Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 201-205

Effects of Filtration Volume Using Powdered Activated Charcoal on the Recovery of L (+)-Lactic Acid from Sago Starch

Kopli B Bujang*, Ang Shin Ying and Dayang Salwani Bt Awang Adeni

Resource Biotechnology Program, Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia.

Abstract

In this preliminary study, fine powdered activated charcoal (PAC) was used to recover lactic acid from the impurities existed in fermentation broth with different volume size of crude lactic acid. 200 g of activated charcoal in 1N hydrochloric acid (HCl) was packed into glass columns (49mm in diameter), followed by deionized water until pH around 6. Next, 0.5 L (or 2.5 mL/g PAC) and 1.0 L (or 5.0 mL/g PAC) of autoclaved cell-free fermentation broth was added to the glass columns, followed by 0.5 L and 1.0 L of deionized water respectively. It is observed that the efficiency of UV light absorbing compounds removal at λ_{200} and λ_{260} (%) is 94.42, 97.61 and 92.55, 97.56 respectively for 0.5L and 1.0L of fermentation broth. Color removal efficiency is observed to be the same (more than 99%) whereas lactic acid recovery increases from 13.56% to 46.43% with the ascend of sample (fermentation broth) volume loaded. On the other hand, the recovery of lactic acid by deionized water (0.5 and 1.0L) is 84.04% and 51.86% respectively. It gives a total recovery of 97.60 and 98.29% respectively for both parameters. Protein and glucose recoveries are observed to be around zero percent, which improves the purity of the permeates.

Keywords: Powdered Activated Charcoal (PAC), Lactic Acid Recovery, Decolorization

1.0 Introduction

Activated charcoal, also known as activated carbon is widely used to adsorb odorous or colored substances from gases or liquids. Besides, it is an antidote to toxic substances, as soil amendment in agriculture, and as a component of nursery of transplant media (OMRI, 2002). Mohan and Karthikeyan (1997) used activated charcoal to remove lignin and tannin color while Nakano *et al.* (1996) performed fermentation coupled with propionic acid removal system using activated charcoal. Activated charcoal, which is prepared from wood and vegetables, occurs in either powder or granular form. It is a solid, porous, tasteless, black carbonaceous material, which has large surface area and pore volume that gives it a unique adsorption capacity. Moreover, activated carbon can be recycled, reactivated or regenerated from spent carbon (OMRI, 2002).

Due to its high potential and broad applications, powdered activated charcoal (PAC) is employed in this study to determine the effects of crude lactic acid filtration volume on the recovery of lactic acid as well as the efficiency of organic compounds removal

* *Corresponding author: E-mail address: bkopli@pps.unimas.my*

including proteins, color and glucose since lactic acid from fermentation contains high impurities.

2.0 Materials & Method

2.1 Microorganism

Lactococcus lactis IO-1 (JCM 7638) was used throughout the study. Cells from stock cultures were incubated in Thioglycolate (TGC) medium without dextrose (Difco) for 18 hours at 37°C. 10% (v/v) was used for inoculum preparation.

2.2 Sago Hydrolysate

Sago hydrolysate was prepared by enzymatic hydrolysis as detailed earlier (Bujang *et al.*, 1999).

2.3 Activated Carbon

Fine activated carbon powder, PAC (PC laboratory reagent, West Malaysia) was used in the adsorption studies.

2.4 Glass Column

Glass columns (Labglass, LG-4569T-160, 49 x 700 mm) with a bottom layer of superfine glass wool (Cope, UK) were used.

2.5 Fermentation broth (Crude Lactic Acid)

Fermentation broth containing lactate was obtained by performing batch fermentation in a 2L benchtop fermentor (Biostat-B, B. Braun Intrnational, Germany) at 37ºC and pH 6 by automatic titration with 10 M NaOH. Constant stirring at 500 rpm was controlled throughout the fermentation. The media for inoculum and fermentation has the following compositions in g/L: glucose (sago hydrolysate), 60; polypeptone, 5 (Becton Dickinson); yeast extract, 5 (Difco), and sodium chloride, 5 (BDH) except 10 g/L of glucose for inoculum. Fermentation broth was autoclaved at 121ºC (15 psi) for 15 min prior to centrifugation at 7000 rpm and filtered using Whatman 0.45 µm cellulose nitrate membrane filters. Then, the crude lactic acid was autoclaved again prior to the recovery with powdered activated charcoal.

2.6 Recovery of Lactic Acid using Powdered Activated Charcoal (PAC): Experimental Design

Glass columns (49 x 700 mm, Labglass) with a bottom layer of superfine glass wool (Cope, UK) were packed with 200 g of sterilized fine activated carbon powder (PC laboratory reagent, West Malaysia) in 1 N hydrochloric acid (HCl) to give a height of approximately 22cm. The columns were then washed with sterile deionized water until pH was around 6. Water remaining in columns was sucked out using a peristaltic pump. These columns were then further treated with UV light. Next, 0.5L (or 2.5 mL/g PAC)

and 1.0 L (or 5 mL/g PAC) of sterile cell-free fermentation broth was added to the columns, followed by 0.5 and 1.0 L of sterile deionized water into the respective columns. Permeates and eluates are kept at 4ºC for further analysis.

