A simplified procedures for cellulase filter paper assay

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Abstract

A new procedures to minimize labor intensiveness and complexity that has long been recognized in cellulase filter paper activity measurement (FPAase) described by the international union of pure and applied chemistry (IUPAC) was developed. It follows the main idea of IUPAC with only exception finding at least two cellulase dilutions have optical densities slightly more and less than a reference optical density of an arbitrary fixed 2 mg absolute glucose amount after a red-ox color reaction and due to cellulase-filter paper hydrolysis. The yielding glucose amount difference as compared to this reference is expressed in terms of absorbance difference percent determined by 3,5-dinitrosalicylic acid (DNS), in case of cellulase is cellubiase rich. If not, an external supplemental portion should be added. The intersection of the line of these two cellulase dilutions with abscissa intersect a vertical at a hypothetical 0% absorbance difference percent corresponding to a critical cellulase dilution exactly release this fixed arbitrary 2 mg glucose amount value. The factor 0.37 of this critical cellulase dilution equals to its filter paper units expressed in FPU per ml.

Key words: Cellulase, activity, Filter paper unit, absorbance difference.

Abbreviations:

FPAase: cellulase filter paper activity

IUPAC: International union of pure and applied chemistry

DNS: 3,5-dinitrosalicylic acid

FPU: Filter paper unit

NS: Nelson-somogyi

AED: 2-aminoethanaminium 2-(ethoxycarbonyl)-4,6-dinitrophenolate

A(R): absorbance of releases sugar from unknown cellulase-filter paper reaction

A(f): absorbance of an arbitrary fixed 2 mg glucose

Introduction:

Dwindling fossil fuel resources as well as global climate change makes enzymatic cellulase research is of interest to a growing scientific community seeks a green and renewable bio fuels based on biomass valorization. cellulose is the most abundant renewable homogeneous polymer on earth and ideal substrate for cellulase system comprises of exo-glucanase, endo-glucanase and cellobiase acts synergistically or competitively in a manner still not fully understood [1]. It is naturally interconnected with hemicellulose and both of them embedded in lignin structure found in a variety of lignocellulosic biomass. It comprises of glucose units through 1,4-b-glucosidic linkage with amorphous and crystalline regions, its degree of polymerization and accessibility to cellulase system is totally depend on the pretreatment method that affects its composition, morphological features and crystallinity in order to find a low cellulytic dose necessitate efficient hydrolysis into glucose [2,3]. It is always a challenge to determine FPAse activity without reduction in accuracy and without labor intensiveness. Briefly according to IUPAC an arbitrary glucose amount 2 mg liberated from 50 mg filter paper in a fixed time by a critical cellulase dilution which is determined and translated into a cellulase concentration then filter paper units is calculated. among the methods

determine reducing sugars Nelson-Somogyi (NS) assay [4, 5] and 3,5- dinitrosalicylic acid (DNS) assay [6] recommended by IUPAC. Other methods such as those based on the use of sodium 2,2′ - bicinchoninate [7], p-hydroxybenzoic acid hydrazide [8], or potassium ferricyanide [8] and recently AED [10,11].

Theory and calculation:

Due to cellulase-filter paper reaction, a two cellulase dilutions must be determined have absorbance difference slightly more and less than 2 mg an arbitrary absolute glucose amount after red-ox reaction as the same idea of IUPAC finding cellulase dilution slightly release more or less than a fixed 2 mg glucose.

in case of cellulase is rich in cellobiase levels, glucose amounts as an end products was released at the same volumes (v, 0.5 ml), same absorbance extinction coefficient (ε) and same path length (b), so weight difference is reasonable expressed in absorbance difference according to Beer-Lambert law.

$\Delta A\% = [A(R)-A(f)/]A(f)\ [100]$

Where A(R) is the absorbance of the released sugar in a cellulase dilution after red-ox reaction.

A(f) is the absorbance of fixed arbitrary glucose amount and equals 2 mg after red-ox reaction.

The cellulase shall be tested must contain high levels of cellobiase either naturally or supplemented externally.

Results and discussion:

As a consequent of the cellulase activity is not directly proportional to sugar amount released, as explained by ghose [12] twice cellulase amount would not be expected to yield twice sugar amount in a same incubation time, therefore a cellulase dilutions must be made reacting a filter paper as a cellulosic substrate have both of crystalline and amorphous regions yielding glucose as an end product if the cellulase under investigation has been isolated form a source able to secrete high levels of cellobiase, if not, a supplemental amounts must be added that is essential for assay response, glucose undergoes Red-ox reaction giving a color shift their absorbances should be closer to a standard glucose sample as a reference of 4 mg/ml undergoes the same color reaction under the same assay conditions. plot of a certain two cellulase dilutions intersects abscissa at 0% point ordinate represents the critical cellulase dilution exactly release 2 mg absolute glucose amount. 0.37 this value represents the filter paper activity expressed as FPU/ml of native cellulase.

Materials:

According to IUPAC procedures,

-(1M) Citrate buffer: 210 gm citric acid mono hydrate, 750 ml distilled water, sodium hydroxide 50-60 gm (add until pH equals 4.3), dilute to 1000 ml and if necessary add sodium hydroxide till pH 4.5. Dilute this buffer to 0.05 M and do needful till pH becomes 4.8

-DNS reagent: Mix very well 10.6 gm 3,5-dinitrosalicylic acid, 1416 ml distilled water and 19.8 gm sodium hydroxide then add sodium potassium tartrate 306 gm, phenol (melts at 50 C) 7.6 ml and sodium bisulfite 8.3 gm.

titrate 3 ml sample of solution above, phenolphithaleine indicator versus 0.1 M HCl. it should take 5-6 ml.

-Glucose standard solution : prepare 4 mg/ml glucose standard solution.

-Filter paper Whatman No.1, 50 mg (1cm, 6 cm).

Proposed Procedures:

1-prepare a series of cellulase dilutions in citrate buffer and express them in dilution factors.

2-incubate cellulase dilution (0.5 ml cellulase dilution, 1 ml buffer and 50 mg filter paper), cellulase blank and cellulase control at 50 C for one hour.

Stop the reaction by adding 3 ml DNS to all three tubes and heat them as well as glucose standard (0.5 ml glucose standard, 4 mg/ml), 1 ml buffer and 3 ml DNS) and glucose blank (1.5 ml citrate buffer and 3 ml DNS) at 90 C for 5 minutes. Each absorbance should be taken in regard to its blank and substract cellulase dilutions absorbances from its control absorbance.

cellulase blank: 1.5 ml buffer, 50 mg filter paper and 3 ml DNS.

cellulase control: 0.5 ml cellulase dilution, 1 ml buffer and 3 ml DNS.

3-Cool them and add 10 ml distilled water to each tube.

4-Measure absorbance for glucose standard and cellulase dilutions at 540 nm

5-calculate absorbance difference percent $\Delta A\%$ and choose two values more and less than 0%. plot absorbance difference percent on ordinate versus dilution factors on

abscissa, Intersection of the regression line with abscissa at 0% point absorbance difference represents critical dilution factor. 0.37 this critical dilution factor represents filter paper activity in FPU/ml.

Conclusion:

A new methodology for cellulase assay has been developed based on absorbance difference between an arbitrary glucose known sample and the released glucose from unknown cellulase-filter paper reaction. the factor 0.37 of the critical dilution factor is the cellulase activity expressed in FPU/ml.

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-Availability of Data and Material: available in different ways.

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