THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE
Ozioko, Paul C et al, The *Experiment, 2013, July, Vol.12. (2), 778-785*

REVIEW ARTICLE: CELLULASES, THEIR SUBSTRATES, ACTIVITY AND ASSAY METHODS

ABSTRACT

At present, the problems of energy and environment have been a large obstacle for the development of human civilization. The current predominant energy source, which is the fossil fuel supply, is limited in that it causes environmental pollution such as global green house effect. This was necessitated by the wide use of chemical means in sourcing for energy need of the steadily increasing human population. Hence, continuous efforts have been emphasized towards the solution of the energy supply depletion problems and environmental impacts caused by undwindling human activity, thus, the quest for alternative and environmentally friendly sources of energy, especially the use of enzymes such as cellulose necessited this survey.

Keywords: Cellulase, Energy source, environmental pollution and global green house.

1.0 INTRODUCTION

Cellulase refers to a class of hydrolases produced chiefly by fungi, bacteria, protozoans, and termites, which catalyzes the hydrolysis of cellulose (Lee *et al*., 2000; Watanabe *et al.*, 1998). The important of this enzyme cannot be underscored in that it can be produced by micro-organisms, termites as well as animals (Watanabe and Tokuda, 2001). Also, the use of cellulases to biodegrade cellulose containing biomass helps in mopping up agricultural biomas littered in every environment. Thus, converting waste to wealth.

Cellulase is a complex enzyme composed of three catalytic subunits which work in synergy to bring about the conversion of cellulose to a monomeric unit, glucose, which can subsequently be fermented for bio-ethanol production. These cellulase components are Endo-1, 4-β-glucanase, Exo-1, 4- β-glucanase, and 1, 4- β-D-Glucosidase (Bayer et al., 1998; Henrissat, 1994).

2.0 Cellulases

Cellulase (EC 3.2.1.4) refers to a class of hydrolases produced mainly by fungi, bacteria, protozoans, and termites, which catalyzes the hydrolysis of cellulose (Lee *et al*., 2000; Watanabe *et al.*, 1998). However, there are also cellulases produced by other types of organisms such as plants, molluscs, animals (Watanabe and Tokuda, 2001). This type of cellulase is produced mainly by symbiotic bacteria in the ruminating chambers of herbivores.

Recently, following the report of an endogenous cellulose gene in termites, which were previously considered to digest cellulose exclusively through symbiotic protists (Watanabe et a., 1998), endogenous genes have also been found in many invertebrates such as insects, nematodes and molluscs (Watanabe and Tukuda, 2001). These findings contradict previously held notions that cellulose can only be degraded by micro organisms.

Cellulose decomposition or degradation requires the multiple emzymes, celluloses. In general, cellulose is degraded to cellodextrins or glucose by the sequential synergistic action of three cellulose systems: end-1-4-β-glucanase, exo-1,4-β-glucanase, and β–glucosidase (Bayer et al., 1998; Henrissat, 1994).

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE
Ozioko, Paul C et al, The *Experiment, 2013, July, Vol.12. (2), 778-785*

Endo-1, 4-β-glucanase (EC 3.2.1.4)

Endo-1, 4-β-glucanase (EG), simply called endoglucanase, cleave randomly intermolecular β-1, 4-glucosidic linkages within the cellulose chain. The endoglucanases are commonly assayed by viscosity reductions in carboxymethyl cellulose (CMC) solution. The modes of actions of endoglucanases and exoglucanases differ in that endoglucanases decrease the specific visocosity of CMC significantly with little hydrolysis due to intramolecular cleavages, whereas exoglucanases hydrolyze long chains from the ends in a progressive process (Teeri, 1997; Zhang and Lynd, 2004).

