Wheat Bran as Substrate for Enzyme Production and its Application in the Bio-deinking of Mixed Office Waste (MOW) Paper

Pallavi Biswas,* Amit K. Bharti, Ashish Kadam, and Dharm Dutt

Judicial utilization of various low-cost agro-industrial wastes and optimization of various process parameters can reduce production costs of enzymes. Wheat bran was found to be the best carbon source among various agro-industrial wastes explored for *Penicillium citrinum* NCIM-1398. Additionally, ammonium sulphate was found as the optimum nitrogen source at moisture content 70%, pH 5.5, and temperature 30 °C for enzyme production. The maximal enzyme activities of endo β-1,4-glucanase, xylanase, FPase, and amylase were 21.0 IU/gds, 3140 IU/gds, 3.59 FPU/gds, and 73.4 IU/gds, respectively. Bio-deinking of mixed office waste paper improved the pulp brightness by 9.5%, and the effective residual ink concentration were decreased by 26.3% in comparison to MOW after pulping. Similarly, dirt counts were reduced from 4880 to1360 ppm at an enzyme dose of 6.0 IU/g compared to deinking without enzyme. The strength properties of enzymatically deinked pulp such as tear index, tensile index, and burst index increased by 6.92%, 11.31%, and 7.61%, respectively, compared with the control.

Keywords: Wheat bran; Endo β-1,4-glucanase; Xylanase; White Mixed Office Waste (MOW) paper; Bio-deinking

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INTRODUCTION

Wheat, being one of the staple foods of Indian population, is extensively cultivated throughout the nation. In India during crop year 2017-18, wheat witnessed the record production of 99.7 MT (Das et al. 2019). Wheat milling is used in industries for making processed food items such as semolina, all-purpose flour, pasta, noodles, *etc.* During such processing, wheat bran is produced as a by-product. Flour industries produced around 796 thousand metric tons of wheat bran in India during fiscal year 2018 (Statista 2018). Wheat bran mainly consists of starch (10 to 20%), non-starch polysaccharides (41 to 60%), and protein (15 to 20%) (Ralet *et al.* 1990; Bergmans *et al.* 1996; Liu *et al.* 2010). Unfortunately, wheat bran is referred to as 'waste', even though it has nutritional values and is perfectly reusable. These low cost and easily available agro-industrial wastes may serve as excellent substrates for fermentation to obtain various valuable secondary metabolites such as enzymes, organic acids, cellular proteins, and prebiotics of microbial origin (Arotupin *et al.* 2015).

Enzymes produced by microbes have various applications in industries including food, biofuel, detergents, animal feed, textile, leather, and paper processing. High enzyme production cost is a critical factor that limits applications and basically governs by the cost of substrate used as nitrogen or carbon sources and cost of medium used for fermentation

(Singh *et al.* 2012). For enzyme production, solid state fermentation (SSF) is preferred over submerged fermentation (SmF) because it exhibits higher yield, less energy consumption, and low production cost (Pandey 2003). Various other process parameters such as moisture content, temperature, incubation time, and pH are crucial factors for enzyme production (Dutt and Kumar 2014; Gautam *et al.* 2015). Many fungi, bacteria, and actinomycetes produce various enzymes. However, fungi are the source of higher yields of cellulase and hemicellulase enzymes (El-Hadi *et al.* 2014). Fungal species such as *Penicillium*, *Aspergillus*, and *Trichoderma* are mainly used for enzyme production (Iyer 2018).

Various research reports revealed that conventional techniques of deinking such as washing, dispersion, and flotation are less effective to remove the ink from waste papers. During chemical deinking, the use of chemicals such as sodium silicate, sodium hydroxide, hydrogen peroxide, chelating agents, and surfactants causes environmental pollution and long-term health hazards. To overcome these problems, waste water treatment is required, which increases the cost of overall process (Woodward *et al.* 1994; Marques *et al.* 2003). The enzymatic approach of deinking is more efficient and effective in comparison to conventional chemical deinking for mixed office waste (MOW) paper (Singh *et al.* 2019). Additionally, the enzymatic approach reduces the energy requirements along with waste minimization. In this paper, various process parameters were optimized for cost-effective enzyme production by *P. citrinum* NCIM-1398. The enzyme obtained was studied for its potential application in the bio-deinking of MOW paper.

The aim of the present study is to isolate and optimize process parameters for enzyme production by *P. citrinum* NCIM-1398. Crude enzyme obtained from *P. citrinum* NCIM-1398 was studied for its application in bio- deinking of MOW paper. In present study the use of wheat bran as the substrate significantly reduces the enzyme production cost.

EXPERIMENTAL

Isolation and Identification of Microorganism

A total of 157 fungal cultures were isolated from various samples including degrading lignocellulosic materials, soil, and cow dung manure. Samples were collected from Ichhapur- West Bengal, Hauz Khas- Delhi, Dehradun—Uttarakhand, Saharanpur- Uttar Pradesh, Port Blair and Baratang Islands- Andaman and Nicobar Islands, India. Isolates were further screened for potent endo β-1,4-glucanase, xylanase, and amylase production *via* zone clearance assays (Teather and Wood 1982; Deb *et al.* 2013). Scanning electron microscopy (SEM; TESCAN, model MIRA 3 LMH, Libusia, Czech Republic) was used for the detailed morphological studies of the selected strains. The Basic Local Alignment Search Tool (BLAST) and gene sequencing by fungal specific ITS r-RNA were used for molecular identification, and the sequences were submitted to the National Collection of Industrial Microorganisms (NCIM), Pune, Maharashtra, India. The fungal culture was identified as *Penicillium citrinum* and submitted with accession number NCIM-1398.

Agro-industrial Waste as Carbon Source

Various agro-industrial wastes including sugarcane bagasse, rice straw, wheat straw, rice husk, wheat bran, and corn cob were used as substrates for enzyme production. All selected agro-industrial wastes were washed thoroughly, dried, and ground into

particles ranging in size between 250 and 1400 µm.

