Environmental Science and Pollution Research (2018) 25:16743–16753
https://doi.org/10.1007/s11356-018-1807-7

RESEARCH ARTICLE

Experimental assessment and validation of quantification methods for cellulose content in municipal wastewater and sludge

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Received: 8 September 2017 / Accepted: 19 March 2018 / Published online: 2 April 2018

Abstract

Cellulose, mostly in the form of toilet paper, forms a major component of the particulates in raw municipal wastewater, which could lead to significant consequences due to the potential accumulation of cellulosic fibers and slow biodegradability. Despite the sparse reports on cellulose content and degradation in wastewater and sludge, an accurate and validated method for its quantification in such matrices does not exist. In this paper, four different methods were compared including dilute acid hydrolysis, concentrated acid hydrolysis, enzymatic hydrolysis, and the Schweitzer reagent method. The Schweitzer reagent method, applied to municipal wastewater and sludge, was found to be a very robust and reliable quantification method in light of its reproducibility, accuracy, and ideal (100%) recovery. The determination of cellulose content is critical to understand its fate in wastewater treatment plants as well as improve sludge management and enhance resource recovery.

Keywords Cellulose · Toilet paper · Wastewater · Sludge · Resource recovery · Schweitzer reagent

Introduction

The wastewater treatment industry is evolving from the traditional goals of effective control of environmental and health impacts of wastewater discharge to increased sustainability and decreasing costs by minimizing energy costs and resource recovery (Ruiken et al. 2013). Typically, organic matter in wastewater is characterized by surrogate parameters like chemical oxygen demand (COD), total organic carbon (TOC), and biochemical oxygen demand (BOD) and the main organic contaminants have been identified as protein, carbohydrates, and lipids (Raunkjær et al. 1994). Of the insoluble pollutants in wastewater treatment plant influents, cellulose, in the form of toilet paper, has been reported to be a major component which inadvertently ends up in sewage sludge (Edberg and Hofsten 1975; Verachtert et al. 1982). Toilet paper consumption in North America amounts to around 1.9 kg per capita per month (Ruiken et al. 2013). Based on 400 L wastewater produced per person per day, 220 mg total-suspended-solids (TSS) per liter wastewater, and the abovementioned statistics on toilet paper consumption, wastewater can contain up to 158 mg toilet paper/L, that is, about 72% of the TSS. The determination of cellulose in wastewater is thus indispensable to understand its fate in wastewater treatment facilities as well as its recovery potential.

Cellulose is the most abundant organic polymer on earth and is intimately associated with numerous aspects of human advancements including fuel, shelter, clothing, food, and paper (Bauer and Ibanez 2014; Harris et al. 2010; Olsson and Westman 2013; Thoorens et al. 2014). Cellulose is considered a complex carbohydrate very similar to starch and is a linear polymer of β-1,4-glycosidic bond linked with β-D-glucose units (Olsson and Westman 2013; Rinaldi and Schüth 2009; Thoorens et al. 2014). The degree of polymerization (DP), which is directly related to solubility, is the number of glucose units in a cellulose chain. Lack of branching and unique conformation of hydroxyl groups causes chains of cellulose to form, and the dense intramolecular hydrogen bonds provide

Responsible editor: Philippe Garrigues

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chain stiffness forming crystalline structures that are insoluble in water and most of the common solvents (Bauer and Ibanez 2014; Rinaldi and Schüth 2009). Of the three classes of cellulose, α-cellulose is the pure form of cellulose with high (greater than 200) DP whereas β-cellulose (DP less than 30) and γ-cellulose (DP 50–200) are associated with the hemicellulose constituent of plant material (Bolam 1965). Microcrystalline cellulose, also known as Avicel (brand name derived from the original company name—*A*merican *Vi*scose *Cel*lulose), is a partially depolymerized α-cellulose, prepared by treating α-cellulose with mineral acids (Thoorens et al. 2014).

Cellulose is a valuable resource which if recovered can be used for various other applications such as production of fuels and chemicals, building materials, bioplastics, and flocculants (Pellizzer 2016; Rinaldi and Schüth 2009). Accordingly, when it is recovered, sludge disposal costs could be reduced substantially (Faust et al. 2014; Honda et al. 2002) and oxygen consumption and concomitant energy use for biodegradation are eliminated. To this end, new processes and technologies have been developed and validated at full scale such as the one based on the Cellvation™ concept, recently developed through a number of Horizon 2020 European projects (http://www.cirtec.nl/en/gebruikt-toiletpapier-krijgt-tweede-leven/). This process, based on the use of the microsieving technology (e.g., Salsnes Filter), has shown significant potential for cellulose recovery from raw wastewater with potential downstream increase in biological processing capacity due to the removal of COD. Moreover, due to the low extent of cellulose biodegradability in the aeration tank, the removal of cellulose and other fiber-like material is expected to lead to additional operational savings such as lower aeration energy consumption and secondary sludge production.

However, in order to investigate the fate of cellulose during wastewater treatment, the lack of accuracy for cellulose determination in wastewaters and sludges must be addressed. Of the different methods studied in the literature, acid hydrolysis and enzymatic hydrolysis of cellulose are the most widely studied methods. Both methods are based on the principle of hydrolyzing cellulose to monosaccharides, with the glucose yield indicating the cellulose content in the sample.

Table 1 summarizes some of the literature studies that explored one-stage and two-stage acid hydrolysis of cellulose. Updegraff (1969) observed 100% glucose yield using concentrated (72%) sulfuric acid as the hydrolyzing agent. On the other hand, Camacho et al. (1996), also using concentrated (70%) sulfuric acid, observed only 32% glucose yield from microcrystalline cellulose. Gavila et al. (2015) and Kim et al. (2001) used diluted sulfuric acid for hydrolysis at high temperatures (120 and 205 °C, respectively) but only achieved about 60% yield of microcrystalline cellulose and α-cellulose, respectively. Orozco et al. (2007) also studied dilute acid hydrolysis of cellulose at higher temperature but by using phosphoric acid at 7.5% acid concentration at 160 °C and observed 55% yield. As a final one-step hydrolysis method, Chimentao et al. (2014) used oxalic acid at 65 and 120 °C for a prolonged treatment and achieved 85% yield.

Yoon et al. (2014) reported 90% yield in microcrystalline cellulose using a two-stage acid hydrolysis method (National Renewable Energy Laboratory, i.e., NREL method). This NREL method was developed to determine the structural carbohydrates and lignin in biomass. The procedure uses a two-step acid hydrolysis to fractionate biomass into easily quantifiable forms (Sluiter et al. 2012). The first-stage 1-h hydrolysis uses 72% sulfuric acid that disrupts the crystalline structure of cellulose resulting in release of glucose units. The 1- to 2-h second-stage hydrolysis utilizes 4% sulfuric acid digestion which yields hemicellulosic sugars, i.e., xylose, arabinose, mannose, and galactose (Bauer and Ibanez 2014; Gao et al. 2014). The glucose yield of these two-stage methods was 90–93% for pure cellulose and microcrystalline cellulose, respectively.

