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ORIGINAL ARTICLE

Utilization of bottle gourd vegetable peel waste biomass in cellulase production by *Trichoderma reesei* and *Neurospora crassa*

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Abstract

Enormous waste has been generated from the vegetable and fruit processing industries, which are a good source of carbohydrates. Such unused remnant imposes huge disposal and severe pollution problems. Due to the presence of cellulose, hemicellulose, pectin, minerals, and vitamins, these waste materials have a great prospective for its bioconversion into useful products, viz., acids, enzymes, fuels, and value-added products. To reveal their possible potential, separate sets of experiments have been conducted by using bottle gourd peel waste biomass as a carbon source for cellulase production. It was observed from experimental findings that 30 °C temperature and 0.56 g/l of inoculum dosages are the most promising situations for cellulase production by both the fungal strains. FPase and CMCase activity considerably increases by the inclusion of whey as well as starch hydrolysates in the media used in the production study. The present study portrays the utility of bottle gourd peel waste, whey, and starch-based hydrolysates in cellulase production by *Trichoderma reesei* and *Neurospora crassa*. The exploitation of cost-effective, cheap, bottle gourd vegetable peel waste for cellulase production could be an innovative, effective, sustainable, and green approach in cellulase production.

Keywords Bottle gourd peel · Vegetable peel wastes · FPase · CMCase · Cellulases

1 Introduction

The most abundant and renewable resources in the earth for the production of and value-added chemicals and biofuels are lignocellulosic biomass [39]. Agro-industrial waste materials are made up of complex polysaccharide that fortifies the growth and development of industrially important microbes. During the agriculture raw material processing for food, a bulk quantity of agro solid wastes was generated [7, 22, 37]. Economical and efficient depolymerization of lignocellulosic biomass is the basic necessity for large-scale production of biomass originated fuels and chemicals [34]. Due to awareness of the health welfares of fruit and vegetable, the demand for fruits and vegetables has increased considerably. Sound knowledge about the physicochemical properties of lignocellulosic derived biomass and further analytical characterization for those properties plays a vital role in the effective biomass conversion technology [7].

Bottle gourd is an annual climbing vine that belongs to cucurbitaceae family and also known by other names as calabash, lauki, trumpet gourd, calebassier, cojombro, guiro amargo, talayag, gucuzzi, and zucca melon [13]. This plant contains triterpenoid, Cucurbitacins, antioxidants, flavones, C-glycosides, ß-glycosides, vit C, thiamin, riboflavin, and niacin in fruits. Thus, it is a good source of vitamins, irons, and minerals as well as an excellent diet for people having digestive problems [23]. Bottle gourd fruits are conventionally used as a nutritive thing having cardioprotective, cardiotonic, purgative diuretic, and antidote to certain poisons [13]. It is also beneficial in insanity, epilepsy, and other nervous diseases.

These plants were found to retain anti-inflammatory activity, anti-diabetics, anti-hyperlipidemic, and anticancer activity [32, 35]. Bottle gourd peels are also utilized in the detection and quantification of lethal metals in industrial effluents and groundwater [1]. Bottle gourd peel based activated carbon acts as an adsorbent for the exclusion of leather dye (Direct Black 38) from aqueous solution [12]. It has also been used for the adsorption of hazardous Reactive red 195-A (RRD) and Reactive blue 222 (RBD) from aqueous solution [30]. Bottle

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gourd peel extract-based magnetic nanoparticles are also used for organic dyes degradation [33]. Behera and Gupta [6] reported the production of few edible mushroom cultures by the utilization of bottle gourd peel biomass. Waste produced after processing of fruit and vegetable is difficult to manage. Therefore, utilization of these wastes for the production of value-added chemicals not only valorizes waste biomass but also helpful in the reduction of environmental pollution [48]. Due to the importance of bottle gourd vegetables in various processes industries, effective and economical utilization of their peels is also important for complete exploitation. In the present investigation, a novel concept has been used to study the application of bottle gourd peel as a carbon and energy source for cellulase production.

Chemically and enzymatically processed starch are used in the food and pharmaceutical industry in different forms such as starch hydrolysates, glucose syrups, fructose, maltodextrin derivatives, or cyclodextrins as substitutes, mixtures, thickening agents, and fillers. In addition to that, the sugars produced can be fermented to produce value-added chemicals [4, 8, 15]. Starch is a glucose-based polymer mainly comprised of two main fractions: these are amylose and amylopectin, both having different structural properties. Amylose is highly hydrophilic, due to more number of hydroxyl groups. Amylose is a much smaller molecule than amylopectin, and it also contains alpha amylase linkage [2, 56]. Amylopectin entails of short α-1,4 linkage, linked to linear chains of glucose units and α-1,6 linkage, linked to side chains. Branched amylopectin contains both α-amylase and β-amylase linkages [3, 8]. Starch is plentiful renewable resources and used as an important feedstock for industrial applications [36]. Arrangement of starch molecules in the plant is in the form of semicrystalline granules with a unique granular size. Granular microstructure and the nanostructure of the growth rings collectively affect the enzymatic digestibility of granular starches [8, 50]. Granules of rice starch are relatively smaller (about 2 μm) than potato starch granules (up to 100 μm). Wheat starch grains are bimodal in size, smaller B-starch (15–20%) ,and the larger A-starch granules (80–85%) [25, 31]. Water-insoluble protein complex (wheat gluten) is present in the wheat endosperm. As literature suggested, the water-soluble starch hydrolysates also act as a better inducer for cellulase production. It stimulates the enzymatic system to the same extent as pure cellulose [10, 51].

