Carbohydrate Polymers 99 (2014) 22–30

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Bioconversion of industrial solid waste—Cassava bagasse for pullulan production in solid state fermentation

K.R. Sugumaran, P. Jothi, V. Ponnusami*

School of Chemical and Biotechnology, SASTRA University, Thanjavur 613401, Tamilnadu, India

ARTICLE INFO

Article history:
Received 13 July 2013
Received in revised form 16 August 2013
Accepted 16 August 2013
Available online 22 August 2013

Keywords:
Pullulan
Aureobasidium pullulans
Cassava bagasse
Morphology of cells
Gel permeation chromatography

ABSTRACT

The purpose of the work was to produce commercially important pullulan using industrial solid waste namely cassava bagasse in solid state fermentation and minimize the solid waste disposal problem. First, influence of initial pH on cell morphology and pullulan yield was studied. Effect of various factors like fermentation time, moisture ratio, nitrogen sources and particle size on pullulan yield was investigated. Various supplementary carbon sources (3%, w/w) namely glucose, sucrose, fructose, maltose, mannose and xylose with cassava bagasse was also studied to improve the pullulan yield. After screening the suitable supplement, effect of supplement concentration on pullulan production was investigated. The pullulan from cassava bagasse was characterized by FTIR, ^{1}H-NMR and ^{13}C-NMR. Molecular weight of pullulan from cassava bagasse was determined by gel permeation chromatography. Thus, cassava bagasse emerged to be a cheap and novel substrate for pullulan production.

1. Introduction

Approximately about 600–650 tons per day of cassava bagasse are disposed from sago industry in India (Srivas & Anatharaman, 2005). Owing to the balanced nutritional composition (50% by wt starch), low ash content and high organic content, cassava bagasse is used as a low cost substrate for several biochemical processes (Carta, Soccol, Ramos, & Fontana, 1999). Cassava bagasse disposed from sago industry causes high pollution owing to its organic content and more biodegradability (Singh, Saini, & Kennedy, 2008). As a result, biotransformation of these industrial solid wastes to value added products is helpful to our society to minimize the environmental pollution as well as cost of fermentation.

Extra cellular biodegradable polysaccharide, pullulan is produced by *Aureobasidium pullulans*. It has a linear mixed attachment α-D-glucan, having repeating unit of matotriose attached by α-(1 → 6) linkage. Pullulan can able to form oil resistant, transparent and oxygen impermeable thin films. Pullulan can be used as a starch replacement during low calorific food formulation in food industry, packing material in pharma industry. Pullulan is comparatively costlier than other exo-polymers such as xanthan and dextran (Pandey, Nigam, Soccol, et al., 2000). Therefore it is essential to use cost effective and high nutritional waste material for microorganism growth as well as pullulan production due to cut down the cost of fermentation (Wu, Jin, Tong, & Chen, 2009). Potato starch waste (Barnett, Smith, Scanlon, & Israilides, 1999), brewery wastes (Roukas, 1999), jaggery (Vijayendra, Bansal, Prasad, & Nand, 2001), beet molasses (Lazaridou, Roukas, Biliaderis, & Vaikousi, 2002), sweet potato (Wu et al., 2009), coconut by-products (Thirumavalavan, Manikkadan, & Dhanasekar, 2009), hydrolyzed potato starch waste (Göksungur, Uzunoğullarí, & Dağbağlí, 2011), jack fruit seed (Sugumaran, Sindhu, et al., 2013) and Asian palmyra palm kernel (Sugumaran, Gowthami, et al., 2013) had been used as a cost-effective substrate for microbial pullulan production. In order to reduce the cost of fermentation, solid state fermentation is preferred over submerged fermentation (Pandey, Soccol, & Mitchell, 2000). Only few researchers had reported pullulan production using cassava bagasse in solid state fermentation (Ray & Moorthy, 2007).

Therefore, microbial pullulan production was investigated from cassava bagasse as a solid substrate in order to reduce the cost of fermentation. Various factors like initial pH, fermentation time, moisture ratio, nitrogen source, particle size and supplementation on pullulan production were studied. Then pullulan sample was characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and gel permeation chromatography.

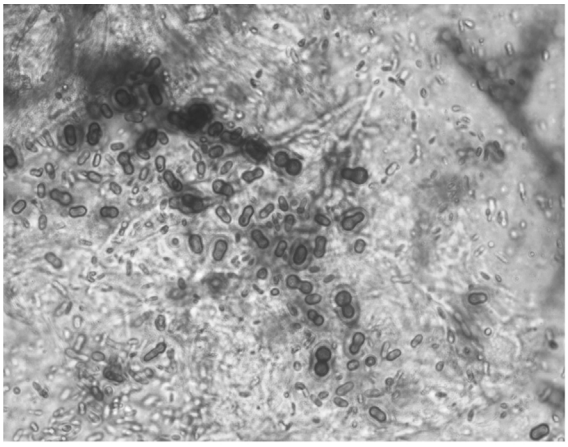
2. Materials and methods

2.1. Microorganisms and culture conditions

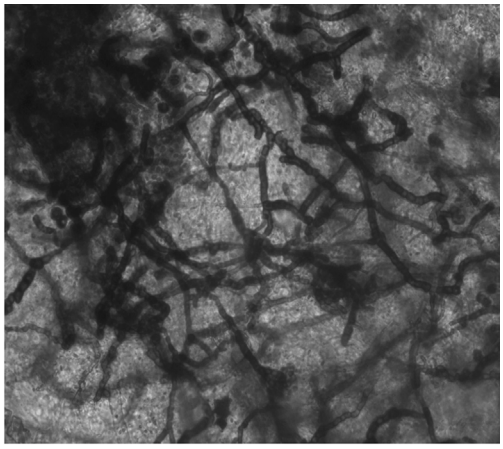
A. pullulans MTCC 2670 was purchased from Microbial Type Culture Collection, Chandigarh, India. Stock culture was sustained on potato dextrose agar (PDA) at 4 °C and revived every 3 weeks.

* Corresponding author. Tel.: +91 4362 264101; fax: +91 4362 264120.
E-mail addresses: vponnu@chem.sastra.edu, ponnusamiv@gmail.com (V. Ponnusami).

