Carbohydrate Polymers 93 (2013) 95–101

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Utilization of corn steep liquor for biosynthesis of pullulan, an important exopolysaccharide

Nishat Sharma [a], G.S. Prasad [b], Anirban Roy Choudhury [a,*]

[a] Biochemical Engineering Research & Process Development Centre (BERPDC), CSIR-Institute of Microbial Technology (IMTECH), Council of Scientific and Industrial Research (CSIR), Sector 39A, Chandigarh 160 036, India

[b] Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology (IMTECH), Council of Scientific and Industrial Research (CSIR), Sector 39A, Chandigarh 160 036, India

ARTICLE INFO

Article history:
Received 27 October 2011
Received in revised form 29 May 2012
Accepted 22 June 2012
Available online 29 June 2012

Keywords:
Pullulan
Exopolysaccharides
Aureobasidium pullulans
Corn steep liquor (CSL)
Green technology
Agri-industrial wastes

ABSTRACT

Five different agricultural wastes viz. rice bran oil cake, soya bean oil cake, cotton seed oil cake, mustard seed oil cake and corn steep liquor (CSL) were evaluated for their use as nutrient along with 15% (w/v) glucose as carbon source for biosynthesis of pullulan using *Aureobasidium pullulans* RBF 4A3. Among the selected agricultural wastes, CSL was found to be the best and supported production of 77.92 g L^{-1} pullulan under un-optimized conditions. Single point optimization technique resulted in increase in 18% pullulan (88.59 g L^{-1}) production. The process was successfully validated in a 7-L fermenter and a process economic analysis has suggested that use of CSL as nutrient may result in 3-fold reduction of cost of raw materials for pullulan production as compared to a process where conventional nitrogen sources were used. These observations may be helpful in development of a cost effective process for pullulan production.

1. Introduction

Pullulan is an important exopolysaccharide having applications in several industrial sectors like pharmaceutical, food and cosmetic industries. This extra cellular homo-polymer of maltotriose sub-units is produced by yeast-like fungus *Aureobasidium pullulans* and has unique linkage patterns of repeating units of α-1,4 and α-1,6 glucans which render it special physico-chemical properties like mechanical flexibility, oxygen impermeability, easy derivatibility, etc. and all these properties have made it potential candidate for several industrial applications. In spite of these unique properties and potential applications, this polymer is not very popular for industrial uses because of its cost. It is three times costlier than other biopolymers such as xanthan gum, which has similar application like pullulan in food and cosmetic industries. A suitable cost effective bioprocess for pullulan production may bring down the cost of pullulan and make it attractive for industrial applications.

Several reports have been published on production of pullulan via fermentation (Jiang, 2010; Ravella et al., 2010; Singh & Saini, 2008). Recently, we have also reported high pullulan production (66.79 g L^{-1}) using glucose, yeast extract and peptone as the substrate by an osmo-tolerant strain of *A. pullulans* RBF 4A3 (Choudhury, Saluja, & Prasad, 2011). Published reports indicate that media components used add significant cost to the production, and it may even reach up to 30% of the total production cost (Miller & Churchill, 1986). Therefore, it is utmost important to find cheap substrates for production of pullulan which will make the process economically viable. Alternative nitrogen sources like urea, ammonium salts, etc. have been used with limited success for pullulan production (West & Reed-Hamer, 1991, 1994).

There are also reports of using cheap raw materials like soybean pomace (Seo et al., 2004), hydrolysed potato starch for pullulan production (Ksungur, Uzunoğulları, & Dağbağlı, 2011). However, in those cases, the yield was not significantly high and hence the economics of the process was also not much favorable. Therefore, it is required to evaluate low cost substrates to develop a cost effective process. Agri-industrial residues are used as feed stock for production of different chemicals and biochemicals like ethanol, citric acid, lactic acid due to their easy availability of large quantity, low cost and high nutrient content (Leathers, 2003). Hence, it may also be possible to develop a cost-effective process for production of pullulan by using agri-industrial residues.

The aim of the present study was to examine potential of agri-industrial residues as nutrient in place of conventional media components like yeast extract and peptone for production of pullulan by *A. pullulans* RBF-4A3. Previously, soyabean pomace was utilized as nitrogen source for production of pullulan, in which the yield was very low (Seo et al., 2004). In the present

* Corresponding author. Tel.: +91 172 6665328; fax: +91 172 2695215.
E-mail address: anirban@imtech.res.in (A.R. Choudhury).

0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.06.059

study, five different agri-industrial wastes namely rice bran oil cake, soya bean oil cake, cotton seed oil cake, mustard seed oil cake and corn steep liquor (CSL) were examined for their use as nutrient sources for production of pullulan using *A. pullulans* RBF 4A3. Among the selected agri-industrial residues CSL was found to be the most suitable as nutrient and the pullulan produced was also high as compared to earlier published reports (Ksungur et al., 2011; Shengjun, Jin, Tong, & Chen, 2009).

