

# A consistency mapping for the effects on enzymatic hydrolysis sugar yield using two sugar yield definitions in cellulosic biofuel manufacturing



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## ABSTRACT

Two different sugar yield definitions (cellulose-based and biomass-based) were used in reported studies investigating the relationship between biomass particle size and enzymatic hydrolysis sugar yield. It is noticed that these reported relationships are not consistent if sugar yield is defined differently. The literature does not contain any reports on the effects of sugar yield definition on the relationship between biomass particle size and enzymatic hydrolysis sugar yield. This paper presents a consistency mapping to show under what conditions the relationships are consistent (or inconsistent) when these two definitions are used. The application of this mapping is illustrated via an experimental study with poplar wood biomass on the relationship between biomass particle size and enzymatic hydrolysis sugar yield using both sugar yield definitions. The application of this mapping is also illustrated via data reported in the literature. Not limited to particle size, this mapping is applicable to investigations of the relationships between a variety of parameters (biomass type, pretreatment condition, etc.) and enzymatic hydrolysis sugar yield.

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## 1. Introduction

Biofuels have been recognized as promising alternatives to petroleum-based liquid transportation fuels [1–3]. Cellulosic biomass can be converted into biofuels through biochemical pathway. Before biochemical conversion, cellulosic biomass has to go through a size reduction step to make it easier to handle and to make the biofuel production process more efficient [4]. Cellulosic biomass biochemical conversion consists of two major processes. First, biomass particles produced by size reduction are depolymerized to fermentable sugars through pretreatment and enzymatic hydrolysis. Second, the fermentable sugars are converted into biofuel (ethanol) through fermentation [5].

Cellulosic biomass ethanol yield is highly dependent on the cellulose conversion rate during enzymatic hydrolysis [6]. Extensive research has been conducted to enhance the digestibility of cellulosic biomass in order to increase the enzymatic hydrolysis sugar yield [7,8]. Cellulosic biomass consists of mainly three

different polymers, namely cellulose, hemicelluloses, and lignin. Cellulose is trapped in the shield formed by lignin and hemicelluloses [7,9,10].

The size of particles produced after biomass size reduction (referred as particle size in the following content) is an important input parameter affecting enzymatic hydrolysis sugar yield [11,12]. The literature contains many studies investigating the relationship between particle size and sugar yield. However, the reported relationships are inconsistent. As shown in Table 1, many publications reported that smaller biomass particles had higher enzymatic hydrolysis sugar yield than larger biomass particles. However, there are also publications that did not support such a relationship.

It was found that two different sugar yield definitions were used in the related publications. One definition is cellulose-based sugar yield, and calculated as the percentage of cellulose in biomass converted to fermentable sugar (glucose) by enzymatic hydrolysis. The other definition is biomass-based sugar yield, and calculated as the ratio of the glucose produced by enzymatic hydrolysis to the initial dry weight of the biomass. In this paper, these two definitions are so called for the purpose of easy comparison and discussion. These concepts might be called differently elsewhere.

It is interesting to note that, when cellulose-based sugar yield definition was used, all (except one) publications reported the

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**Table 1**  
Reported relationship between particle size and sugar yield.

Biomass material	Smaller particles produced higher sugar yield	Sugar yield definition	Reference
Douglas fir	Yes	Cellulose based	[13]
Douglas fir	Yes	Cellulose based	[14]
Corn stover	Yes	Cellulose based	[15]
Red oak	Yes	Biomass based	[16]
Spruce wood	Yes	Cellulose based	[17]
Lodgepole pine	Yes	Cellulose based	[18]
Switchgrass	No	Biomass based	[19]
Corn stover	No	Biomass based	[20]
Wheat straw	No	Biomass based	[21]

relationship that smaller biomass particles had a higher sugar yield. In the three publications that did not support such a relationship [19–21], biomass-based sugar yield definition was used. Furthermore, the literature does not contain any reports on the effects of sugar yield definition on the relationship between biomass particle size and enzymatic hydrolysis sugar yield.