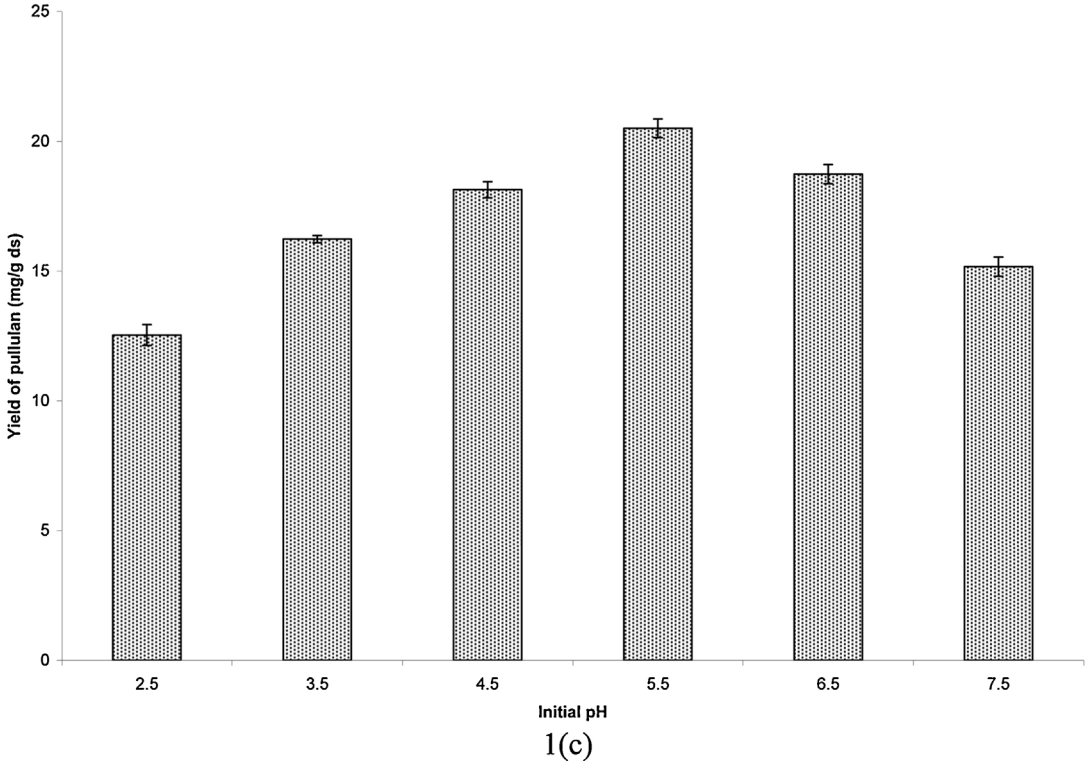
0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.08.039



1(a)



1(b)



1(c)

Fig. 1. (a) Morphology of cells at 5.5 pH; (b) morphology of cells at 2.5 pH; and (c) effect of initial pH on pullulan yield.

Seed medium containing the following composition in g/L: sucrose – 30; $(NH_4)_2SO_4$ – 0.6; yeast extract – 0.4; K_2HPO_4 – 5.0; $MgSO_4{\cdot}7H_2O$ – 0.2; NaCl – 1.0 and pH 5.5 (Goksungur, Ucan, & Guvenc, 2004) was prepared, sterilized at 121 °C for 15 min and cooled. Two loops of *A. pullulans* were transferred to 100 ml of sterilized medium and it was incubated in an orbital shaker at 200 rpm for 3 days. Temperature was maintained at 30 °C.

2.2. Preparation of solid substrate

Cassava bagasse disposed from sago industry, Salem, Tamilnadu, India was grinded by ball mill and then sieved. Cassava bagasse (4.76 mm size) was taken, de-watered, sun-dried for a week and then dried in a hot air oven at 80 °C for 24 h to prevent microbial contamination. Then dried material was kept in a container at room temperature.

2.3. Solid state fermentation

Production medium used in this study had the following composition (in g/L): $(NH_4)_2SO_4$ – 0.6; yeast extract – 0.4; K_2HPO_4 – 5.0; $MgSO_4{\cdot}7H_2O$ – 0.2 and NaCl – 1.0. pH of the medium was adjusted to 7.5 using 1 N NaOH or HCl as required. Cassava bagasse was used as a carbon source in the production medium. Twenty gram of cassava bagasse was taken in 250 ml Erlenmeyer flask in which above medium was introduced with a moisture ratio of 1:2 (substrate:moisture), sterilized and cooled. 5% (v/v) of 48 h-old culture from seed medium was added to sterilized production medium. Solid state fermentation was carried out at room temperature.

2.4. Extraction and purification of pullulan

Six volume of ice cool water was added to the heterogeneous fermentation medium and was incubated in a rotary shaker at 200 rpm

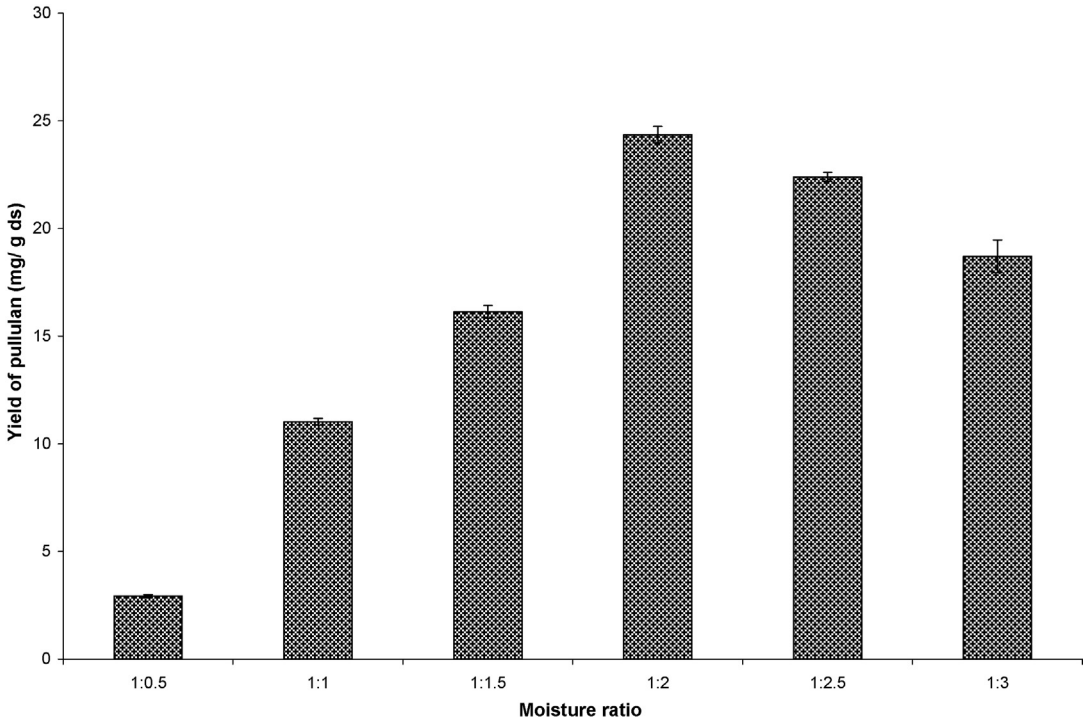


Fig. 2. Effect of moisture ratio on pullulan yield.

for 2 h (Ray & Moorthy, 2007). Then sample was centrifuged at 10,000 rpm for 15 min to separate cells and solid particle from supernatant. It was confirmed that no microorganism available in the supernatant using light microscope (Sugumaran, Gowthami, et al., 2013). Solid particles with cells were dried in a hot air oven at 90 °C to constant weight. The supernatant was added with equal volume of ethanol and was kept at 4 °C for 1 day for precipitation. After 1 day, the precipitated sample was again centrifuged at 10,000 rpm for 10 min to separate precipitate efficiently and precipitate was dried to 90 °C in a hot air oven to constant dry weight (Sugumaran, Gowthami, et al., 2013; Sugumaran, Sindhu, et al., 2013; Vijayendra et al., 2001). The yield of pullulan was estimated by gravimetrically and expressed as mg of precipitate per gram of dried fermented substrate. Experiments were carried out in triplicates and data was reported as mean ± SEM using minitab statistical software, version 16. The level of significance was

