ISSN : 0974 - 7435 Volume 9 Issue 2

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 9(2), 2014 [79-82]

Downstream processing studies for pullulan recovery in solid state fermentation using asian palmyra palm kernel - inexpensive substrate

K.R.Sugumaran, V.Ponnusami*

School of Chemical and Biotechnology, SASTRA University, Thanjavur – 613401, Tamilnadu, (INDIA)

E-mail : vponnu@chem.sastra.edu

ABSTRACT

Production of industrially important biopolymer, pullulan was investigated using asian palmyra palm kernel in solid state fermentation. After fermentation, polysaccharide was recovered by harvesting the cells at 121ρ C in autoclave, centrifugation followed by solvent precipitation. Recovery of pullulan was tried with single step solvent precipitation method. Screening of solvents such as acetone, isopropanol, ethanol and butanol on pullulan recovery was studied. After screening the suitable solvent, effects of volume ratio of solvent & supernatant and precipitation time on pullulan recovery were carried out.

INTRODUCTION

Microbial pullulan, biodegradable, colorless, tasteless, non toxic and odorless powder decomposes at 250^0 C[1]. Pullulan is not soluble in many solvents such as isopropanol, methanol and ethanol. Pullulan is soluble in water to form viscose solution, which is able to form transparent, oxygen impermeable thin films and fibers[2,3]. Pullulan is used as a standard material for estimating molecular weight of polymers in chromatography column[4], as a plasma expander due to biodegradability and molecular weight[5], control release of genetic material[6]. In recent years, pullulan is widely used in food, biomedical and pharmaceutical industry[7].

Market price of microbial pullulan is higher than other polysaccharides like dextran and xanthan gum[8] due to production cost and recovery cost. Cost of fermentation was considerably reduced by various low cost materials such as jaggery[9], beet molasses[10], sweet potato[11], hydrolysed potato starch waste[12], asian palmyra palm kernel[13], jack fruit seed[14] and cassava bagasse[15,16]. Generally, pullulan is recovered from fermentation medium using centrifugation followed by solvent precipitation. Low volume of solvents having low hydrophilicity and high molecular weight such as propanol, iso propanol and tetrahydrofuran are appropriate for precipitation than low molecular weight and high hydrophilicity[17], however further purification steps such as ultra filtration and ion exchange are required[18].

The objective of the work was to produce pullulan under low cost fermentation. An attempt had been made to recover the polymer from fermentation medium with one step, cost effective, precipitation method.

MATERIALS AND METHODS

Microorganisms and culture conditions

Aureobasidium pullulans MTCC 2670 was pur-

FULL PAPER

chased from Microbial Type Culture Collection, Chandigarh, India. Stock culture was maintained using PDA agar at 4°C and revived once in three weeks.

Seed medium consisting of sucrose-30 g/L; $(NH_4)_2SO_4$-0.6 g/L; yeast extract- 0.4 g/L; K_2HPO_4-5.0 g/L; $MgSO_4.7H_2O$-0.2 g/L; NaCl -1.0 g/L was prepared[19]. pH of the medium was adjusted to 5.5 using 1N NaOH or 1N HCl. Medium was autoclaved at 121° C for 15 min and cooled. Two loops of cells from stock culture were transferred to 100 ml of sterilized medium which was incubated in an orbital shaker at 200 rpm for 3 days at 30°C.

Solid state fermentation

Palmyra palm seeds were collected from Pudukottai and Sivagangai districts, Tamilnadu, India. Outer shell of seed was removed. Palm kernel was carefully taken and washed with distilled water in three times to remove the impurity. Then kernel was cut into pieces, air dried for seven days. Twenty gram of palmyra palm kernel was taken in 250ml Erlenmeyer flask, in which basal medium $(NH_4)_2SO_4$-0.6 g/L; yeast extract- 2.5 g/L; K_2HPO_4- 5.0 g/L; $MgSO_4.7H_2O$-0.2 g/L and NaCl -1.0 g/L) was added. Production medium pH was adjusted to 6.8 before sterilization using 1N NaOH or 1N HCl. Solid:moisture ratio was maintained at 1:1 and medium was sterilized and inoculated with 5% (v/v) of seed culture having 0.8 optical density. Then, heterogeneous fermentation medium was shacked for 10-15 min for achieving uniform humidity on solid substrate. The fermentation was carried out at room temperature.