Enzyme Production

First, 15 mL of nutrient salt solution (NSS) prepared at pH 5 was used to soak 5 g of wheat bran, taken in flasks, and autoclaved for 15 min at 15 psi. The NSS was composed of 4.0 g of NH_4Cl, 1.5 g of KH_2PO_4, 1.0 g of yeast extract, 0.5 g of KCl, and 0.5 g of $MgSO_4$ dissolved per liter of distilled water, supplemented with 0.04 mL/L of trace element solution composed of µg/L as 20 µg/L $MnSO_4 \cdot 7H_2O$, 180 µg/L $ZnSO_4.7\ H_2O$, and 200 µg/L $FeSO_4 \cdot 7\ H_2O$ at pH 6 (Singh and Garg 1996). Four discs each of 5 mm diameter were aseptically cut from actively growing fungal culture from plates of wheat bran agar and used for inoculation. Inoculated flasks were incubated at 30 ºC for 5 days. For crude enzyme extraction, the fermented contents of the flask were crushed in 25 mL of distilled water followed by shaking for 60 min at 150 rpm on a shaker incubator.

Enzyme Assay

Standard methods approved by International Union of Pure and Applied Chemistry (IUPAC) were used in determining activities of endo β-1,4-glucanase and FPase. One filter paper unit is defined as the amount of enzyme that liberates 1µmol glucose per mL per min under assay conditions (Ghosh *et al.* 1987). Similarly, one unit of endo β-1,4-glucanase activity is defined as the amount of enzyme that release 1 µmol of glucose under assay conditions. Xylanase activity was determined according to Bailey *et al.* (1992). The amount of reducing sugars released from 1% (w/v) of beech wood xylan (Sigma, St. Louis, MO, USA) was estimated to determine xylanase activity. One unit xylanase activity is defined as the amount of enzyme that release 1 µmol of xylose per ml per min under reaction conditions (Bailey *et al.*1992). Amylase activity was analyzed using DNS method (Shaik *et al.* 2017). Enzyme activities were expressed as activity unit per mass of initial dry solid substrates (gds). DNS method was used for estimation of reducing sugar.

Optimization of various process parameters for enzyme production

The effect of various parameters such as carbon source, nitrogen sources, temperature, pH, moisture content, incubation period, and surfactant were studied using a one factor at a time (OFAT) approach. Enzyme dose optimization for enzymatic deinking is utmost important to provide suitable conditions for enzyme to work most efficiently.

Bio-deinking of Mixed Office Waste (MOW) paper

Manually torn MOW paper with a size of 1.5 to 2.0 cm^2 was soaked in water at 50 °C for 30 min. Pulping was performed in a hydrapulper at 60 °C and 650 rpm for 20 min. An enzyme cocktail extracted from *P. citrinum* NCIM-1398 was used for the deinking of pulp produced under optimum pulping conditions (Table 1). MOW pulp was treated with 2 IU to 10 IU/g of endo β-1,4-glucanase dosing. The dosing of xylanase and amylase present in crude enzyme varied according to endo β-1,4-glucanase activity. Enzymatic treatment of MOW pulp was carried out by maintaining 10% pulp consistency, 0.1% polyoxyethylene sorbitan monooleate surfactant (Tween 80) dose, pH 5.5 ± 2, temperature 55 ± 2 °C, and reaction time 60 min. Thereafter the pulp was subjected to ink flotation for 10 min in a flotation cell, where 1% pulp consistency, pH 7.2 ± 2, and temperature 35 ± 2 °C was maintained. The pulp was washed in tap water before the evaluation of bio-deinking efficacy of enzyme treatment.

Pulp brightness was determined according to TAPPI T452 om-02 (2007) (Stevenson *et al.* 2012). Effective residual ink concentration (ERIC) was also determined by infra-red reflectance measurement.

Pulp pads were prepared for evaluation of physical properties sheets (TAPPI T 218 sp-02, 2007) and then subjected for evaluation of tensile index (TAPPI T494 om-01, 2007), burst index (TAPPI T 403 om-97, 2007), double fold (TAPPI T 423 cm-98, 2007), tear index (TAPPI T 414 om-98, 2007).

To evaluate dirt count, laboratory-made hand sheets were prepared (TAPPI T 213 om-01, 2007). The deinkability factors D_E (deinkability based on ERIC) and D_B (deinkability based on brightness) were evaluated (Dutt *et al.* 2012).

Statistical analysis

All experiments were carried out in triplicate and experimental results were represented as the mean ± standard deviation of values.

RESULTS AND DISCUSSION

Enzyme Production

Of the 157 isolates examined, *P. citrinum* NCIM-1398 was the most potent producer of endo β-1,4-glucanase, xylanase, and amylase. The SSF of wheat bran was carried out at 30 ± 2 °C, initial pH 5.0, and initial moisture content of 75% for 5 days incubation with *P. citrinum* NCIM-1398. Initially, *P. citrinum* NCIM-1398 was found to produce endo β-1,4-glucanase 11.16 IU/gds, FPase 1.53 IU/gds, xylanase 2178.58 IU/gds, and amylase 52.32 IU/gds.

Optimization of Various Parameters for Enzyme Production

Effect of carbon source

Wheat bran as a carbon source exhibited the highest endo β-1,4-glucanase activity of 11.2 IU/gds (Fig. 1). Likewise, the maximum xylanase activity of 2180 IU/gds and 1.53 IU/gds FPase was observed in wheat bran (Fig.1). Solid substrates provide a base for the growth of microbes and space between substrate particles, provide aeration during SSF (Bharti *et al*. 2018).

Wheat bran consists of starch (10 to 20%), non-starch polysaccharides (41 to 60%), and protein (15 to 20%) along with vitamins and minerals (Stevenson *et al.* 2012). Its high nutritive value, particle size suitability, and good porosity provide better anchorage and ease of enzyme excretion (Kar *et al.* 2013). These virtues make it a good choice of substrate for enzyme production.

Other researchers have reported the potential of wheat bran as a suitable substrate for enzyme production (Gawande and Kamat 1999; Chandra *et al.* 2007; Kavya and Padmavathi 2009).