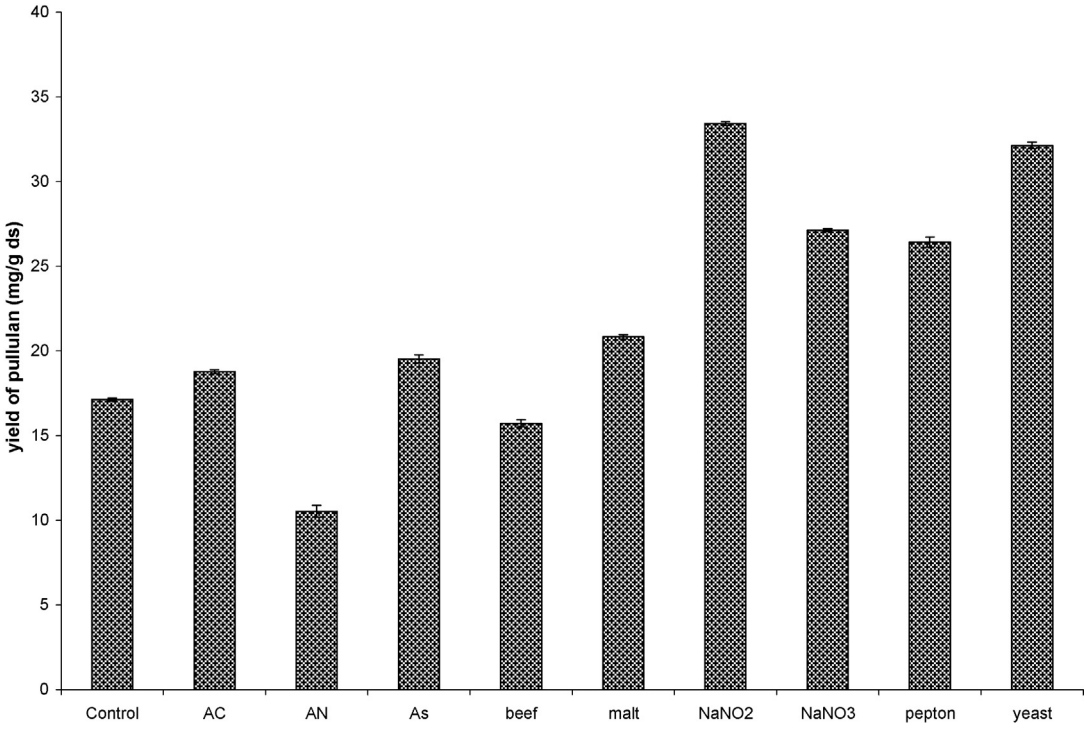


Fig. 3. Screening of nitrogen source on pullulan yield.

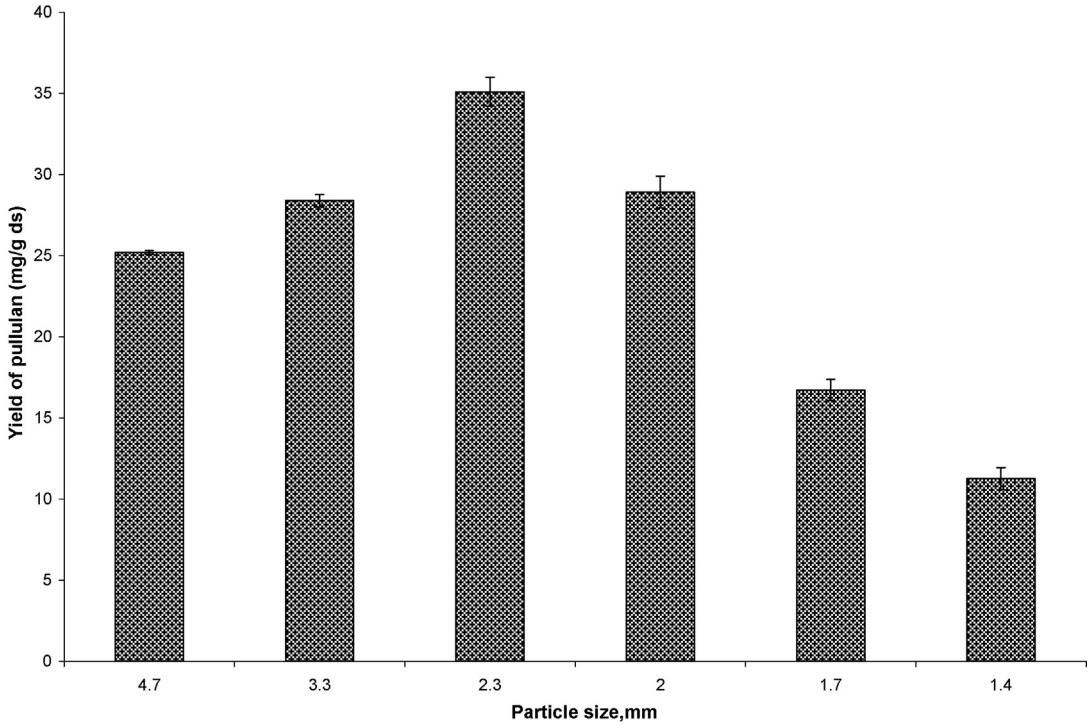


Fig. 4. Effect of particle size on pullulan yield.

considered as 99% confidence level. Using light microscopy (Carl Zeiss Inc., Germany), cell morphology in solid state fermentation was observed

2.5. Determination of various factors on pullulan production in solid state fermentation

Effect of initial pH ranging from 2.5 to 7.5 on cell morphology and pullulan production was studied using phosphate citrate buffer with the moisture ratio of 1:2. Influence of fermentation time (1–8 days), moisture ratio (1:0.5–1:3) on pullulan production was investigated. Then effects of nitrogen source ($NaNO_2$, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, peptone, malt extract, beef extract, and yeast extract), particle size, supplement carbon source (glucose, sucrose, fructose, maltose, mannose, and xylose) with solid substrate on pullulan production were investigated to improve the yield.

2.6. Characterization of pullulan

The polymer sample from cassava bagasse was first characterized using FTIR spectroscopy (Perkin-Elmer 1600 spectrophotometer). For FTIR spectra, sample was blended with KBr pellet (Thirumavalavan et al., 2009; Sugumaran, Gowthami, et al., 2013; Sugumaran, Sindhu, et al., 2013). For ^{1}H-NMR spectroscopy, 10 mg of purified sample was dissolved in sufficient volume of DMSO-d6 solvent and tetramethylsilane was used an internal standard for ^{1}H-NMR spectroscopy (Zhang et al., 2009).

Pullulan average molecular weight, polydispersity index were estimated by gel permeation chromatography (Agilent 1200 series) with a PL gel column and a RID detector. Polystyrene was a used as a standard to construct a calibration curve. HPLC grade DMSO used as a mobile phase was introduced to the chromatography at a rate of 0.375 ml/min. The sample concentration and injection volume were about 1.0 mg/ml and 20 μL, respectively (Sugumaran, Sindhu, et al., 2013).

3. Results and discussion

3.1. Effect of Initial pH on cell morphology and pullulan production

The pH of fermentation media highly influences the morphology of *A. pullulans*, cultivation rate and production of pullulan (Goksungur et al., 2004; Seviour, Kristiansen, & Harvey, 1984). Therefore, effect of initial pH ranging from 2.5 to 7.5 (before sterilization) in the production medium on cell morphology (Fig. 1(a) and (b)) and pullulan production was studied in solid state fermentation using cassava bagasse substrate with a moisture ratio of 1:2 (Fig. 1(c)). Tukey's test confirmed that difference in yield of pullulan with respect to initial pH was statistically significant ($p < 0.01$). Pullulan yield increased gradually with increase in initial pH from 2.5 to 5.5 and then decreased after pH 5.5. Yeast like morphology was predominant in the production medium at 5.5 pH and yield of pullulan achieved to be 20.5 mg/g ds at same pH. This result is consistent with the previous reports (Campbell, Siddique, McDougall, & Seviour, 2004; Catley, 1980; Heald & Kristiansen, 1985; Li et al., 2009; Ronen, Guterman, & Shabtai, 2002; Simon, Bouchet, & Caye-Vaugien, 1995). Maximum pullulan production was obtained by Heald and Kristiansen (1985) when 60% yeast like cells were available in the production medium at pH 6.3 (Heald & Kristiansen, 1985). 16.39 g/L of pullulan was obtained from Asian palmyra palm kernel at an initial pH of 6.5 with predominant yeast like morphology in the production medium (Sugumaran, Gowthami, et al., 2013).

