 1,3-PD [25]. To investigate their effects on colority, single salt was added to 450.0g SCFB placed in a round-bottom
35 flask for vacuum distillation. The flask was then heated at 4.00kPa of absolute pressure using an electric heating jacket until the temperature reached 180.0 ^{o}C and kept for 30 min. The pH of the bottom liquid was not controlled during heating. Samples of distillate and bottom liquid were then taken and the absorbance
40 was measured at 460 nm for comparing the colority [26] using a spectrophotometer (772E, Spectrum Shanghai, China).

Scraped thin-film evaporation (STFE)

The STFE experiments were performed using real fermentation broth obtained according to our previous work [25].
45 Before STFE, the fermentation broth was first treated by membrane filtration to remove protein and bacteria cells followed by vacuum evaporation to remove a part of the water. The concentrated fermentation broth consisted of 242.1g/L 1,3-PD, 48.6 g/L 2,3-BD, 67.3g/L glycerol, 30.0g/L
50 sodium acetate, 36.2 g/L sodium succinate, 28.1 g/L sodium lactate, and the rest was water. A lab-scale scraped thin-film evaporator was used in the experiments. Its structure parameter and operation conditions are shown in Table 1. About 5000 g concentrated broth was continuously pumped
55 into the evaporator at a speed of 16.7 ml/min (≈25.0 g/min). The condensate at the top of evaporator was analyzed for colority and compositions.

Table 1. Operation conditions of the scraped thin-film evaporation

Experimental conditions	Value
evaporator area (m^2)	0.2
feeding flow (mL/min)	16.7
evaporator temperature (^{o}C)	220.0
condenser temperature (^{o}C)	3.0
pressure (kPa)	0.30
scraper speed (rpm)	390

60 **Analytical methods**

The concentration of 1,3-PD, 2,3-BD and glycerol were determined by a high performance liquid chromatography(HPLC) system (Shimadzu, Japan) equipped with a refractive index detector and an AMINEX HPX-87H column (Bio-Rad, USA).
65 5mM H_2SO_4 was used as a mobile phase at a flow rate of 0.8 mL/min at column temperature of 65 ^{o}C. The recovery yield of 1,3-PD, 2,3-BD and glycerol during STFE is defined as follows:

$$\text{Re cov ery yield } (\%) = \frac{m_d c_d}{m_f c_f} \times 100\% \qquad (1)$$

where m_d and m_f are the weights of distillate and feeding liquids
70 respectively; c_d and c_f are the concentrations of 1,3-PD, 2,3-BD or glycerol in the distillate and feeding liquids, respectively.

The colority of distillate and bottom liquid was characterized by the absorbance at 460 nm [25]. However, the colority of the final product 1,3-PD after decoloration by active charcoal was
75 determined by colorimetric method with platinum-cobaltic standard solution [26] because that the absorbance at 460 nm is too low.

Result and discussion

Effect of several salts on the colority

80 Several inorganic and organic salts were singly added into the SCFB to study their effects on the colority of distillate and bottom liquid obtained in distillation. The concentrations of the added salts were selected based on the experimental results of distillation of real fermentation broth. The determined
85 absorbances at 460 nm are shown in Table 2. Corresponding photos of the bottom liquids are shown in Figure 2.

Table 2. The A_{460} of the distillate and the bottom liquid after heating

Salts	Concentration (g/L) *	A_{460} (distillate)	A_{460} (Bottom liquid)
No salts before heating	0	0.006	0.006
No salts after heating	0	0.033	0.121
$(NH_4)_2SO_4$	20.00	0.047	0.993
K_2HPO_4	3.45	0.030	0.204
KH_2PO_4	1.25	0.027	0.163
$MgSO_4$	1.00	0.034	0.104
sodium succinate	36.17	0.041	0.133
sodium acetate	28.11	0.042	0.177
sodium lactate	30.00	0.032	0.151