Xiang et al. (2003) described acid hydrolysis of cellulose as a complex heterogeneous reaction involving hydrolytic chemical reaction factors as well as nonreaction factors impacted by various factors such as state of hydrogen bonding, crystallinity, diffusion barrier, chemical composition, and swelling state of cellulose. In addition to the abovementioned factors, decomposition of hydrolysis products (by dehydration) as a second step following hydrolysis is another challenge (Rinaldi and Schüth 2009). Based on the aforementioned studies, it appears that acid hydrolysis is not the most reliable method for cellulose determination.

Similarly, varying glucose yields have been observed with enzymatic hydrolysis depending on the cellulose source tested. While promising and reliable results are obtained using model cellulosic substrates (like α-cellulose), the results cannot be extrapolated to "real" samples. A number of substrate-related and enzyme-related effects and their interactions play an important role in the hydrolysis efficiency and are the most challenging aspect of this method (Mansfield et al. 1999; Yang et al. 2011). For instance, cellulose's structure, crystallinity, DP, and accessible surface area impact enzyme adsorption which directly correlates to hydrolysis yields (Mansfield et al. 1999; Yang et al. 2011). Similarly, enzyme-related factors, such as thermal instability, product inhibition, and enzyme inactivation, have been reported to impact the hydrolysis of cellulose (Yang et al. 2011). Consequently, numerous studies performed different pre-treatments (such as hydrogen peroxide, potassium hydroxide, sulfuric acid, hydrochloric acid, and HCl/KOH), prior to enzymatic hydrolysis to depolymerize cellulosic fibers into products with low DP which facilitate substrate-enzyme contact (Alkasrawi et al. 2016; Camacho et al. 1996; Champagne and Li 2009; Rinaldi and Schüth 2009). These pre-treatments have been reported to enhance end-product yields from 31% to 69% by facilitating swelling of cellulose that alters the crystalline structure of

Table 1 Literature review of cellulose determination methods

Cellulose type	Acid	Contact time (h)	Temperature (°C)	Yield (%)	Reference
α-Cellulose	72% sulfuric acid	1	Room temperature	100	(Updegraff 1969)
Microcrystalline cellulose (Avicel)	70% sulfuric acid	20	40 °C	32	(Camacho et al. 1996)
Microcrystalline cellulose (Avicel)	3% sulfuric acid	4	120 °C in a microwave reactor system	57	(Gavila et al. 2015)
α-Cellulose	0.07% sulfuric acid	0.5	205 °C	62	(Kim et al. 2001)
Cellulose (type unknown)	7.5% phosphoric acid	0.08	160 °C in a microwave reactor system	55	(Orozco et al. 2007)
Microcrystalline cellulose	6% oxalic acid	6	120 °C	85	(Chimentao et al. 2014)
Microcrystalline cellulose (Avicel) (two-stage acid hydrolysis)	72% sulfuric acid 4% sulfuric acid	1 2	30 °C 100 °C	90	(Yoon et al. 2014)
Microcrystalline cellulose (Avicel) (two-stage acid hydrolysis)	72% sulfuric acid 4% sulfuric acid	1 1	Room temperature 121 °C	93	(Bauer and Ibanez 2014)

cellulose, decreases the DP, and expands the specific surface area for enzyme accessibility.

The majority of the research done on acid and enzymatic hydrolysis treatment has been focused on the industrial hydrolysis of cellulose to glucose and cellodextrins (short-chain cellulose oligomers) with the ultimate goal of producing fuels and chemicals (Rinaldi and Schüth 2009), and accordingly, the reliability and accuracy of cellulose measurement was secondary to final product yield quantification. The Schweitzer method, named after the Swiss chemist Matthias Eduard Schweizer (1818–1860), who invented the Schweizer also called Schweitzer reagent (cuprammonium hydroxide solution) (Kauffman 1984), developed by Hurwitz et al. (1961) was originally intended to determine cellulose in sewage sludge but despite promising recovery of cellulose and good reproducibility, this method was never further explored in the literature for wastewater-related research. The aforementioned authors focussed only on temporal variation of cellulose measurements in activated sludge to correlate that with an operational problem of fibrous heat-dried activated sludges causing problems with mechanical equipment, with no attempt of method validation. In the authors' opinion, two potential reasons for the lack of further interest in the Schweitzer method for wastewater applications could be that there was no interest in determining cellulose in wastewater before and the issue has only recently garnered attention due to transition in the wastewater treatment industry towards resource recovery. Additionally, the authors also believe that researchers nowadays no longer search into older journal articles that are not readily accessible through internet search engines. Although this reagent did not garner attention in wastewater research, the Schweitzer reagent has been used successfully in experimental botany research (Fuller and Barshad 1960) as well as to isolate cellulose from soil samples (Gupta and Sowden 1964). The most widely used application of the Schweitzer reagent is in the textile industry, i.e., in the production of synthetic cellulose products such as rayon (Seymour and Johnson 1976). In contrast to the aforementioned methods, the Schweitzer method does not depend on the hydrolysis to glucose. The Schweitzer reagent is an excellent solvent for cellulose and forms a complex with the cellulose that upon acidification or in alcoholic conditions, precipitates, allowing the cellulose to be measured gravimetrically.

The objective of this work was to compare the different cellulose measurement methods and to determine the most reliable method for accurate quantification of cellulose in a complex matrix of wastewater and sludge. A good method should be reproducible, accurate (no bias with actual cellulose content), have fixed recovery (preferably 100%), quick or with little hands-on time, and cheap in terms of chemicals and equipment. Four different methods were tested for the abovementioned criteria including dilute acid hydrolysis, concentrated acid hydrolysis, enzymatic hydrolysis, and the Schweitzer method. The underlying principle of the three hydrolytic methods is that cellulose is hydrolyzed to glucose.

Materials and methods

For the determination of cellulose, in this paper, four methods were tested, three of which used hydrolysis followed by soluble product determination, and one gravimetric measurement. For the identification of the best method for cellulose determination, the tests were first performed using α-cellulose (Sigma Aldrich, Ontario, Canada) as a standard to avoid interferences. Thereafter, primary clarifier sludge and sieved primary sludge (sludges arising from sieving raw wastewater through a 350-μm sieve) (Sarathy et al. 2015) sample was used to confirm the performance of the methodology. The sludge sample was collected from the Greenway WWTP, located in London, Ontario, Canada. The average total solid content of primary clarifier sludge and sieved primary sludge was $3 \pm 0.01\%$ and $5 \pm 0.24\%$, respectively. Both the sludge

samples were dried at 105 °C (VWR Gravity Convection Oven, Ontario, Canada) overnight prior to testing.