Catley (1971) found that the optimum pH for cell growth and pullulan production was different (Catley, 1971). The favorable pH for pullulan production was reported to be in the range of 5.5–7.5 (Cheng, Demirci, & Catchmark, 2009; Lee & Yoo, 1993; Shingel, 2004). Lacroix, LeDuy, Noel, and Choplin (1985) reported maximum polysaccharide production at pH 5.5, while no pullulan was obtained at pH 2 while using sucrose synthetic medium (Lacroix et al., 1985). The maximum pullulan concentration of 29.43 g/L was achieved at pH of 5.5 using sweet potato (Wu et al., 2009). The

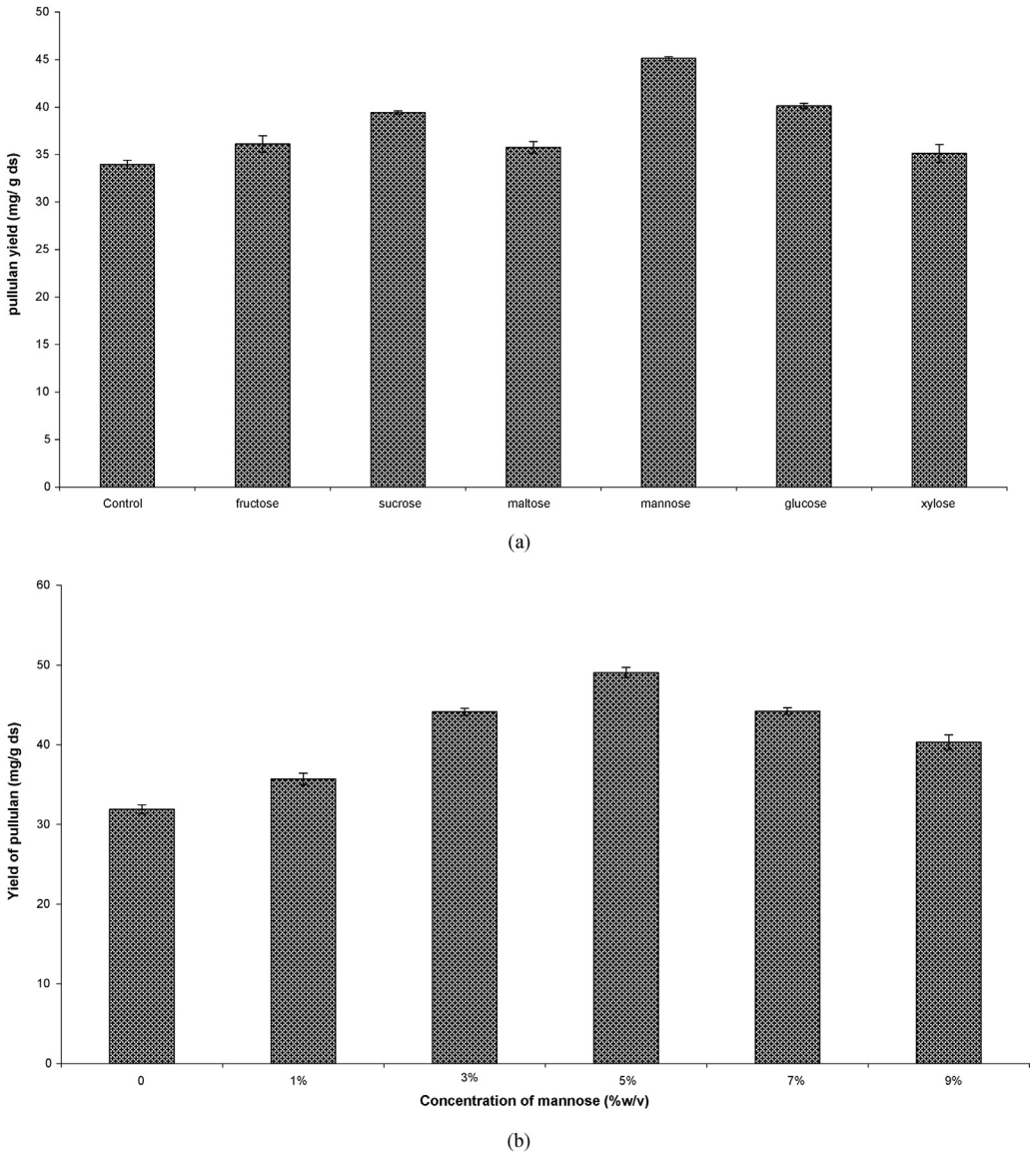


Fig. 5. (a) Screening of supplement carbon source on pullulan yield and (b) effect of concentration of mannose on pullulan yield.

optimum initial pH for maximizing the pullulan production (30.28 g/L) was found to be 5.5 using response surface method (Jiang, 2010). Vijayendra et al. (2001) produced 2.313% (w/v) of pullulan using jaggery at initial pH of 5.0 (Vijayendra et al., 2001). This was consistent with our findings. However, Thirumavalavan et al. (2009) obtained high concentration of pullulan (58 g/L) at 7.0 pH using coconut by products (Thirumavalavan et al., 2009). Maximum concentration of exopolymer (6.5 g/L) from carob pod extract obtained at pH 6.5 (Israilides et al., 1998). Auer and Seviour (1990) observed a high concentration of polysaccharide at initial pH of 7.5 (Auer & Seviour, 1990). Therefore, difference in value of optimum pH reported from literature could be due to the types of *A. Pullulans* strain, age of inoculum used, composition of fermentation medium and nature of fermentation (Pandey, Nigam, Soccol, et al., 2000; Pandey, Soccol, & Mitchell, 2000; Pandey, Soccol, Nigam, et al., 2000).

3.2. Effect of fermentation time on pullulan production

Influence of fermentation time on yield of pullulan using cassava bagasse at initial pH of 5.5 in the moisture ratio of 1:2. Tukey's test confirmed that difference in yield of pullulan with respect to fermentation time was statistically significant ($p < 0.01$). Pullulan concentration increased till 4 days and then the yield decreased (figure not shown). Yield of pullulan was maximum (23.6 mg/g ds) on fourth day. Similarly, Göksungur et al. (2011) observed high concentration of pullulan (19.2 g/l) after 111.8 h at pH 7.2 (Göksungur et al., 2011). Maximum pullulan production (30.28 g/L) was achieved on fifth day with an initial pH of 5.5 at 28 °C using sucrose as a carbon source (Jiang, 2010). Maximum concentration of pullulan (54 g/L) obtained using coconut by products on sixth day (Thirumavalavan et al., 2009). Sugumaran, Gowthami, et al. (2013), reported high concentration of pullulan on seventh day using Asian palmyra palm kernel (Sugumaran, Gowthami, et al., 2013; Sugumaran, Sindhu, et al., 2013). The yield of pullulan decreased due to enzymatic hydrolysis of pullulan by glucoamylase in later stages of fermentation (Thomas & Strohfus, 1996).