2.1 Exo-1, 4-β-glucanase (EC 3.2.1.91)

Exo-1, 4-β-glucanases (exo-1, 4-β-D-glucan cellobiohydrolases, CBH), simply called exoglucanases, cleave the accessible ends of cellulose modules to liberate glucose and cellobiose. *Triochoderma reesei* cellobiohydrolase I and II act on the reducing and non-reducing cellulose chain ends respectively (Teeri, 1997; Teeri, *et al*., 1998). Avicel has been used for measuring exoglucanase activity among insoluble cellulosic substrates. Unfortunately, amorphous cellulose and soluble collodextrins are substrates for both purified exoglucanases and endoglucanases. Therefore, unlike endoglucanases and β-glucosidases, there is no substrates specific for exoglucanases within the cellulase mixtures (Sharrock, 1988). However, the enzymatic deploymerization step performed by endoclucanases and exoglucanases is the rate-limiting step for the cellulose hydrolysis process.

1,4-β-Glucosidase (EC 3.2.1.21)

β-D-glucosidases hydrolyze soluble cellobiose and other cellodextrins with a degree of polymerization (DP) up to six to produce glucose in the aqueous phase. The hydrolysis rate markedly decreases as the substrate degree of polymerizations increases (Henrissat et al., 1989; Zhang and Lynd, 2004). The term "cellobiase" is often misleading due to this key enzymes broad specificity beyond a DP of two. Relative to endoglucanaises and celobiohydroloses, low levels of the *T. reesei* β-glucosidase are selected in submerged culture.

3.0 Substrates for Cellulase Activity Assays

Substrates for cellulase activity assays can be divided into two categories, based on their solubility in water as shown in Table I

Substrates	**Detection**	**Enzymes**
(a) Soluble:		
Short chain (low DP):		
Cellodextrins	RS, HPLC, TLC	Endo, Exo, BG.
Radio-labelled cellodextrins	TLC plus liquid scintillation	Endo, Exo, BG.
Cellodextrin derivatives:		
β -methylumbelliferyl- oligosaccharides.		
p-nitrophenol-oligosaccharides.	Fluorophore liberation,	Endo, Exo, BG.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY
ISSN 2319-2119
REVIEW ARTICLE

	TLC.	
Long chain cellulose derivatives:	Chromophore liberation,	Endo, Exo, BG.
CMC, HEC.	TLC.	
Dyed CMC.		
	RS, Viscosity	Endo
(b) Insoluble:	Dye liberation	Endo
Crystalline cellulose:		
cotton microcrystalline		
Cellulose (Avicel), velonia		
Cellulose, bacterial cellulose.	RS, TSS, HPLC.	Total, Endo,
Amorphous cellulose:		Exo.
PASC, alkali-swollen cellulose,		
RAC.		
	RS, TSS, HPLC.	Total, Endo,
Dyed cellulose.		Exo.
Fluorescen cellulose.		
Chromogenic and Fluorophoric	Dye liberation.	Total, Endo.
Derivatives:	Fluorophore liberation.	Total.
TNP – CMC.		
Fluram-cellulose.		
Practical cellulose-containing substrate:	Chromophore liberation.	Endo.
	Fluorophore liberation.	Total, Endo.
α -cellulose, pretreated lignocellulosic		

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE

biomass.	HPLC, RS.	Total.

Table I: substrates containing β-1, 4-glucosidic bonds hydrolyzed by cellulases and their detections (Percival-Zhang *et al*., 2006).

Keys: RS = reducing sugars; TSS = total soluble sugars; CMC = Carboxymethyl Cellulose; HEC = Hydroxymethyl cellulose; TNP-CMC = Trinitrophenyl-carboxymethyl cellulose; PASC = Phosphoric acid-swollen cellulose; RAC = Regenerated amorphous cellulose; HPLC = High performance liquid Chromatography; TLC = Thin layer chromatography; Endo = endoglucanase, Exo = exoglucanase; Total = total cellulase activity.

3.1 Soluble Substrates

Soluble substrates include low DP cellodextrins from two to six sugar units and their derivatives, as well as long cellulose derivatives. They are often used for measuring individual cellulase component activity (Table 1).