This paper presents a consistency mapping to show under what conditions the relationships are consistent (or inconsistent) when these two definitions are used. The application of this mapping is then illustrated via an experimental study with poplar wood biomass the relationship between biomass particle size and enzymatic hydrolysis sugar yield using both sugar yield definitions. The application of this mapping is also illustrated via data reported in the literature.

## 2. Development of the consistency mapping

### 2.1. Two sugar yield definitions

#### 2.1.1. Cellulose-based sugar yield

Cellulose-based sugar yield was used to evaluate the efficiency of enzymatic hydrolysis. It is expressed in terms of the percentage of cellulose converted to fermentable sugar (glucose), and calculated by the following equation:

$$\text{Cellulose-based sugar yield (\%)} = \frac{c \times V}{1.11 \times m \times C_b} \times 100\% \quad (1)$$

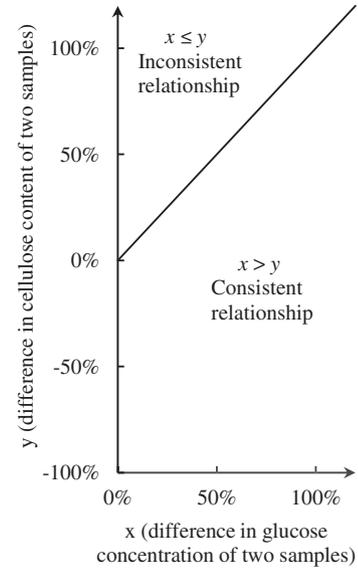
where  $c$  is the concentration (g/L) of glucose in the hydrolysis slurry,  $V$  (L) is the total volume of the slurry,  $m$  (g) is the dry weight of the biomass loaded into the hydrolysis flask, and  $C_b$  (%) is the cellulose content in the biomass before hydrolysis. The factor 1.11 is the cellulose-to-glucose conversion factor, which reflects the weight gained in converting cellulose to glucose in hydrolysis.

#### 2.1.2. Biomass-based sugar yield

Biomass-based sugar yield evaluates the glucose yield (g) per unit dry weight of biomass loaded into the hydrolysis process. It is calculated by the following equation:

$$\text{Biomass-based sugar yield (g glucose/g dry biomass)} = \frac{c \times V}{m} \quad (2)$$

where  $c$  is the concentration (g/L) of glucose in the hydrolysis slurry,  $V$  (L) is the total volume of the slurry, and  $m$  (g) is the dry weight of the biomass loaded into the hydrolysis process.



**Fig. 1.** Consistency mapping.

### 2.2. Derivation of the consistency mapping

The formulas for sugar yield calculation using the above-mentioned two definitions involve two variables. One is glucose concentration  $c$  (g/L) in two samples under comparison after hydrolysis ( $c_1$  and  $c_2$ ), and the other is cellulose content  $C_b$  (%) in the two samples before hydrolysis ( $C_{b1}$  and  $C_{b2}$ ). To simplify the derivation, the sample with a higher glucose concentration is subscripted as “1”.

Whether the relationships between particle size and sugar yield using the two sugar yield definitions are consistent or not is determined by the relative values of  $x$  and  $y$ . Where, “ $x$ ” is the difference in glucose concentration, and calculated as  $x = [(c_1 - c_2)/c_2] \times 100\%$ ,  $x > 0$ , and “ $y$ ” is the difference in cellulose content, and calculated as  $y = [(C_{b1} - C_{b2})/C_{b2}] \times 100\%$ ,  $y > -1$ . The statement that the relationships using these two definitions are consistent is equivalent to the following inequality:

$$\left( \frac{c_1 \times V_1}{1.11 \times m_1 \times C_{b1}} \times 100\% - \frac{c_2 \times V_2}{1.11 \times m_2 \times C_{b2}} \times 100\% \right) \cdot \left( \frac{c_1 \times V_1}{m_1} - \frac{c_2 \times V_2}{m_2} \right) > 0 \quad (3)$$