* The initial pH was the natural pH of SCFB. No pH adjustment was performed after salt was added
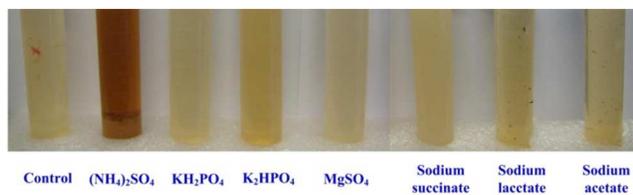

Fig. 2 The effects of different salts on the colority of bottom
5 liquid

It is clear that (NH$_4$)$_2$SO$_4$ showed significant negative effect on the colority of distillate and bottom liquid. The color of the bottom liquid after distillation became dark yellow compared with light yellow for other samples. It indicated that (NH$_4$)$_2$SO$_4$
10 might play as a catalyst or reactant in the colorization reaction.

To further investigate the possible mechanisms for the increase of colority, FTIR spectra of concentrated broth, distillate and control (solution prepared using pure 1,3-PD, 2,3-BD, glycerol and water) were recorded as shown in Figure 3. It is
15 clear that two new peaks appeared for the concentrated broth at 1711 cm^{-1} and 1572 cm^{-1}, respectively. The peak at 1711 cm^{-1} is attributed to the carbonyl group in carboxyl or ketone, while the absorption peak at 1572 cm^{-1} might be attributed to C=N or C=C. It indicated that oxidation reaction might take place during
20 distillation, and the ammonium salt might take part in or facilitate the reaction. However, no significant difference between the spectra of distillate and control were found even if the initial pH of the liquid increased from 7 to 12. It was probably because that the concentration of chromophores in the distillate was too low to
25 be detected by FTIR. To further study the new compounds formed in the distillation, GC-MS (Gas chromatography-Mass spectrometry) was used to analyze the concentrated broth. It was found that 3-hydroxy-2-butanone and 2,3-butanedione were detected, which was in accordance with the FTIR results.
30 However, no amide compound was detected probably due to the very low concentration.
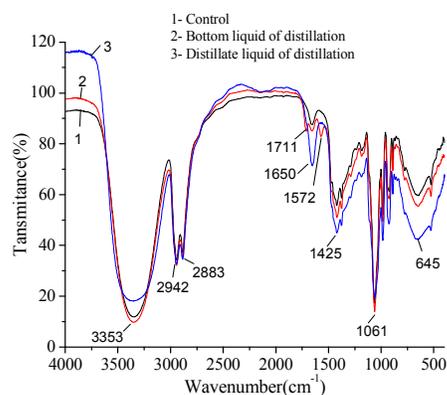

Fig. 3 FTIR spectra of concentrated broth, distillate and control sample (prepared by mixing pure 1,3-PD, 2,3-BD, glycerol and
35 water)

3-hydroxy-2-butanone is one of the main co-products during the biological synthesis of 2,3-BD by *Klebsiella pneumonia*. It is the precursor of 2,3-BD and can be transformed into 2,3-BD by acetoin reductase [27]. Since 2,3-BD is produced as a co-product
40 during the biological synthesis of 1,3-PD, the presence of 3-

hydroxy-2-butanone is also of great possibility. 3-hydroxy-2-butanone is easy to convert into 2,3-butanedione which is a yellow liquid. Another possible mechanism for the increase of colority during distillation is the Maillard reaction. Maillard
45 reaction usually take place between an amino group and an α-hydroxyl carbonyl moiety of a reducing sugar [28]. The amino acid and ammonium salt in the fermentation broth could provide amino groups, while the reducing sugar and other carbonyl compound in the broth, such as 3-hydroxy-2-butanone, could
50 provide α-hydroxyl carbonyl moiety. However, according the GC-MS results, Maillard reaction probably is not a main reason for the increase of colority. Moreover, It is also possible that the presence of ammonium salt increases the acidity of the solution, and thus the formation of 2,3-butanedione can be facilitated from
55 3-hydroxy-2-butanone or even from 2,3-BD. It also has been reported that the acids in the broth can promote the conversion of chromophore precursors to chromophores [29]. It thus can be concluded that the increase of the colority of the concentrated broth was mainly due to the presence of ammonium salt, which
60 increase the acidity of solution by hydrolysis (Eqn. 2) and facilitate the formation of chromophores.