Acid hydrolysis

Acid hydrolysis, using 5% sulfuric acid and a cellulose concentration of 20 g/L, was performed. An initial test where 0.2 g of α-cellulose, toilet paper, and sieved solids were added to 10 mL of 5% sulfuric acid solution in a lightly capped glass vial was performed. The reaction was carried out at 100 °C. One milliliter of samples was taken at predetermined time intervals, and the glucose concentration was determined using glucose kits (Biopacific Diagnostics, Ontario, Canada). A second test was done, and the reaction volume was increased to 100 mL. The cellulose yield was computed as the measured glucose concentration divided by the cellulose mass added (Eq. 1) as follows:

$$\text{Cellulose yield}\ (\%) = \frac{\text{Glucose concentration}\left(\frac{\text{g}}{\text{L}}\right) \times \text{volume}\ (\text{L})}{\text{substrate added}\ (\text{g})} \times 100\% \quad (1)$$

Enzymatic hydrolysis

Enzymatic hydrolysis was conducted following the method of Champagne and Li (2009). Although Champagne and Li (2009) recommended using 10% (by weight) cellulase concentration, in this work different cellulase concentrations ranging from 1 to 20% cellulase-to-cellulose concentration ratios were tested. The first test was carried out on α-cellulose where the equivalent weight of α-cellulose (2 g, dry mass) and cellulase enzyme corresponding to the respective enzyme loading were added to 100 mL of sodium citrate buffer (pH 4.8) in a 125-mL batch bottle. The batches were placed in a shaker where the temperature was maintained at 40 °C and shaken (Thermo Scientific MaxQ4000 Shaker, Ontario, Canada) at 160 rpm. Samples were withdrawn at predetermined time intervals, and the glucose concentrations were determined using glucose kits. Equation 1 was used to calculate the % cellulose yield. The method was also tested on sieved primary sludge samples.

NREL method

As a third alternative, the National Renewable Energy Laboratory (NREL) method was tested to measure for its potential to measure cellulose in wastewater and sludge. This method uses a two-step acid hydrolysis to hydrolyze the sludge into soluble forms, which can be quantified using HPLC (Sluiter et al. 2012). In the first step, 0.3 g of sample (dry mass) was added to a glass vial and 3 mL of 72% sulfuric acid was added. The mixture was stirred using a glass tube and placed in a water bath set at 30 °C for 1 h. After 1 h incubation, the tubes were removed from the water bath and diluted to 4% sulfuric acid by adding 84 mL of deionized water. The samples were thoroughly mixed and placed in an autoclave at 121 °C in the liquid setting for 1 h. After autoclaving, the samples were allowed to cool to near room temperature. The samples were filtered through a 0.45-μm filter paper, and 20 mL of filtrate was collected in a 50-mL vial. Calcium carbonate was used to neutralize the sample to pH 5–6. The neutralized samples were subsequently filtered through a 0.2-μm syringe filter and analyzed for glucose, cellobiose, xylose, galactose, arabinose, and mannose using an HPLC (Hewlett Packard Model 1090 HPLC with a refractive index detector; HPLC column: BioRad Aminex7 HPX-87C). In order to assess if the method could differentiate between cellulose and starch, an initial test was also conducted with different cellulose: starch mass ratios including 0:1, 1:3, 1:1, 3:1, and 1:0. The method was also tested on toilet paper and sieved sludge samples.

Schweitzer method

Cellulose forms a soluble complex with the Schweitzer reagent but precipitates in an alcohol solution (Hurwitz et al. 1961). The Schweitzer reagent was prepared by adding 5.5 g of cupric hydroxide to 1 L of 28 to 29% ammonium hydroxide, and the mixture was stirred for 30 min. The reagent has a deep blue color. The following procedure was applied to determine the cellulose content using the Schweitzer method. First, the sample was pretreated to remove protein and other impurities. 0.1 to 0.3 g of sample (dry mass) was added to an Erlenmeyer flask and diluted to 200 mL with distilled water. To this sample, 1.25 mL of 50% NaOH solution and 5 mL antifoaming agent (Sigma Aldrich, Ontario, Canada; diluted in proportion of one part defoamer to five parts water) was added. The mixture was boiled for 30 min. The mixture was then cooled, and 300 mL of distilled water was added. The diluted mixture was transferred to a centrifuge bottle, and a centrifugal force of 724×*g* was applied for 20 min (Beckman Coulter Allegra 6 Centrifuge). The supernatant was decanted; the pellet was washed with 300 mL of distilled water and centrifuged again. The supernatant was discarded, and 100 mL of the Schweitzer reagent was added to the pellet. The pellet was broken using a spatula, and the bottle was placed on a mechanical shaker for 60–90 min at 120 rpm. The bottle was centrifuged, and the supernatant was collected into another centrifuge bottle containing 300 mL of 80% ethyl alcohol. The mixture was stirred and allowed to stand for 30 min. After 30 min, the bottle was centrifuged, and the supernatant was discarded. The pellet was washed with 1.25% HCl (breaking up the pellet using a spatula) until the blue copper color of the precipitate disappeared completely. The solution was filtered on pre-washed and weighed 1.2-μm

glass fiber filters (VWR, Ontario, Canada). The precipitate was washed with distilled water, followed by 10–20 mL of 80% ethyl alcohol. The filter was dried in a 105 °C oven overnight and weighed. The filter was ignited in a muffle furnace (Lindberg Blue Box Furnace) at 550 °C for 60 min and weighed. The % cellulose in the sample was calculated using the following equation (Eq. 2):

$$\%\text{cellulose} = \frac{\text{wt.dried residue} - \text{wt.ignited residue}}{\text{wt.of sample}} \times 100 \quad (2)$$

Results and discussion

Acid hydrolysis

Acid hydrolysis is the most widely used method for hydrolyzing carbohydrates. In an initial test, different cellulose sources were tested in triplicates including α-cellulose and toilet paper at 20 g/L (dry mass) in 10-mL reaction volume. As can be seen from Fig. 1a, the replicates were not reproducible. The highest yield of 50% was observed for toilet paper and α-cellulose samples, after 45 h of hydrolysis. It is noteworthy that cellulose yields for two α-cellulose samples were 50 and 42% and for the three toilet paper samples were 50, 25, and 23%.