Dairy industry waste (whey) is the byproduct of a cheese manufacturing process [38]. It is a severe pollutant that enforces excessive BOD of 30,000–50,000 mg/lit. Disposal of whey creates a substantial loss of possible nutrients in the form of lactose. It also promotes the process of eutrophication, causing excessive growth of microorganisms and aquatic plants [5, 27, 45]. Application of lactose (soluble carbon source) present in the whey for cellulase production consents much control on the environment, simplifies the fermentation operational process, and accelerates the cellulase production [46]. The present experimental work illustrates the effectiveness of innovative and cheap bottle gourd peel waste as an energy source for cellulase production, along with the utilization of dairy industry waste (whey) as well as starch hydrolysates on its production by *Trichoderma reesei* and *Neurospora crassa*.

2 Materials and methods

2.1 Materials

Chemicals, biochemicals, and reagents consumed to execute the present work were of Himedia, Sigma-Aldrich, and Merck make. *Trichoderma reesei* NCIM 1186 and *Neurospora crassa* NCIM 1021 were acquired from the National Chemical Laboratory (NCL) Pune, India. Whey was procured from the local dairy industry, whereas the local vegetable market was a center for the collection of bottle gourd.

2.2 Methods

2.2.1 Preparation of raw material

After peeling off the bottle gourd, the peel was dried, ground, and further sieved with a mesh screen. The ground raw material (850 μm) was used as a solid bed for cellulase production analysis.

2.2.2 Estimation of holocellulose and lignin content in bottle gourd Peel waste

Holocellulose and acid-insoluble lignin in raw materials were assessed by the TM1-A-9 and TM1-A-7 test method, respectively, as stated in the laboratory guide of Central Pulp and Paper Research Institute (CPPRI) Saharanpur, U.P., India [24].

2.2.3 Determination of ash and moisture content

Ash and moisture content were determined by the prescribed methods, as stated in the laboratory guide of Central Pulp and Paper Research Institute (CPPRI) Saharanpur, U.P., India [24].

2.2.4 FTIR/XRD/SEM analysis of bottle gourd Peel

Nicolet 6000 spectrophotometer was used to carry out Fourier transform infrared (FTIR) spectroscopic analysis. To perform this, oven-dried samples were mixed with KBr in the proportion of 1:200 mg (raw material: KBr) and further pressed

under vacuum to form the pellets. Transmittance was quantifying over a scale from 4000 to 500 cm^{-1}.

XRD (x-ray diffraction) analysis of *Bottle gourd* peels was estimated on a Bruker AXS D8 Advance diffractometer. The samples were imaged in the range from 0 to 70° angle.

Scanning electron microscopy (SEM) was used to determine the surface properties of treated and untreated raw materials. In this study, the samples were glazed with a gold film. The samples were then investigated using scanning electron microscope model LEO-435 VP.

2.2.5 Pretreatment of starch

Acid pretreatment of starch was performed by using a 2% HCl (v/v) solution. Ten grams of wheat, rice, and potato starch powdered biomass were taken separately after that 40 mL of diluted HCl solution were added separately in each starch sample, to maintain the slurry of about 25% consistency. Afterwards, starch hydrolysates were exposed to heat treatment in a pressure of 15 psi at 121 °C for 1 h time duration. The pretreated starch hydrolysates with different volumes were consumed in the production medium.

2.3 Inoculum development

Inoculum development experiments have been performed in respective culture media by the methods previously used by Verma et al. [47].

2.3.1 Dry weight determination

Cell dry weight of microbial suspensions was determined by the procedure used by Verma et al. [47]. The determination of fungal growth by cell dry weight was expressed as the mean of three independent readings.

2.3.2 Preparation of Production Media & Solid State Fermentation

Three types of production medium were used for production studies. (I) Normal basal salt media was used for production studies having the following constituents (g/L): urea, 0.3; $(NH_4)_2SO_4$, 1.4; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.3; peptone, 1.0; Tween 80, 0.2; $FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2.2H_2O$, 0.4; $CoCl_2.6H_2O$, 0.02. (II) Whey containing production media: 15, 30, and 50% (v/v) whey was incorporated separately in the earlier described production media. (III) Whey + starch hydrolysates containing production media: 30% (v/v) whey along with 2 and 5% (v/v) of 2% HCl-treated starch hydrolysate (potato, wheat and rice starch) was incorporated in the earlier described production media.

