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VALUE-ADDED MULTIPLE PRODUCTS FROM DEINKED WASTE PAPER FOR SUSTAINABLE DEVELOPMENT THROUGH BIOTECHNOLOGICAL APPROACH

ANIL KUMAR H. S[1], GANGA GAMANA[2], SULAKSHANA PAI[3] AND MELITA RASHMI REBELLO[4]

[1,2,3,4]*Department of Biotechnology Engineering, Nitte 574 110, Udupi, Karnataka, India*

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ABSTRACT

The high cost of input energy and increased environmental concerns are paving way for the biotechnological conversion of waste biomass which is a potentially sustainable approach to develop novel bioprocess and products. Toxic chemicals like pesticides, heavy metals, dyes contaminated waste waters which remain unseen are environmentally most hazardous. Due to their high solubility in the aquatic environments; it can lead to bio-magnification over time. The present study involves preparation of deinked waste paper and its fermentation in both submerged and solid state fermentation. Further the residue of fermentation was developed as bio-adsorbent for removal of water contaminant. ***Deinking of waste paper was prepared by treating sodium hydroxide and hydrogen peroxide.*** **Dried deinked samples are used as a carbon source for submerged and solid state fermentation using** ***Aspergillus niger*** **and** ***Trichoderma viridae*****. Reducing sugar and cellulase thus produced is estimated. Removal of methylene blue and ampicillin were investigated using treated waste paper and fermented waste paper residue as bio-adsorbent. Treated waste paper columns are used to check the removal efficiency of methylene blue and ampicillin from its respective solutions. A significant reduction in methylene blue and ampicillin was observed through adsorption studies.** ***Effective biotechnological approach leads to generate multiple valuable products from a single waste resource. Future study has scope in optimizing its capacity for the scale up and reusability. The results shown in the present investigation is a promising lead for the sustainable development of solutions for the environmental problems in the most economic and eco-friendly manner.***

KEY WORDS: Bio-adsorbent, Bioproducts, Cellulase, Deinking, Fermentation, Waste paper.

INTRODUCTION

The paper waste is degradable in nature; the majority of the paper waste is either dumped or incinerated. It has been estimated that out of 2.6 million tonnes of dry recyclables generated by households per annum; paper waste contributes to 1.3 million tonnes. The recycling rate of paper and paper-based products in India was 27 %; which is much lower when compared to Germany (73 %), Sweden (69 %), Japan (60 %) and USA (49 %) (Ahluwalia and Patel, 2018). The very first step in waste paper recycling is the process of deinking. The conventional method involves the use of chemicals such as sodium hydroxide, sodium silicate, sodium carbonate, hydrogen peroxide, surfactants and so on. The effluent coming out of such deinking systems is environmentally hazardous and sometimes fails to comply with the environmental standards. The study undertaken by various researchers in this field brought out alternative solutions that involve the use of enzymes such as cellulases, hemicellulases, pectinase, lipase, esterase, α amylase, lignolytic enzymes (Pathak *et al*., 2011); laccases (Singh and Kumar, 2019); xylanases (Viesturs *et al*., 1999) etc. In the recycling sector; deinked waste paper is used for the production of paper boards, notebooks and such similar products. The problem with recycling is that it can be done only for four cycles. The cellulose