3.3. Effect of initial moisture content on pullulan production

Moisture content is an important factor in solid state fermentation due to oxygen transfer, microorganism growth and metabolic activity. High moisture content may cause the

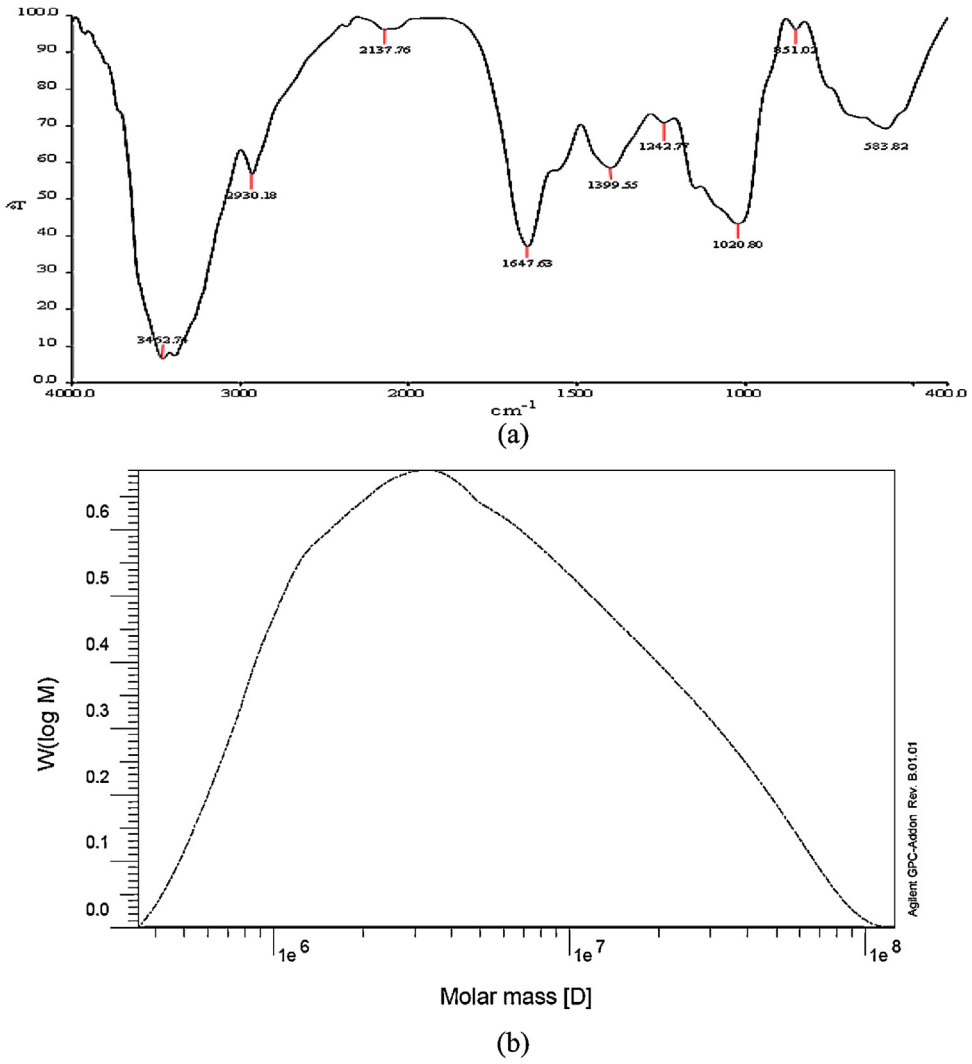


Fig. 6. (a) FTIR and (b) gel permeation chromotogram for pullulan from cassava bagasse.

reduction of oxygen transfer rate due to low substrate porosity, whereas low moisture content may lead poor diffusion of nutrients due to water-tightness of the solid substrate (Pandey, 2003). Fig. 2 explains the influence of moisture ratio on pullulan production using cassava bagasse at initial pH 5.5 on forth day. Tukey's test confirmed that difference in yield of pullulan with respect to moisture ratio was statistically significant ($p < 0.01$). Pullulan yield gradually increased with increase in moisture ratio until 1:2 and then pullulan yield decreased. Pullulan yield was maximum (24.3 mg/g ds) with 1:2 moisture ratio. Similarly, production of exo polysaccharide (27.5 g/kg ds) was investigated with 1:2 moisture ratio using cassava starch residue. (Ray & Moorthy, 2007). Sugumaran, Gowthami, et al. (2013), observed maximum pullulan concentration of 17 g/L of basal medium in solid state fermentation with 50% moisture content (Sugumaran, Gowthami, et al., 2013; Sugumaran, Sindhu, et al., 2013).

3.4. Screening of nitrogen source on pullulan production

Exo-polymer production from *A. pullulans* was strongly affected by source of nitrogen in the production medium (Goksungur et al., 2004). Screening of nitrogen source namely $NaNO_2$, $NaNO_3$, NH_4NO_3, $(NH4)_2SO_4$, NH_4Cl, peptone, malt extract, beef extract, yeast extract on pullulan yield was carried out using cassava bagasse at initial pH of 5.5 with 1:2 moisture ratio. The results are depicted in Fig. 3. Tukey's test confirmed that difference in yield of pullulan with respect to nitrogen source was statistically significant ($p < 0.01$). The maximum concentration of pullulan was observed in the production media containing $NaNO_2$ as a nitrogen source (33.4 mg/g ds) followed by yeast extract (32.1 mg/g ds). There was no difference in significant between yeast extract and $NaNO_2$. On the other hand, low pullulan yield (10.5 mg/g ds) was observed in the medium containing ammonium nitrate. The yield in this case was lower than that of control.

Lin, Zhang, and Thibault (2007) demonstrated influence of nitrogen source on the molecular weight of the pullulan (Lin et al., 2007). Thirumavalavan et al. (2009) had observed high concentration of pullulan (58.6 g/L) using yeast extract (Thirumavalavan et al., 2009). Pullulan concentration was significantly affected by different concentration of $NaNO_2$ (Campbell, McDougall, & Seviour, 2003; Orr, Zheng, Campbell, McDougall, & Seviour, 2009). Pullulan production (22.46 g/L), activity of UDPG-pyrophosphorylase (45.07 mU/mg of protein) and yeast like cells ($81 \pm 3.2\%$) were highly improved by $NaNO_2$ in the production medium (Jiang, Wu, & Kim, 2011).

Numerous researchers had also reported the influence of nitrogen source on pullulan production (Auer & Seviour, 1990; Lazaridou et al., 2002; Reed-Hamer & West, 1994; Schuster, Wenzig, & Mersmann, 1993; Sugumaran, Gowthami, et al., 2013; Thirumavalavan et al., 2009). Auer and Seviour (1990) observed