$$NH_4^+ + H_2O \rightleftharpoons NH_3 \cdot H_2O + H^+ \qquad (2)$$
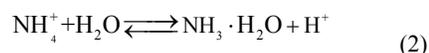
Therefore, a novel strategy can be made by adjusting the pH of the solution to weaken or eliminate the chromophoric reaction
65 during distillation. The formed salts can be simultaneously removed by using a scraped thin-film evaporator. The effects of several factors on simple distillation and STFE were thus further investigated as follows.

Effects of initial pH and pressure on simple distillation

70 Since 1,3-PD, 2,3-BD and glycerol have high boiling points of 210°C, 183 ^{o}C, and 290 ^{o}C, respectively, it is better to recover them by distillation under low pressure to reduce the chromophoric reactions. Therefore, after adjusting the pH of solution, vacuum distillaiton was carried out at three pressure
75 levels, namely 0.14kPa, 2.00kPa and 4.00kPa respectively. Six pH levels in the range of 7-12 were considered. The results on the yields of main products (1,3-PD, 2,3-BD) and glycerol are shown in Fig. 4. Corresponding effects of pH and pressure on distillate colories (A$_{460}$) are shown in Figure 5. It can be known that the
80 recovery yields of 1,3-PD and 2,3-BD were generally higher than 95%, while the yield of glycerol was 15-55% depending on distillation pressure. Decreasing pressure was beneficial to the recovery of 1,3-PD and 2,3-BD. pH showed no significant effects on the yields of the main products. However, the distillate
85 colority indeed decreased with increase of pH. It indicated that alkali pH could really reduce the chromophoric reaction to form the chromophores. This result further corroborated that the formation of chromophores during distillation were acid-catalyzed reactions.
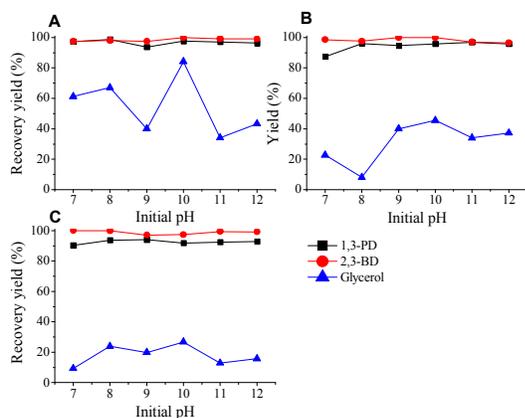
Fig. 4 The recovery yields of 1,3-PD, 2,3-BD and glycerol at different inital pH and pressure in a simple distillation. (**A**) at 0.14kPa, (**B**) at 2.00kPa, (**C**) at 4.00kPa.
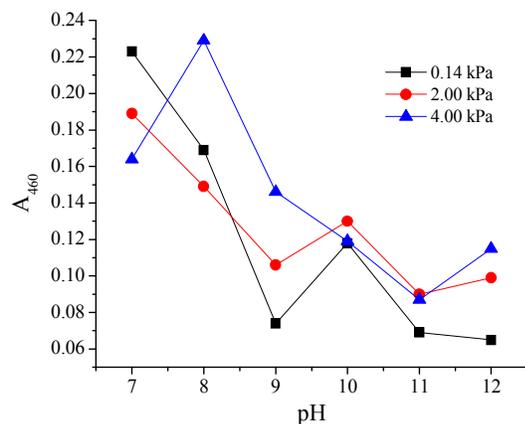

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Fig. 5 The absorbance of distillate at 460nm (A_{460}) at different initial pH obtained by a simple distillation.