Extraction of biopolymer

After fermentation, sample was mixed with six volume of distilled water and kept in an orbital shaker for 2 h at 200 rpm[15]. Then medium was harvested at 121° C for 15 min in autoclave. Then harvested medium was centrifuged at 10000 rpm for 15 min at 4° C. Solid particles with cell debris were carefully removed and dried at 90° C to constant weight. Supernatant was blended with equal volume of cold ethanol at 4° C for 24h for precipitation. Then precipitated sample was dissolved by the addition of 10 ml deionized water, heated to 80° C for 20 min, cooled. Then sample was again centrifuged at 10000 rpm for 15 min at 4° C to recover the precipitate. Precipitated sample was dried at 90° C to constant weight[9,13,14,16]. Yield of pullulan was expressed as mg of pullulan per g of dry substrate (mg/gds). Experiments were carried out in triplicates and values are expressed by mean ± SD using minitab statistical software, version 16. The level of significance was considered as $p < 0.01$[8].

Down stream processing

The effect of different solvents such as acetone, isopropanol, ethanol and butanol on recovery of pullulan (%w/v) was studied. This study was carried out by the addition of various solvent with supernatant in the volume ratio of 1:1 and sample was kept at 4° C for 24 h for precipitation. After screening the suitable solvent for precipitation, the effect of volume ratio of solvent on pullulan recovery (%w/v) was investigated at 4° C for 24 h. effect of precipitation time on pullulan recovery (%w/v) was carried out after finding the suitable solvent and volume ratio of supernatant & solvent.

RESULTS AND DISCUSSIONS

Screening of solvent

Various solvents such as ethanol, acetone, butanol and isopropanol were used as to precipitate pullulan after centrifugation. Comparison of various solvent on % recovery of pullulan (%w/v) is shown in Figure 1. Tukey's test conformed that difference in % recovery of pullulan using various solvent was statistically significant ($P<0.01$). maximum pullulan recovery (1.27 %w/v) was obtained by ethanol. Similarly, Kachhawa et al. 2003 studied screening of various solvents on amount

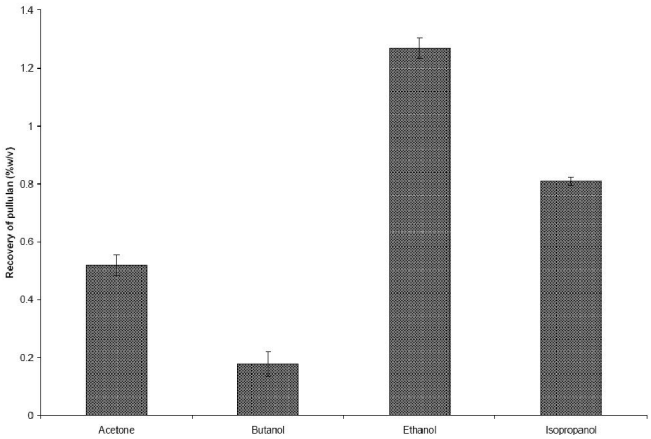

Figure 1 : Screening of solvent on recovery of pullulan (mean ± SD, n=3)

of precipitation of pullulan and observed ethanol to be a best solvent for pullulan precipitation and resulted 0.45 g pullulan[18]. Addition of ethanol to pullulan precipitation at 4^0 C was reported by several researchers[20-23]. Roukas and Biliaderis (1995) used ethanol in a second stage precipitation of pullulan, where in the first stage pullulan was precipitated by acetone and dissolved in water[24].