Cellodextrins are soluble for DP≤6, and very slightly soluble for 6<DP<12 (Zhang and Lynd, 2005). Their solubility decreases drastically with increasing DP because of strong intermolecular hydrogen bonds and system entropic effects. Cellodextrins are often prepared through cellulose hydrolysis by fuming hydrochloric acid, sulphuric acid, acetylation or mixed acids (HCl and H_2SO_4) (Zhang and Lynd, 2003). Cellodextrins are also prepared through biosynthesis using *clostridium thermocellium* cellobiose and cellodextrin phosphorylases (Zhang and Lynd, 2006), or *T. reesei* β–glucosidase. Cellodexrin mixtures can be separated into single components using chromatographic methods such as charcoal-celite, thin layer, cation-exchange, or size-exclusion (Shintate *et al.*, 2003).

Chromogenic p-nitrophenyl glycosides and fluorogenic methylumbelliferyl-D-glycosides derived from soluble cellodextrins are very useful for the study of initial cellulase kinetics (Tuohy *et al*., 2002), reaction specificity (Zverlov et al., 2002), and binding site thermodynamics (Barr and Holewinski, 2002). They are also used to determine the inhibition constants of cellulase in the presence of added collobiose and glucose (Tuohy *et al*., 2002), because chromophores released form substituted glycosides can be easily measured independently of sugars.

Long DP cellulose derivatives can be dissolved in water because of their chemical substitutions. Ionic-substituted carboxymethyl cellulose (CMC) is often used for determining endoglucanase activity, called CMCase, because endoglucanases cleave intramolecular β-1,4-glucosidic bond randomly, resulting in a drastic reduction in the DP (i.e., specific viscosity) of CMC. Carboxymethyl cellulose has two very important physical parameters – the degree of substitution (DS) and DP. The solubility of CMC is closely associated with the DS that has a maximum stoichiometric value of three. Carboxymethyl cellulose is soluble in water when DS>0.3-0.7 (Kerlsson et al., 2001). Commercial CMCs usually have a DS<1.5. It is strongly recommended that a reducing assay or viscosity assay using CMC as substrate should be limited to the first 2% hydrolysis of substrate when DS = 0.7. This is important because only non-substituted glucose units are accessible to cellulase, and hydrolysis action requires at least two or three contiguous non-substituted residues. Carboxymethyl cellulose dissolution in water should be done by gentle swirling to avoid DP reduction (Sharrock, 1988). Also, the viscosity of ionic CMC is influenced by pH, ionic strength and polyvalent cation concentration. Therefore, it is recommended to use non-ionic substituted celluloses, such as hydroxymethyl cellulose (HEC), for determining endoglucanase activity.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE

3.2 Insoluble Substrates

Insoluble cellulose-containing substrates for cellulase activity assays include nearly pure celluloses (Cotton linter, Whatman No.1 filter paper, bacterial cellulose, microcrystalline cellulose and amorphous cellulose) and impure cellulose – containing substrates (dyed cellulose, α-cellulose, and pretreated lignocellulose). Native cellulose, referred to as cellulose I, has two distinct crystalline forms- Iα, which is dominant in bacterial and algal cellulose, Iβ which is dominant in higher plants (Atalla and Vanderhart, 1984). Native cellulose (cellulose I) can be converted to other crystalline forms (II-IV) by various treatments (O'Sullivan, 1997). Several very physical values such as crystalinity index (CrI), degree of polymerization, and cellulose accessibility to cellulose, can be estimated based on maximum cellulase adsorption (Zhang and Lynd, 2004).

Cotton, bacterial cellulose, and *Valonia ventricosa* algal cellulose are examples of highly crystalline cellulose (Boisset *et al*., 1999), whereas amorphous cellulose is at the other extreme. Microcrystalline cellulose, filter paper, α-cellulose, and pretreated cellulosic substrates have modest crystallinity index (CrI) values and can be regarded as a combination of crystalline fraction and amorphous fraction, but there is no clear borderline between the two fractions.