Separate sets of batch experiment were performed in 250 mL Erlenmeyer flasks comprising sieved bottle gourd peels as the carbon source for the growth and production of organisms impregnated with the normal basic salt media. Bottle gourd peel waste bed soaked with basal salt media were autoclaved and then separately inoculated with 0.36, 0.46, 0.56, 0.66, and 0.76 (g/L) of potato dextrose (PD) broth culture solution of *Trichoderma reesei* and M_2 broth culture solution of *Neurospora crassa* for 6 days. Further in another set of experiment, production medium containing flasks was put in an incubator at 25, 27, 30, 32, and 35 °C for 6 days. To investigate the influence of various initial pH (3 to 8) of the basal salt medium, separate sets of experiments were performed at 30 °C. Another set of experiments was carried out to study the effect of whey, wheat, potato, and rice starch hydrolysate. About this 15, 30, and 50% (v/v), whey was incorporated separately in the earlier described production media and now the whey containing supplementary production media was further expended for impregnation of bottle gourd peel based solid bed. Alternatively untreated, 2% and 5% (v/v) acid-treated wheat, potato and starch hydrolysate solution were incorporated separately in 30% (v/v) whey containing basal salt media which was further used for impregnation of Bottle gourd peel solid bed. All the bottle gourd peel bed containing production flasks inoculated with culture solution to study the effect of whey, wheat, potato, and rice starch hydrolysates was placed in an incubator at 30 °C for 6 days.

2.3.3 Extraction of enzyme

Extraction of enzyme was performed by the method previously used by Verma et al. [47]. The subsequent supernatant was collected and used as a crude enzyme source. All extractions were performed in duplicate.

2.3.4 Total Cellulase (filter paper activity) and CMCase activity

Filter paper (FPA) and carboxymethyl cellulase (CMCase) activity were analyzed by the method recommended by Ghose [16].

3 Results and discussions

3.1 Characterization of bottle gourd peel waste biomass

3.1.1 Evaluation of bottle gourd peel waste biomass

To establish the major constituent of bottle gourd peel waste biomass, proximate evaluation has been executed. To resolve the appropriateness and effectiveness of waste biomass for

cellulase production, a distinct set of experiments and investigations have been performed.

It has been observed from the proximate analysis of dried bottle gourd peel waste that holocellulose (66.35 ± 4.65) component stated as major constituent followed by lignin (21.80 ± 3.38) on percentage (w/w%) basis.

3.1.2 XRD pattern of bottle gourd peel waste biomass

The crystallinity and surface area of peel based biomass are evaluated as the most important factors to interpret the structural evolution of biomass [20]. To determine the accessibility and nature of cellulose present in the bottle gourd peel waste biomass, XRD analysis has been executed.

XRD pattern of bottle gourd peel shows the lesser number of peaks with broader peak heights which proves its lower crystallinity as viewed from Fig. 1. Therefore, we can suggest that cellulose present in Bottle gourd peel is easily accessible for fungal attacks.

3.1.3 FTIR spectra of bottle gourd peel waste biomass

To identify the constituents of lignocellulosic waste materials, FTIR spectroscopy has been performed. This is a well-established analytical method for process monitoring and identifying the chemical species. It gives a total simultaneous chemical analysis of lignocellulosic waste material.

FTIR spectroscopy was used for recognizing the components of lignocellulosic biomass. The lignocellulosic constituents of bottle gourd peel could be examined from the peak existence in between 3448 cm^{-1} and 895 cm^{-1}. Several peaks were observed by FTIR spectra of bottle gourd peel (3448 cm^{-1}, 3343 cm^{-1}, 3313 cm^{-1}, 2925 cm^{-1}, 2360 cm^{-1}, 1641 cm^{-1}, 1539 cm^{-1}, 1426 cm^{-1}, 1326 cm^{-1}, 1247 cm^{-1}, 1065 cm^{-1}, and 895 cm^{-1}) as shown in Fig. 2.

Strong bands have been observed in the FTIR spectra of bottle gourd peel at 3448 cm^{-1}, 3343 cm^{-1}, and 3313 $cm^{-1}$with a higher percentage of absorbance. These bands are allied to the –OH distending vibration of hydroxyl groups present in the phenolics of lignin and aliphatic compounds [18].

FTIR spectra of bottle gourd peel at 2925 cm^{-1} shows extreme band at this region with a higher percentage of absorption, may be consigned to the (C–H) stretch band of methyl groups present in the lignin [19]. The bands in the region of 1326 cm^{-1} in bottle gourd peel spectra may be endorsed to phenolic syringyl ring C–O stretching of phenol, while bands at 1247 cm^{-1} in bottle gourd peel spectra may probably be due to C–O stretching in the acetyl and phenolic groups [44]. The peak in the spectrum near 1539 cm^{-1} in bottle gourd peel may be owing to the aromatic skeletal vibrations C=C present in the lignin.

The spectral band at 1641 cm^{-1} in Bottle gourd peel may be attributed primarily due to the C=O stretching vibration of alpha-keto carbonyl for cellulose [17, 19]. The presence of the vibrational peak at 1065 cm^{-1} in the spectra of bottle gourd peel may be due to the C–OH stretching vibration of the cellulose backbone [41]. The absorption band at 895 cm^{-1} in the spectra of bottle gourd peel may be assigned to the C–H distortion of cellulose as well as ß-glucosidic linkage between sugars [44, 54].