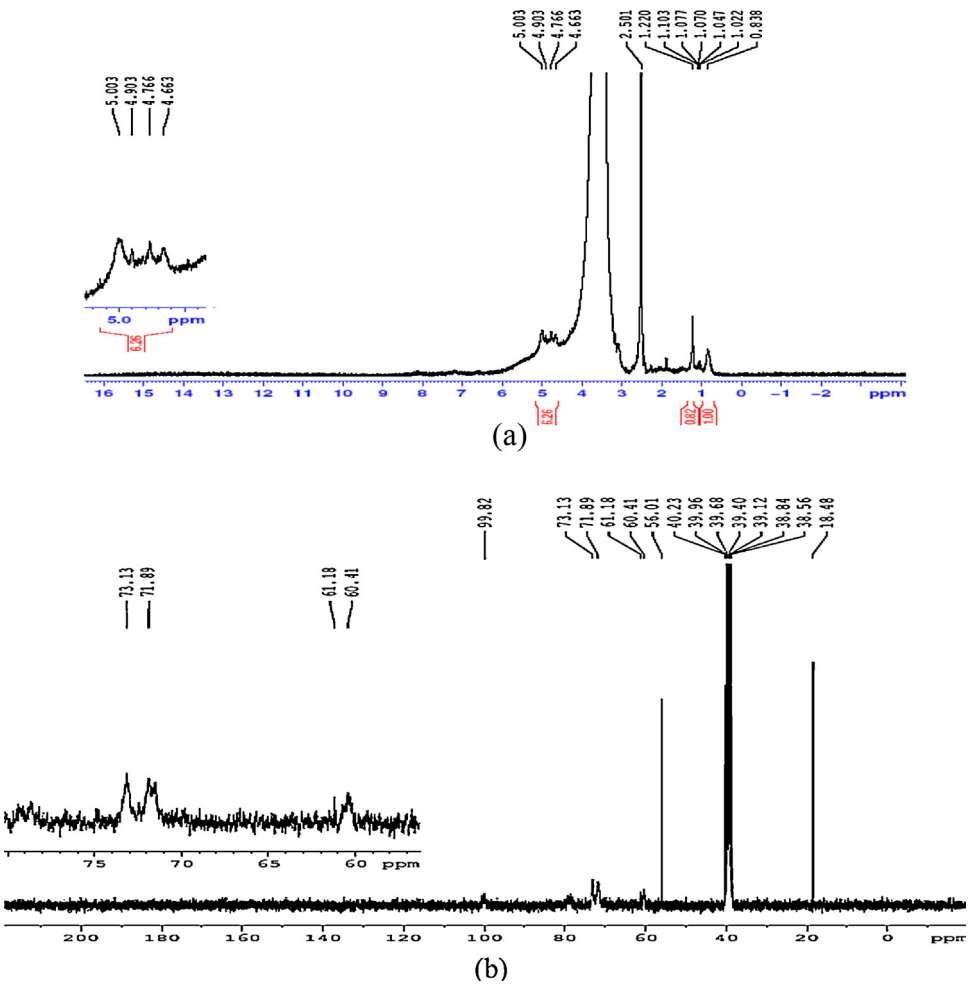


Fig. 7. (a) ^{1}H-NMR for pullulan from cassava bagasse and (b) ^{13}C-NMR for pullulan from cassava bagasse.

ammonium nitrate and ammonium sulfate as favorable nitrogen source for enhancing pullulan production (Auer & Seviour, 1990).

3.5. Effect of particle size on pullulan production

In solid-state fermentation, particle size is the crucial factor to affect the microorganism attachment, diffusion of various substrates and metabolites production (Pandey, 1991; Prakasham, Rao, & Sarma, 2006; Zadrazil & Puniya, 1995). Effect of particle size of cassava bagasse on pullulan production was investigated (Fig. 4). Tukey's test confirmed that difference in yield of pullulan with respect to particle size was statistically significant ($p < 0.01$). The optimum particle size of cassava bagasse was found to be 2.38 mm and corresponding pullulan yield was found to be 35.1 mg/g ds. Even though very small particle sizes (1.68 mm and 1.41 mm) provided very high surface area for the particle, low yield of pullulan was obtained due to insufficient support for microbial growth. Therefore maximum pullulan yield obtained at 2.38 mm particle size.

Normally, smaller size particles offer a greater specific surface area for microbial attachment. But, substrate agglomeration may result due to very small size particle. Small particle size may interfere with aeration owing to small void volume and result in lower microbial attachment on surface. Larger size particles offer good aeration due to large void volume but provide small specific surface area for microbial attachment (Pandey, Nigam, Soccol, et al., 2000; Pandey, Soccol, & Mitchell, 2000). Our results are consistent with previous findings.

3.6. Effect of supplement carbon source on pullulan production

In our work, effect of different co-substrates (3%, w/w) namely glucose, sucrose, fructose, maltose, mannose, xylose along with the main carbon source on pullulan yield was studied at pH 5.5 in the moisture ratio of 1:2 with $NaNO_2$ as a nitrogen source on forth day. The results are shown in Fig. 5(a). Mannose and glucose had resulted high yield of pullulan of 45.1and 40 mg/g ds, respectively. In our study, low yield of pullulan obtained from fructose and xylose containing production medium. This may due to longer biosynthesis pathway starting from fructose and xylose to UDP-glucose. UDP glucose is the precursor for pullulan synthesis. Eventhough UDP-glucose present in xylose and fructose containing medium after long metabolic pathway, it could not be fully utilized for pullulan production due to low glucosyltransferase activity (Duan, Chi, Wang, & Wang, 2008). Fig. 5(b) shows the effect of mannose concentration on pullulan production in solid state fermentation. Pullulan yield was increased with respect to mannose concentration up to 5% (w/v), and then yield of pullulan decreased. Pullulan yield was improved to 49 mg/g ds at 5% (w/w) mannose concentration. Our findings are consistent with previous reports. Pullulan production decreased due to excess carbon source more than 5% (w/w) in the production medium. The reason may be owing to suppression effect of sugars on enzymes namely UDPG-pyrophosphorylase and

α-phosphoglucose mutase involved in pullulan biosynthesis (Kim, Kim, Lee, Lee, & Kim, 2000; Shin, Kim, Lee, Cho, & Byun, 1987).

3.7. Characterization of pullulan

Fig. 6(a) shows FTIR spectra for the pullulan sample from cassava bagasse. Bands appeared at 3462.7 cm^{-1} and 2930.1 cm^{-1} were the characteristics of O—H stretching and C—H stretching, respectively. Remaining characteristic signals observed at 1647.6, 1399.5 and 1020.8 cm^{-1} were due to O—C—O stretching, C—O—H bending and C—O stretching respectively (Sugumaran, Gowthami, et al., 2013; Sugumaran, Sindhu, et al., 2013; Thirumavalavan et al., 2009). A small hump observed at 851 cm^{-1} was due to α-configuration of D-glucopyranose units (Barker, Bourne, Stacey, & Whiffen, 1954).

The number average molecular weight, weight average molecular weight and polydispersity index which is the ratio of weight average molecular weight and number average molecular weight of pullulan sample was estimated by gel permeation chromatography. GPC chromatogram is shown in Fig. 6(b). The weight average molecular weight, number average molecular weight and poly-dispersity index were found to be 9.8×10^6 g/mol, 2.5×10^6 g/mol and 3.8, respectively. For pullulan, polydispersity index was ranging from 2.1 to 4.1 (Roukas & Mantzouridou, 2001; Wiley, Ball, Arcidiacono, & Kaplan, 1993). Depending on the medium composition, culturing conditions, type of strain, and the average molecular weight of pullulans varied (Pollock, 1992; Wiley et al., 1993). It has also been reported that pullulan molecular weight is affected by initial pH and nature of substrate used (Kim et al., 2000; Lee et al., 2001). The molecular weights of exo-polymers from agro wastes were higher than that obtained from glucose containing production medium (Israilies, Scanlon, Smith, Harding, & Jumel, 1994).