2.7 Analyses

Concentration of lactic acid in fermentation broth and permeats was determined by Waters HPLC (USA) using fermentation monitoring column (Biorad). Samples were eluted with 1 mM H_2SO_4 at 60 ºC and flowrate of 0.6 ml/min. Glucose concentrations in samples were detected by an instant glucose analyzer (Advantage II Accu-chek). Total protein was determined by Biorad DC Protein Assay kit. True color of samples was determined using the Hach DR/2010 spectrophotometer (1999). Absorbance of samples was determined using UV-Vis spectrophotometer (Ultrospec 1100 Pro, Biochrom, UK).

3.0 Results and Discussion

The effects of recovery with powdered activated charcoal are shown in, Table 1, 2 and Figure 1. The UV absorption spectrum before and after the recovery with activated charcoal shows that most of the absorption occurs at the range of 200-300 nm where organic compounds including DNAs, amino acids and proteins absorb these wavelengths. It is observed that the efficiency of UV light absorbing compounds removal in permeates from fermentation broth at λ_{200} and λ_{260} (%) is 94.42, 97.61 and 92.55, 97.56 respectively for 0.5 L (2.5 mL/g PAC) and 1.0 L (5.0 mL/g PAC). However, the UV absorbance may be interfered by the presence of lactic acid or sodium lactate, which has absorptions at 200-250 nm regions causing higher absorbance obtained. Color removing efficiency is the same for both filtration volumes, which is 99.74% and 99.30% respectively.

Lactic acid recovery in permeates from crude lactic acid is observed to ascend from 13.56% to 46.43% with the increase of sample volume loaded (0.5 to 1.0 L). However, lactate recovery in permeates from deionized water descends with the increase of sample volume loaded (84.04 to 51.86%). This is, perhaps, due to the adsorption of lactic acid by the activated charcoal in glass column loaded with 0.5 L fermentation broth in the earlier phase and deionized water with a higher affinity, elutes lactic acid from the absorbent. Besides, protein recovery for both sample volumes is zero percent except for eluate from 1.0 L deionized water, which has a recovery of 1.39%. In addition to that, 100% removal of glucose for both volumes is obtained and average flow rate in mL/min is 0.27 and 0.32 for permeates from fermentation broth for 0.5 and 1.0 L respectively. This may be due to the pressure incurred by higher volume height in glass column for 1.0L.

Table 1 Effects of different sample volume loaded on the efficiency of color and UV light absorbing compounds removal

Parameters	**0.5 L (2.5 mL/g)**	**1.0 L (5.0 mL/g)**
Initial True Color (Units PtCo)	2636.67	
True Color After Treatment (Units PtCo)	6.67	18.33
Color Removing Efficiency (%)	99.74	99.30
Initial UV Absorption, λ_{200} & λ_{260} (cm^{-1})	356.9 / 35.2	
UV Absorption After Treatment, λ_{200} & λ_{260} (cm^{-1})	19.9 & 0.84	26.6 & 0.86
UV Light Absorbing Compounds Removing Efficiency (%)	94.42 & 97.61	92.55 & 97.56

Table 2 Effects of different sample volume loaded on the recovery of lactic acid

Parameters	**0.5 L (2.5 mL/g PAC)**		**1.0 L (5.0 mL/g PAC)**	
	FB*	**DI Water****	**FB***	**DI Water****
Average Flowrate (mL/min)	0.27	0.21	0.32	0.28
Volume Recovery (%)	96	96	99.90	95.90
Lactate Recovery (%)	13.56	84.04	46.43	51.86
Protein Recovery (%)	0.00	0.00	0.00	1.39
Glucose Recovery (%)	0.00	0.00	0.00	0.00

* FB = Fermentation broth
** DI Water = Deionized water

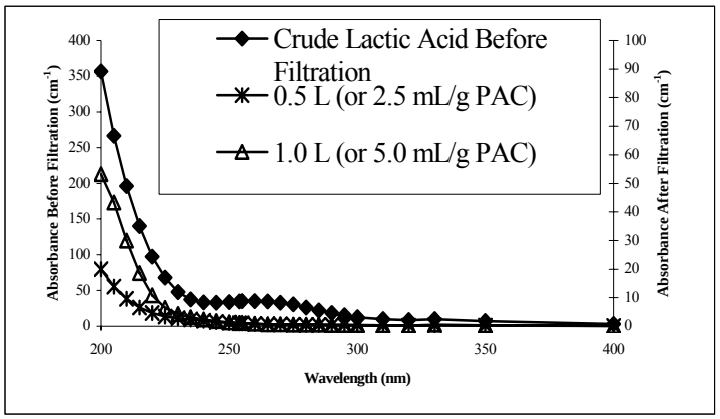

Figure 1 UV absorption spectrum of permeate before and after filtration by PAC

4.0 Conclusion

In conclusion, lactic acid in fermentation broth can be recovered utilizing activated charcoal powder as well as decolorizing the broth and improving the purity of the

permeate recovered. Higher sample volume loaded increases the amount of lactic acid recovered compared to lower volume but possesses the higher amount of impurities which is depicted by the UV absorption spectrum. Lactic acid absorbed to the activated charcoal or trapped in the glass columns can be eluted using deionized water.

Acknowledgement

The authors wish to express their gratitude to Universiti Malaysia Sarawak, for their assistance in these experiments. This research was funded by IRPA Grant No. 09-02-09 1024 EA001 and UNIMAS Fundamental Grant No. 1/66/387/2003(124).

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