2. Materials and methods

2.1. Media components

Media components like glucose, yeast extract, peptone, agar, etc. were obtained from Hi Media Laboratories (Mumbai, India) and standard pullulan was procured from Sigma (St. Louis, USA). Corn steep Liquor was obtained from Bharat Starch Industries Limited (Yamunanagar, India) and rice bran oil cake, soya bean oil cake, cotton seed oil cake and mustard seed oil cake were obtained from local market.

2.2. Yeast strain, culture conditions and inoculum development

The strain, *A. pullulans* RBF 4A3, used in this study was isolated from inflorescence of *Caseulia axillaries* (Choudhury et al., 2011). It was maintained at 28 °C using yeast peptone dextrose (YPD) agar media and for long term preservation, glycerol vials were stored at −70 °C.

Inoculum development was carried out by using fresh cultures grown on YPD agar plate for 24 h followed by incubation at 28 °C for 24 h at an agitation speed of 200 rpm on a YPD (1% yeast extract, 2% peptone and 2% dextrose) broth. This inoculum (2.5 mL) was used to inoculate 50 mL of production medium in a 250 mL conical flask.

2.3. Screening of different agri-industrial wastes for their use as nutrient in production media

Five different agri-industrial wastes, namely, rice bran oil cake, mustard oil cake, soybean oil cake, cotton seed oil cake and corn steep liquor were evaluated as nutrients for pullulan production. The production media composed of 2% of the agricultural waste, and 15% dextrose in each case. Fermentation was carried out in 250 mL shake flask containing 25 mL media at 28 °C in an orbital shaker at 200 rpm for 120 h. Samples were taken at 24-h interval and pullulan content was examined.

2.4. Optimization of pullulan production in shake-flask

The yield of product in case of any fermentation process may be enhanced by optimizing various parameters. In present study single point optimization technique was used to optimize CSL concentration, incubation temperature, agitation speed, inoculum size and initial pH of the media for pullulan production by *A. pullulans* RBF 4A3.

2.4.1. Effect of concentration of corn steep liquor on pullulan production

Among the selected agri-industrial residues CSL was found to be the best. In further experiments CSL was used as nutrient along with dextrose. The concentration of corn steep liquor was varied in the range of 1% (v/v) to 8% (v/v) in the production media and the dextrose concentration was maintained at 15% (w/v) in all cases. The initial pH was maintained at 4.5 and the media was inoculated using 20.8 mg fresh weight biomass per mL of the production medium. The flasks were incubated at 28 °C and 200 rpm. Samples were withdrawn periodically (at every 24-h interval up to 120 h) and analyzed for pullulan content.

2.4.2. Effect of incubation temperature on pullulan production

Effect of incubation temperature on pullulan production was studied by varying the same in the range of 15–30 °C using a production media consisting of 2% CSL and 15% dextrose. All other process conditions were maintained same as described earlier. Samples were withdrawn at every 24 h and analyzed for pullulan production, residual sugar content and pH of the media.

2.4.3. Effect of agitation speed

In aerobic fermentation processes agitation plays a key role in product formation. The agitation speed was varied from 100 to 350 rpm to understand its effect on pullulan production. All other parameters were maintained as optimized in earlier experiments. The fermentation was carried out till 120 h and periodic samples were obtained to determine pullulan production.

2.4.4. Effect of inoculum size

Inoculum size is another factor that substantially affects the production of the product in fermentation processes. In the present study the production media was inoculated using an inoculum containing 41.6 mg mL^{-1} biomass (on fresh weight basis) and varying the inoculum size from 2% to 10% (v/v) level. All other parameters were kept same as optimized during earlier batches.

2.4.5. Effect of initial pH

pH plays a very important role in product formation in all fermentation processes. In case of present study, the initial pH of the medium was varied from 3.5 to 6.5 to obtain optimum pullulan production. The remaining process conditions were same as optimized during earlier experiments.

2.5. Analysis, purification and characterization of pullulan

The fermentation broth was centrifuged at 16,000 × *g* for 20 min at 4 °C using a Sigma 6K-15 centrifuge. This cell free broth was subjected to solvent precipitation using 2 volumes of ethanol at 4 °C. The precipitate thus obtained was once again separated by centrifugation at 16,000 × *g* for 20 min at 4 °C and was re-dissolved in de-ionized water and subjected to dialysis (MWCO 20,000) for removal of small molecular weight compounds. Then the polymer was re-precipitated using 2 volumes of ethanol at 4 °C and the precipitate was separated by centrifugation and dried at 80 °C till constant weight. Pullulan content in the exopolysaccharide was determined by enzymatic method (Chi & Zhao, 2003). The dried and purified precipitate was dissolved in de-ionized water (3 mL) and used as substrate for enzymatic hydrolysis. Reaction was carried out at 40 °C for 2 h in 1 mL volume (0.5 mL of the substrate; 0.4 mL of 0.2 (M) phosphate buffer (pH 5.0) and 0.1 mL (0.84 U) of pullulanase enzyme (Sigma, USA) solution. The residual sugar released during enzymatic hydrolysis was measured by the method of Miller (1959) using a Hitachi U-2900 UV-visible spectrophotometer. This was compared with the residual sugar released during hydrolysis of standard pullulan obtained from Sigma, USA. The pullulan content was expressed in terms of grams of pullulan (dry weight) produced per liter of fermentation broth.