The reaction volume in the above test was too small, and therefore, the test was repeated in 100-mL reaction volume at 20 g/L α-cellulose concentration (Fig. 1b). The results obtained in this test, i.e., the 25% cellulose yield was much lower than the 50% yield observed in the initial test and was not very encouraging due to the lack of reproducibility. Several studies have reported overall cellulose yields of 50–60% at higher temperatures of > 200 °C in typical batch reactors (Kim et al. 2001; Wyman et al. 2005). Nevertheless, pyrolysis and other side reactions occur at higher temperatures, leading to charring or caramelization of glucose (Orozco et al. 2007; Wyman et al. 2005). A black residue was observed in this study, which evidently may explain the low cellulose yields. There is abundant literature (Table 1) that has studied acid hydrolysis, using various acids (sulfuric acid, hydrochloric acid, oxalic acid, acetic acid-water-nitric acid, phosphoric acid, etc.) at varying temperatures and conditions, and every study achieved different cellulose yields (Bauer and Ibanez 2014; Chimentao et al. 2014; Kim et al. 2001; Orozco et al. 2007; Schell et al. 2003; Yoon et al. 2014). The majority of the research done on dilute acid treatment has been conducted to hydrolyze cellulose to glucose and cellodextrins (short-chain cellulose oligomers) (Olsson and Westman 2013). However, since the objective of this study was to quantify cellulose itself, the inability to duplicate the results of the test does not make this method reliable, and therefore, it is not suitable for determining cellulose concentrations.

Enzymatic hydrolysis

Enzymatic hydrolysis is the other widely studied method for cellulose hydrolysis. Although Champagne and Li (2009) recommended using 10% cellulase-to-cellulose concentration (on a mass basis), in this study, different cellulase concentrations ranging from 1 to 20% (Fig. 2a) were tested. It can be observed from Fig. 2a that although the 20% cellulase condition had the highest rate of cellulose conversion, the yield plateaued at 46% after 2 days. The highest yield of 67% cellulose was achieved by the 10% cellulase.

In order to develop a standard calibration curve for cellulose, the 20% cellulase dose was selected due to its high rate and another experiment was run using different cellulose concentrations as shown in Fig. 2b. We see a similar trend in this test, with the yield plateauing at 47 ± 3% after 2 days. The test was terminated after 7 days.

The standard curve was plotted at different time intervals, and a good linear relation was observed between the cellulose concentration and the measured glucose concentrations with $R^2 > 0.99$ (Fig. 3), but the slope of the linear relation was different at different times which makes it extremely difficult to standardize.

Hereafter, the enzymatic hydrolysis method was tested on sieved primary sludge samples and 20% cellulase dose (Fig. 4). The aforementioned standard curves (Fig. 3a, b) at 1 and 2 days were used to estimate the cellulose concentrations at different concentrations of sieved primary sludge. Table 2 tabulates these results which highlight the inconsistencies in % cellulose estimated in the same sample of sieved primary sludge at different concentrations. Unlike the experiment above that tested α-cellulose, varying yields (ranging from 40 to 83%) were observed at different concentrations of sieved primary sludge (Table 2). It is interesting to observe that the higher the concentration of sieved primary sludge, the higher the glucose yields (Fig. 4).

Theoretically, the specific surface area available for enzyme activity should not be different; however, higher recoveries maybe an artifact of biomass activity and hydrolysis of other carbohydrates to glucose. Champagne and Li (2009) conducted a similar study where enzymatic hydrolysis of dried primary sludge (4% TS) was performed, and 25 ± 0.8% conversion was reported after 24 h. This conversion efficiency increased to 37 ± 1% when the primary sludge was pretreated with both HCl and KOH (Champagne and Li 2009). Champagne and Li (2009) also emphasized that the differences in the percentage conversion were due to the cellulose fibers in the sludge being inaccessible to the enzyme due to the complex matrix of the primary sludge, and therefore, pre-treatment with HCl-KOH prior to enzymatic

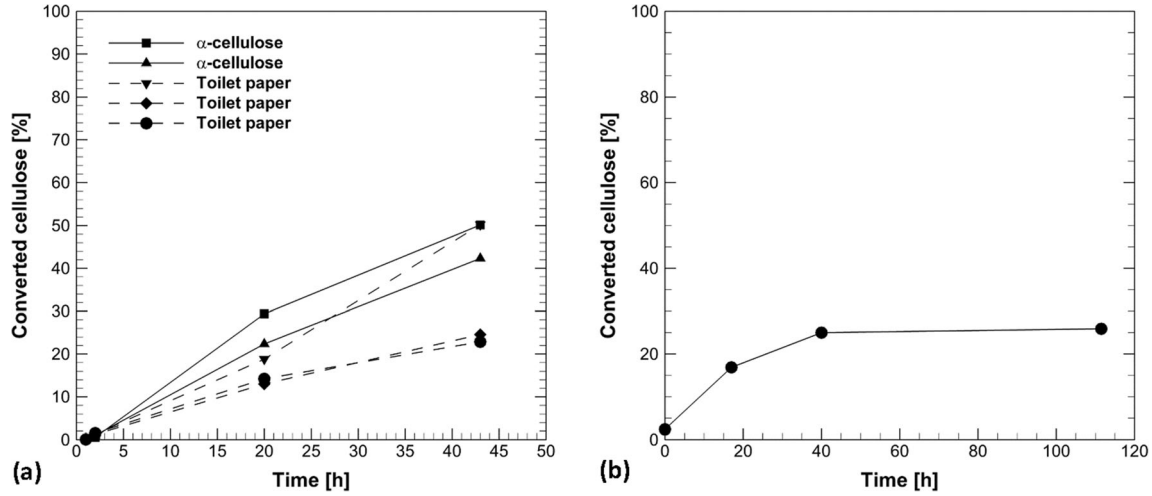

Fig. 1 Acid hydrolysis method at 100 °C (**a**) using different cellulose sources in 10 mL reaction volume and (**b**) at 20 g/L α-cellulose in 100 mL reaction volume

hydrolysis helped isolate cellulosic content from non-cellulosic constituents.

Thus, although enzymatic hydrolysis showed good reproducibility while testing with α-cellulose (Fig. 2b), it was not effective with sieved primary sludge samples due to its complex composition. Additionally, Mansfield et al. (1999) emphasized that the results obtained using "purer" model cellulosic substrates cannot be extrapolated to "real' substrates. The efficacy of enzymes in hydrolyzing substrates is intimately linked to the structural characteristics of the substrate such as DP, crystallinity, fiber size, accessible surface area, and the extent of fibrillation (Mansfield et al. 1999).

NREL method

The NREL method was another method that was tested to measure cellulose. In order to assess whether the method could differentiate between cellulose and starch, an initial test was conducted with different cellulose-to-starch mass ratios including 0:1, 1:3, 1:1, 3:1, and 1:0. Figure 5a shows the mass fraction of soluble sugars to the sum of cellulose and starch added, and it is observed that glucose was the predominant sugar detected in all the tests irrespective of the applied cellulose-to-starch mass ratio. The inability to differentiate cellulose from other carbohydrates is the biggest drawback of this method since the aggressive acidic hydrolysis solubilizes both cellulose and starch to glucose.

