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ACID HYDROLYSIS AS A METHOD TO VALORIZE CELLULOSIC FILTER CAKE FROM INDUSTRIAL CARRAGEENAN PROCESSING

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ABSTRACT

Wastes generated from carrageenan processing industry include cellulosic filter cakes (CFC) which are mainly composed of structural sugar (0.25 w/w) and ash (0.75 w/w, primarily perlite). This study investigated the possible valorization of CFC by recovering the available sugars as glucose through direct acid hydrolysis. Five different sulfuric acid concentrations (5% v/v to 15% v/v) were used as catalyst for hydrolysis done at constant temperature and solvent-to-solid ratio of 95°C and 8 mL/g, respectively, over a reaction time of 5 to 300 minutes, to determine the effect of acid concentration on the hydrolysis yield. The maximum sugar yield achieved was only ~0.06 w/w, corresponding to a recovery of ~24%, for hydrolysis done with 10% v/v sulfuric acid for 120 minutes. Although the amount of sugar recovered was relatively low, hydrolysates obtained have a sugar concentration of ~7 g/L, a level considered adequate for substrates in some fermentation processes. In addition, none of the inhibitory compound, 5-hydroxymethylfurfural, was present in the hydrolysate. Drying of residual solids obtained after hydrolysis was found to result in the sulfonation of the remaining organic fraction, producing a sulfonated residue (with total acid density of 4 to 7 mmol H+/g) which may be used as solid acid catalyst.

1. INTRODUCTION

Philippines leads the global carrageenan production with a production capacity of at least 36, 400 MT, sharing 40% of the total world production (Nobleza, 2013). Refined carrageenan is produced by alkali treatment of red seaweeds genera Gigartina, Eucheuma (previously Kappaphycus), Chondrus, and Hypnea, using sodium hydroxide or potassium hydroxide for carrageenan extraction, followed by separation of the cellulosic residue from the extract through filtration (Rhein-Knudsen et al., 2015; Stanley, 1987). During filtration, perlite is added at a ratio of 500 kg for every 1700 kg of raw seaweeds processed (Lhonneur, 1992), which results to about 31% of perlite in the cellulosic filter cake generated if assumed that the carrageenan extraction yield is ~35 kg per 100 kg of raw seaweed (Manuhara et al., 2016). Considering that the annual production of red seaweeds is ~1.6 million tons, with 22% processed to produce refined carrageenan (FAO, 2015; Philippine Bureau of Investments, 2011), there is about 16.2 ktons of perlite-containing cellulosic filter cake waste that needs to be disposed. A relevant action would be to find ways of valorizing said waste as an approach to solving the disposal problem and make possible a zero-waste carrageenan production industry.

Cellulosic biomass, like that of macroalgae for carrageenan proprduction are composed of cellulose, hemicellulose and lignin (Fan et al., 1987). However, compositional analysis done by Tan and Lee (2015) found that 68% of macroalgae carrageenan residue (MCR) is cellulose and has no acid insoluble lignin and hemicellulose, making MCR attractive for hydrolysis and subsequent fermentative processes, since delignification would not be required. Cellulose is composed of anhydrous glucose which are linked together by glycosidic bonds (Badger, 2002). Glucose is a useful fermentation substrate in the production of bioethanol and other high-value platform chemicals like 1,2-propanedieol (Douglas and Cooney, 1986), lactic acid and acetol (Hang, 1989), 2-keto-L-gulonic acid (Matsuda and Kageyama, 1982), and succinic acid (Li et al., 2010; Zheng et al., 2009). It can be recovered from cellulosic materials by breaking down β-1,4-glycosidic linkages through a process called hydrolysis (Kumar et al., 2009). However, the

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ease by which cellulose can be hydrolyzed is a function of its crystallinity and intermolecular bonding. The structure of cellulose can be grouped into six polymorphs namely cellulose I, II, III_1, III_{11}, IV_1, IV_{11} and VII. Only cellulose I is found in nature and is further classified into I_α and I_β (Rinaldi and Schüth, 2009). Cellulose I_α, a dominant structure in algal biomass, have a metastable triclinic structure, whereas, cellulose I_β which is commonly found in wood and cotton fibers have a stable monoclinic structure (O'Sullivan, 1997; Rinaldi and Schüth, 2009). In a triclinic structure, only one cellulose chain is present per unit cell whereas in monoclinic structure, there are two chains in each unit cell. The latter is more packed, resulting to more interactions between cellulose molecules. Thus, algal biomass have lesser degree of crystallinity and are relatively easier to hydrolyze than wood and cotton fibers (O'Sullivan, 1997). Hydrolysis of algal biomass can be enzymatically or chemically catalyzed.

Enzymatic hydrolysis uses a mixture of different types of enzymes called endoglucanase, exoglucanase, and β-glucosidase (Zhang and Zhang, 2013). Enzymatic hydrolysis of algal biomass have been investigated by Kumar et.al (2013), Trivedi et.al (2013) and Tan & Lee (2014). Masarin et.al (2016) and Tan & Lee (2014) particularly looked into the enzymatic hydrolysis of carrageenan residue. A mixture of enzymes (92 FPU/ml Cellic Ctec II and 1800 UI/ml β-glucosidase) were used by Masarin et.al (2016) to hydrolyze the Eucheuma cottonii extraction residue for 72 hours at temperature and pH of 45°C and 4.8, respectively, achieving a sugar yield of 65%w/w (g sugar per 100 g residue). Tan & Lee (2014) achieved ~64%w/w sugar yield from the hydrolysis of residue using an enzyme loading of 82.08 FPU/ml (Celluclast 1.5L) and 326.12 U/ml (Novozyme 188) for 54 hours at a temperature and pH of 50°C and 4.8, respectively. Although high sugar yield was achieved and no inhibitors (5-HMF and furfural) were formed during hydrolysis at relatively mild conditions, the long reaction times and the cost of enzymes are considered the main drawbacks to actual industrial application.