Therefore, it can be known that a relative high initial pH and low pressure should be employed in distillation to decrease the
10 colority. However, there are still several issues limiting the application of simple distillation in the downstream processing of 1,3-PD recovery, one of which refers to the high salinity of the fermentation broth. Moreover, NaOH was consumed and new salt was formed by adjusting the initial pH of the broth. The salts not
15 only has a negative effect on the colority of distillate but also limits the continuous operation for 1,3-PDO recovery. Therefore, desalination is important for the subsequent rectification for 1,3-PDO purification, and thus a scraped thin-film evaporator was employed to recover 1,3-PDO and 2,3-BD and remove salts
20 continuously.

Effects of pH on scraped thin-film evaporation (STFE)

During STFE, concentrated fermentation broth was continuously pumped into the evaporator. The effects of pH on the recovery yields of 1,3-PD, 2,3-BD and glycerol at pressure of
25 0.30 kPa are shown in Figure 6. The yields of 1,3-PD and 2,3-BD were higher than 97%, and pH showed no significant effects on the yields. This result was similar to that of simple distillation. However, glycerol yield was much higher than that of simple distillation. This was mainly because that scraped thin-film

30 evaporator could provide more surface to evaporate the liquid, and thus higher efficiency for evaporation was achieved.
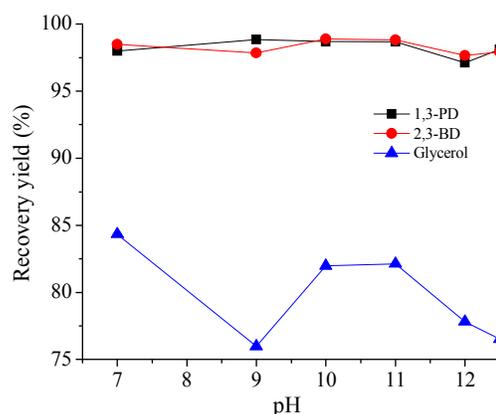

Fig. 6 The recovery yields of 1,3-PD, 2,3-BD and glycerol in the scraped thin-film evaporation at pressure of 0.30 kPa

35 The effects of pH on the colority (A_{460}) of distillate obtained by STFE are shown in Fig. 7. The A_{460} decreased significantly with increase of pH from 7.0 to 12.0, and the lowest colority was obtained at pH 12. It was also found that the impurity concentrations in distillate were influenced by the pH of feeding
40 liquid. For example, acetic acid, which was a by-product in the fermentation broth produced by the metabolism of the bacteria used for 1,3-PD production, was detected as an impurity in the distillate. When the pHs of feeding liquid were 7, 9 and 10, the acetic acid concentrations were 15.24，8.06 and 5.16 g/L,
45 respectively. No acetic acid was detected when pH was higher than 11. This is because the acetic acid concentration in the concentrated broth was controlled by the following equilibrium reaction:

$$CH_3COOH \rightleftharpoons CH_3COO^- + H^+ \qquad (3)$$

50 The pKa of acetic acid is 4.76 at 25°C, corresponding to disscociation constant (K_a) of 1.76×10^{-5}. In the experiments, the total concentration of acetic acid and acetate in the feeding liquid was 0.747mol/L. It thus can be calculated that the concentrations of acetic acid ([HAc]) at pHs of 7, 8, 9, 10, 11,12, and 12.5, are
55 about 0.255, 0.0255, 0.00255, 0.000255, 2.55×10^{-5}, 2.55×10^{-6} and 8.05×10^{-7} g/L, respectively. [HAc] decreased by 10 times when pH increased by 1 unit. Therefore, alkali condition is very good for reducing the hydrolysis of acetate to form acetic acid. [HAc] actually can be neglected when pH is 11. Therefore, under acid
60 condition (low pH), most of acetic acid exists as the forms of molecule, and thus it was easy to evaporate into the distillate. Increasing pH moved the equilibrium of Eqn. 3 to the right and thus less acetic acid was detected in the distillate. Removing acetic acid is very important to increase the quality of 1,3-PD
65 product, because acetic acid has a negative effect on co-polymerization of 1,3-PD and terephthalic acid to produce PTT. The existence of acetic acid may terminate the polymerization reaction. According to experimental results, when initial pH of the feeding liquid was higher than 11, no acetic acid or acetate
70 was detected in the distillate. Therefore, adjusting initial pH before STFE is also of great importance on this aspect.
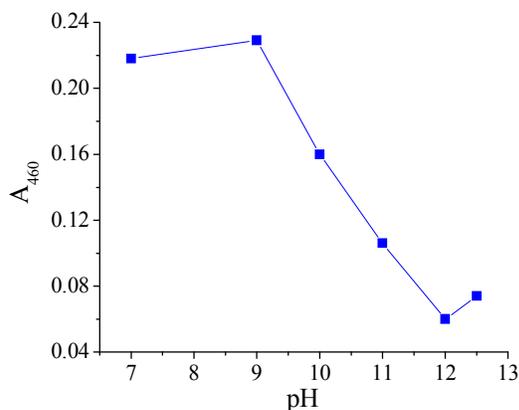
Fig. 7 A_{460} of distillate obtained by STFE at different pH