Volume ratio of supernatant to ethanol on % recovery of pullulan

Different volume ratios of supernatant to ethanol from 1:0.5 to 1:3.5 on recovery of pullulan were studied (Figure 2). Better precipitation and % recovery of pullulan (1.49 %w/v) were observed with volume ratio of 1: 2 (supernatant: solvent). Tukey's test confirmed that difference in % recovery of pullulan with respect to volume ratio of supernatant to ethanol was statistically significant ($p<0.01$). Several reports explained pullulan precipitation using two volumes of ethanol[18,25]. Maximum pullulan precipitation was achieved by 2-3 volumes solvent added per volume of fermentation medium[26].

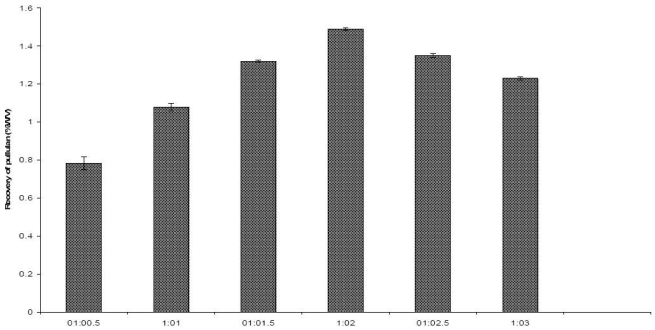

Figure 2 : Effect of volume ratio of solvent on recovery of pullulan (mean ± SD, n=3)

Effect of precipitation time on pullulan recovery

Figure 3 explains the effect of precipitation time on pullulan recovery. In this study, pullulan recovery increased with precipitation time till 18th h using ethanol as a solvent, used in the volume ratio of 1:2 (supernatant:solvent) at 4^0 C. After 18th h, recover of pullulan was decreased. Maximum pullulan recovery of 1.42 % w/v was obtained at 18th h. Results obtained from this study were found to be statistically significant by tukey's test ($P < 0.01$). Chi and Zhao (2003) obtained maximum recovery of polysaccharide for 12 h at 4p C[21]. Youssef et al., 1999 recovered microbial pullulan by *A. pullulans* using two volumes of ethanol at 4^0 C for 6 h[26]. Pullulan precipitation using two volumes of ethanol for 1 h had been reported by various researchers[23,25]. Forabosco et al. (2006) obtained pullulan from filtered supernatant with three volumes of cold ethanol for 24 h[22].

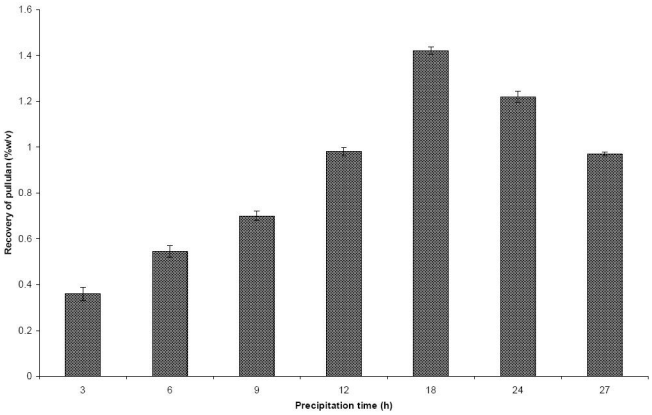

Figure 3 : Effect of precipitation time on recovery of pullulan (mean ± SD, n=3)

CONCLUSION

Pullulan was successfully produced from asian palmyra palm kernel as a low cost solid waste in solid state fermentation. Single step purification strategy was developed. Maximum pullulan recovery of 1.27 %w/v was obtained by ethanol. 1.49%w/v pullulan recovery was achieved by ethanol with volume ratio of 1: 2 (supernatant: solvent). Then, Maximum pullulan recovery of 1.42 % w/v was collected at 18th h with ethanol in the volume ratio of 1: 2.