where,  $m_1 = m_2 = m$ , and  $V_1 = V_2 = V$ . Taking  $c_1 = (1 + x)c_2$ , and  $C_{b1} = (1 + y)C_{b2}$  into the inequality gives

$$\left( \frac{(1 + x) \cdot c_2 \times V}{1.11 \times m \cdot (1 + y) \cdot C_{b2}} \times 100\% - \frac{c_2 \times V}{1.11 \times m \times C_{b2}} \times 100\% \right) \cdot \left( \frac{(1 + x) \cdot c_2 \times V}{m} - \frac{c_2 \times V}{m} \right) > 0 \quad (4)$$

Inequality (4) is reduced to

$$\frac{V^2 \times c_2^2}{1.11 \times m^2 \times C_{b2}} \cdot \frac{x \cdot (x - y)}{(1 + y)} > 0 \quad (5)$$

Because  $x > 0$ ,  $1 + y > 0$ , and others are positive constants, the solution to the inequality is  $x > y$ . To summarize, if  $x > y$ , the relationships are consistent; on the other hand, if  $x \leq y$ , the relationships are inconsistent. The above derivation is based on the scenario that  $x > 0$ . In the special scenario that  $x = 0$ , it is easy to

**Table 2**  
Chemical composition of poplar wood chips.

Component	Percentage on dry weight basis
Cellulose	41.1 ± 0.4
Hemicellulose	22.9 ± 0.3
Lignin	24.0 ± 0.7
Ash	2.9 ± 0.1

find out that if  $y = 0$ , the relationships are consistent; if  $y \neq 0$ , the relationships are inconsistent.

A consistency mapping ( $x > 0$ ), as shown in Fig. 1, is developed to show under what conditions the relationships between particle size and sugar yield using these two sugar yield definitions are consistent (or inconsistent).

### 3. Experimental study to illustrate the application of the mapping

#### 3.1. Material and methods

##### 3.1.1. Material

Poplar wood chips were purchased from Petco Animal Supplies, Inc. (Manhattan, KS, USA). The moisture content of the wood chips was 7.1%. The wood chips were placed in sealed Ziploc® bags and stored at room temperature before size reduction by mills. Table 2 lists the chemical composition of the wood chips.

##### 3.1.2. Biomass size reduction

Two types of mills were used for size reduction of poplar wood chips: a knife mill (Model SM 2000, Retsch, GmbH, Haan, Germany) and a hammer mill (Model No. 5, Meadows Mills, Inc., North Wilkesboro, NC, USA). Sieves of two sieve sizes (with openings of 1 and 4 mm on the sieves) were used in both mills to produce poplar biomass particles with two levels (– and +) of particle size. Wood chips remained in the milling chamber until they were small enough to pass through the openings on the sieve. After milling, particles were collected and kept in sealed Ziploc® bags and stored in a refrigerator at 4 °C until further processing. Table 3 lists the experimental conditions in biomass size reduction.

##### 3.1.3. Biomass extraction

The purpose of biomass extraction is to remove extractives from wood particles produced by mills because these extractives could potentially interfere with subsequent analysis. The two-step extraction process was conducted by following National Renewable Energy Laboratory procedure (NREL/TP-510-42619) [22]. In the first step, distilled water was used (for 24 h) to remove water-soluble extractives. In the second step, ethyl alcohol (190 proof) was used (for 24 h) to remove alcohol-soluble extractives. After biomass extraction, wood particles were dried in an oven at 40 °C for 24 h and stored in individual self-seal sample bags.