The EPS produced was characterized by using FT-IR spectroscopy as described earlier (Choudhury et al., 2011). The sample for FT-IR was prepared by blending 2 mg of EPS with 60 mg of potassium bromide powder. The mixture was desiccated overnight at 50 °C under reduced pressure. Pullulan from Sigma, USA was used as a standard. Fourier transform infrared (FTIR) spectra were recorded with a Perkin Elmer spectrophotometer over a range of

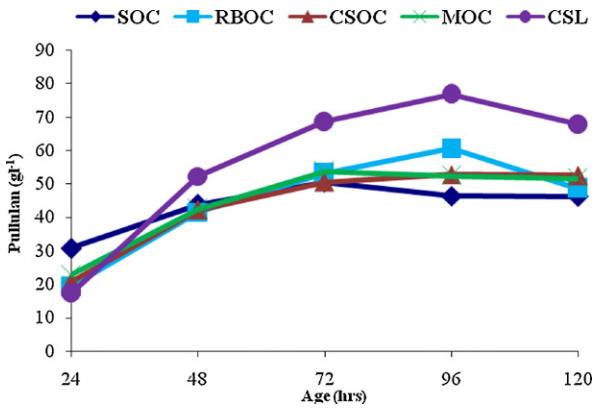

Fig. 1. Effect of different agri-industrial wastes on pullulan production by *Aureobasidium pullulans* RBF 4A3 under un-optimized conditions.
2% concentration of each agri-industrial wastes used by using 2.08 mg fresh weight biomass per mL of fermentation media at 200 rpm, incubated at 28 °C: (♦) SOC; (■) RBOC; (▲) CSOC; (×) MSOC; and (●) CSL represents pullulan production using representative agri-industrial wastes as nutrients.

4000–400 cm^{-1}, 16 scans with a resolution of 2 cm^{-1} were acquired and averaged.

2.6. Validation of shake flask experiments in a laboratory scale fermenter

A fermentation run was carried out in a 7-L Applikon fermenter with 5 L working volume to validate the results obtained during optimization of the process in shake flask. Fermentation medium containing 2% (v/v) CSL and 15% (w/v) dextrose was inoculated with 2.08 mg mL^{-1} fresh weight biomass per mL of the fermentation medium. The fermentation was carried out at 20 °C for 120 h and under 250 rpm and 1VVM aeration; samples were withdrawn at 24 h interval to measure pullulan content, pH and residual sugar content.

3. Results and discussions

3.1. Screening of different agri-industrial wastes for their use as nutrient in production media

Five selected agri-industrial residues were screened for their ability to be used as a nutrient for pullulan production. The result obtained indicates that CSL supported better pullulan production (77.92 g L^{-1}) as compared to all other agri-industrial wastes (Fig. 1) after 96 h of incubation. This may be attributed to the fact of presence of suitable ratio of carbohydrate and protein in case of CSL as compared to other agri-industrial wastes used. The pullulan obtained in present case is significantly high as compared to previous published reports (Kuan-Chen, Demirci, & Catchmark, 2011).

3.2. Optimization of pullulan production in shake flask

One of the major bottlenecks for popularizing pullulan is the cost of the product. The cost of the product may significantly be brought down by increasing the yield of the same. To obtain enhanced pullulan production different parameters like CSL concentration in media, incubation temperature, agitation speed, inoculum size and initial pH of the media were optimized using single point optimization technique.