Dutta *et al.* (2007) found 2.04 IU/mL of endo β-1,4-glucanase activity and 0.64 IU/mL of FPase by *P. citrinum* when wheat bran was used as the sole carbon source. Muthukrishnan (2017) also reported that the optimum carbon source for the cellulase production (91 U/mL) is wheat bran.

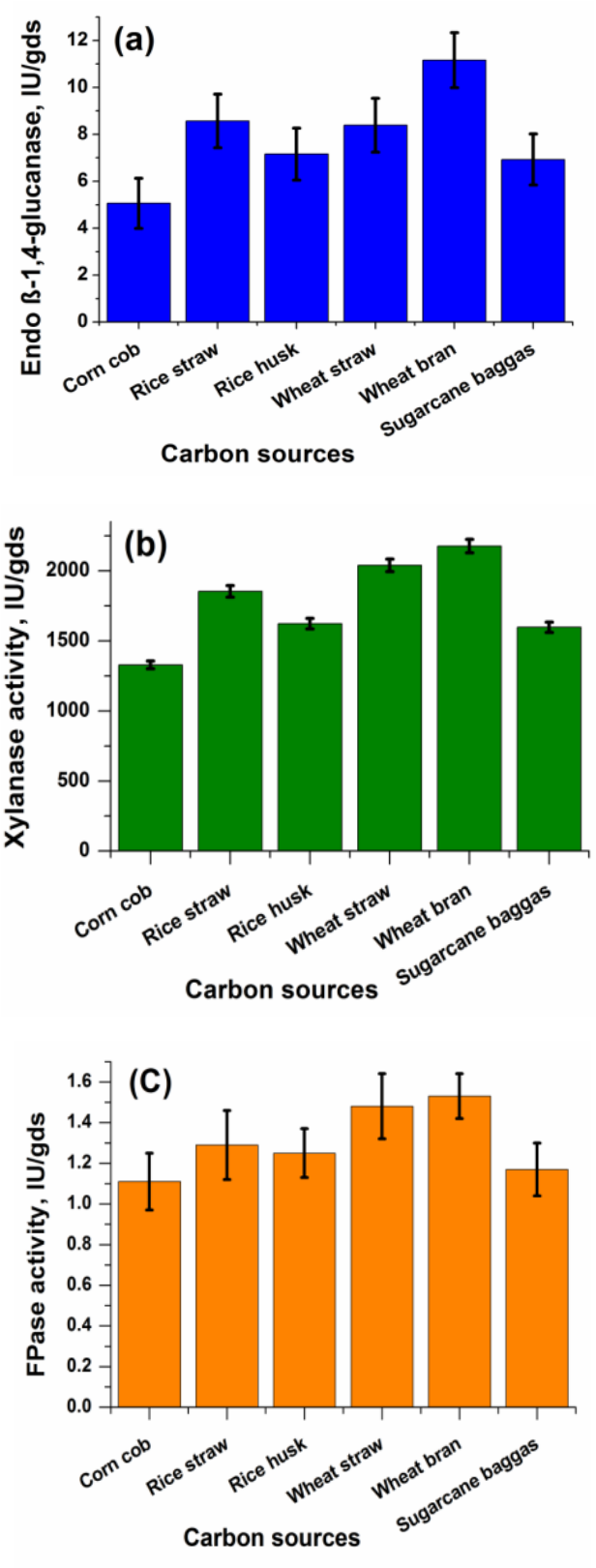

Fig. 1. Effect of carbon sources on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds), (b) Xylanase activity (IU/gds), (c) FPase activity (FPU/gds)

Effect of temperature

The present study revealed the maximum cellulose activity (11.23 IU/gds), xylanase activity (2186 IU/gds), and FPase activity (1.91 FPU/gds) at 30 °C (Fig. 2). Temperature changes above or below 30 °C led to decreased enzyme activities. Temperature plays a vital role in enzyme production. Fungi grow well in the temperature range of 25 °C to 32 °C (Chandra 2016; Bharti *et al.* 2018). However, temperature variation from optimum results in growth deficits, and thus it deteriorates enzyme production. Gautam *et al.* (2015) and Kavya and Padmavathi (2009) reported that higher temperature leads to thermal denaturation of metabolic enzymes, which results in poor enzyme production by microorganisms. Saha and Ghosh (2014) reported the optimum temperature (30 °C) resulting in enhanced xylanase production to 2834 IU/mL by *P. citrinum*. Montani *et al.* (1988) also reported 30 °C as optimum temperature for endo β-1,4-glucanase and xylanase production for *P. citrinum*. Moreover, Bharti *et al*. (2018) reported that at 30 °C *Talaromyces stipitatus* MTCC 12687 produced the maximum β-glucosidase (62.6 IU/gds), FPase (4.51 FPU/gds), and endoglucanase (53.3 IU/gds). Other fungi, namely *Aspergillus niger* (Mrudula and Murugammal 2011), *Aspergillus flavus* (Gautam *et al.* 2015) *Penicillium oxalicum* (Dwivedi *et al.* 2011), show 30 °C as the optimum temperature for their growth.

Effect of incubation time

This study found that various enzyme activities of *P. citrinum* NCIM-1398 increased gradually and achieved maximum endo β-1,4-glucanase activity (13.24 IU/gds), xylanase activity (2245 IU/gds), and FPase activity (2.31 FPU/gds) at day 7 of incubation (Fig. 3), and after that, these activities slowly decreased (Fig. 3). Kar *et al.* (2013) and Kavya and Padmavathi (2009) stated that this reduction in the enzyme activity might be because of diminution of nutrients and production of toxic compounds in the fermentation medium which hinders the fungal growth and enzyme production. Similarly, Dutta *et al.* (2008) reported that *P. citrinum* on utilization of wheat bran as substrate, produced 1.89 ± 0.12 IU/mL endoglucanase after 168 h (7days) of incubation time.