Structural characterization of the purified pullulan from cassava bagasse was carried out by ^{1}H-NMR spectroscopy (Fig. 7(a)) and it was compared with commercial pullulan (Ram, Gaganpreet, & Kennedy, 2009; Sugumaran, Gowthami, et al., 2013; Zhang et al., 2009). Four chemical signals were displayed in the anomeric region at 5.003, 4.903, 4.766 and 4.663 ppm due to four sugar repeating unit in the polysaccharide (Tako et al., 2013). Signal observed at 1.2 and 1.1 ppm was due to 6-deoxy-D-altrose present in the polysaccharide (Tako et al., 2012). C-NMR for pullulan from cassava bagasse is shown in Fig. 7(b). The signal at 18.48 ppm explained availability of 6-deoxy sugar (Tako et al., 2012). Splitting of C-6 (60.41 and 61.18 ppm) and C-4 (71.89 and 73.13 ppm) were due to C-1 of (1 → 4) linked glucose unit (Lazaridou et al., 2002). The absence of signals between 82 and 88 ppm explained that all the sugar residue available in pyranose form in biopolymer (Ye et al., 2008). Anomeric α (1 → 6) was appeared by peak at 99.82 ppm (Ram et al., 2009).

4. Conclusion

Pullulan production was investigated by low cost industrial waste – cassava bagasse in solid state fermentation. Effect of initial pH on morphology of cells and pullulan yield was studied. Then effects of fermentation time, moisture ratio, nitrogen source, supplement carbon source on pullulan yield were investigated. Finally effect of supplement concentration on pullulan production was carried out. It was found that the suitable conditions for pullulan yield using cassava bagasse are: initial pH −5.5; Fermentation time – 4th day, moisture ratio – 1:2, nitrogen source – sodium nitrite, supplement carbon source – mannose and 5% (w/w) mannose concentration. Microbial pullulan produced in this study was characterized by FTIR, ^{1}H-NMR and ^{13}C-NMR. Using gel permeation chromatography, the molecular weight of pullulan was found to be 9.8×10^6 g/mol.

References

Auer, D. P. E., & Seviour, R. J. (1990). Influence of varying nitrogen sources on polysaccharide production by *Aureobasidium pullulans* in batch culture. *Applied Microbiology and Biotechnology*, *32*, 637–644.

Barker, S. A., Bourne, E. J., Stacey, M., & Whiffen, D. H. (1954). Infrared spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose. *Journal of the Chemical Society*, *75*, 171–176.

Barnett, C., Smith, A., Scanlon, B., & Israilides, C. J. (1999). Pullulan production by *Aureobasidium pullulans* growing on hydrolysed potato starch waste. *Carbohydrate Polymers*, *38*, 203–209.

Campbell, S. B., McDougall, M. B., & Seviour, J. R. (2003). Why do exopolysaccharide yields from the fungus *Aureobasidium pullulans* fall during batch culture fermentation? *Enzyme and Microbial Technology*, *33*, 104–112.

Campbell, B. S., Siddique, A. B., McDougall, B. M., & Seviour, R. J. (2004). Which morphological forms of the fungus *Aureobasidium pullulans* are responsible for pullulan production? *FEMS Microbiology Letters*, *232*, 225–228.

Carta, F. S., Soccol, C. R., Ramos, L. P., & Fontana, J. D. (1999). Production of fumaric acid by fermentation of enzymatic hydrolysates derived from cassava bagasse. *Bioresource Technology*, *68*(1), 23–28.

Catley, B. J. (1971). Utilization of carbon sources by *Pullularia pullulans* for the elaboration of extracellular polysaccharides. *Applied Microbiology*, *22*, 641–649.

Catley, B. J. (1980). The extracellular polysaccharide pullulan, produced by *Aureobasidium pullulans*: A relationship between elaboration rate and morphology. *Journal of General Microbiology*, *120*, 265–268.

Cheng, K. C., Demirci, A., & Catchmark, M. J. (2009). Current knowledge of pullulan from production to application. *Current Top Biotechnology*, *5*, 29–48.

Duan, X., Chi, Z., Wang, L., & Wang, X. (2008). Influence of different sugars on pullulan production and activities of α-phosphoglucose mutase. UDPG-pyrophosphorylase and glucosyltransferase involved in pullulan synthesis in *Aureobasidium pullulans* Y68. *Carbohydrate Polymers*, *73*(4), 587–593.

Goksungur, Y., Ucan, A., & Guvenc, U. (2004). Production of pullulan from beet molasses and synthetic medium by *Aureobasidium pullulans*. *Turkish Journal of Biology*, *28*, 23–30.

Göksungur, Y., Uzunoğullarí, P., & Dağbağlí, S. (2011). Optimization of pullulan production from hydrolyzed potato starch waste by response surface methodology. *Carbohydrate Polymers*, *83*, 1330–1337.

Heald, P. J., & Kristiansen, B. (1985). Synthesis of polysaccharide by yeast like forms of *Auerobasidium pullulans*. *Biotechnology and Bioengineering*, *27*, 1516–1519.

Israilides, C. J., Smith, A., Harthill, J. E., Barnett, C., Bambalov, G., & Scanlon, B. (1998). Pullulan content of the ethanol precipitate from fermented agro-industrial wastes. *Applied Microbiology and Biotechnology*, *49*, 613–617.

Israilies, C., Scanlon, B., Smith, A., Harding, S. E., & Jumel, K. (1994). Characterization of pullulans from agro-industrial wastes. *Carbohydrate Polymers*, *25*, 203–209.

Jiang, L. F. (2010). Optimization of fermentation conditions for pullulan production by *Aureobasidium pullulans* using response surface methodology. *Carbohydrate Polymers*, *79*, 414–417.

Jiang, L., Wu, S., & Kim, J. M. (2011). Effect of different nitrogen sources on activities of UDPG-pyrophosphorylase involved in pullulan synthesis and pullulan production by *Aureobasidium pullulans*. *Carbohydrate Polymers*, *86*(2), 1085–1088.

Kim, J. H., Kim, M. R., Lee, J. H., Lee, J. W., & Kim, S. K. (2000). Production of high molecular weight pullulan by *Aureobasidium pullulans* using glucosamine. *Biotechnology Letters*, *22*, 987–990.

Lacroix, C., LeDuy, A., Noel, G., & Choplin, L. (1985). Effect of pH on the batch fermentation of pullulan from sucrose medium. *Biotechnology and Bioengineering*, *27*, 202–207.

Lazaridou, A., Roukas, T., Biliaderis, C. G., & Vaikousi, H. (2002). Characterization of pullulan produced from beet molasses by *Aureobasidium pullulans* in a stirred tank reactor under varying agitation. *Enzyme and Microbial Technology*, *31*, 122–132.

Lee, K. Y., & Yoo, Y. J. (1993). Optimization of pH for high molecular weight pullulan. *Biotechnology Letters*, *15*, 1021–1024.

Lee, J. H., Kim, J. H., Zhu, I. H., Zhan, X. B., Lee, J. W., Shin, D. H., et al. (2001). Optimization of conditions for the production of pullulan and high molecular weight pullulan by *Aureobasidium pullulans*. *Biotechnology Letters*, *23*, 817–820.

Li, B. X., Zhang, N., Peng, Q., Yin, T., Guan, F. F., Wang, G. L., et al. (2009). Production of pigment-free pullulan by swollen cell in *Aureobasidium pullulans* NG which cell differentiation was affected by pH and nutrition. *Applied Microbiology and Biotechnology*, *84*, 293–300.

Lin, Y., Zhang, Z., & Thibault, J. (2007). *Aureobasidium pullulans* batch cultivations based on a factorial design for improving the production and molecular weight of exopolysaccharides. *Process Biochemistry*, *42*, 820–827.