The FTIR spectral map of bottle gourd peel recommends that lignin and phenolic components are present in good amount in the bottle gourd peel which creates impediment to the uptake of cellulose by the fungal system even that microbes grow well under bottle gourd peel due to the presence of sizeable amount of cellulose which provides favorable conditions for fungal attack.

3.2 Bottle gourd peel used in cellulase production

Separate sets of batch experiments were performed for the cellulase production studies by *T. reesei* and *N.crassa* for

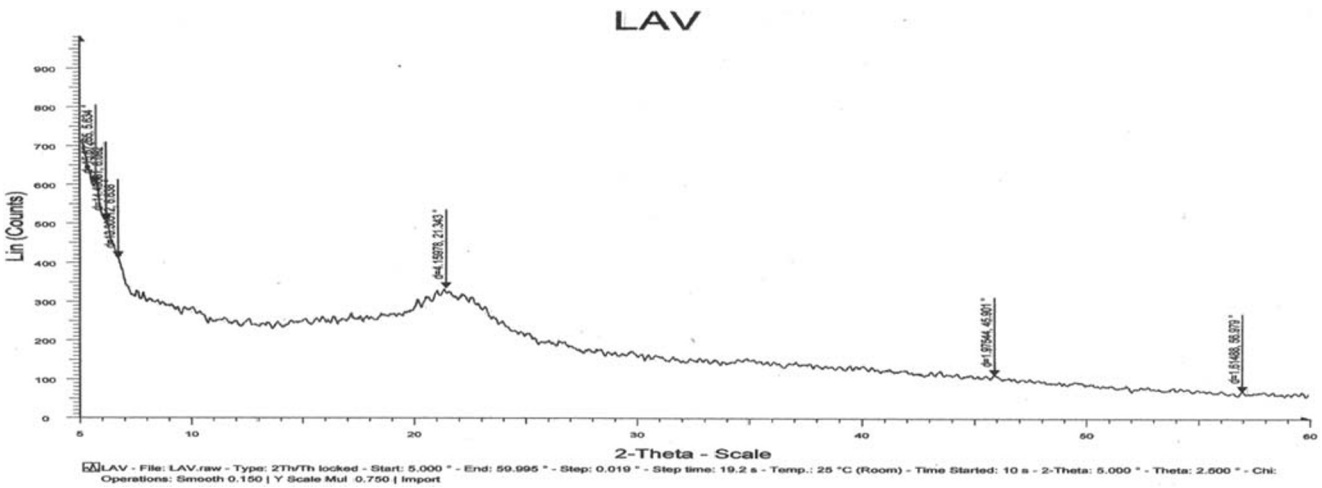

Fig. 1 XRD pattern of peel waste

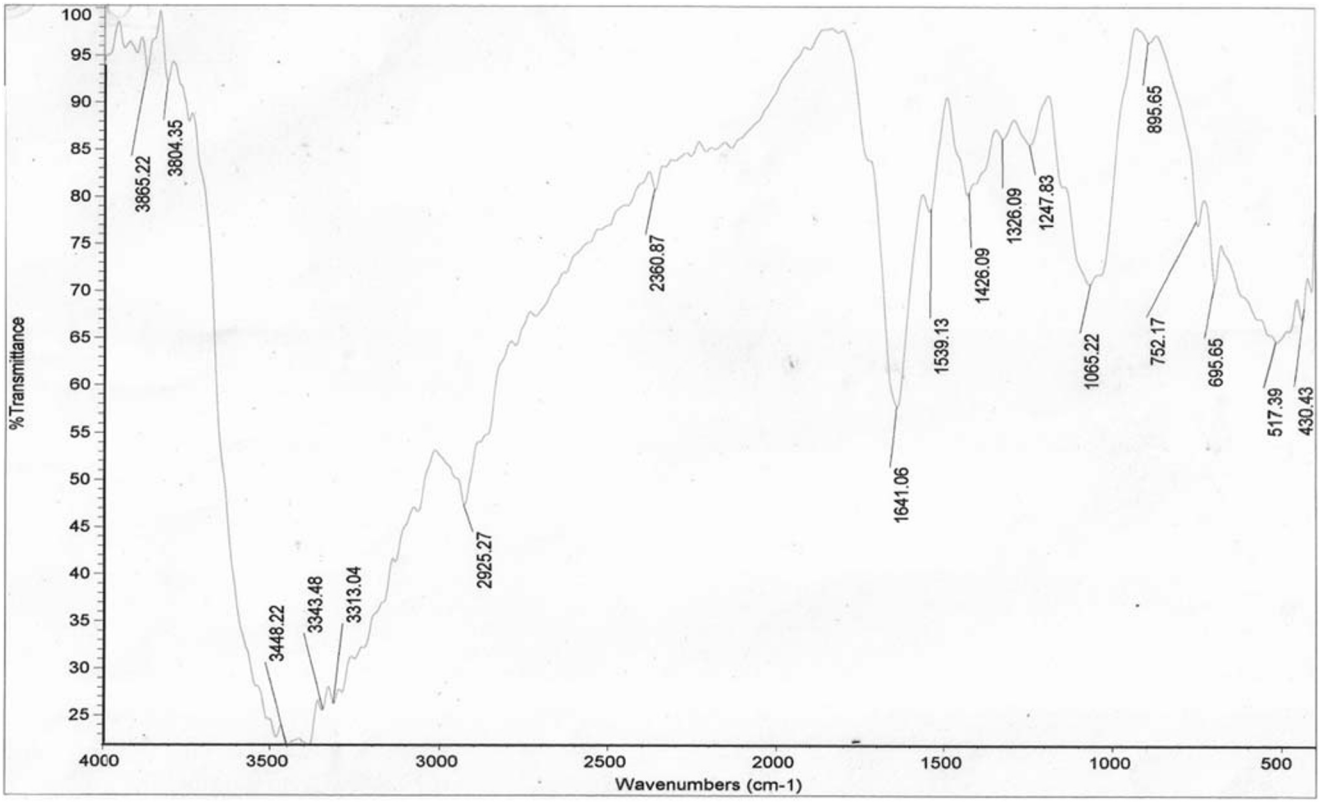

Fig. 2 FTIR spectral diagram of dried, grounded bottle gourd peel waste

utilizing bottle gourd peel as raw material for solid support. Bottle gourd peels are soft and light green as observed from Fig. 3. It was observed that *Trichoderma* grew better than *N.crassa* under bottle gourd peel-based solid bed as shown by Fig. 3d, e.

This observation evidences that *T. reesei* can grow and produce cellulases under phenolic-based raw materials. On the other hand, *N.crassa* showed lesser tolerance under such harsh conditions. During heat treatment in autoclaving disruption of raw material cell membranes as well as cell walls are observed which hydrolyzes the bonds and making more accessible the cellulosic as well as other constituents such as antioxidants and phenolics. As literature reported, *T. reesei* has the forbearance to grow in the recalcitrant pollutants of a certain level [43]. Scanning electron microscopy has proved to be a precious and invaluable tool for analyzing the growth of the fungal system [47]. To study the amplified view of untreated and microbial treated bottle gourd peel, scanning electron microscopic analysis of the desired samples has been performed.