In order to dismiss this method as a reliable method for cellulose measurement, the NREL test was performed on toilet paper and sieved primary sludge samples with the results depicted in Fig. 5b which shows the mass fraction of cellobiose and arabinose relative to the mass of dry sieved primary solids added. Cellobiose (C6) and glucose (C6) are soluble products of cellulose whereas arabinose (C5) is a soluble product of hemicellulose. The sieved primary sludge showed 44 ± 2% cellobiose as compared to the toilet paper which showed 24 ± 3%

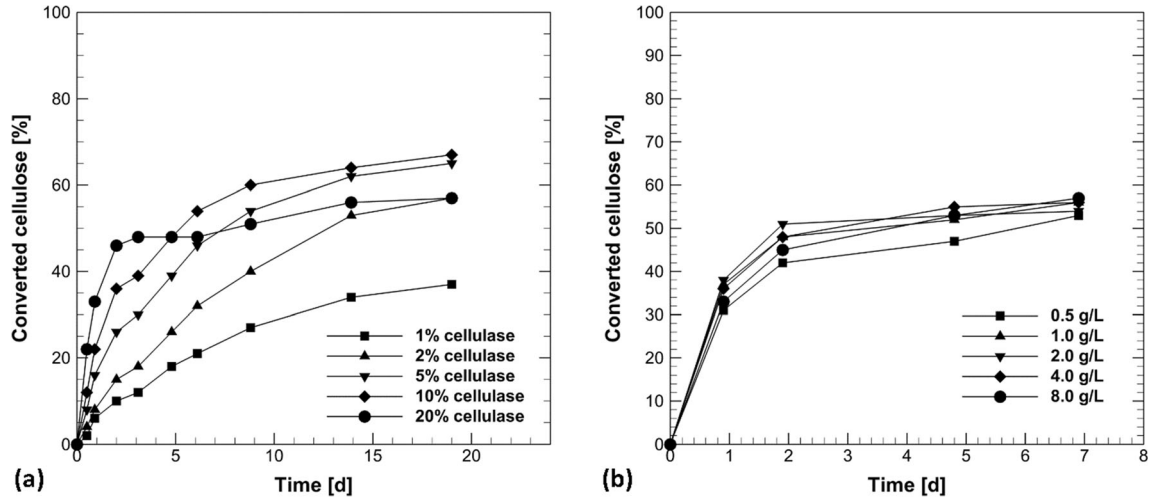

Fig. 2 Enzymatic hydrolysis (**a**) at different cellulase dose and 20 g/L α-cellulose and (**b**) with 20% cellulase dose at different α-cellulose concentrations

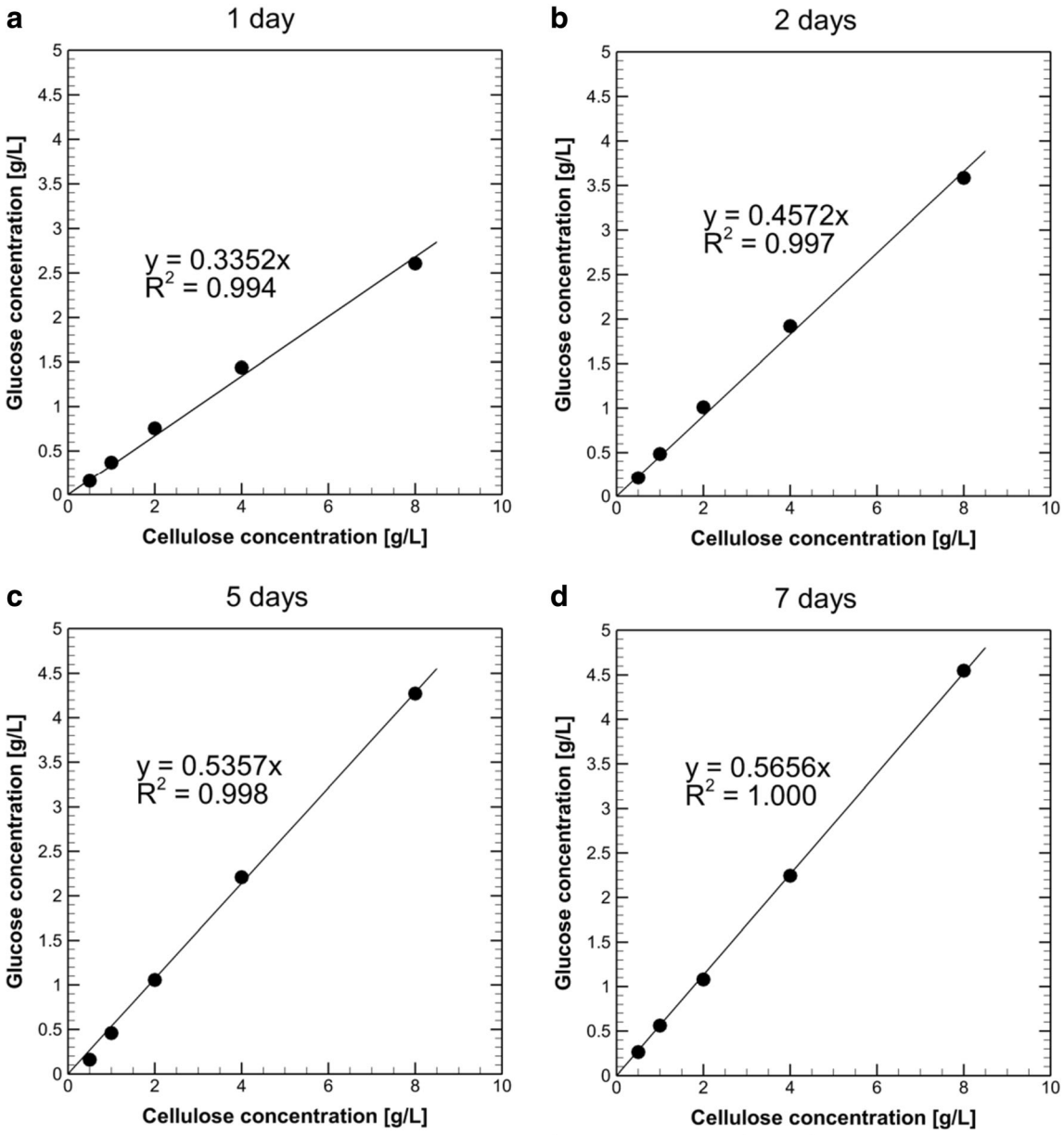

Fig. 3 Standard curves at different time intervals: (**a**) 1 day; (**b**) 2 days; (**c**) 5 days; and (**d**) 7 days