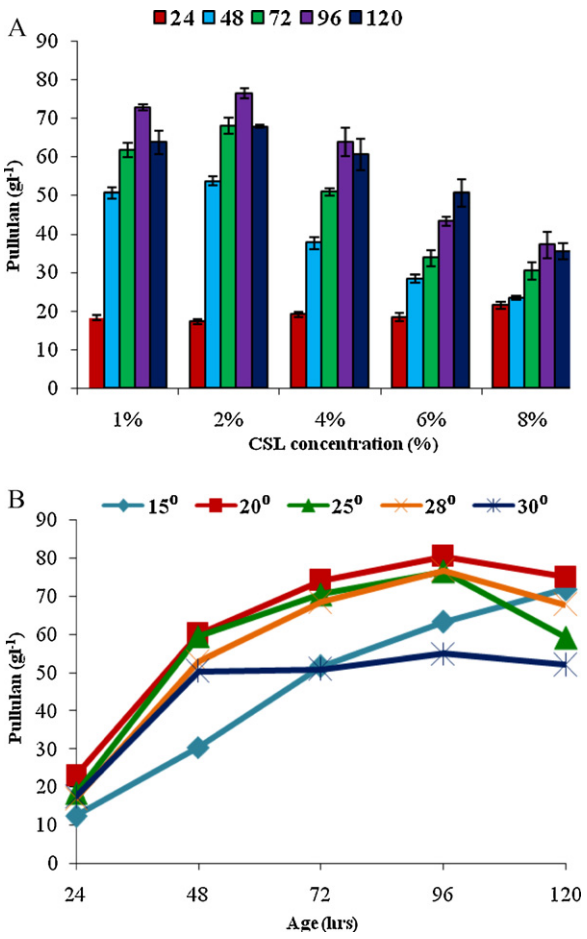

Fig. 2. (A) Effect of CSL on pullulan production by *Aureobasidium pullulans* RBF 4A3. CSL concentration was varied from 1%to 8% by using 2.08 mg fresh weight biomass per mL of fermentation media at 200 rpm. 24 h, 48 h, 72 h, 96 h and 120 h represent pullulan production at the respective age of fermentation. Initial pH of the medium was 4.5. (B) Effect of incubation temperature on pullulan production by *Aureobasidium pullulans* RBF 4A3. In this case incubation temperature varied from 15 °C to 30 °C. CSL concentration was maintained at 2%. The flasks were inoculated with 2.08 mg fresh weight biomass per mL of fermentation media and incubated at 200 rpm: (♦) 15 °C; (■) 20 °C; (▲) 25 °C; (×) 28 °C; and (*) 30 °C represents pullulan production at the respective temperatures of incubation.

3.2.1. Effect of concentration of corn steep liquor in production media

Corn steep liquor is an inexpensive by product obtained from corn starch production industries and produced in large quantity (CRA 2006). CSL contains 40% protein, 21% lactic acid and 16% nitrogen free extract (Mirza & Mushtaq, 2006) and is also very rich in amino acids and vitamins which make it an excellent but inexpensive source of essential nutrients. However, there are very few reports published on production of exopolysaccharides using CSL as nutrient (Matsuyama, Kawasaki, & Yumoto, 1999; Papaspyridi, Katapodis, Zagou, & Kapsanaki, 2011). In order to find out optimum concentration of corn steep liquor for pullulan production, shake flask fermentations were carried out by varying corn steep liquor concentration from 1% to 8% (v/v) in the production media. The pullulan production was observed to increase from 72.71 g L^{-1} to 76.43 g L^{-1} after 96 h of fermentation when corn steep liquor concentration was increased from 1% (v/v) to 2% (v/v). However with increase in concentration of CSL pullulan production was reduced (Fig. 2A) and even went down to 37.17 g L^{-1} when 8% CSL was used. This observation indicated that although corn steep liquor is very rich in essential nutrients but higher concentrations of CSL may

have negative effect on production due to presence of inhibitory components like of lactic acid, etc.

3.2.2. Effect of temperature on pullulan production

Incubation temperature has significant effect on product formation and biomass development in case of any fermentation process (McNeil & Kristiansen, 1990). Hence, it is important to obtain optimum temperature for product formation to maximize the yield of pullulan from the fermentation broth. As per published literatures, optimum temperature for pullulan production is different for strains in the range of 25–30 °C (Kuan-Chen et al., 2011). In present study, it was observed that 20 °C is optimum temperature of incubation for pullulan production and 80.47 g L^{-1} pullulan was produced when the shake flasks were incubated at 20 °C for 96 h (Fig. 2B). It was also observed that with the increase in temperature of incubation beyond 20 °C pullulan production was decreased and only 55.11 g L^{-1} pullulan was produced after 96 h at 30 °C. Earlier Roukas and Biliaderis (1995) have also reported similar observations. However, there is other report which shows optimal pullulan production at 25 °C (Chi & Zhao, 2003). These reports indicate that the temperature optimum for production of pullulan is strain specific.