Cotton fiber is made from natural cotton after impurities, such as wax, pectin and coloured matter have been removed (Wood, 1988). Whatman No. 1 filler paper is made from long fiber cotton pulp with a low CrI, approximately 45% (Dong *et al*., 1998). Microcrystalline cellulose, called hydrocellulose or avicel (the commercial name), is made through the following steps: hydrolysis of wood pulp by dilute hydrochloric acid to remove the amorphous cellulose fraction, formation of colloidal dispersions by high shear fields, followed by spray drying of the washed pulp slurry (Fleming *et al*., 2001; Zhang and Lynd, 2004). However, microcrystalline cellulose still contains a significant fraction of amorphous cellulose. Avicel is a good substrate for exoglucanase activity assay, because it has a low DP and relatively low accessibility. Therefore, some researchers feel that "avicelase" activity is equivalent to exoglucanase activity (Wood and Bhat, 1998). However, some endoglucanases can release considerable reducing sugars from avicel (Zhang and Lynd, 2004).

Amorphous cellulose is prepared by converting the crystallins fraction of cellulose to the amorphous form by mechanical or chemical methods. These celluloses include mechanically made amorphous cellulose, alkali- swellen cellulose and phosphoric acid swollen cellulose (PASC). Alkali-swollen amorphous cellulose is made by swelling cellulose powder in a high concentration of sodium hydroxide (e.g., 16% wt/wt) producing the cellulose type II from type I (O'Sullivan, 1997). Phosphoric acid swollen cellulose is most commonly made by swelling dry cellulose powder by adding 85% o-phosphoric acid (Wood, 1988). High concentration of phosphoric acid treatment could result in some degree of conversion of type II cellulose from type I. Amorphous cellulose should be kept in hydrated condition; simple air-drying dehydration results in a loss of substrate reactivity (Zhang and Lynd, 2004).

Lignocellulose pretreatment breaks up the recalcitrant structure of lignocellulose so that cellulase can hydrolyze pretreated lignocelluose faster and more efficiently. Current leading lignocelulose pretreatment technologies, including dilute acid, hot water, flow through ammonia fiber explosion (AFEX), ammonia recycle percolation, and lime, have been recently reviewed (Mosier *et al.*, 2005; Wyman *et al*., 2005).

Other insoluble substrates include α–cellulose which contains major cellulose, and a small amount of hemicelluloses and dyed cellulose. Insoluble cellulose derivatives can be chemically substituted with trinitrophenyl groups to produce chromogenic trinitrophenyl-carboxymethyl cellulose (TNP-CMC).

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE

4.0 Methods of Cellulase Activity Assays

The two basic approaches to measuring cellulase activity are (1) measuring the individual cellulase (endoglucanase, exoglucanase, and β-glucosidase) activities, and (2) measuring the total cellulase activity. In general, cellulase enzyme activities are expressed in the form of the initial hydrolysis rate for the individual enzyme components within a short time, or the end-point hydrolysis for the total enzyme mixture to achieve a fixed hydrolysis degree within a given time.

4.1 Endoglucanase Activity Assay

Endoglucanase activities can be measured on a reduction in substrate viscosity and/or an increase in reducing ends determined by a reducing sugar assay. Because exoglucanase also increases the number of reducing ends, it is strongly recommended that endoglucanase activities be measured by both methods. As the carboxymethyl substitutions on CMC make some glucosidic bonds less susceptible to enzyme action, a linear relationship between initial hydrolysis rates and serially diluted enzyme solutions requires (a) dilute enzyme preparation, (b) a short incubation period, or a very low enzyme loading, (c) a low degree of substitution CMC, and (d) a sensitive reducing sugar assay. Many workers agree that the 2, 2-bicinchroninate (BCA) method of reducing sugar assay is superior to the dinitrosalicyclic acid (DNS) method (Carcia *et al*., 1993).