cellobiose. No glucose was detected in either sample; however, a significant amount of arabinose was detected in toilet paper (70 ± 1%) and sieved primary sludge (38 ± 2%). However, the reported 70% hemicellulose content in toilet paper seems to be unrealistically high. Few online sources (http://en.fenjie.com/news/show_223.html (accessed April 28, 2017); http://www.perinijournal.it/Items/en-US/Articoli/PJL-34/New-strength-additive-for-tissue-offers-much-promise (accessed April 28, 2017)) indicate the addition of hemicellulose to cellulose pulp in the making of toilet paper, but the precise composition of toilet paper is not available to the best of the authors' knowledge. Alternatively, the authors speculate that perhaps it is not arabinose that is detected but another degradation product. Yoon et al. (2014) studied different second hydrolysis reaction temperatures and observed lower cellulose to glucose conversion at higher temperature of 120 °C (~ 70%) but higher conversion of cellulose to formic acid due to further degradation of glucose in acidic medium to 5-hydroxymethylfurfural (HMF) and then to formic acid and levulinic acid. Similarly, the aforementioned authors also studied combinations of cellulose, xylan, and lignin and observed different conversion efficiencies compared to cellulose alone. The same argument made regarding the effect of structural characteristics on enzymatic hydrolysis applies to the two-stage acid hydrolysis (Mansfield et al. 1999).

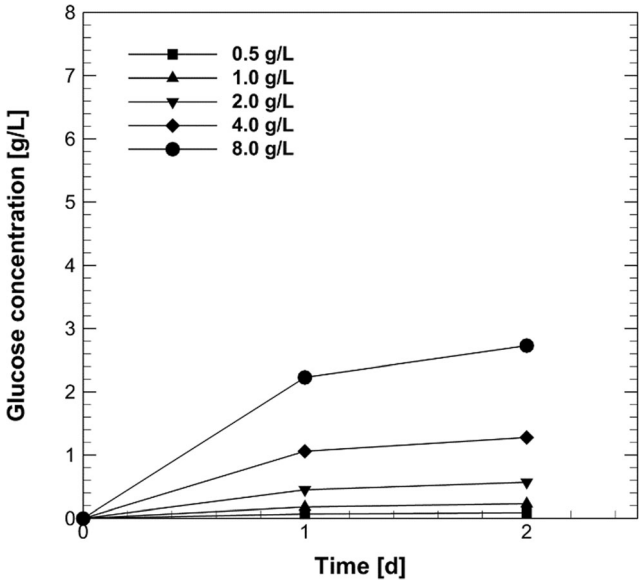

Fig. 4 Enzymatic hydrolysis with 20% cellulase dose at different sieved primary sludge concentrations

Schweitzer method

Figure 6 illustrates the % cellulose by dry mass in different cellulose sources as measured by the Schweitzer method. All tests were done in duplicates and showed excellent reproducibility, as evidenced by the minimal range of error bars. Toilet paper and α-cellulose were used as standards and showed 100% recovery, which was extremely encouraging. To confirm that the reagent does not bind to starch, two additional tests were run: starch only and combination of starch and cellulose (50%–50% by mass). The starch-only condition recovered < 1% cellulose which was anticipated, while the 50/50 starch and cellulose combination yielded 48 ± 1% of cellulose, re-affirming the cellulose specificity of the test method.

After the successful results obtained, the test was performed on primary clarifier sludge and sieved primary sludge, which showed 18 ± 0% and 37 ± 1% on dry basis, respectively. To further validate the method, known amounts (0.1 and 0.2 g) of α-cellulose were added to 0.3 g of dry primary sludge, and the recovery of the added α-cellulose was estimated by the difference between measured cellulose in the amended sample and raw primary sludge sample (Eq. 3). According to Fig. 6, % cellulose in standard addition test where 0.3 g of primary sludge was incorporated with 0.2 g of α-cellulose was measured to be 49 ± 1%. Therefore, the difference between the amended sample and the un-amended sample should be the known amount (in this case 0.2 g cellulose) to be recovered:

$$\text{Recovered\%cellulose} = \frac{\text{Amended sample-primary sludge}}{\text{Known cellulose added}} \times 100\%$$

$$\text{Recovered\%cellulose} = \frac{(0.49 \times 0.5\ \text{g amended sample}) - (0.18 \times 0.3\ \text{g primary sludge})}{0.2\ \text{g cellulose}} \times 100\% = 95\% \quad (3)$$

Similarly, the % cellulose in standard addition test where 0.3 g of primary sludge was mixed with 0.1 g of α-cellulose was measured to be 37 ± 1%, i.e., 92% of added cellulose was recovered.

The Schweitzer method thus satisfies the criteria for a reliable analytical method to quantify cellulose in wastewater and sludge samples as proven based on reproducibility, accuracy, and fixed 100% recovery. It is noteworthy that all other methods tested in this study with the exception of the Schweitzer method rely on measurement of soluble sugars after hydrolysis and implicitly assume that the original concentration of soluble sugars in the samples is negligible. Furthermore, soluble sugars could be produced by hydrolysis of other carbohydrates not specifically cellulose. Thus, all other methods theoretically should overestimate the cellulose content. Despite the aforementioned, it is evident that the recoveries of cellulose using the Schweitzer method are much greater which is essentially because the Schweitzer method does not depend on the hydrolysis efficiency and reduced product analysis, but instead uses a dissolution-extraction method with gravimetric quantification of the precipitate formed. The complete recovery of both standards used, i.e., toilet paper and α-cellulose, as well as the relative quickness and ease of the Schweitzer method renders it the most ideal method for cellulose determination in wastewater and sludge samples. Although Hurwitz et al. (1961) originally developed this method

Table 2 Estimated % cellulose of sieved primary sludge

	Sieved sludge concentration dosed				
	0.5 g/L	1 g/L	2 g/L	4 g/L	8 g/L
Glucose conc. (g/L) after 1 day	0.07	0.18	0.45	1.06	2.23
Corresponding cellulose conc. (g/L) using Fig. 3a standard curve	0.2	0.5	1.3	3.2	6.7
Estimated % cellulose	40	54	67	79	83
Glucose conc. (g/L) after 2 days	0.09	0.23	0.57	1.28	2.73
Corresponding cellulose conc. (g/L) using Fig. 3b standard curve	0.2	0.5	1.3	2.8	6.0
Estimated % cellulose	41	50	63	70	75