Effect of initial pH

Production of enzyme is greatly affected by the pH of SSF. At pH 5.5, the maximum endo β-1,4-glucanase (14.15 IU/gds) and FPase (2.45 FPU/gds) activities were obtained, whereas maximum xylanase (2475.36 IU/gds) activities were obtained at pH 6 (Fig. 4). The fungus grows well and efficiently produces enzyme at moderately acidic range of pH 5.5 to 6.0 (Fig. 4). During SSF pH maintenance is difficult due to the lack of mixing. Solid substrate also has a buffering effect in the system (Raimbault 1998; Bharti *et al.* 2018). Production or assimilation of organic acid by micro-organisms during SSF may change the pH of the medium (Vandenberghe *et al.* 1999). Beguin and Aubert (1992) reported that fungi prefer a slightly acidic medium. Muthukrishnan (2017) reported the production of 71 U/mL of endo β-1,4-glucanase at pH 5.5 by *P. citrinum*. Oyedeji and Ojekunle (2018) reported that pH 6.0, a slightly acidic environment, is optimum for the production of endo β-1,4-glucanase by *P. citrinum*.

Effect of initial moisture content

Fungal culture grows over and around the solid substrate matrix during SSF. Different microorganisms have different moisture requirements for their optimal growth.

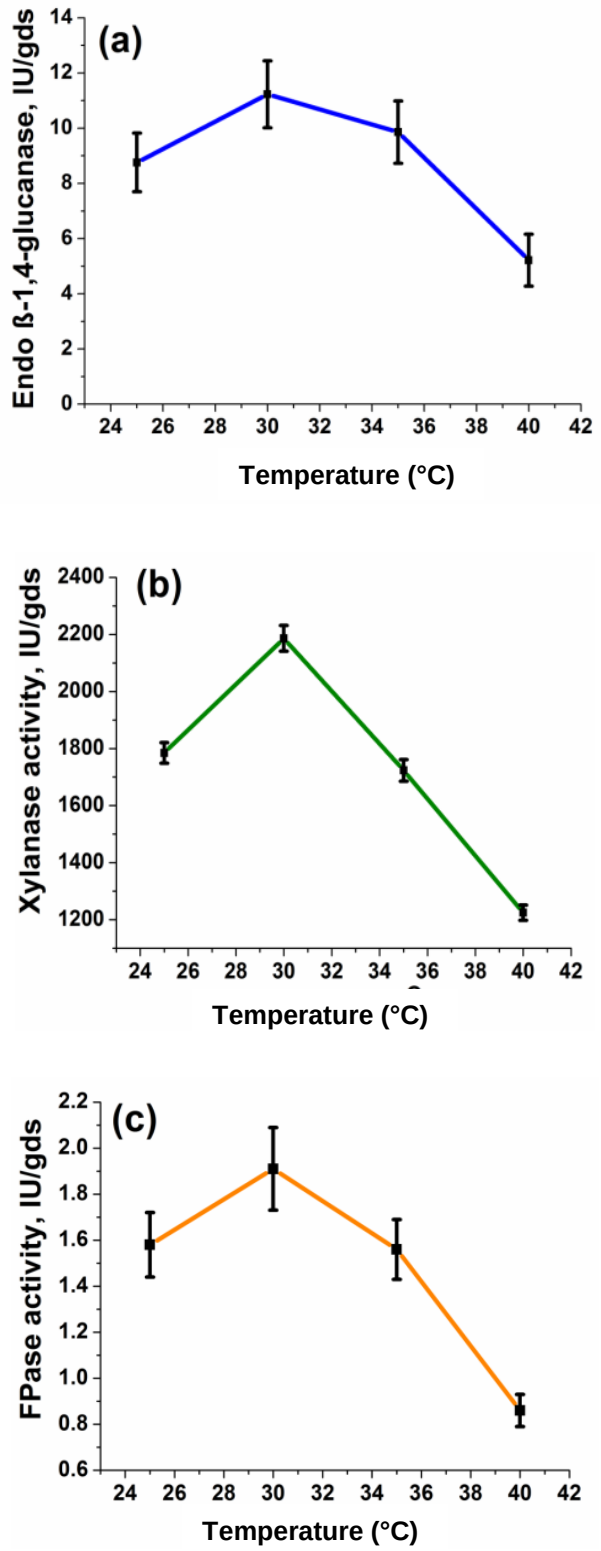

Fig. 2. Effect of temperature (ºC) during incubation on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds) (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