**Table 3**  
Particle size levels and size reduction conditions.

Condition no.	Particle size level	Mill type	Sieve size (mm)
1	–	Knife	1
2	+	Knife	4
3	–	Hammer	1
4	+	Hammer	4

#### 3.1.4. Biomass pretreatment

Dilute sulfuric acid pretreatment was employed in this study. Ten grams of extractive-free biomass particles and 200 mL of 2% (w/v) sulfuric acid were loaded in the 600-mL vessel of a Parr pressure reactor (Model 4760A, Parr Instrument Co., Moline, IL, USA), and treated at 140 °C for 30 min.

The pretreated biomass particles were washed with hot distilled water using a centrifuge (Model Marathon 2100, Thermo International Equipment Co., Needham, MA, USA) to remove dissolved sugars, acid residues, and inhibitors (substances that would decrease enzymes' ability to depolymerize cellulose to glucose [23]) formed during pretreatment. The rotation speed of the centrifugal was 4000 rpm. Each biomass sample was washed three times, and each time lasted for 15 min. The solid biomass after centrifugal was carefully collected. For each test condition, a small portion of the collected solid biomass was used for chemical composition analysis, and the rest was used for subsequent enzymatic hydrolysis.

#### 3.1.5. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in eight 125-mL flasks in a water bath shaker (Model C76, New Brunswick Scientific, Edison, NJ, USA) with agitation speed of 110 rpm at 50 °C for 48 h. There were two flasks containing biomass particles collected under each of the four size reduction conditions. Each flask contained 50 mL of hydrolysis slurry. The slurry consisted of 5% (w/v) biomass on dry weight base, sodium acetate buffer (50 mM, pH = 4.8), and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. Accellerase 1500™ enzyme complex (Danisco USA, Inc., Rochester, NY, USA) was used. The enzyme loaded was 1 mL for each gram of dry biomass.

After hydrolysis for 48 h, 0.1 mL of the hydrolysis slurry was withdrawn from each flask, and mixed with 0.9 mL of double distilled water in a 1.5-mL micro-centrifuge tube. The capped tubes were placed into boiling water for 15 min to deactivate the enzyme. Afterwards, the tubes were centrifuged at 10,000 rpm for 15 min to separate supernatant liquid from solid biomass residues using a micro-centrifuge (Model RS-102, Revolutionary Science, Shafer, MN, USA). Supernatant liquid from each tube was filtered through a 0.2- $\mu$ m hydrophilic PTFE syringe filter (EMD Millipore, Billerica, MA, USA). Filtered supernatant liquid was kept in 1.5-mL autosampler vials at 4 °C in a refrigerator before sugar concentration measurement.

#### 3.2. Measurement procedures

##### 3.2.1. Moisture content and dry weight

Biomass moisture content was measured by following National Renewable Energy Laboratory procedure (NREL/TP-510-42621) [24]. About 2.5 g of biomass was placed in an aluminum weighing dish and dried in an oven at 105 °C for 24 h. The loss in weight of the biomass after oven drying was recorded. Moisture content was calculated as follows:

$$\text{Moisture content (MC)(\%)} = \frac{\text{Loss in weight}}{\text{Weight of biomass before drying}} \times 100\% \quad (6)$$

Knowing the moisture content, dry weight could be calculated as follows:

$$\text{Dry weight (g)} = (1 - \text{MC}) \times \text{weight of biomass with moisture} \quad (7)$$

Biomass weight reported in this study is dry weight.

### 3.2.2. Chemical composition

The chemical composition of biomass (wood chips before size reduction or biomass particles collected after pretreatment) was measured by following the National Renewable Energy Laboratory procedure (NREL/TP-510-42618) [25]. Two duplications for each test condition were employed. Structural carbohydrates in biomass were reported as the percentages of cellulose and hemicellulose. Lignin, the major non-carbohydrate component, was reported as the percentage of the sum of acid-insoluble and acid-soluble lignin. The percentage of ash content was also reported.

### 3.2.3. Sugar concentration

Sugar concentration was measured using high performance liquid chromatography (HPLC). The HPLC (Shimadzu, Kyoto, Japan) system was equipped with an RPM-monosaccharide column ( $300 \times 7.8$  mm; Phenomenex, Torrence, CA, USA) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL/min of degassed double-distilled water, and the column oven temperature was  $80^\circ\text{C}$ .