Scanning electron microscopic analysis also proves fruitful growth of *T.reesei* as compared with *N.crassa* under bottle gourd-based solid state fermentation as shown by Fig. 4.

Fungal production of cellulases was compared under various operating parameters such as temperature, inoculum dosages, and pH. As shown in Table 1, a temperature higher or lower than 30 °C was somewhat less favorable for the production of cellulase by *T. reesei* and *N. crassa*. Under high temperature, decrement in the enzyme activity has been perceived, which might be because at higher temperature, thermal deactivation enzymes as well as microbes have been occurred [52]. At higher temperature, the hyphae appears with warped, reduced branches and thereby concentrates the cell nucleus which affect and diminish the microbial production [55], while at lower temperature, the substrates affinity for cells in microbial system is lowered, because of the thickening of lipids of the membrane and due to this the microbial production of enzyme is decreased [29]. Good cellulase activities were observed in terms of FPase and CMCase by *T.reesei* (3.38 ± 0.09 IU/mL, 3.45 ± 0.11 IU/mL) *N.crassa* (1.52 ± 0.06 IU/mL, 4.42 ± 0.13 IU/mL) under bottle gourd-based solid bed at 30 °C.

Alternatively, pH 5.0 was found to be the most suitable for cellulase production by *T.reesei* while *N.crassa* performs better at pH 6.0. pH regulates the speciation and concentrations of electron donors, acceptors, and reaction products, which in turn establish the energy yields of redox reactions [21]. Operational pH stimulates a stress response and eliciting a pH signal pathway to regulate the expression [49]. In higher or lower pH-based production medium, cellulase activity was substantially decreased. This indicates that a highly acidic or alkaline condition becomes unsuitable for fungal growth and production system.