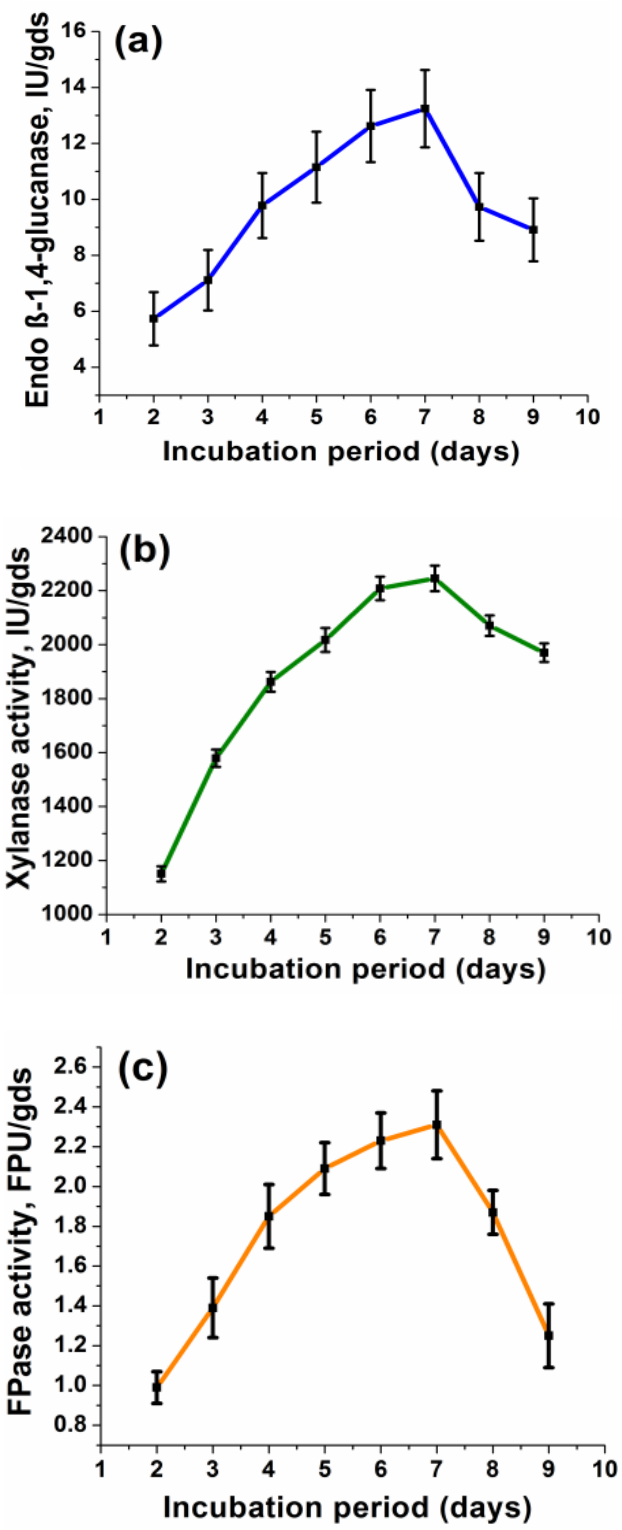

Fig. 3. Effect of incubation period on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds), (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

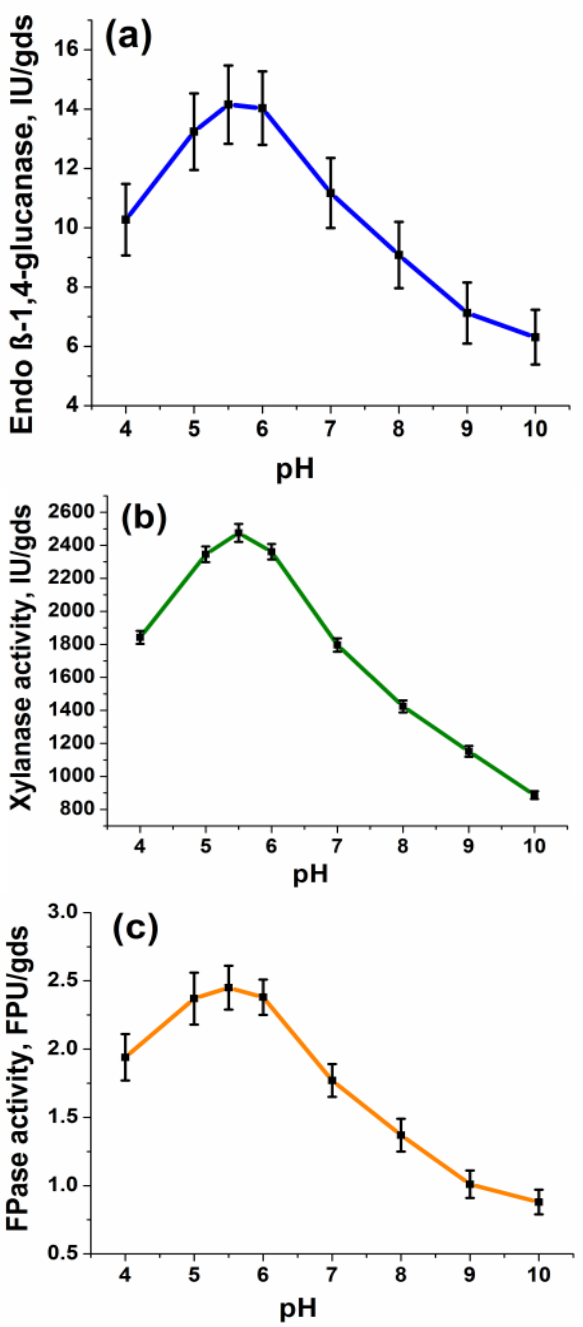

Fig. 4. Effect of pH on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds) (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

This study recorded the maximum endo β-1,4-glucanase (17.82 IU/gds), xylanase (2651.97 IU/gds), and FPase (3.23 FPU/gds) activities at 70% initial moisture content (Fig. 5). A small variation in moisture content from the optimum value drastically reduced the enzyme activity. It is well documented that an increase in the moisture content from its optimum value leads to lack of pores available for oxygen supply for growth of fungal culture and thus causes declines in the enzyme production. However, moisture content that is lower than the optimum value also hampered the enzyme production because insufficient moisture content inhibits substrate to swell properly which, in turn, prevents the transfer of

nutrients. Gautam *et al.* (2018) reported similar results, where a maximum endoglucanase production of 6720 IU/gds was found at 70% moisture content. Moisture content in the range of 55 to 70% increased enzyme production with the increase in moisture content. Even poor xylanase production was reported on further increases in moisture content (beyond 70%). Bharti *et al.* (2018) also reported that 70% moisture content was optimum for growth of *Talaromyces stipitatus* MTCC 12687.

Effect of nitrogen source

Among the various nitrogen sources used in the study, ammonium sulphate expressed the maximum activities of endo β-1,4-glucanase (15.62 IU/gds), xylanase activity (2460 IU/gds), and FPase (2.97 IU/gds) (Fig. 6). Ammonium sulphate was an effective nitrogen source for enzyme production (Fig. 6).

Nitrogen is the building block of enzymes (proteins) and is the major constituent of protoplasm. Stimulation of endoglucanase activity by ammonium salt may be due to the direct entry of ammonium salt in protein synthesis (Vyas *et al.* 2005), whereas sulphate is required for the synthesis of important biomolecule such as amino acids. Vyas *et al.* (2005) reported identical results where ammonium sulphate was found optimum for endo β-1,4-glucanase (2.833 IU/mL) and exoglucanase activity (0.282 FPU/mL) production by *Aspergillus terreus* AV49. *A. nidulence* and *P. citrinum* also use ammonium sulphate as the optimum nitrogen source (Kumar *et al*. 2016; Muthukrishnan 2017).