fiber becomes so weak that after the fourth cycle of recycling and it has to be discarded (Adhikari *et al*., 2008). Biotechnology can provide solution for the better utilization of the waste paper. Waste paper contains cellulose; which is one of the most abundant and renewable biopolymers found on the earth's surface (Huda *et al*., 2005). Cellulose mainly derives from agricultural, municipal solid waste and forestry residues (Hoong and Feng, 2011). A potential strategy for the effective utilization of such a source is the hydrolysis of substrate for the production of reducing sugar which is the most basic raw material for numerous bioproducts (Sukumaran *et al*., 2005). Bacteria and fungi during their growth on cellulose substrate by solid and submerged state fermentation produce cellulase (Kuhad *et al*., 2011). Fungi; particularly *Aspergillus sp*., *Penicilliumsp.* and *Trichoderma sp*. are well known efficient producers of cellulases (Jayant *et al*., 2011; Rathnan *et al*., 2015). Cellulases thus produced by these microorganisms are commercially available for various industrial and agricultural applications (Jayant *et al*., 2011). Water is an important resource and one of the basic requirements for human survival. It is an important element for geological, ecological and biological sustainability. A life without water seems unimaginable. Through millennia of continuous commercial, industrial and agricultural activities; we are currently witnessing the change in water profile. With the rapid development of industries such as textile, mining, fertilizer, pesticides, tanneries, batteries, paper and pulp, jewellery, coinage, pharma and so on; the heavy metals, dyes and antibiotics are getting mixed into the natural environment (Sarma *et al*., 2019). New components are being added which are proven to be disrupting the normal functioning of the ecology. The treatment capacity of domestic sewage in India is far below the quantity of sewage generated and only 31% of the total sewage produced in 908 cities were treated as on 2008 (Subedi *et al*., 2015). Though conventional treatments filter out dyes to some reasonable extent; antibiotics still manage to pass through the systems as the treatment plants are not designed to eliminate pharmaceutical residues. Bioadsorbents are emerging as a potential natural alternativesfor low cost targeted pollutant removal when compared to expensive chemical based systems (Gupta *et al*., 2009). Bio-adsorbents like bagasse (Saad *et al*., 2005); rice husk, coconut coir, tea leaves and cow dung, wool bre and cotton bre, chitosan (Juang and Tseng, 2008); sawdust, neem husk (Alau *et al*., 2010); silk cotton hull (Kadirvelu 2003); tuberose sticks (Alam, 2006); corn cob, barley husk (Robinson *et al*., 2002); rice straw, wheat (Abbas *et al*., 2014) orange peel, eggshell, coffee residue, coconut waste (Renu *et al*., 2017) and tamarind fruit shell (Saha, 2010) are cheap, highly effective and environmentally safe. The focus of the present study is to investigate the utilization of waste paper as a substrate to carry out a comparative study of cellulase production by solid and submerged state fermentation incorporating *Aspergillus niger* and *Trichoderma viridae.* The fermented waste paper residue that was left behind and chemically treated waste paper were used to study the removal of synthetic solutions of methylene blue and ampicillin capacity.

MATERIALS AND METHODS

The required waste paper was obtained from NMAM Institute of Technology, Nitte campus. All chemicals and reagents used were of analytical grade. Distilled water (DW) was used for the preparation of media and solutions. Sodium hydroxide (NaOH), dipotassium hydrogen phosphate (K_2HPO_4), disodium hydrogen phosphate (Na_2HPO_4), sodium hydrogen carbonate ($NaHCO_3$) and glucose ($C_6H_{12}O_6$) were obtained from Loba Chemie, hydrogen peroxide (H_2O_2), potassium chloride **(**KCl), calcium chloride ($CaCl_2$), potassium dihydrogen phosphate (KH_2PO_4), magnesium sulfate ($MgSO_4$), ammonium chloride (NH_4Cl), ammonium sulfate (NH_4SO_4) and carboxymethyl cellulose (CMC) were obtained from Merck; sodium chloride (NaCl) was obtained from Nice, ferrous sulfate ($FeSO_4$), ampicillin, peptone agar; and methylene blue were obtained from Himedia.

De Inking

Deinking was carried out as reported by (Kumar *et al*., 2016) 5 g of paper was treated with 100 mL of 2% (w/v) NaOH for 15 minutes under agitation followed by heat treatment at 70 °C for 30 minutes and 1%(v/v) hydrogen peroxide treatment with agitation for 15 minutes. Deinked paper was washed several times with water until it reached neutral pH and dried at room temperature.

Fermentation

Aspergillus niger and *Trichoderma viridae* were propagated on solidified CMC agar plate (Table 1)

to produce conidial inoculums for submerged cultivation. The propagated strains were inoculated to the cellulase production media (Table 2) for the preparation of starter culture. 100 mL of cellulase production media was prepared. Negative control with just media and two positive controls (carboxy methyl cellulose as carbon source); one each for *A.niger* and *T.viridae* were prepared. Two conical flasks with paper as carbon source and 0.05 g of CMC as an enhancer were prepared for *A. niger* and *T.viridae*. After inoculation; the flasks were incubated at room temperature for a week. Reducing sugar test (Miller, 1959) and cellulase assay (Sharrock, 1988)was carried out at the end of the incubation period. 30 g each of the deinked printed paper samples was taken in 6 glass bottles of 250 mL capacity; moistened with 100 mL of Mineral salt media (MSM) (Table 3) and were inoculated with 10 mL of *A.niger*. After inoculation, the flasks were incubated at room temperature for a week. At the end of the incubation period 100 mL of 100 mM Tris buffer was added to each of the flasks; shaken well and the filtrate was collected. The filtrate was subjected to reducing sugar test and cellulase assay.