3.2.3. Effect of agitation speed on pullulan production

Agitation helps in mass transfer in the fermentation system by mixing and maintaining homogeneity in the fermentation broth. However, too much agitation may also be harmful to the microbial system because higher level of agitation will increase the shear inside the fermentation broth which in turn may lead to cell damage and affect the polymer production. Hence, it is important to optimize agitation speed to obtain higher pullulan production. In the present study, effect of agitation speed was varied at 5 different levels (100, 200, 250, 300, 350 rpm). The results obtained indicated that pullulan production increased with increase in agitation speed in the early hours of fermentation (till 72 h) in all cases except in case of 100 rpm (Fig. 3A). At an agitation speed of 100 rpm very little (34.37 g L^{-1}) pullulan was produced indicating that this agitation speed was too low to provide proper mass transfer within the system. As already mentioned, pullulan production was almost similar till 72 h of fermentation in case of all other agitation speed. However, an agitation speed of 300 rpm was found to be the best for pullulan production (88.13 g L^{-1} pullulan was produced after 96 h of fermentation). Earlier reports showed that pullulan production increases with increase in agitation speed up to a critical level, beyond which it starts to decline (Gibbs & Seviour, 1996). In this context, it also may be noted that critical level of agitation speed depends on several factors including type and volume of fermentation, characteristics of media components and microbial strain used for pullulan production (Lazaridou, Roukas, Biliaderis, & Vaikousi, 2002). In the present study, pullulan production increased along with increase in agitation speed till 300 rpm and further increase in agitation speed caused decline in pullulan production, indicating that 300 rpm is optimal for pullulan production in this case.

3.2.4. Effect of inoculum size on pullulan production

Inoculum size also affects pullulan production significantly because it not only determines the initial microbial load but also determines the duration of lag phase and have effect on cell morphology and their growth pattern which in turns affect the pullulan production (Vinroot & Torzilli, 1988). Hence it is important to optimize the inoculum size to achieve higher production of pullulan. The results indicated that pullulan production increases with increase in inoculum size from 2% (v/v) to 5% (v/v) using an inoculum, having 41.6 mg mL^{-1} of fresh weight biomass per mL inoculum, resulting in production of 88.46 g L^{-1} pullulan after 96 h fermentation (Fig. 3B). Higher inoculum sizes did not increase pullulan production any further; therefore, optimum inoculum concentration (5% (v/v)) may help in balancing between biomass production and maximum product formation in present case.

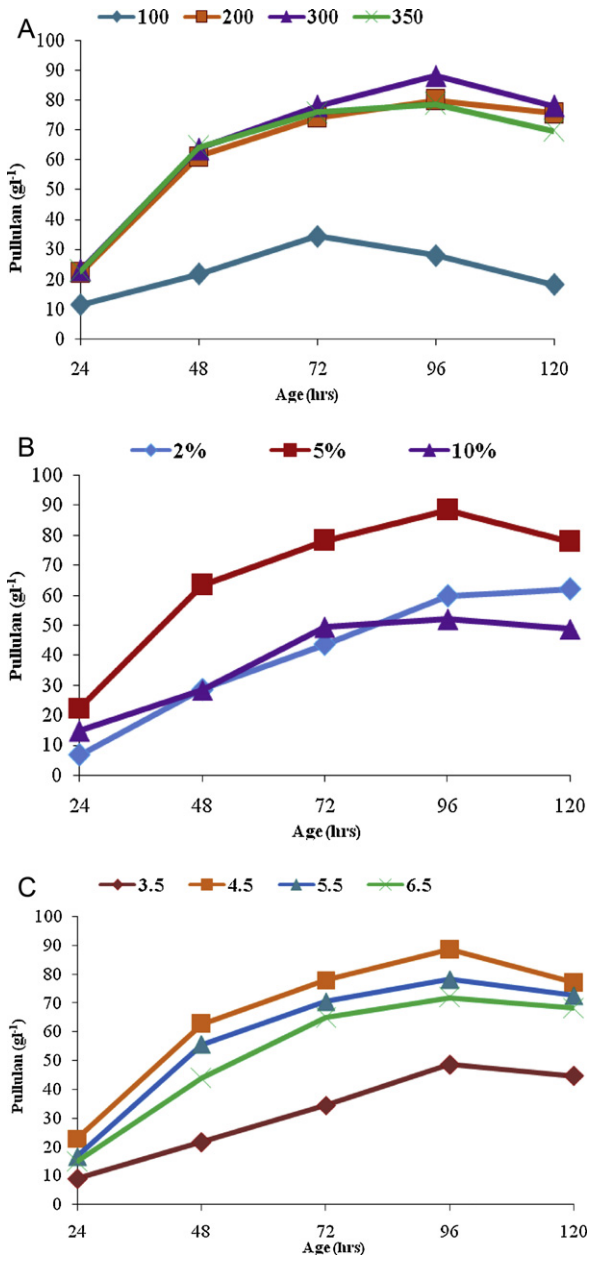

Fig. 3. (A) Effect of agitation speed on pullulan production by *Aureobasidium pullulans* RBF 4A3. In this case agitation speeds were varied from 100 to 350. The CSL was used at 2% concentration and the flasks were incubated at 20 °C: (♦) 100; (■) 200; (▲) 300; (×) 350 represents pullulan production at the respective shaker rpm. (B) Effect of inoculum volume on pullulan production by *Aureobasidium pullulans* RBF 4A3. The inoculum sizes were varied from 2% (v/v) to 10% (v/v) and incubation was done at 20 °C with a media containing 2% CSL: (♦) 2%; (■) 5%; and (▲) 10% denotes pullulan production at the specific inoculum volume. (C) Effect of initial pH on pullulan production by *Aureobasidium pullulans* RBF 4A3. Initial pH was varied from 3.5 to 6.5 and the flasks were incubated with a media containing 2% CSL: (♦) 3.5; (■) 4.5; (▲) 5.5 and (×) 6.5 represents pullulan production at the specified initial pH.