It should be noted that decolorization by activated charcoal adsorption is necessary for post-treatment of rectification product as shown in Fig. 1 in order to obtain qualified 1,3-PD for producing PTT. It seems that colority control is not necessary for STFE if a post-decolorization was used. However, we have found that the inirial pH of concentrated broth showed significant effects on the amount of activated charcoal used for post-treatment (Fig. 8) to produce qualified 1,3-PD (colority <10Hazen units). The consumption of activated charcoal was greatly reduced when pH was adjusted from 7 (natural pH of the broth at the end of fermentation) to 12.5. For example, the loading of activated charcoal can be reduced by 60% when initial pH was increased from 7 to 12.5. Nevertheless, pH showed no significant effect on the purity of 1,3-PD. One reason for this was that the rectification column used in the experiments was efficient enough to purify 1,3-PD with high purity (99.8%). The other reason was that the amount of chromophores present in the crude 1,3-PD product was very low even if a dark yellow color was observed. Therefore, the production cost would be reduced greatly by adjusting the initial pH of concentrated broth before desalination using STFE.
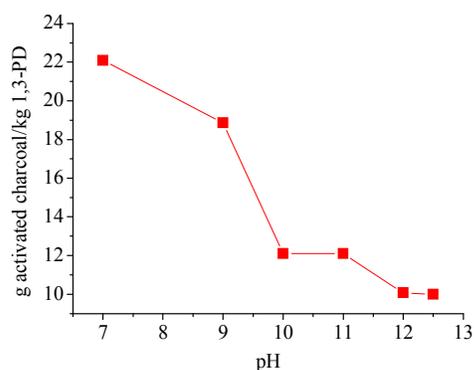

Fig. 8 Effect of pH on the weight of activated charcoal used for the decoloration of crude 1,3-PD product

Conclusions

The salts present in the fermentation broth of 1,3-PD production by microbial fermentation showed negative effects on downstream processing. One of the problems is the increase of product colority after distillation. It was found that $(NH_4)_2SO_4$ showed the most negative influence on the increase of colority, which probably due to the decrease of pH (increase of acidity) caused by hydrolysis of $(NH_4)_2SO_4$. A novel strategy was thus made by adjusting the initial pH of concentrated broth before distillation and desalination. The experimental results demonstrated that the colority of distillate indeed was decreased at high pH. The optimal pH was found to be 12. Scraped thin-film evaporation was further used for desalination and recovering the main product 1,3-PD and by-product 2,3-BD. Increasing pH from 7 to 12.5 showed no negative effects on the recovery yields of 1,3-PD and 2,3-BD, but greatly decreased the colority of distillate. It was also found that the acetic acid concentration in the distillate was greatly reduced by increasing the pH. No acetic acid was detected at initial pH higher than 11. The reduction of colority greatly reduced the amount of activated charcoal used in post-treatment to obtain qualified 1,3-PD. The novel strategy is thus very promising for recovering and purifying 1,3-PD from fermentation broth, especially for industrial processing.

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