Table 1. Cellulose agar medium for screening (Apun *et al*., 2000)

Ingredients	Composition (%)
Peptone	1
CMC	1
K_2HPO_4	0.2
$MgSO_4$	0.03
$(NH_4)_2SO_4$	0.25
Agar	1.5

Table 2. Composition of cellulase production media (Mandels, 1985)

Composition	Weight (g/100mL)
CMC	0.05
Paper	0.5
Peptone	0.075
$FeSO_4$	0.001
KH_2PO_4	0.05
$MgSO_4$	0.05

Adsorption studies

Pre-treatment of waste paper with Na_2HPO_4 was carried out as reported by Takaaki and Kenzo, 2011 with slight modifications. 30 g of printed paper and newspaper were treated with 300 mL of 5%(w/v) $NaHCO_3$. It was maintained at 70 °C for 30 minutes; followed by washing several times with tap water until pH 7 was reached. This was to ensure that the alkalinity is removed. It was later filtered and the soaked paper was kept for air drying. 2 conical flasks were taken; treated printed paper sample was added to the first one and newspaper sample to the other. After the addition of 300 mL of 5%(w/v) of Na_2HPO_4; both the samples were refluxed for 4 hours using a reflux condenser. The samples were then washed with tap water and air dried.The fermented waste paper residue was obtained by filtering the fermentation broth. The waste paper samples which were inoculated with fungus during fermentation were taken as a bio-adsorbent in this case.

Table 3. The composition of MSM media

Ingredients	Composition (g/mL)
Sodium chloride	0.8
Potassium chloride	0.8
Calcium chloride	0.1
Disodium hydrogen phosphate	2.0
Magnesium sulfate	0.2
Ferrous sulfate	0.1
Glucose	8.0
Ammonium chloride	2.0

Batch adsorption studies and analysis

0.1% methylene blue and ampicillin solutions were prepared. 2 g each of chemically treated printed and newspaper samples and 2 g of fermented waste paper residue were taken in separate columns. It was first saturated with distilled water. After saturation the columns were tested for the removal of antibiotic traces. 10 mL each of the ampicillin solution was added to the respective columns and the absorbance of the eluent was checked. 1 mg/mL ampicillin stock solution was prepared. 0.4 mL; 0.8 mL; 1.2 mL; 1.6 mL; 2 mL of the stock solution were taken in a test tube and made up to 2 mL with distilled water. The standard calibration curve was prepared from the absorbance values obtained from the UV – Visible spectrophotometer. Unknown samples were analyzed. Percentage adsorption was found out through the calculations by making use of concentration values obtained from the standard graph of ampicillin. The procedure was repeated for methylene blue. The standard calibration curve for methylene blue was prepared from the absorbance values obtained from the colorimeter. Unknown samples were analyzed. Percentage

adsorption was found out through the calculations by making use of concentration values obtained from the standard graph of methylene blue. The percentage adsorption was calculated using Eq. 2.

$$\text{Concentration adsorbed} = \text{Initial concentration} - \text{final concentration} \quad ..(1)$$

$$\text{Percentage adsorption (\%)} = \frac{\text{Concentration adsorbed}}{\text{Initial concentration}} \times 100 \quad .. (2)$$

RESULTS AND DISCUSSIONS

Fermentation

Negative control was prepared by sterilizing the cellulase production media. Positive control has the same components as cellulase production media.Paper media has the same composition as cellulase production media with the modification in the carbon source as CMC-0.05 g and deinked printed paper-0.5 g. A maximum release of 183.33 µg of reducing sugar was seen in positive control of *A. niger* when compared to 66.67 µg of paper media in submerged state fermentation (Fig. 1). Under solid state fermentation with *A. niger*; a release of 1766.67 µg of reducing sugar was seen (Fig. 2). 66.67 µg of reducing sugar was seen in positive control of *T. viridae* when compared to 33.33µg of paper media in submerged state fermentation.Under solid state fermentation with *T. viridae*; a release of 1733.33 µg of reducing sugar was seen. From the results; it is evident that both *T. viridae*and *A.niger* grows well when solid state fermentation is employed.