## 3.3. Experimental results

### 3.3.1. Cellulose-based sugar yield

In order to calculate cellulose-based sugar yield, the content of cellulose in biomass samples before hydrolysis was acquired through chemical composition analysis and is listed in Table 4 together with other chemical components. It can be seen that cellulose contents for the two particle size levels are approximately the same.

The relationship between particle size and cellulose-based sugar yield is shown in Fig. 2. Smaller biomass particles had a higher sugar yield than larger particles, for both knife milling and hammer milling methods. This can be interpreted as that cellulose in smaller biomass particles were more efficiently hydrolyzed into glucose by enzymes in hydrolysis.

Mooney et al. [13] hydrolyzed Douglas fir woody biomass of two particle size levels. Their results showed that cellulose-based sugar yield of smaller particles was 24% higher than that of larger particles after 72-h hydrolysis (Fig. 3). The same trend was also reported by Zhu et al. [17] using a shorter hydrolysis time (12 h) to convert spruce woody biomass of four particle size levels (Fig. 4). This trend was also reported for herbaceous biomass. As an example, Zeng et al. [15] milled corn stover and separated milled particles into two particle size levels. They found that when using cellulose-based sugar yield definition, smaller particles produced higher yield (Fig. 5).

### 3.3.2. Biomass-based sugar yield

Fig. 6 shows the relationship between particle size and biomass-based sugar yield. For both knife milling and hammer milling methods, smaller biomass particles have a higher sugar yield than larger biomass particles. Dasari and Benson [16] reported a similar trend for red-oak (Fig. 7). Smaller particles had a higher sugar yield than larger particles.

Not all related publications support this relationship. Zhang et al. [21] found that larger wheat straw particles milled using a

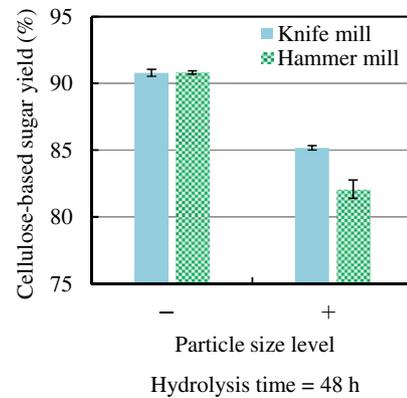


Fig. 2. Relationship between particle size and cellulose-based sugar yield in this study.

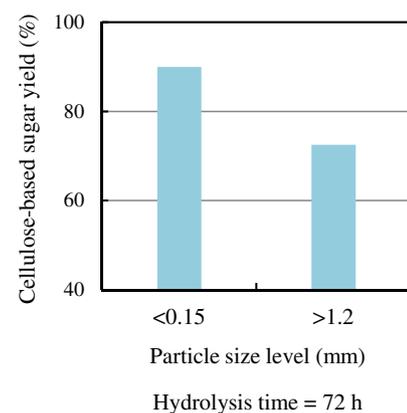


Fig. 3. Relationship between particle size and cellulose-based sugar yield reported by Mooney et al. [13].

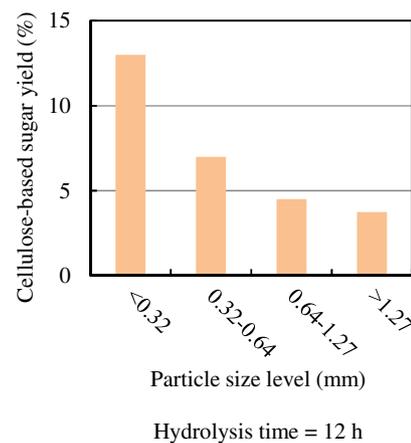


Fig. 4. Relationship between particle size and cellulose-based sugar yield reported by Zhu et al. [17].