3.2.5. Effect of initial pH on pullulan production

pH is one of the most important parameter in any fermentation process because it may change the metabolic activity of the cells which in turn affect the level of enzyme or metabolite productions (Krishna, 2005). Earlier, Roukas and Biliaderis (1995) reported maximum pullulan production at a pH of 6.5 and in another report

Table 1
Pullulan production by *Aureobasidium pullulans* using different substrates.

Name of microorganism	Substrates		Fermentation mode	Fermentation volume	Pullulan production ($g\,L^{-1}$)	Productivity of Pullulan ($g\,L^{-}$ h)	Yields of Pullulan (g pullulan/g sugar consumed)	References
	Carbon source	Nitrogen source						
Aureobasidium pullulans	Sucrose	Ammonium sulphate	Continuous	1.5 L	23.1	0.9	0.6	Cheng, Demirci, and Catchmark (2011)
Aureobasidium pullulans	Glucose	Yeast extract, peptone	Batch	50 mL	66.8	0.7	0.4	
Aureobasidium pullulans	Molasses	Yeast extract	Batch	5 L	35	0.3	0.7	Ksungur, Ucan, and Gubenc (2004)
Aureobasidium pullulans	Hydrolysed potato starch waste	Yeast extract	Batch	50 mL	19.2	0.2	0.3	Ksungur et al. (2011)
Aureobasidium pullulans	Beet molasses	K_2HPO_4, L-glutamic acid, olive oil, Tween-80	Batch	100 mL	32.0	0.3	0.5	Roukas (1998)
Aureobasidium pullulans	Glucose	Soyabean pomace	Batch	100 mL	7.6	0.1	0.4	Seo et al. (2004)
Aureobasidium pullulans	Jaggery	Yeast extract	Batch	50 mL	51.9	0.7	0.5	Vijayendra, Bansal, Prasad, and Nand (2001)
Aureobasidium pullulans	Dextrose	Corn steep liquor	Batch	25 mL	88.6	0.9	0.6	Current study
Aureobasidium Pullulans	Sucrose	Ammonium sulphate	Central Composite Design	50 mL	44.4	0.2	0.8	Singh, Singh, and Saini (2009)

Table 2
Process economic analysis for raw material cost for production of pullulan using corn steep liquor.

Raw material	Process I[a]					Process II[a]				
	Requirement ($g\,L^{-1}$ media)	Rate ($/kg)	Cost L^{-1} media ($)	Pullulan produced ($g\,L^{-1}$)	Cost of raw material/kg Pullulan produced ($)	Requirement ($g\,L^{-1}$ media)	Rate ($/kg)	Cost L^{-1} media ($)	Pullulan produced ($g\,L^{-1}$ media)	Cost of raw material/kg Pullulan produced ($)
Glucose	–				–	150	0.8	0.1	88.6	1.3
Sucrose	53.1	1.00	0.1	44.4	2.3					
Yeast extract	0.7	70	0.05		1.1	–				
Di potassium hydrogen phosphate	0.5	26	0.01		0.23					
Ammonium sulphate	1.1	8	0.01		0.23	–				
Sodium chloride	1.5	6	0.01		0.23					
CSL	–				–	20	0.5	0.01		0.1
Total					4.09					1.4

Process I: Singh et al. (2009).
Process II: Present study.
[a] The prices were obtained as per the rate of supply of commercial grade bulk materials by Hi-Media, India and Merck, Germany (1USD = 50INR).