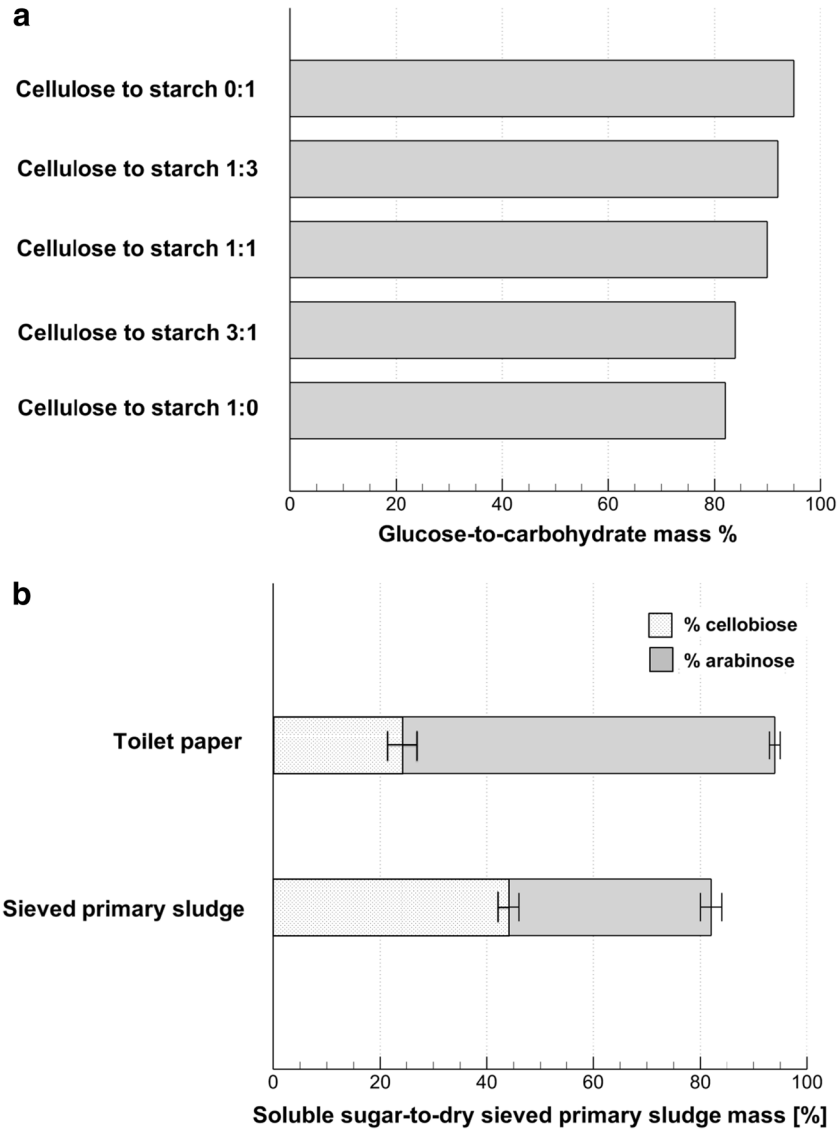

Fig. 5 NREL method results on (**a**) different cellulose-to-starch ratios and (**b**) toilet paper and sieved primary sludge

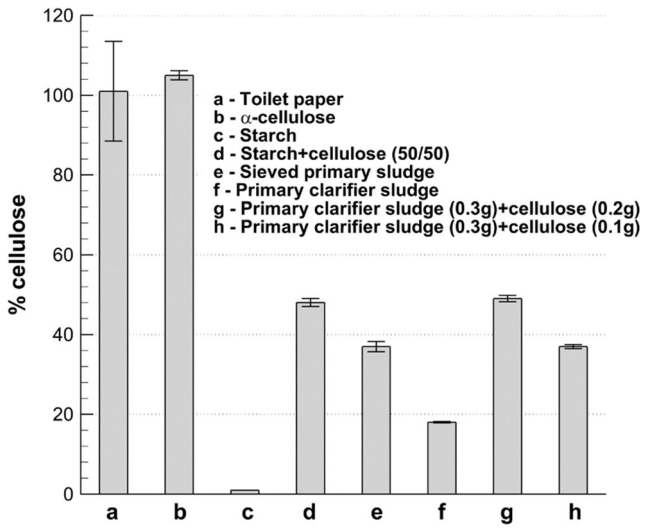

Fig. 6 Schweitzer method results for different cellulose sources

for cellulose determination in sewage sludge and reported similar recovery of cellulose with high reproducibility (97.5 and 98%), they did not provide any proof of validation for the method. In this study, extensive validation tests using different cellulose standards such as α-cellulose and toilet paper were undertaken. Additionally, this study also confirmed that starch (another common carbohydrate found in wastewater) does not interfere with the cellulose measurements.

Conclusions

After evaluation of the results, it can be concluded:

- Of the four methods tested for cellulose determination in wastewater/sludges, the Schweitzer reagent method is the

only reliable method. Acid hydrolysis was not reproducible even with pure cellulose, and enzymatic hydrolysis showed poor reproducibility with sieved primary sludge despite its high reliability with pure cellulose. The inability to differentiate cellulose from other carbohydrates limited the reliability of the NREL for sieved primary sludge.
- The advantage of the Schweitzer method is its simplicity, thanks to its specificity to cellulose, reproducibility, the 100% recovery, and relative quickness of the test as well as its independence from hydrolysis reactions.
- Having a reliable method to quantify cellulose in wastewater will have great implications on wastewater research and will aid the already emerging trend to increase sustainability and resource recovery.

Acknowledgements This research was funded by Natural Sciences and Engineering Research Council (NSERC) of Canada—Collaborative Research and Development (CRD) (grant number CRDPJ-488704-15). Dr. Peter A. Vanrolleghem holds the Canada Research Chair on Water Quality Modeling.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Alkasrawi M, Al-Hamamre Z, Al-Shannag M, Abedin MJ, Singsaas E (2016) Conversion of paper mill residuals to fermentable sugars. Bioresources 11(1):2287–2296

Bauer S, Ibanez AB (2014) Rapid determination of cellulose. Biotechnol Bioeng 111(11):2355–2357. https://doi.org/10.1002/bit.25276

Bolam F (1965) Stuff preparation for paper and paperboard making. Pergamon Press Inc, New York

Camacho F, Gonzalez-Tello P, Jurado E, Robles A (1996) Microcrystalline-cellulose hydrolysis with concentrated sulfuric acid. J Chem Technol Biotechnol 67(4):350–356. https://doi.org/10.1002/(SICI)1097-4660(199612)67:4<350::AID-JCTB564>3.0.CO;2-9

Champagne P, Li C (2009) Enzymatic hydrolysis of cellulosic municipal wastewater treatment process residuals as feedstocks for the recovery of simple sugars. Bioresour Technol 100(23):5700–5706. https://doi.org/10.1016/j.biortech.2009.06.051