**Table 4**  
Chemical composition (percentage on dry weight basis) for biomass particles before hydrolysis.

Condition no.	Particle size level	Mill type	Cellulose	Hemicellulose	Lignin	Ash
1	-	Knife	62.9 ± 1.1	4.2 ± 0.1	30.7 ± 0.2	1.6 ± 0.1
2	+	Knife	62.8 ± 0.1	4.5 ± 0.1	31.0 ± 0.2	1.6 ± 0.2
3	-	Hammer	64.1 ± 0.1	4.6 ± 0.1	29.3 ± 0.1	1.6 ± 0.1
4	+	Hammer	63.2 ± 0.7	4.4 ± 0.1	31.9 ± 0.7	1.3 ± 0.1

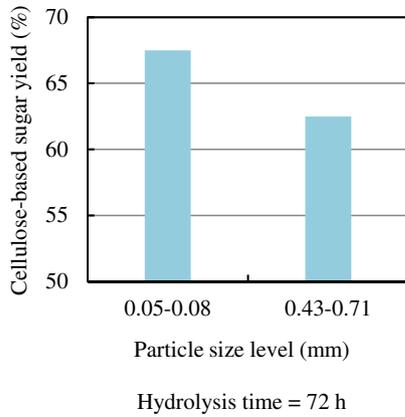


Fig. 5. Relationship between particle size and cellulose-based sugar yield reported by Zeng et al. [15].

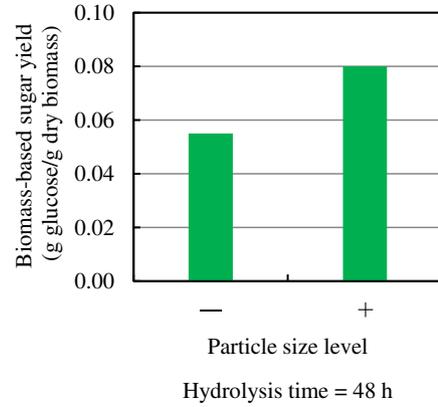


Fig. 8. Relationship between particle size and biomass-based sugar yield reported by Zhang et al. [21].

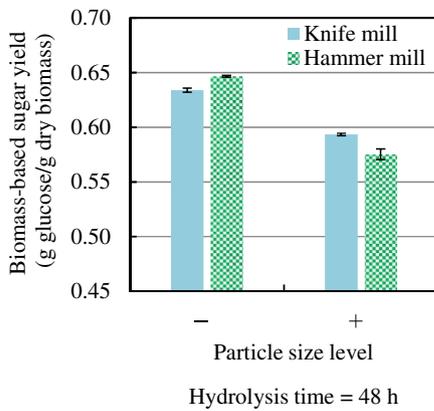


Fig. 6. Relationship between particle size and biomass-based sugar yield in this study.

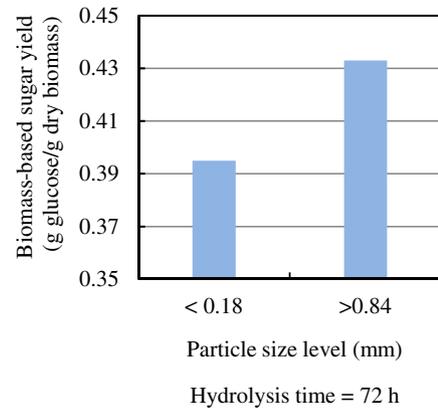


Fig. 9. Relationship between particle size and biomass-based sugar yield reported by Kaar and Holtzapfle [20].

2-mm sieve had higher cellulose-based sugar yield than smaller particles milled using a 1-mm sieve (Fig. 8). It is noted that, in this work, before pretreatment, a pelleting process was employed to agglomerate milled biomass particles into pellets. Kaar and Holtzapfle [20] found that cellulose-based sugar yield of smaller corn stover particles was lower than that of larger particles (Fig. 9). Chang et al. [19] found that, though switchgrass particles with particle size of 0.40–0.84 mm had 18% higher cellulose-based sugar

yield than particles with particle size of 0.84–2 mm, reducing particle size below 0.4 mm did not increase sugar yield (Fig. 10).