Orr, D., Zheng, W., Campbell, B. S., McDougall, B. M., & Seviour, R. J. (2009). Culture conditions affecting the chemical composition of the exopolysaccharide synthesized by the fungus *Aureobasidium pullulans*. *Journal of Applied Microbiology*, *107*, 691–698.

Pandey, A. (1991). Effect of particle size of substrate on enzyme production in solid-state fermentation. *Bioresource Technology*, *37*(2), 169–172.

Pandey, A. (2003). Solid-state fermentation. *Biochemical Engineering Journal*, *13*, 81–84.

Pandey, A., Nigam, P., Soccol, C. R., Soccol, V. T., Singh, D., & Mohan, R. (2000). Advances in microbial amylases. *Biotechnology and Applied Biochemistry*, *31*(2), 135–152.

Pandey, A., Soccol, C. R., & Mitchell, D. (2000). New developments in solid-state fermentation: I – Bioprocess and products. *Process Biochemistry*, *35*, 1153–1169.

Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P. S., & Mohan, R. (2000). Biotechnological potential of agro-industrial residues II: Cassava bagasse. *Bioresource Technology*, *74*, 81–87.

Pollock, T. J. (1992). Pullulan from polymorphic *Aureobasidiulll pullulans*. *Society for Industrial Microbiology News*, *42*, 147–156.

Prakasham, R. S., Rao, Ch. S., & Sarma, P. N. (2006). Green gram husk – An inexpensive substrate for alkaline protease production by *Bacillus* sp. in solid-state fermentation. *Bioresource Technology*, *97*(13), 449–1454.

Ram, S. S., Gaganpreet, K. S., & Kennedy, J. F. (2009). Downstream processing and characterization of pullulan from a novel colour variant strain of *Aureobasidium pullulans* FB-1. *Carbohydrate Polymers*, *78*(1), 89–94.

Ray, R. C., & Moorthy, S. N. (2007). Exopolysaccharide (pullulan) production from cassava starch residue by *Aureobasidium pullulans* strain MTTC 1991. *Journal of Scientific and Industrial Research*, *66*, 252–255.

Reed-Hamer, B., & West, T. P. (1994). Effect of complex nitrogen sources on pullulan production relative to carbon source. *Microbios*, *80*, 83–90.

Ronen, M., Guterman, H., & Shabtai, Y. (2002). Monitoring and control of pullulan production using vision sensor. *Journal Biochemistry and Biophysics Methods*, *3*, 243–249.

Roukas, T. C. (1999). Pullulan production from brewery wastes by *Aureobasidium pullulan*. *World Journal of Microbiology and Biotechnology*, *15*, 447–450.

Roukas, T., & Mantzouridou, F. (2001). Effect of aeration rate on pullulan production and fermentation broth rheological properties in an airlift reactor. *Journal of Chemical Technology and Biotechnology*, *76*, 371–376.

Schuster, R., Wenzig, E., & Mersmann, A. (1993). Production of the fungal exopolysaccharide pullulan by batch-wise and continuous fermentation. *Applied Microbiology and Biotechnology*, *39*, 155–158.

Seviour, R. J., Kristiansen, B., & Harvey, L. (1984). Morphology of *Aureobasidium pullulans* during polysaccharide elaboration. *Transactions of the British Mycological Society*, *82*, 350–356.

Shin, Y. C., Kim, Y. H., Lee, H. S., Cho, S. J., & Byun, S. M. (1987). Production of exopolysaccharide pullulan from inulin by a mixed culture of *Aureobasidium pullulans* and *Kluyveromyces fragilis*. *Biotechnology Letters*, *9*, 621–624.

Shingel, K. I. (2004). Current knowledge on biosynthesis, biological activity, and chemical modification of the exopolysaccharide, pullulan. *Carbohydrate Research*, *339*, 447–460.

Simon, L., Bouchet, B., & Caye-Vaugien, C. (1995). Pullulan elaboration and differentiation of the resting forms in *Aureobasidium pullulans*. *Canadian Journal of Microbiology*, *40*, 35–45.

Singh, R. S., Saini, G. K., & Kennedy, J. F. (2008). Pullulan: Microbial sources, production and applications. *Carbohydrate Polymers*, *73*, 515–531.

Srivas, T., & Anatharaman, M. (2005). *Cassava marketing system in India, Technical Bull ser No. 43*. Thiruvananthapuram: Central Tuber Crops research Institute., 102 pp.

Sugumaran, K. R., Gowthami, E., Swathi, B., Elakkiya, S., Srivastava, S. N., Ravikumar, R., et al. (2013). Production of pullulan by *Aureobasidium pullulans* from Asian palm kernel: A novel substrate. *Carbohydrate Polymers*, *92*(1), 697–703.

Sugumaran, K. R., Sindhu, R. V., Sukanya, S., Aiswarya, N., & Ponnusami, V. (2013). Statistical studies on high molecular weight pullulan production in solid state fermentation using jack fruit seed. *CarbohydratePolymers*, http://dx.doi.org/10.1016/j.carbpol.2013.06.071

Tako, M., Dobashi, Y., Tamaki, Y., Konishi, T., Yamada, M., Ishida, H., et al. (2012). Identification of rare 6-deoxy-D-altrose from an edible mushroom (*Lactarius lividatus*). *Carbohydrate Research*, *350*, 25–30.

Tako, M., Dobashi, Y., Shimabukuro, J., Yogi, T., Uechi, K., Tamaki, Y., et al. (2013). Structure of a novel α-glucan substitute with the rare 6-deoxy-D-altrose from *Lactarius lividatus* (mushroom). *Carbohydrate Polymers*, *92*(2), 2135–2140.

Thirumavalavan, K., Manikkadan, T. R., & Dhanasekar, R. (2009). Pullulan production from coconut by-products by *Aureobasidium pullulans*. *African Journal of Biotechnology*, *8*, 254–258.

Thomas, P. W., & Strohfus, B. A. (1996). Pullulan degrading enzyme activity of *Aureobasidium pullulans*. *Journal of Basic Microbiology*, *36*, 377–380.

Vijayendra, S. V. N., Bansal, D., Prasad, M. S., & Nand, K. (2001). Jaggery: A novel substrate for pullulan production by *Aureobasidium pullulans* CFR-77. *Process Biochemistry*, *37*, 359–364.

Wiley, B. J., Ball, D. H., Arcidiacono, S. M., & Kaplan, D. L. (1993). Control of molecular weight distribution of the biopolymer pullulan produced by *Aureobasidium pullulans*. *Journal of Environmental Polymer Degradation*, *1*, 3–9.

Wu, S., Jin, Z., Tong, Q., & Chen, H. (2009). Sweet potato: A novel substrate for pullulan production by *Aureobasidium pullulans*. *Carbohydrate Polymers*, *76*, 645–649.

Ye, L., Zhang, J., Ye, X., Tang, Q., Liu, Y., Gong, C., Du, X., & Pan, Y. (2008). Structural elucidation of the polysaccharide moiety of a glycopeptide (GLPCW-II) from Ganoderma lucidum fruiting bodies. *Carbohydrate Research*, *343*(4), 746–752.

Zadrazil, F., & Puniya, A. K. (1995). Studies on the effect of particle size on solid-state fermentation of sugarcane bagasse into animal feed using white-rot fungi. *Bioresource Technology*, *54*, 85–87.

Zhang, H.-z., Gao, F.-p., Liu, L.-r., Li, X.-m., Zhou, Z. m., Yang, X.-d., et al. (2009). Pullulan acetate nano particles prepared by solvent diffusion method for epirubicin chemotherapy. *Colloids Surface B: Biointerfaces*, *71*, 19–26.