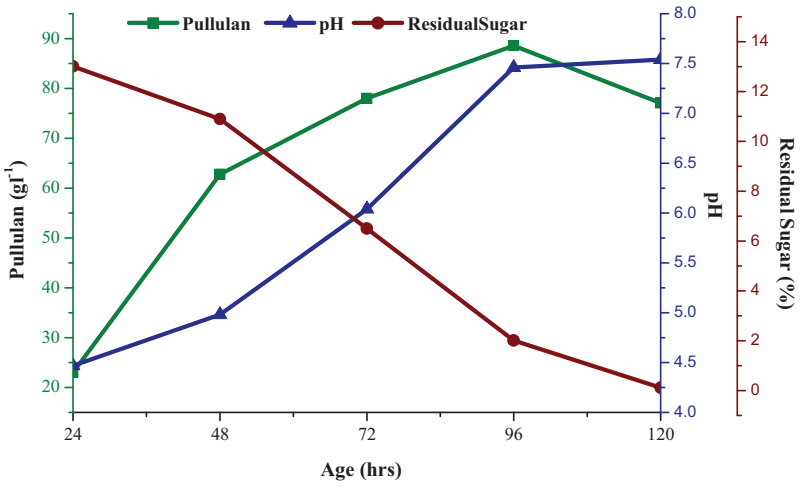

Fig. 4. Fermentation kinetics of the optimized batch. The optimum conditions of optimized batch are 2% (w/v) CSL, 15.5% (w/v) dextrose, 2.08 mg fresh weight biomass per mL of fermentation media, rpm 200, incubated at 20 °C, and pH adjusted to 6.0: (■) Pullulan, (▲) pH, (●) residual sugar were measured periodically and plotted herewith.

Auer and Seviour (1990) observed maximum pullulan production at an initial pH of 7.5, indicating that the pH optima depend on the strain as well as on the substrate used for production of polymer. The initial pH of the media was varied from 3.5 to 6.5 in present study. It was observed that the pullulan production increases with increase in the initial pH of the media from 3.5 to 4.5, however, further increase in pH affects pullulan production adversely (Fig. 3C), suggesting that an initial pH of 4.5 is most suitable for pullulan production (88.59 g L^{-1} after 96 h of fermentation). In the present study, the significant change in pullulan concentration in the production media with the change in pH also indicates the importance of optimization of the pH for development of a commercially feasible process for the production of the polymer.

3.3. Validation of shake flask experiments in a laboratory scale fermenter

The optimized batch was run in a 7-L Applikon fermenter with 5-L working volume to validate the results obtained during shake flask studies and also to understand scalability of the process. The fermentation kinetics of the optimized batch demonstrates that the rate of polysaccharide production is directly proportional with consumption of carbon source (Fig. 4). At the end of 96 h of fermentation. 88.59 g L^{-1} of polymer was produced, after which the pullulan in the fermentation broth depleted. This observation is in agreement with earlier observations made by Roukas (1999) and may be due to the fact that, at the end phase of fermentation, reducing sugar content in the media was very low and hence the product formed got degraded by the producing organism (Thomas & Strohfus, 1996) to support their metabolic activity. This also indicates that the process optimized in the shake flask level may be scaled up easily and it may lead to development of a successful cost effective technology for pullulan production. It should also be noted that optimum production obtained in fermenter is significantly high as compared to earlier reports (Table 1). FT-IR data showed that the spectra obtained for standard pullulan (Sigma) and the pullulan produced via fermentation is identical and thus confirms the chemical structure of the polymer produced in this study is same as standard pullulan (supplementary figure).

3.4. Process economics

Cost analysis of raw materials indicated that the high costs of conventional nitrogen sources are responsible for major part of the raw material cost in pullulan production and therefore substituting those with a cheaper raw material may bring down the raw material cost for pullulan production. CSL is less expensive as compared to the conventional nitrogen sources like yeast extract and a peptone. Therefore, substituting those costly nutrients by CSL can reduce the cost of raw materials for pullulan production by around 3-fold (Table 2).

4. Conclusion

Corn Steep liquor is a by product of corn starch processing industries and its utilization as a nutrient may add significant value to this extensively produced by product. In the present study we have used single point optimization technique and showed that 88.59 g L^{-1} pullulan can be produced after 96 h of fermentation under optimized conditions using 2% (v/v) CSL as the nutrient along with 15% (w/v) dextrose. This is around 14% increase as compared to initial non-optimized conditions for pullulan production. A cost analysis data clearly indicated that it was possible to reduce the raw material cost by around 6-fold by replacing conventional media components like yeast extract and peptone by CSL. This may help in reduction of production cost of pullulan in future and may also lead to popularization of this polymer having versatile use. Further process scale up studies using CSL as the nutrient is under progress in our laboratory.

Acknowledgements

The authors are thankful to Council of Scientific and Industrial Research (CSIR) for providing funding for carrying out experiments. The authors also thankful to Dr. Chanderpaul, Manager R&D, Bharath Starch Industries, Yamunanagar, India for providing CSL samples required for the experimentation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.06.059.

References

Auer, D. P., & 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.

Cheng, K. C., Demirci, A., & Catchmark, J. M. (2011). Continuous pullulan fermentation in a biofilm reactor. *Applied Microbiology and Biotechnology*, *90*, 921–927.

Chi, Z., & Zhao, S. (2003). Optimization of medium and cultivation conditions for pullulan production by a new pullulan-producing yeast strain. *Enzyme and Microbial Technology*, *33*, 206–211.

