Saudi Journal of Biological Sciences (2016) 23, S93–S98

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Measurement of filter paper activities of cellulase with microplate-based assay

Xiaoxiao Yu [b,1], Yan Liu [a,1], Yuxiao Cui [a], Qiyue Cheng [a], Zaixiao Zhang [a], Jia Hui Lu [a], Qingfan Meng [a], Lirong Teng [a], Xiaodong Ren [a,*]

[a] School of Life Sciences, Jilin University, Changchun, Jilin 130012, China
[b] Key Laboratory for Molecular Enzymology and Engineering of Ministry of Education, Jilin University, Changchun 130023, China

Received 7 June 2015; revised 19 June 2015; accepted 20 June 2015
Available online 15 July 2015

KEYWORDS

Cellulase;
Filter paper assay;
IUPAC;
Microplate-based assay

Abstract It is always a challenge to determine the total cellulase activity efficiently without reducing accuracy. The most common total cellulase activity assay is the filter paper assay (FPA) established by the International Union of Pure and Applied Chemistry (IUPAC). A new procedure to measure the FPA with microplate-based assay was studied in this work, which followed the main idea of IUPAC to dilute cellulase preparation to get fixed glucose release. FPAs of six cellulase preparations were determined with the microplate-based assay. It is shown that FPAs of cellulase Youtell, RCconc, R-10, Lerkam, Yishui and Sinopharm were 67.9, 46.0, 46.1, 27.4, 7.6 and 8.0 IU/ml respectively. There was no significant difference at the 95% confidence level between the FPA determined with IUPAC and the microplate-based assay. It could be concluded that the FPA could be determined by the microplate-based assay with the same accuracy and much more efficiency compared with that by IUPAC.

1. Introduction

Lignocelluloses are the most abundant renewable bioresource in the world. The cellulase, which has been widely used in the food processing, is the critical enzyme which could catalyze cellulose to oligosaccharides and glucose (Patindol et al., 2007; Kapasakalidis et al., 2009; Renouard et al., 2010; Abbès et al., 2011). In fact, the cellulase is a system consisting of endoglucanases, exoglucanases, and β-D-glucosidases, all of which hydrolyze crystalline cellulose synergically. The cellulase activities are always measured using insoluble cellulose. The heterogeneity of insoluble cellulose and the complexity of the cellulase system cause formidable problems in measuring total cellulase activity (Mullings, 1985; Criquet, 2002; Helbert et al.,

* Corresponding author at: School of Life Science, Jilin University, Changchun, Jilin 130012, China. Tel.: +86 43185155138; fax: +86 43185168637.
E-mail address: renxiaodong@jlu.edu.cn (X. Ren).
[1] These authors contributed to the work equally and should be regarded as co-first authors.
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2015.06.018
1319-562X

2003; Eveleigh et al., 2009; Farnet et al., 2010; Dashtban et al., 2010). 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 International Union of Pure and Applied Chemistry (IUPAC) (Zhang et al., 2006; Batool et al., 2015). The main idea of the IUPAC method is that cellulase must be diluted until the amount of product plotted against cellulase concentration is reasonably linear. The assay requires a fixed amount (2 mg) of glucose released from a 50-mg filter paper (1 × 6 cm). A series of cellulase dilution solutions is required to achieve a fixed degree of hydrolysis (Ghose, 1987; Butt et al., 2015).

Though the IUPAC method is accepted worldwide, there are still some shortcomings for FPA assays, such as labor-intensiveness, low-throughput, and requiring a large quantity of substrate, cellulase and chemicals. Several methods were developed for the purpose of high-throughput cellulase activity screening these years (Boyer et al., 2002; Goddard and Reymond, 2004; Xiao et al., 2005; Kasana et al., 2008; King et al., 2008; Peralta et al., 2008). Decker found that replacing the filter paper with Solka-floc, Sigmacell-20, Avicel and cotton linters, the assay for a rather similar substrate in hydrolytic properties to the filter paper could be automated on a Cyberlabs C400 robotics deck (Ashraf et al., 2013; Decker et al., 2003). Berlin used the disk made from yellow poplar to estimate the hydrolysis of cellulose to glucose in a 96-well microplate. The assay shows considerable time and cost benefits over the standard assay (Berlin et al., 2005). Chundawat developed a procedure with the 96-well Biomass Conversion Research Lab microplate method for the high-throughput