Chimentao RJ, Lorente E, Gispert-Guirado F, Medina F, Lopez M (2014) Hydrolysis of dilute acid-pretreated cellulose under mild hydrothermal conditions. Carbohydr Polym 111(13):116–124. https://doi.org/10.1016/j.carbpol.2014.04.001

Edberg N, Hofsten B (1975) Cellulose degradation in wastewater treatment. J Water Pollut Control 47:1012–1020

Faust L, Krooneman J, Euverink GJW (2014) A new reliable method to measure cellulose in wastewater and sludge. Products and processes for biotechnology in the Biobased economy, University of Groningen. Poster presented at cellulose symposium

Fuller MS, Barshad I (1960) Chitin and cellulose in the cell walls of Rhizidiomyces sp. Am J Bot 47(2):105–109

Gao X, Kumar R, Wyman CE (2014) Fast hemicellulose quantification via a simple one-step acid hydrolysis. Biotechnol Bioeng 111(6):1088–1096. https://doi.org/10.1002/bit.25174

Gavila L, Constanti M, Medina F (2015) D-Lactic acid production from cellulose: dilute acid treatment of cellulose assisted by microwave followed by microbial fermentation. Cellulose 22(5):3089–3098. https://doi.org/10.1007/s10570-015-0720-1

Gupta UC, Sowden FJ (1964) Isolation and characterization of cellulose from soil organic matter. Soil Sci 97:328–333

Harris D, Bulone V, Ding S-Y, DeBolt S (2010) Tools for cellulose analysis in plant cell walls. Plant Physiol 153:420–426. https://doi.org/10.1104/pp.110.154203

Honda S, Miyata N, Iwahori K (2002) Recovery of biomass cellulose from waste sewage sludge. J Mater Cycles Waste 4(1):46–50. https://doi.org/10.1007/s10163-001-0054-y

Hurwitz E, Beck AJ, Sakellariou E, Krup M (1961) Degradation of cellulose by activated sludge treatment. J Water Pollut Control Fed 33(10):1070–1075

Kauffman GB (1984) Eduard Schweizer (1818-1860): the unknown chemist and his well-known reagent. Profiles Chem 61(12):1095–1097. https://doi.org/10.1021/ed061p1095

Kim JS, Lee YY, Torget RW (2001) Cellulose hydrolysis under extremely low sulfuric acid and high-temperature conditions. Appl Biochem Biotechnol 91(1–9):331–340. https://doi.org/10.1385/ABAB:91-93:1-9:331

Mansfield SD, Mooney C, Saddler JN (1999) Substrate and enzyme characteristics that limit cellulose hydrolysis. Biotechnol Prog 15(5):804–816. https://doi.org/10.1021/bp9900864

Olsson C, Westman G (2013) Direct dissolution of cellulose: background, means and applications. Cellul Fundam Asp Intech 143–178. https://doi.org/10.5772/52144

Orozco A, Ahmad M, Rooney D, Walker G (2007) Dilute acid hydrolysis of cellulose and cellulosic bio-waste using a microwave reactor system. Process Saf Environ Prot 85(5):446–449. https://doi.org/10.1205/psep07003

Pellizzer L (2016) Synthesis of cellulose-based flocculants and performance tests. Masters thesis. University of Coimbra. http://hdl.handle.net/10316/37492

Raunkjær K, Hvitved-Jacobsen T, Nielsen PH (1994) Measurement of pools of protein, carbohydrates and lipid in domestic wastewater. Water Res 28(2):251–262. https://doi.org/10.1016/0043-1354(94)90261-5

Rinaldi R, Schüth F (2009) Acid hydrolysis of cellulose as the entry point into biorefinery schemes. ChemSusChem 2(12):1096–1107. https://doi.org/10.1002/cssc.200900188

Ruiken CJ, Breuer G, Klaversma E, Santiago T, van Loosdrecht MCM (2013) Sieving wastewater—cellulose recovery, economic and energy evaluation. Water Res 47(1):43–48. https://doi.org/10.1016/j.watres.2012.08.023

Sarathy S, Ho D, Murray A, Batstone D, Santoro D (2015) Engineered fractionation of primary solids—a comparison of primary treatments using rotating belt filters and primary clarifiers. Proceedings of the Water Environment Federation, WEFTEC, Chicago, USA. doi: https://doi.org/10.2175/193864715819555931

Seymour RB, Johnson EL (1976) The effect of solution variables on the solution of cellulose in dimethyl sulfoxide. J Appl Polym Sci 20(12):3425–3429. https://doi.org/10.1002/app.1976.070201220

Schell DJ, Farmer J, Newman M, McMillen JD (2003) Dilute-sulfuric acid pretreatment of corn stover in pilot-scale reactor: investigation of yields, kinetics, and enzymatic digestibility of solids. Appl Biochem Biotechnol 105(1–3):69–85. https://doi.org/10.1385/ABAB:105:1-3:69

Sluiter A, Hames B, Ruiz C, Scarlata C, Sluiter J, Templeton D, Crocker D (2012) Determination of structural carbohydrates and lignin in biomass. National Renewal Energy Laboratory (NREL), US Department of Energy. Technical Report: NREL/TP-510-42618

Thoorens G, Krier F, Leclercq B, Carlin B, Evrard B (2014) Microcrystalline cellulose, a direct compression binder in a quality

by design environment—a review. Int J Pharm 473(1–2):64–72. https://doi.org/10.1016/j.ijpharm.2014.06.055

Updegraff DM (1969) Semimicro determination of cellulose in biological materials. Anal Biochem 32(3):420–424. https://doi.org/10.1016/S0003-2697(69)80009-6

Verachtert H, Ramasamy K, Meyers M, Bevers J (1982) Investigations on cellulose biodegradation in activated sludge plants. J Appl Bacteriol 52(2):185–190. https://doi.org/10.1111/j.1365-2672.1982.tb04839.x

Wyman CE, Decker SR, Brady JW, Viikari L, Himmel ME (2005) Hydrolysis of cellulose and hemicellulose. In: Polysaccharides, structural diversity and functional versatility, pp. 1023–1062

Xiang Q, Lee Y, Pettersson P, Torget R (2003) Heterogeneous aspects of acid hydrolysis of α-cellulose. Appl Biochem Biotechnol 107:505–514

Yang B, Dai Z, Ding S-Y, Wyman CE (2011) Enzymatic hydrolysis of cellulosic biomass. Biofuels 2(4):421–450. https://doi.org/10.4155/BFS.11.116

Yoon SY, Han SH, Shin SJ (2014) The effect of hemicelluloses and lignin on acid hydrolysis. Energy 77(1):19–24. https://doi.org/10.1016/j.energy.2014.01.104