Effect of surfactants

Among all the employed surfactants (0.1%), the maximum activity of endo β-1,4-glucanase (21.0 IU/gds), xylanase (3140 IU/gds), FPase (3.59 FPU/gds), and amylase 73.4 IU/gds was induced by Tween 80. The primary role of surfactant is to boost the release of enzyme from the solid substrate. The cell membrane permeability is improved by the surfactant, which results in better water penetration into the substrate. The surface area of the substrate increases, which also stimulates the microbial growth during SSF (Vu *et al.* 2011).

Additionally, surfactant also increase microbial cell membrane permeability, which ultimately results into release of more enzymes in the medium Bharti *et al.* (2018). Dutta *et al.* (2008) reported that both ionic and nonionic surfactants induce stimulatory effect on enzyme activity of *Penicillium citrinum*. Bharti *et al.* (2018) reported that 0.10% (w/v) Tween-80 induced the maximum endo β-1,4-glucanase (66.8IU/gds) production by *Talaromyces stipitatus* MTCC 1268. Dutta and Kumar (2014) and Kumar *et al.* (2016) also reported Tween-80 as the better surfactant for achieving maximum enzyme activity for *Aspergillus sp*.

Bio-Deinking of Mixed Office Waste (Mow) Paper

MOW pulp deinked with crude enzyme having endo β-1,4-glucanase (6 IU/g), xylanase (876 IU/g), and amylase (26.5 IU/g) from *P. citrinum* NCIM-1398 improved pulp brightness by 9.54%, compared with MOW paper after pulping. Similarly, ERIC value of the enzymatically treated pulp was mitigated by 35.4% compared to MOW after pulping and dirt count from 4880 to 1360 ppm. The deinkability based on brightness (D_B) improved by 42.0%, whereas deinkability based on ERIC (D_E) improved by 27.1% compared to the respective control.

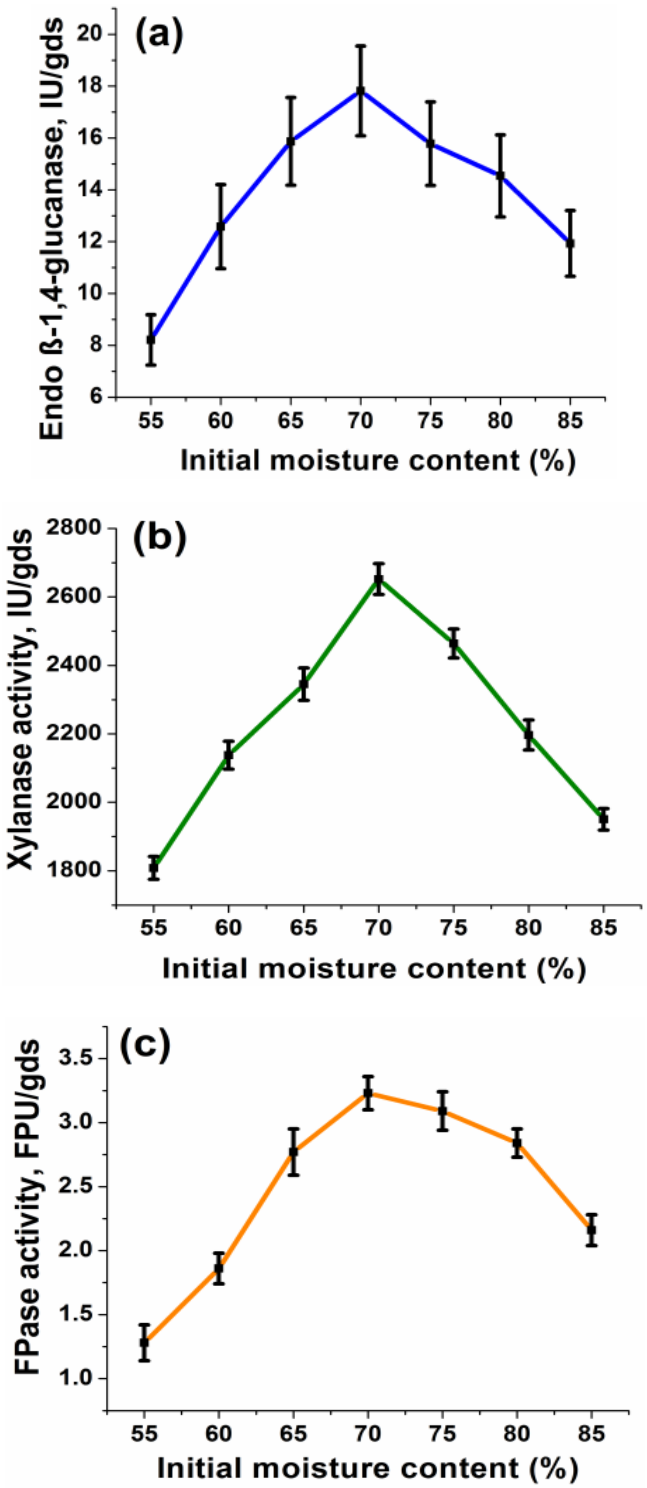

Fig. 5. Effect of moisture content on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds), (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