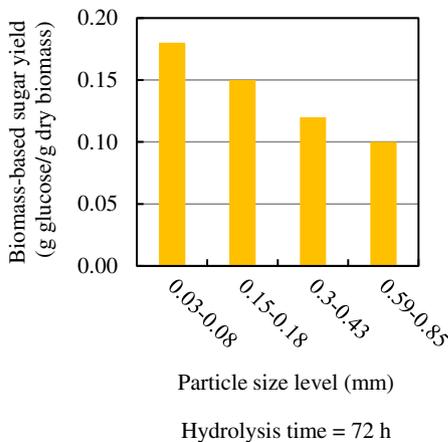


Fig. 7. Relationship between particle size and biomass-based sugar yield reported by Dasari and Benson [16].

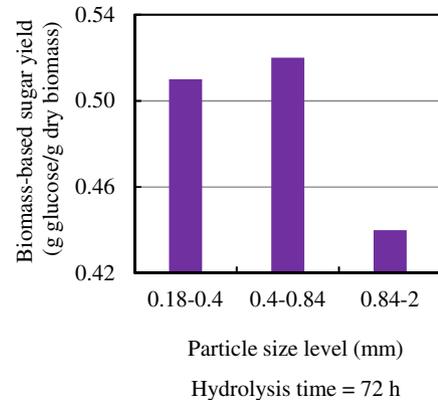


Fig. 10. Relationship between particle size and biomass-based sugar yield reported by Chang et al. [19].

#### 4. Application of the consistency mapping

##### 4.1. Illustration using data from the experimental study

In the present experimental study, the required values to apply the consistency mapping are calculated as  $x = 6.84\%$  and  $y = 0.24\%$

**Table 5**  
Data reported by Ballesteros et al. [26].

	Small particle size	Large particle size
Particle size level (mm)	2–5	5–8
Cellulose-based sugar yield (%)	36	34
Biomass-based sugar yield (g glucose/g dry biomass) <sup>a</sup>	0.14	0.15
Sugar concentration (g/L) <sup>a</sup>	2.76	2.93
Cellulose content (%)	34.4	38.8

<sup>a</sup> Data obtained through calculation based on data provided by Ballesteros et al. [26].

for the small and large particles produced by knife milling;  $x = 12.37\%$  and  $y = 1.40\%$  for the small and large particles produced by hammer milling (values were calculated using the means of the two duplicated tests). Since  $x > y$ , the relationships between particle size and sugar yield using the two sugar yield definitions are consistent.

#### 4.2. Illustration using data from study reported in the literature

Applications of the consistency mapping can also be illustrated using the data published in the literature. A study conducted by Ballesteros et al. [26] was employed as an example. The authors studied the sugar yield of softwood biomass of two levels of particle size. The reported sugar yield was cellulose-based. From the data listed in Table 5, the values needed to apply the consistency mapping are calculated as  $x = 6.16\%$  and  $y = 12.79\%$ . Since  $x < y$ , the relationships between particle size and sugar yield using two sugar yield definitions are inconsistent.

## 5. Conclusions

This paper develops a consistency mapping for the effects on enzymatic hydrolysis sugar yield using two sugar yield definitions. The application of this mapping is illustrated via an experimental study with poplar wood biomass on the relationship between biomass particle size and enzymatic hydrolysis sugar yield. Under the experimental conditions in this study, smaller particles had a higher sugar yield. This relationship remained consistent using both sugar yield definitions. This mapping is not limited to investigations on the relationship between particle size and sugar yield. It is applicable to studying relationships between a variety of parameters (such as biomass type, pretreatment condition, etc.) and sugar yield.

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