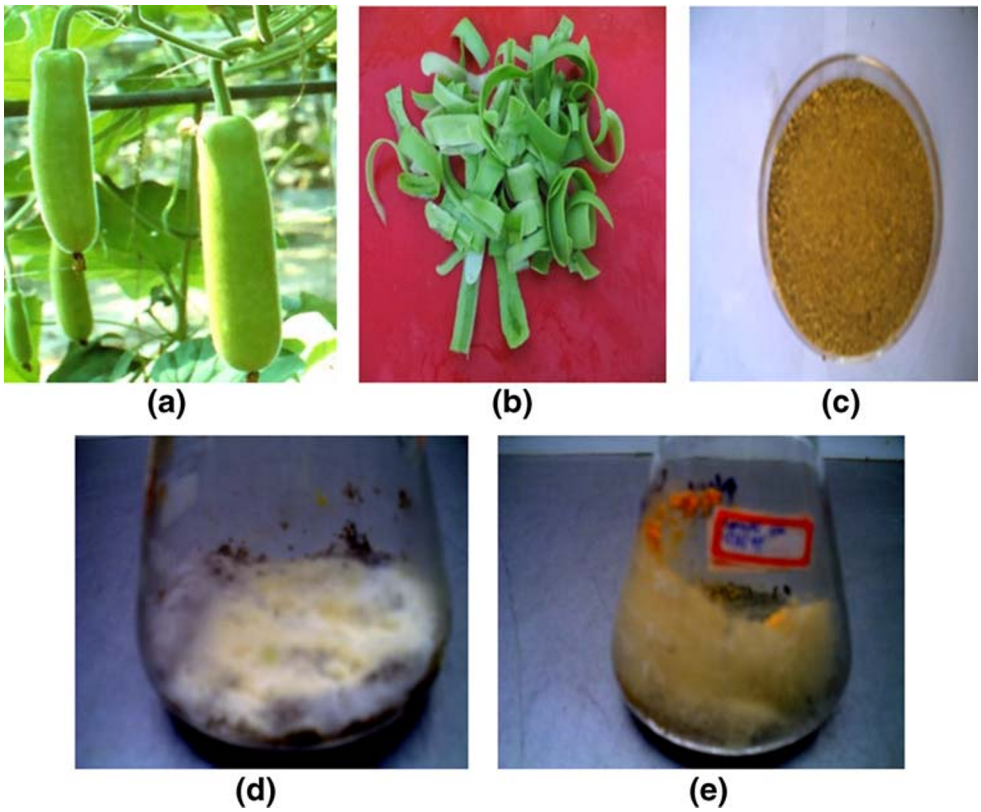

Fig. 3 Bottle gourd whole plant (**a**), bottle gourd peel (**b**), Ground, sieved, and dried bottle gourd peel (**c**),growth of *Trichoderma reesei* (**d**), and *Neurospora crassa* (**e**) on bottle gourd peel

If the pH is not suitable, microbial metabolism would be disturbed and ultimately affects fungal production system or in another word if there is no proper balance of ions, and the shape of the active site of enzyme would be distracted so that the substrate could not be bounded into the enzyme. Such conditions would favor decrement in enzyme activity [52]. Lower pH conditions endorses the dissemination of formic acid, acetic acid, and other short-chain fatty acids across the membrane, which disperses proton drive force across the membrane and deters the microbial growth [21]. An acidic pH favored cellulase production, while this was significantly decreased towards much acidic as well as neutral and slight alkaline pH, as observed from Table 1.

The size of inoculum seems to have a profound effect on microbial growth and enzyme production. Inoculum dosages of 0.56 g/l were found most appropriate dosages for cellulase production by both of the fungal strains. Inoculum size influences the utilization of carbon and nitrogen from the medium by microbial sources. Dhillon et al. [11] stated that maximum enzyme activity was analyzed using 5% inoculum. An increment in inoculum size from 5% showed a progressive decrease in enzyme activity reaching the lowest at 20% inoculums. Smaller inoculum sizes produced a transient mycelial stage, whereas a higher inoculum concentration becomes favorable, probably because of the reduction in the lag phase caused by highly concentrated inocula. Reduction in cellulase

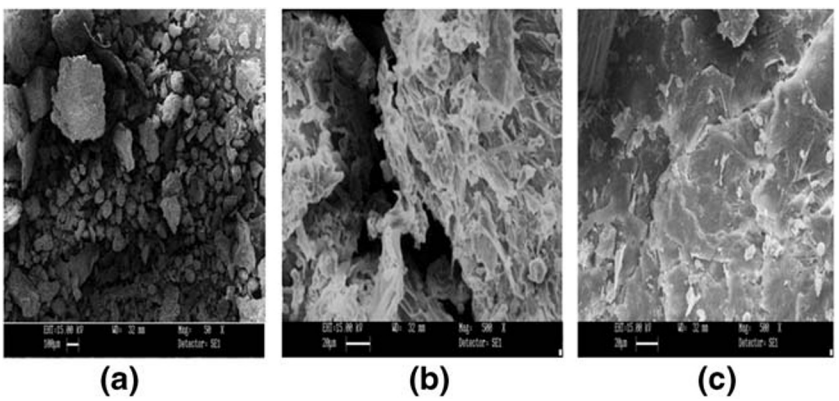

Fig. 4 SEM micrograph of untreated bottle gourd peel under × 50 magnification (**a**), *Trichoderma reesei*-treated bottle gourd peel-based solid bed under × 500 magnification (**b**), *Neurospora crassa*-treated bottle gourd peel-based solid bed under × 500 magnification (**c**)

Table 1 Cellulases activities produced by *Trichoderma reesei* and *Neurospora crassa* under bottle gourd peel-based solid state cultivation at different temperatures, pH, and inoculum dosages