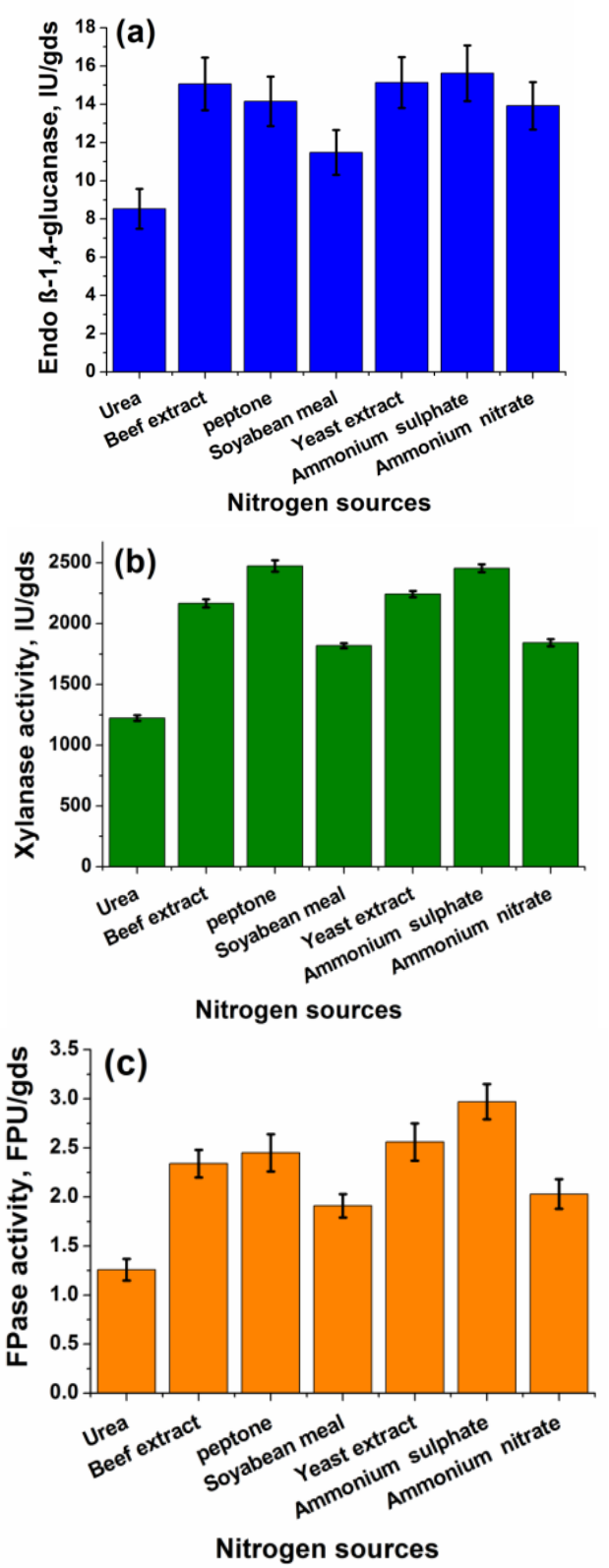

Fig. 6. Effect of nitrogen sources on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds), (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

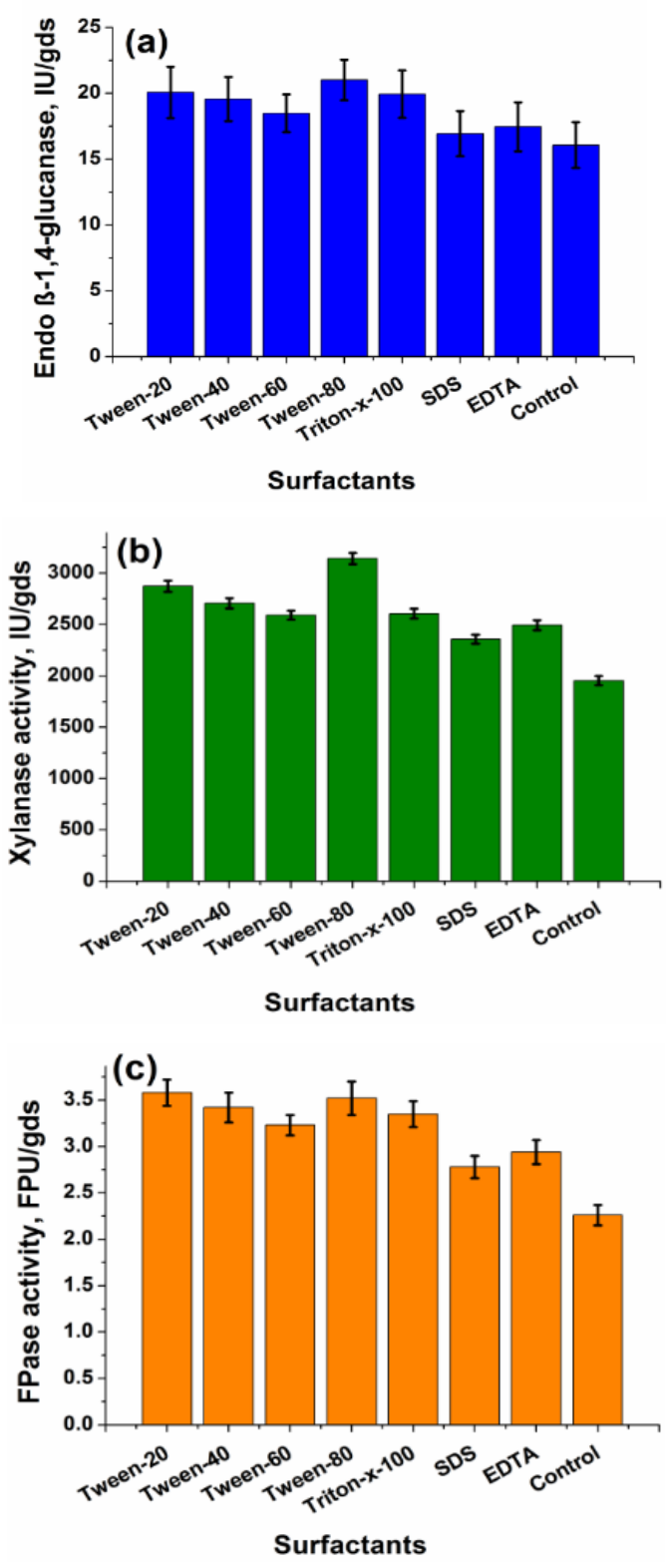

Fig. 7. Effect of surfactants on enzyme production by *P. citrinum* NCIM-1398. (a) Endo β-1,4-glucanase activity (IU/gds) (b) Xylanase activity (IU/gds) (c) FPase activity (FPU/gds)

Table 1. Effect of Enzyme Dose during Enzymatic Deinking of MOW Paper by *P. citrinum* NCIM-1398