Soluble oligosaccharides and their chromophore-substituted substrates, such as p-nitrophenyl glucosides and methylumbelliferyl- β-D-glucosides are also used to measure endo glucanase activities based on the release of chromphores or the formation of shorter oligosaccharides fragments, which are measured by HPLC or TLC (Zverlov et al., 2003; 2005).

4.2 Exoglucanase Activity Assay

Avicel has been used for measuring exoglucanase activity. During chromatographic fractionation of cellulase mixtures, enzymes with little activity on soluble CMC but showing relatively high activity on avicel, are usually identified as exoglucanases. Unfortunately, amorphous cellulose and soluble cellodextrins are substrates for both purified exoglucanases and endoglucanases. Therefore, unlike endoglycanases and β-glucosidases, there is no substrates specific for exoglucanases within the cellulase mixture (Sharrock, 1998; Wood and Bhat, 1988).

4.3 β-D-Glucosidase Activity Assays

β-D-glucosidase are very amenable to a wide range of simple sensitivity assay methods, based on coloured or fluorescent products from p-nitrophenyl- β-D-1,4-glucopyranoside (Strobel and Russell, 1987), β-naphythyl- β-D-glucopyranoside, 6-bromo-2-naphthyl- β-D-glucopyranoside (Setlow *et al.*, 2004). Also, β-D-glucosidase activities can be measured using cellobiose, which is not hydrolyzed by endoglucanases and exoglucanases, and using longer cellodextrins, which are hydrolyzed by endoglucanases and exoglucanases (Ghose, 1987; McCarthyl *et al*., 2004).

4.4 Total Cellulase Activity Assays

The total cellulase system consists of endoglucanases, exoglucanases, and β-D-glucosidases, all of which hydrolyze crystalline cellulose synergistically. Total cellulase activity assays are always measured using insoluble substrates, including pure cellulosic substrates such as Whatman No.1 filter paper, cotton linter, microcrystalline cellulose, bacterial cellulose, algal cellulose and cellulose-containing substrates such as dyed cellulose, α-cellulose and pretreated lignocellulose.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE

The heterogeneity of insoluble cellulase and the complexity of the cellulase system cause formidable problems in measuring total cellulase activity. The most common total cellulase activity assay is the filter paper assay (FPA) using Whatman No.1 filter paper as the substrate, which was established and published by the IUPAC (Ghose, 1987). This assay requires a fixed amount (2.0mg) of glucose released from a 50mg sample of filter paper (i.e, 3.6% hydrolysis of substrate), which ensures that both amorphous and crystalline fractions of the substrate are hydrolyzed. A series of enzyme dilution solutions is required to achieve the fixed degree of hydrolysis. The strong points of this assay are (a) it is based on a widely available substrate, (b) it uses a substrate that is moderately susceptible to cellulases and (c) it is based on a simple procedure.

CONCLUSION

Cellulase is a complex hydrolase with three catalytic subunit, namely, endo-1, 4-β-glucanase, exo-1, 4- β-glocanase, and 1, 4- β-D-glucosidase. The activities of each cellulase components can be assayed and determined independently using different assay procedures. Also, the total cellulase activity, which encompasses the overall activities of the three cellulase components, acting synergistically can also be determined. Similarly, each cellulase components has specific substrates with different affinity.