Choudhury, A. R., Saluja, P., & Prasad, G. S. (2011). Pullulan production by an osmotolerant *Aureobasidium pullulans* RBF-4A3 isolated from flowers of *Caesulia axillaries*. *Carbohydrate Polymers*, *83*, 1547–1552.

Gibbs, P. A., & Seviour, R. J. (1996). Does the agitation rate and/or oxygen saturation influence exopolysaccharide production by *Aureobasidium pullulans* in batch culture? *Applied Microbiology and Biotechnology*, *46*, 503–510.

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

Krishna, C. (2005). Solid-state fermentation systems—An overview. *Critical Reviews in Biotechnology*, *25*, 1–30.

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

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

Kuan-Chen, C., Demirci, A., & Catchmark, J. M. (2011). Pullulan: Biosynthesis, production, and applications. *Applied Microbiology and Biotechnology*, *92*, 29–44.

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.

Leathers, T. D. (2003). Bioconversions of maize residues to value-added coproducts using yeast-like fungi. *FEMS Yeast Research*, *3*, 133–140.

Matsuyama, H. S. R., Kawasaki, K., & Yumoto, I. (1999). Production of a novel exopolysaccharide by *Rahnella aquatilis*. *Journal of Bioscience and Bioengineering*, *87*, 180–183.

McNeil, B., & Kristiansen, B. (1990). Temperature effects on polysaccharide formation by *Aureobasidium pullulans* in stirred tanks. *Enzyme and Microbial Technology*, *12*, 521–526.

Miller, L. G. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*, 426–428.

Miller, T. L., & Churchill, B. W. (1986). Substrates for large-scale fermentations. In A. L. Demain, & L. A. Solomon (Eds.), *Manual of industrial microbiology and biotechnology*. Washington, DC: American Society for Microbiology.

Mirza, M. A., & Mushtaq, T. (2006). Effect of supplementing different levels of corn steep liquor on the post-weaning growth performance of pak-karakul lambs. *Pakistan Veterinary Journal*, *26*, 135–137.

Papaspyridi, L. M., Katapodis, P., Zagou, Z. G., & Kapsanaki, E. (2011). Growth and biomass production with enhanced β-glucan and dietary fibre contents of *Ganoderma australe* ATHUM 4345 in a batch-stirred tank bioreactor. *Engineering in Life Sciences*, *11*, 65–74.

Ravella, S. R., Quiñones, T. S., Retter, A., Heiermann, M., Amon, T., & Hobbs, P. J. (2010). Extracellular polysaccharide (EPS) production by a novel strain of yeast like fungus *Aureobasidium pullulans*. *Carbohydrate Polymers*, *82*, 728–732.

Roukas, T. (1998). Pretreatment of beet molasses to increase pullulan production. *Process Biochemistry*, *33*, 805–810.

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

Roukas, T., & Biliaderis, C. G. (1995). Evaluation of carob pod as a substrate for pullulan production by *Aureobasidium pullulans*. *Applied Biochemistry and Biotechnology*, *55*, 27–44.

Seo, H. P., Son, C. W., Chung, C. H., Jung, D., Kim, S. K., Gross, R. A., et al. (2004). Production of high molecular weight pullulan by *Aureobasidium pullulans* HP 2001 with soybean pomace as a nitrogen source. *Bioresource Technology*, *95*, 293–299.

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

(2006). *Shipments of Products of the Corn Refining Industry-2005*. Corn Refiners Association, CRA.

Singh, R. S., & Saini, G. K. (2008). Pullulan-hyperproducing color variant strain of *Aureobasidium pullulans* FB-1 newly isolated from phylloplane of *Ficus* sp. *Bioresource Technology*, *99*, 3896–3899.

Singh, R. S., Singh, H., & Saini, G. K. (2009). Response surface optimization of the critical medium components for pullulan production by *Aureobasidium pullulans* FB-1. *Applied Biochemistry and Biotechnology*, *152*, 42–53.

Thomas, P. W., & Strohfus, B. (1996). A 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 productin by *Aureobasidium pullualans* CFR-77. *Process Biochemistry*, *37*, 359–364.

Vinroot, S., & Torzilli, A. P. (1988). Interactive effects of inoculum density, agitation and pH on dimorphism in a salt marsh isolate of *Aureobasidium pullulans*. *Mycologia*, *80*, 376–381.

West, T. P., & Reed-Hamer, B. (1991). Ability of *Aureobasidium pullulans* to synthesize pullulan upon selected sources of carbon and nitrogen. *Microbios*, *67*, 117–124.

West, T. P., & Reed-Hamer, B. (1994). Elevated polysaccharide production by mutants of the fungus *Aureobasidium pullulans*. *FEMS Microbiology Letters*, *124*, 167–172.