ACKNOWLEDGEMENT

We sincerely thank SASTRA University for providing moral support and infra structure to complete our project.

REFERENCES

[1] T.Kimoto, T.Shibuya, S.Shiobara; Food Chem.Toxicol., **35**, 323 **(1997)**.
[2] S.Yuen; Process Biochem., **22**, 7 **(1974)**.
[3] K.I.Shingel; Carbohydr.Res., **339**, 447 **(2004)**.
[4] G.S.Buliga, D.A.Brant; Int.J.Biol.Macromol., **9**, 71 **(1987)**.
[5] Y.Tsijisaka, M.Mitsushashi; Polysaccharides and

their derivatives. San Diego: Academic, 447–460 **(1993)**.
[6] H.Hosseinkhani, T.Aoyama, O.Ogawa, Y.Tabata; J.Controlled Release, **83**, 287 **(2002)**.
[7] R.S.Singh, G.K.Saini, J.F.Kennedy; Carbohydr.Polym., **73**, 515 **(2008)**.
[8] S.S.Ram, K.S.Gaganpreet, J.F.Kennedy; Carbohydr.Polym., **78(1)**, 89 **(2009)**.
[9] S.V.N.Vijayendra, D.Bansal, M.S.Prasad, K.Nand; Process Biochem., **37**, 359 **(2001)**.
[10] A.Lazaridou, T.Roukas, C.G.Biliaderis, H.Vaikousi; Enzyme and Microb Technol., **31**, 122 **(2002)**.
[11] S.Wu, Z.Jin, Q.Tong, H.Chen; Carbohydr.Polym., **76**, 645–649 **(2009)**.
[12] Y.Göksungur, P.Uzuno¢ gullarý, S.Da¢ Gba¢ Glý; Carbohydr.Polym., **83(3)**, 1330–1337 **(2011)**.
[13] K.R.Sugumaran, E.Gowthami, B.Swathi, S.Elakkiya, S.N.Srivastava, R.Ravikumar, V.Ponnusami; Carbohydr.Polym., **92(1)**, 697 **(2013)**.
[14] K.R.Sugumaran, R.V.Sindhu, S.Sukanya, N.Aiswarya, V.Ponnusami; Carbohydr.Polym.In press.
[15] R.C.Ray, S.N.Moorthy; J.Sci.Ind.Res., **66**, 252 **(2007)**.
[16] K.R.Sugumaran, P.Jothi, V.Ponnusami, Carbohydr.Polym., **99**, 22-30 **(2014)**.
[17] K.Kato, T.Nomura; U.S. Patent Office, Method for purifying pullulan, Pat.No.4 004 977 **(1977)**.
[18] D.K.Kachhawa, P.Bhattacharjee, R.S.Singhal; Carbohydr.Polym., **52**, 25 **(2003)**.
[19] Y.Goksungur, A.Ucan, U.Guvenc; Turk.J.Biol., **28**, 23 **(2004)**.
[20] A.Mulchandani, J.H.T.Luong, A.LeDuy; Biotechnol.Bioeng., **33**, 306 **(1989)**.
[21] Z.Chi, S.Zhao; Enzyme and Microb.Technol., **33**, 206 **(2003)**.
[22] A.Forabosco, G.Bruno, L.Sparapano, G.Liut, D.Marino, F.Delben; Carbohydr.Polym., **63**, 535 **(2006)**.
[23] Y.Göksungur, S.Dag¢Bag¢Li, A.Uçan, U.Güvenç; J.Chem.Technol.Biotechnol., **80**, 819 **(2005)**.
[24] T.Roukas, C.G.Biliaderis; Appl.Biochem.Biotechnol., **55**, 27 **(1995)**.
[25] A.A.Lazaridou, T.Roukas, C.G.Biliaderis, H.Vaikousi; Enzyme and Microb.Technol., **31**, 122 **(2002)**.
[26] F.Youssef, T.Roukas, C.G.Biliaderis; Process Biochem., **34**, 355 **(1999)**.