REFERENCES

1. Barr, B. K. and Holewinski, R. J. (2002). 4-methylumbelliferyl-β-D-cellobioside: a fluorescent, non-hydrolysable substrate analogue for cellulases. *Journal of Biochemistry.* 41:4447-4452.
2. Bayer, E., Chanzy, H., Lamed, R. and Shoham, . (1998). Cellulose, cellulase and cellulosomes. *Curr. Opin. Struct. Biology.* 8:548-557.
3. Boisset, C., Chanzy, H., Henrissat, B., Lamed, R., Shoham, Y. and Bayer, E. A. (1999). Digestion of crystalline cellulose substrates by *clostridium thermocellum*: Structural and morphological aspects. *Biochemical Journal.* 340:829-835.
4. Dong, X. M., Revol,. J. F. and Gray, D. G. (1998). Effect of microcrystalline preparation conditions on the formation of colloid crystals of cellulose. *Cellulose*. 5:19-32.
5. Fleming, K., Gray, D. G. and Mathews, S. (2001). Cellulose crystallites. *Chemistry.* 7:1831-1835.
6. Ghose, T. K. (1987). Measuring of cellulase activities. *Pure and Applied Chemistry.* 59:257-268.
7. Henrissat, B. (1994). Cellulases and their interaction with cellulose. *Cellulose.* 1:169-196.
8. Henrissat, B., Claeyssens, M., Tomme, P., Lemesle, L. and Mornon, J. P. (1989). Cellulase families revealed by hydrophobic cluster-analysis. *Gene.* 81:83-95.
9. Karlsson, J., Momecilovic, D., Wittgren, B., Schulein, M., Tjemeld, F. and Brinkmalm, G. (2001). Enzymatic degradation of carboxylmethyl cellulose, hydrolyzed by the endoglucamases Cel5A, Cel12A, and Cel45A from *Humicola insolens,* and Cel7B, Cel12A and Cel45A core from *Trichoderma reesei. Biopolymers*. 62:32-40.
10. Lee, I., Evans, B.R. and Woodword, J. (2000). The mechanism of cellulase action on cotton fibres: evidence from atomic force microscopy. *Ultramicroscopy*. 82:213-221.
11. McCarthy, J. K., Uzelac, A., Davis, D. F. and Eveleigh, D. E. (2005). Improved catalytic efficiency and active site modifictaoin of 1,4- β-D-glucan glucohydrolase A from *Thermotoga neapolitana* by directed evolution. *Journal of Biological Chemistry.* 179: 11495-11502.
12. Morsier, N., Wyman, C. E., Dale, B. E., Elander, R. T., Lee, Y. Y. and Holtzapple, M. (2005). Featuring of promising technologies for pretreatment of lignocellulosic biomass. *Bioresources Technology*. 96:673-686.
13. O'Sullivan, A. C. (1997). Cellulose: the structure slowly unravels. *Cellulase.* 4:173-207.
14. Percival-Zhang, Y.H., Himmel, M.E. and Miehenz, J.R. (2006). Outlook for cellulase improvement: Screening and selection strategies. *Biotechnology Advances.* 24:452-481.
15. Polacheak, I., Mealamed, M., Bercovier, H. and Salkin, I. F. (1987). Beta-Glucosidase in Candida albicans and its application in yeast identification. *Journal of clinical Microbiology.* 25:907-910.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