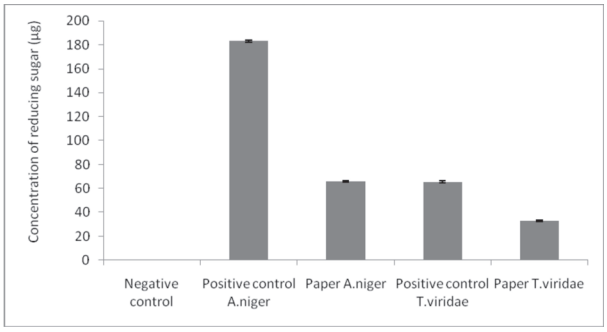

Fig. 1. Production of reducing sugar in submerged state fermentation by *A.niger* and *T.viridae*

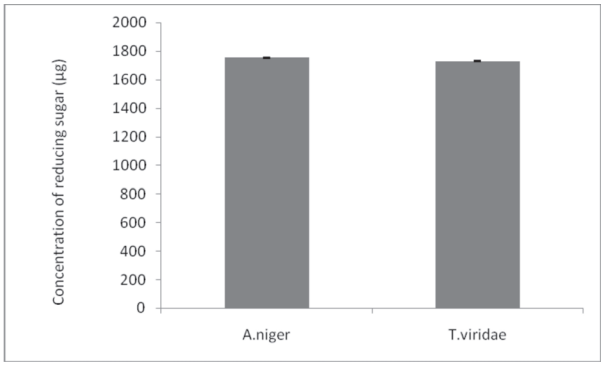

Fig. 2. Production of reducing sugar in solid state fermentation by *A.niger* and *T.viridae*

Determination of cellulase activity

Negative control was prepared by sterilizing the cellulase production media. Positive control has the same components as cellulase production media. Paper media has the same composition as cellulase production media with the modification in the carbon source as CMC-0.05 g and deinked printed paper-0.5 g. The cellulase activity of 0.01233 IU was seen in positive control of *A. niger* when compared to the cellulase activity of 0.01541 IU in paper media when submerged state fermentation was carried out (Fig. 3). Under solid state fermentation with *A. niger*; an activity of 0.02313 IU of cellulase was seen. A maximum of 0.0003 IU of cellulase activity was seen in positive control of *T. viridae* when compared to 0.00154 IU of cellulase activity of paper media in submerged state fermentation.

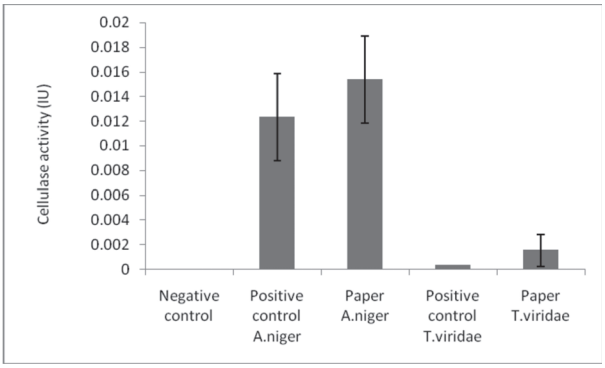

Fig. 3. Cellulase activity as seen in submerged state fermentation by *A.niger* and *T.viridae*

Adsorption analysis

Under solid state fermentation with *T. viridae*; an activity of 0.03083 IU of cellulase was seen (Fig.4). Solid state fermentation results for cellulase activity of *A. niger* and *T. viridae* were almost the same.