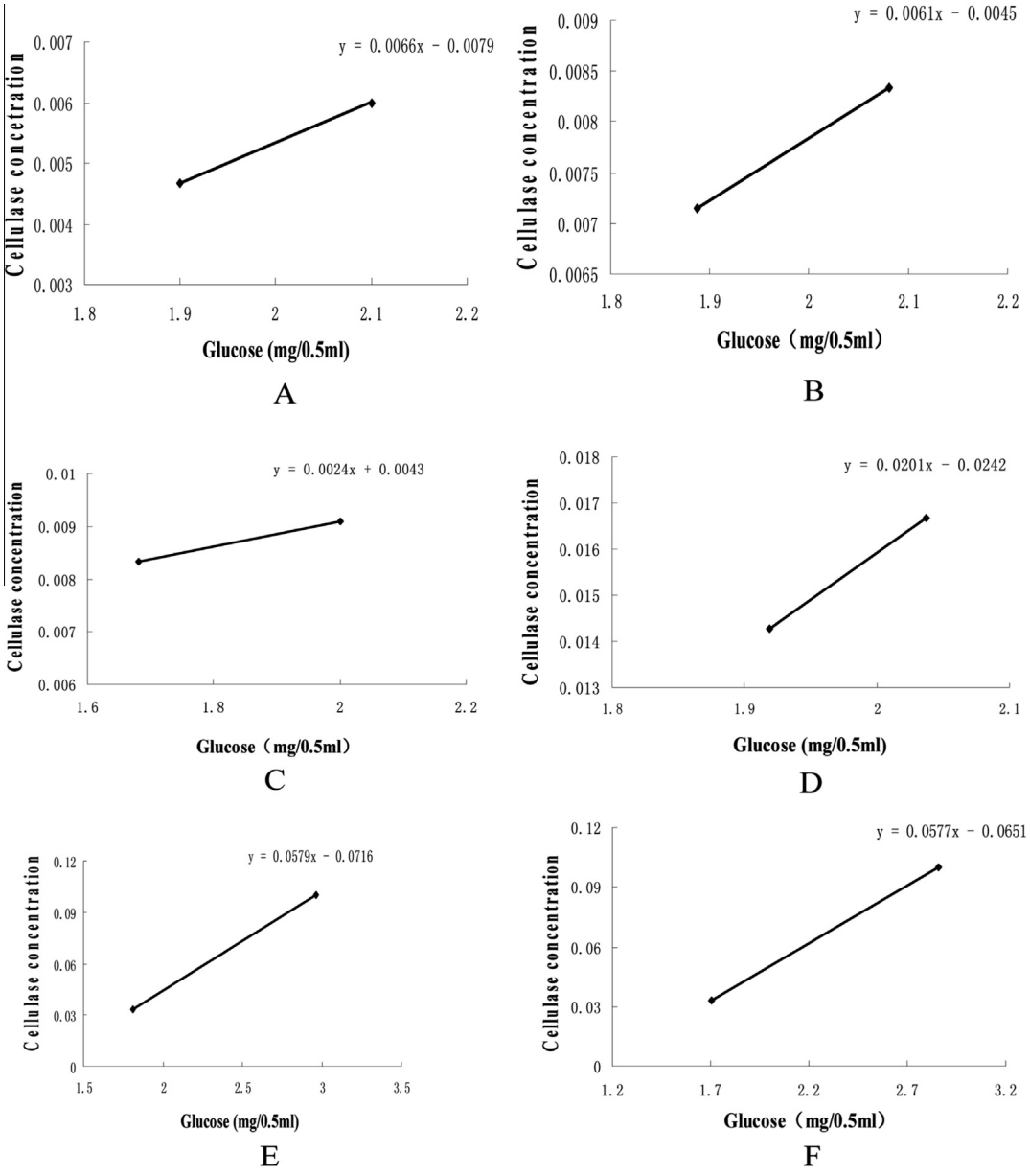

Fig. 1 The curves for fixing the cellulase concentration that produce 2 mg of glucose release with IUPAC. (A) Youtell, (B) RCconc, (C) R-10, (D) Lerkam, (E) Yishui, (F) Sinopharm. *Only a series of results within 14 parallel tests of each cellulase are shown in Figs.1 and 2 and Tables 1 and 2.