	Parameters		*Trichoderma reesei* NCIM 1186		*Neurospora crassa* NCIM 1021	
			FPase(IU/mL)	CMCase(IU/mL)	FPase(IU/mL)	CMCase(IU/mL)
Bottle gourd peel waste	Temp (°C)	25	1.98 ± 0.16	2.10 ± 0.13	1.09 ± 0.13	3.49 ± 0.04
		27	2.74 ± 0.21	2.81 ± 0.20	1.21 ± 0.10	3.93 ± 0.16
		30	3.38 ± 0.09	3.45 ± 0.11	1.52 ± 0.06	4.42 ± 0.13
		32	2.96 ± 0.19	3.08 ± 0.17	1.03 ± 0.03	3.38 ± 0.11
		35	2.45 ± 0.13	2.97 ± 0.21	0.76 ± 0.19	2.97 ± 0.08
	pH	3.0	2.59 ± 0.11	2.26 ± 0.18	0.89 ± 0.05	2.86 ± 0.13
		4.0	2.83 ± 0.20	2.79 ± 0.09	1.03 ± 0.11	3.15 ± 0.06
		5.0	3.38 ± 0.09	3.45 ± 0.11	1.29 ± 0.09	3.89 ± 0.09
		6.0	3.03 ± 0.05	2.83 ± 0.19	1.52 ± 0.06	4.42 ± 0.13
		7.0	2.51 ± 0.18	2.34 ± 0.14	1.41 ± 0.03	4.05 ± 0.05
		8.0	2.07 ± 0.15	2.11 ± 0.06	1.07 ± 0.13	3.31 ± 0.17
	Inoculum dosages(g/L)	0.36	1.78 ± 0.19	1.87 ± 0.11	1.06 ± 0.11	2.93 ± 0.14
		0.46	2.54 ± 0.16	2.43 ± 0.08	1.37 ± 0.09	3.69 ± 0.03
		0.56	3.38 ± 0.09	3.45 ± 0.11	1.52 ± 0.06	4.42 ± 0.13
		0.66	3.13 ± 0.22	3.27 ± 0.17	1.29 ± 0.17	3.29 ± 0.09
		0.76	2.83 ± 0.13	2.91 ± 0.19	0.81 ± 0.04	2.78 ± 0.12

Data are reported as mean ± standard deviation based on the repeated trails

production on increasing the inoculum size could be due to competition between microorganism colonies for nutrients and probably the non-availability of nutrients for the large population limits the fungal growth [26]. Therefore, a suitable and appropriate inoculum size or dosages required for healthier fungal propagation and their enzyme production.

When compared the cellulase activities produced by *T.reesei* and *N. crassa* under bottle gourd peel-based solid state fermentation, it was observed that cellulases are produced from *T.reesei* having higher FPase activity as compared with *N.crassa*. On the other hand, cellulases produced from *N.crassa* showed higher CMCase activity as observed from Table 1.

To evaluate the effects of dairy industry waste (whey) and starch hydrolysates, separate set of experiments has been conducted by utilizing modified basal salt medium.

It was observed from Table 2 that FPase activities were enhanced by the incorporation of 30% whey in BSM. The highest increment in FPase was observed by *T. reesei* (3.80 ± 0.13 IU/mL) strain, and the least increment was observed with *N.crassa* (1.66 ± 0.08 IU/mL) on bottle gourd peel-based solid state fermentation, which suggests that *T. reesei* quite effectively utilized whey as inducer and carbon source. Morikawa et al. [28] reported that lactose may function as an inducer for cellulase formation if it is taken up in the mycelium of *T. reesei* PC-3-7. Induction capacity of whey was very low or negligible by *N.crassa* system. It has been observed from Table 2 that CMCase activities (3.58 ± 0.06; 4.58 ± 0.09) were not much enhanced by incorporation of whey in BSM. These finding insinuate that whey are not much effective inducers for CMCase in comparison to FPase activity produced by microbial system.

FPase activities were not improved by most of the untreated starch-based production systems. They imply that untreated starch having very little or nearly zero cellulase induction capability. FPase of *T.reesei* was further improved by inclusion of (2% v/v) wheat starch hydrolysate (2% HCl treated with 1 h pretreatment time) in whey based basal salt medium.

By increasing the wheat starch hydrolysate concentration (5% v/v), additional improvement in the FPase (4.45 ± 0.11 IU/mL) was observed, which suggests that wheat starch hydrolysates contain few sugars which induce the *Trichoderma reesei* cellulase production system. Growth and cellulase stimulation both take place in the hydrolyzates containing medium, apparently due to the presence of few dimeric sugars in the hydrolyzates which ultimately induces cellulase production [9]. Gao et al. [14] reported that transglycosylation products have been successfully used as the cellulase inducer by *Trichoderma reesei*. Earlier studies showed that starch itself was poor inducer for the cellulase induction, but it was declared highly operative by acid hydrolysis. This was due to the formation of reversion products, such as sophorose (disaccharide) during acid hydrolysis [42].