In all the samples taken for the adsorption studies of methylene blue; percentage adsorption decreased with the trials. This may be due to the saturation of the adsorbent with the methylene blue molecules. Untreated newspaper samples showed better results when compared to treated newspaper

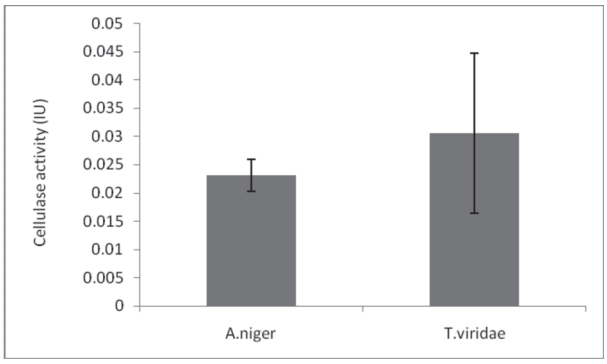

Fig. 4. Cellulase activity seen in solid state fermentation by *A. Niger* and *T.viridae*

samples. This may be due to the loss of cellulose fibers on chemical treatment. Treated printed samples showed better adsorption of methylene blue molecules when compared to untreated printed samples. This may be because of the loosening of the cellulose fibers on treatment leading to the greater exposure of adsorption sites. Fermented waste paper residue showed a greater percentage of adsorption when compared to the rest of the samples. From the Table 4; it is evident that only one trial using the adsorbents gives a satisfactory result which is acceptable as the waste paper is available in large quantities. For the adsorption of ampicillin (Table 5); all of the samples showed similar results. Ampicillin was adsorbed almost completely through the trials conducted.

CONCLUSION

When compared to submerged state, solid state fermentation provides better environment for fungi growth. Treated paper when used as a substrate, stimulates the fungi to release cellulase to breakdown the complex form of carbon which results in the increase in cellulase activity. This process may not happen as predominantly in positive control as there is readily available carbon source. The process of release of cellulase is slow in media with paper substrate alone which makes addition of CMC as enhancer necessary. CMC as an enhancer supports the proliferation of fungi and release of cellulase to act on treated paper substrates. We assume this basic pathway might be the underlying cause for the variations seen in reducing sugar and cellulase activity. Cellulase activity and reducing sugar release can be optimized further and this process can be exploited for commercial production of high quality and cost effective bioproducts. It is observed that not all of the waste paper substrates are completely utilized during fermentation. This opens an opportunity for the repetitive use of the waste paper substrate in future fermentation cycles. Fermented waste paper residue shows a better percentage of adsorption for

Table 4. Percentage adsorption values of methylene blue

Sample name	Percentage adsorption (%)		
	T1	T2	T3
Control (Untreated Newspaper)	46	10	0
Treated Newspaper	40	19	9
Control (Untreated printed paper)	54	23	11
Treated printed paper	60	30	17
Fermentedwaste paper residue	77	35	10

T_1 = First use of the treated paper T_2 = First reuse of adsorbent, T_3 = Second reuse of adsorbent, T_1; T_2; T_3 conducted on the same waste paper

Table 5. Percentage adsorption values of ampicillin

Sample name	Percentage adsorption (%)		
	T1	T2	T3
Control (Untreated Newspaper)	99.80	99.81	99.81
Treated Newspaper	99.81	99.82	99.82
Control (Untreated printed paper)	99.82	99.82	99.81
Treated printed paper	99.80	99.81	99.81
Fermented waste paper residue	99.81	99.82	99.82

T1= First use of the treated paper, T2= First reuse of adsorbent, T3= Second reuse of adsorbent, T1; T2; T3 conducted on the same waste paper

methylene blue; and antibiotic traces. The use of newspapers can be done without treatment for the removal of methylene blue as the treatment reduces the adsorption of methylene blue molecules. In the case of printed paper; chemical treatment has been observed to increase the removal of methylene blue from the introduced methylene blue solution. Overall, the chemically treated adsorbents can be replaced by fermented waste paper residue. A further study on the fermented waste paper residue is necessary to know its true capacity. The use of waste paper for the removal of methylene blue and ampicillin is promising in the field of waste water treatment by providing a cheap as well as an efficient alternative for the existing techniques which are reported to be lacking in that aspect.

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Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues; including plagiarism; informed consent; misconduct; data fabrication and/or falsification; double publication and/or submission and redundancy has been completely observed by the authors.

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