Particulars	Result after pulping*					
Pulp brightness, % (ISO)	73.66±1.6					
ERIC, ppm	267.57±1.86					
	Enzyme treatment **					
Enzyme dose, IU/g	**0**	**2**	**4**	**6**	**8**	**10**
	Result after ink flotation ***					
Pulp yield, %	84.10±1.38	81.90±1.70	79.70±1.36	**78.05±1.20**	76.±1.30	75.11±1.18
Pulp brightness, % (ISO)	75.42±1.32	78.89±1.38	80.87±1.43	**83.20±1.16**	83.36±1.64	83.47±1.87
% Increase/Decrease (Compared to MOW after pulping)	+1.76	+5.23	+7.21	**+9.54**	+9.70	+9.81
Deinkability (DB), %	9.51±0.14	28.25±0.38	38.95±0.45	**51.54±0.68**	52.40±0.61	53.00±0.68
% Increase/Decrease	–	+18.74	+29.44	**+42.03**	+42.89	+43.49
ERIC, ppm	234.33±1.38	215.40±1.12	195.09±1.15	**172.72±1.11**	170.92±1.17	169.73±1.13
% Increase/Decrease	–	-8.07	-16.74	**-26.29**	-27.06	-27.56
Deinkability (DE), %	14.63±0.21	22.96±0.37	31.90±0.33	**41.75±0.48**	42.54±0.46	43.06±0.59
% Increase/Decrease	–	+8.33	+17.27	**+27.12**	+27.91	+28.43
Dirt count, mm^2/m^2	4879.94±21	2797.09±19	2003.66±15	**1356.03±14**	1291.95±22	1025.77±17
% Increase/Decrease	–	-42.68	-58.94	**-72.21**	-73.52	-78.97
CSF (ml)	680±3.00	690±4.00	720±5.00	**740±4.00**	745±3.00	745±3.00
Tensile index, Nm/g	28.56±0.86	29.85±0.94	31.35±0.91	**31.81±0.50**	31.61±0.79	31.06±0.86
Tear index, mNm^2/g	5.38±0.79	5.50±0.20	5.62±0.43	**5.78±0.15**	5.83±0.56	5.87±0.79
Burst index, $kPam^2/g$	1.97±0.21	2.03±0.17	2.08±0.20	**2.12±0.13**	1.08±0.14	1.03±0.21
Double fold, numbers	8	8	7	**7**	5	5

Note: ± refers to standard deviation

***Pulping conditions:**
Pulping time, min = 20
Temperature, °C = 65±2
pH = 7.2±2
Surfactant (Tween-80) dose, % = 0.05

****Enzymatic Treatment:**
Endo β-1,4-glucanase, IU/g = Varied
Xylanase, IU/g = Varied
Amylase, IU/g = Varied
pH = 5.3±2
Consistency, % = 10
Temperature, °C = 55±2
Reaction time, min = 60
Surfactant (Tween 80) dose, % = 0.1

*****Flotation conditions:**
Flotation time, min = 10
Consistency, % = 1.0
Temperature, °C = 35±2
pH = 7.2±2

The increase in optical properties after enzymatic deinking was attributed to enzymatic action in reduction of residual ink specks. Lee *et al.* (2000) explained that the basic function of cellulase is superficial degradation and hydrolysis of cellulose, which facilitates ink removal. Thus, the surface alteration, due to cellulase activity on pulp fiber, facilitates ink removal during pulping. Xylanase has a tendency to adsorb at the polymeric toner surface. This is due to the interaction of the toner particles with the xylanase chain, where the driving force for this event is an attractive interaction between hydrophobic surfaces. In contrast, the hydrophilic portions of xylanase extend from the toner surface and support the dispersion power of enzyme onto the toner particles. This facilitates the dispersion of the toner particles during washing of pulp (Yehia and Reheem 2012). Starch is widely used as sizing agent and wet end additives in the MOW paper; its degradation by amylase is likely to help cellulase-assisted MOW paper deinking. The fact that the application of crude enzyme cocktail was found to be advantageous in deinking of white MOW paper may be due to the synergistic effect of β-1,4-glucanase, xylanase, and amylase. Dutt *et al.* (2012) reported that when sorted office waste paper (SOP) was deinked with a concoction of enzymes (cellulase, xylanase, amylase, and lipase) at a dosage of 6, 3, 1.5, and 6 IU/g of oven dried pulp, the brightness was improved by 13.3% (ISO), D_B by 37.8%, and D_E by 83.0%, whereas ERIC and dirt count has been reduced by 68.18 and 88.04%, respectively as compared with the control.

Bio-deinking improved the tear index from 5.38 to 5.78 mNm^2/g, and the tensile index from 28.6 to 31.8 Nm/g, whereas burst index was increased from 1.97 to 2.12 $kPam^2/g$ compared to MOW after pulping. The increment in tensile, tear and burst index after enzymatic deinking showed that cellulase, xylanase, and amylase enzymes actions were confined only to surface of paper fiber, which resulted into partial hydrolysis and defibrillation of fiber and thus resulted in an increase of inter fiber bonding (Jeffries *et al.* 1995). Lee *et al.* (2011) reported a 14% increase in tear index, 9.6% decrease in tear index, and 3.4% increase in burst index. Xu *et al.* (2009) reported that the enzymatically deinked pulp having cellulase and xylanase in combination gave a higher brightness, lower ERIC, and improved physical properties.

CONCLUSIONS

1. *P. citrinum* NCIM-1398 was found as a co–producer of endo β-1,4-glucanase, xylanase and amylase. By a process of optimization, enhancements in endoglucanase, xylanase FPase, and amylase titer were achieved by, respectively, 1.92, 1.44, 2.37, and 1.82 times.
2. Wheat bran showed its potential as a low price substrate for higher enzyme production by *P. citrinum*. The co-existence of these enzymes activities in cocktail was found to be advantageous in the application of deinking of MOW paper. Deinking efficiency, brightness, tensile index, burst index, and tear index were improved, whereas the tear index and dirt count were reduced after bio-deinking of MOW paper.

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