Table 2 Cellulase activities produced by *T. reesei* and *Neurospora crassa* on normal basal salt as well as modified basal salt media incorporated on bottle gourd peel waste-based solid bed at 30 °C

Bottle gourd peel waste		*Trichoderma reesei* NCIM 1186		*Neurospora crassa* NCIM 1021	
		FPA (IU/mL)	CMCase (IU/mL)	FPA (IU/mL)	CMCase (IU/mL)
Basal salt media (BSM)		3.38 ± 0.09	3.45 ± 0.11	1.52 ± 0.06	4.42 ± 0.13
BSM + W	Whey 15% (v/v)	3.41 ± 0.03	3.09 ± 0.10	1.55 ± 0.02	4.37 ± 0.06
	Whey 30% (v/v)	3.80 ± 0.13	3.58 ± 0.06	1.66 ± 0.08	4.58 ± 0.09
	Whey 50% (v/v)	3.69 ± 0.08	3.43 ± 0.15	1.69 ± 0.03	4.51 ± 0.05
BSM + W + WSH	W30% + UTWS 2% (v/v)	3.85 ± 0.04	3.67 ± 0.09	1.63 ± 0.06	4.56 ± 0.11
	W30% + 2% (v/v)	4.19 ± 0.17	4.01 ± 0.10	1.79 ± 0.09	4.70 ± 0.03
	W30% + 5% (v/v)	4.45 ± 0.11	4.20 ± 0.08	1.86 ± 0.14	4.81 ± 0.03
BSM + W + PSH	W30% + UTPS 2% (v/v)	3.82 ± 0.19	3.63 ± 0.20	1.65 ± 0.02	4.67 ± 0.08
	W30% + 2% (v/v)	4.10 ± 0.07	3.82 ± 0.11	2.09 ± 0.16	4.89 ± 0.05
	W30% + 5% (v/v)	4.16 ± 0.13	3.93 ± 0.08	2.28 ± 0.07	5.43 ± 0.13
BSM + W + RSH	W30% + UTRS2% (v/v)	3.84 ± 0.16	3.65 ± 0.13	1.60 ± 0.03	4.59 ± 0.11
	W30% + 2% (v/v)	4.16 ± 0.13	3.88 ± 0.19	2.01 ± 0.09	4.83 ± 0.04
	W30% + 5% (v/v)	4.37 ± 0.08	4.05 ± 0.11	2.21 ± 0.05	5.33 ± 0.17

Data are reported as mean ± standard deviation based on the repeated trails

W whey, *WSH* wheat starch hydroysate, *PSH* potato starch hydrolysate, *RSH* rice starch hydroysate, *UTWS* untreated wheat starch, *UTPS* untreated potato starch, *UTRS* untreated rice starch

Mandhania et al. [26] suggested that sophorose, cellobiose, or galactose may provoke a putative lactose permease in *T. reesei* PC-3-7 which may be helpful in the induction process. Reasonable improvement in FPase (4.37 ± 0.08 IU/mL) was also observed by *T.reesei* under (5% v/v) rice starch hydrolyzates medium containing bottle gourd peel-based solid state fermentation, while potato starch hydrolysates were found not much effective for FPase induction by *T.reesei*. No satisfactory enhancement in FPase (4.16 ± 0.13 IU/mL) was observed under potato starch hydrolysate-based production. In contrast, *N.crassa* system showed different behaviors for starch hydrolyzate-based cellulase induction. It has been observed from Table 2 that *N.crassa* showed satisfactorily improvement in the FPase activities (2.09 ± 0.16 IU/mL) (2.01 ± 0.09 IU/mL) under 2% v/v potato and rice starch hydrolyzates containing bottle gourd peel bed, respectively, comparison to FPase activity (1.79 ± 0.09 IU/mL) under wheat starch hydrolysate-based fermentation. Significant improvement in FPase activity (2.28 ± 0.07 IU/mL) was also observed by increasing potato starch hydrolysates dosages (5% v/v). These findings suggest that sugars (maltose, maltodextrins) present in the potato starch hydrolysates may induce transcriptional factors for improved cellulase activity for *Neurospora* system rather than its growth [40] [53].

It has also been observed from Table 2 that CMCase activities were satisfactorily enhanced by incorporation of acid hydrolyzed starch in BSM. Higher increment in CMCase activity (5.43 ± 0.13 IU/mL) was observed by *N.crassa* system under potato starch hydrolysatebased fermentation.

It has been observed from Table 2 that whey and acid hydrolyzed starches induce cellulase activities diversely under bottle gourd peel-based solid state fermentation. Higher cellulase activities produced by *T. reesei* in terms of FPase (4.45 ± 0.11 IU/mL) and CMCase (4.20 ± 0.08 IU/mL) were observed under whey and wheat starch hydrolysate containing bottle gourd solid bed-based fermentation.

On the other hand, higher cellulase activities produced by *N.crassa* in terms of FPase (2.28 ± 0.07 IU/mL) and CMCase (5.43 ± 0.13 IU/mL) were observed under whey and potato starch hydrolysates containing bottle gourd peel bed-based fermentation.

4 Conclusions

Vegetable waste biomass is an easily available, inexpensive, and renewable, natural resource for large-scale production of bio-energy. bottle gourd peel serves as a promising candidate for cellulase production under solid state cultivation. Satisfactory improvement in enzyme activities was observed by *Trichoderma reesei* under whey based solid support as compared with *Neurospora crassa.* Among starch hydrolysates, *Neurospora* performed better under medium supplemented with potato starch hydrolysate. Utilization of starch hydrolysates, as well as dairy industry waste in cellulase production under bottle gourd peel-based fermentation, provides a sustainable, recyclable, green, and eco-friendly approach for solid as well as liquid waste management; therefore, the

generation of renewable energy by the exploitation of vegetable wastes is gaining importance in the present scenario.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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