REVIEW ARTICLE

Ozioko, Paul C et al, The *Experiment, 2013, July, Vol.12. (2), 778-785*

16. Setlow, B., Cabrera-Hernandez, A., Cabrara-Martinez, R. M. and Setlow, P. (2004). Identification of arylphospho-β-D-glucosidase in *Bacilus substilis. Arch. Microbiology.* 181: 60-67.
17. Sharracok, K. R. (1988). Cellulase assay methods; A review. *Journal of Biochemical and Biophysiological Methods.* 17:81-105.
18. Shintate, K., Ktaoka, M., Kim, Y.K. and Hayashi, K. (2003). Enzymatic synthesis of a library of beta-(1,4)-hetero-D-glucose and D-xylose-based oligosaccharides employing cellodextrin phosphorylose. *Carbohydrate Researches*. 338:1981-1990.
19. Strobel, H. J. and Russel, J. B. (1987). Regulation of β-glucosidase in *Bacteriodes ruminicla* by a different mechanism: growth rate-dependent depression. *Applied Environmental Microbiology.* 53: 2505-2510.
20. Teeri, T. T. (1997). Crystalline cellulose degradation: New insights into the function of cellobiohydrolases. *Trends Biotechnology.* 15:160-167.
21. Teeri, T. T., Koivula, A., Linder, M., Wohlfahrt, G., Divine, C. and Jones, T. A. (1998). *Trichoderma reesei* cellobiohydrolases: why so efficient on crystalline cellulose? *Biochemical Society of Trans.* 26:173-178.
22. Tuohy, M. G., Walsh, D. J., Murray, P.G., Claessens, M., Cuffee, M.M. and Savage, A. V. (2002). Kinetic parameters and mode of action of the cellobiohydrolases produced by Talaromyces emersonii. *Biochem. Biopsy. Acta*. 1596:366-380.
23. Watanabe, H. and Tokuda, G. (2001). Animal Cellulases. *Cellular and Molecular Life Science.* 58:1167-1178.
24. Watanabe, H., Noda, H., Tokuda, G. and Lo. N. (1998). A cellulase gene of termite origin. *Nature.* 394:330-331.
25. Wood, T. M. (1988). Preparation of crystalline, amorphous and dyed cellulose substrates. *Methods in Enzmology*. 166:19-45.
26. Wood, T.M. and Bhat, K.M. (1988). Methods of measuring cellulase activities. *Methods in Ezymology.* 160: 87-117.
27. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladish, M.R. and Lee, Y. Y. (2005). Coordinated development of leading biomass pretreatment technologies. *Bioresources Technology.* 96:1959-1966.
28. Zhang, Y.H.P. and Lynd, L. R. (2003). Cellodextrin preparation by mixed acid hyrolysis and chromatographic separation. *Analytical Biochemistry.* 322:225-232.
29. Zhang, Y.H.P. and Lynd, L. R. (2004). Toward an aggregated understanding of enzymatic hyrolysis of cellulose: non-complexed cellulase systems. *Biotechnological Bioengineering.* 88:797-824.
30. Zhang, Y.H.P. and Lynd, L. R. (2005). Determination of the number-average degree of polymerization of cellodextrin and cellulose with application to enzymatic hydrolysis. *Biomacromolecules.* 6; 1510-1515.
31. Zhang, Y.H.P. and Lynd, L. R. (2006). Biosynthesis of radiolabelled cellodextrins by the clostridium thermocellium cellobiose and cellodextrin phosphorylases for measurement of intracellular sugars. *Applied Microbiology and Biotechnology*, 70(1):123-129.
32. Zverlov, V. V., Schantz, N. and Scwarz, W. H. (2005). A major new component in the cellulosome of *clostridium thermocellum* is a processive endo-beta-1,4-glucaanase producing cellodextrose. *FEMS Microbiology Literature.* 249:353-358.
33. Zverlov, V. V., Velifodvorskaya, G. A. and Schwarz, W. H. (2003). Two new cellulosome components encoded downstream of cell in the genome of *clostridium thermocellum:* the non-processive endoglucanase CelN and the possible structural protein CseP. *Microbiology*. 149:515-524.
34. Zverlov, V. V., Velikodvorskaya, G.A. and Schwarz, W. H. (2002). A newly described cellulosomal cellobiohydrolase, Celo, from *clostridium thermocellum*: Investigation of the exo-mode of hydrolysis and binding capacity of crystalline cellulose. *Microbiology.* 148:247-255.

***[1]Ozioko, Paul C. [2]Ikeyi Adachukwu P. and [1]Ugwu, Okechukwu P. C.**

[1]Department of Biochemistry, University of Nigeria, Nsukka Enugu State, Nigeria.

[2]Department of Science Laboratory Technology, Institute of Management and Technology (IMT), Enugu, Nigeria.

Email oky9992000@yahoo.com