Chemical hydrolysis of cellulose is carried out in the presence of acids, commonly sulfuric acid, to catalyze the reaction. Depending on the acid used, hydrolysis reaction can be categorized as heterogeneous or homogeneous. Heterogeneous acid hydrolysis employs solid acid catalyst which can be recovered, regenerated and reused. A study was conducted by Tan and Lee (2015) on a two-step hydrolysis of carrageenan residue. The pre-treatment step done for 30 minutes and at 120°C employed the solid acid catalyst, Dowex ™ Gr-D8, at a solvent-to-solid ratio of 10 mL/g, and 4% w/v catalyst loading. The residue left after pre-treatment was then subjected to enzymatic hydrolysis which achieved a sugar yield of 67.8%w/w after 30 h. Although, the use of solid acid catalyst is a promising technology for sugar recovery because it allows catalyst reuse, its particular application in the hydrolysis of cellulosic filter cake poses a challenge because the recovery of the catalyst would be difficult due to the presence of perlite and residual solids.

Homogeneous acid hydrolysis employs acids in liquid state to catalyze the reaction. Several studies have been reported on the dilute-acid hydrolysis of algal biomass. Hydrolysis of K. alvarezii using sulfuric acid (0.2 M) and hydrochloric acid (0.2 M) at 10 mL/g solvent-to-solid ratio, temperature of 130°C for 15 minutes achieved a sugar yield of 38.5%w/w and 22.7%w/w, respectively (Dyah and Meinita, 2012). Hydrolysis of Gracilaria tenuistipitata, Gracilariopsis chorda, and Gelidium amansii using sulfuric acid at same operating conditions had a sugar yield of 26.6%w/w, 23.4%w/w, and 29.2%w/w, respectively (Meinita et al., 2013). Although acceptable sugar yield is achieved for dilute-acid hydrolysis, the main drawback for this technology is the further degradation of sugars to furans (ranging from 0.81 g/L (Dyah et al., 2014) to 5.9 g/L (Meinita et al., 2012)) which can inhibit the activity of microorganisms used in the subsequent fermentation process. Nonetheless, the process has the advantage of having relatively shorter reaction time and using less expensive catalyst, making it more economically attractive for producing reducing sugar from algal biomass.

Studies reported in literature on the acid hydrolysis of algal biomass focused mainly on raw seaweeds and macroalgae cellulosic residue (MCR), which does not contain the perlite or filter aids that are used in actual industrial process. Perlite is a glassy, volcanic rock (Maxim et al., 2014) mainly composed of silica (72%w/w) and alumina (13%w/w) (Samar and Saxena, Shweta, 2016). Considering that these are basic oxides, these may hydrolyze the acid used and may interfere the hydrolysis of CFC. Hence, this study aimed to produce reducing sugar-containing hydrolysates from CFC through hydrolysis using sulfuric acid as the catalyst. Specifically, the objectives of this study were to determine the effects of acid concentration (5% v/v to 15% v/v) and reaction time (5 to 300 minutes) on the yield and concentration of sugar during the hydrolysis of CFC at a constant temperature and solvent-to-solid ratio of 95°C and 8 mL/g, respectively. In addition, the amount of glucose degradation product (5-hydroxymethyfurfural) formed and the proximate composition of hydrolyzed CFC were also determined. Furthermore, the incidental carbonization and sulfonation of the solid residue after hydrolysis were investigated to look into its possible application as a solid acid catalyst.

2. MATERIAL AND METHODS

Cellulosic filter cake (CFC) samples were collected from a carrageenan processing company in Cebu, Philippines. Chemical reagents used were obtained through local distributors: sulfuric acid (Ajax Finechem, 98% w/w), hydrochloric acid (Ajax Finechem, 36% w/w), anhydrous sodium carbonate (Ajax Finechem, 99.8% w/w), sodium hydroxide pellets (Qualikems, 98% w/w),phenol crystals (Qualikems, 99.5%w/w), and acetonitrile (HPLC grade), glacial acetic acid, Rochelle salt (HiMedia Lab, 99% w/w), dinitrosalicylic acid (HiMedia Lab, 98% w/w), D-glucose, and 5-hydroxymethyl furfural (Sigma).

2.1 Collection, storage and characterization of carrageenan filter cake

Collected filter cake samples from a local carrageenan processing, with a wet, pale-white appearance, had an as received moisture content of ~89% w/w. These were dried

at 105°C in a convection oven (Memmert UM 500 F) until a moisture content of <10% was achieved. The dried samples were milled using Wiley mill, determined of its mean particle size following ASTM D1762-84 (ASTM, 2011) and then stored at room temperature (25 to 28 ºC) in a sealable plastic container for further analyses.

2.1.1 Proximate analysis and chemical composition

Filter cake samples were characterized for its proximate composition (moisture, volatile matter, ash and fixed carbon) following NREL/TP-510-42622 (Sluiter et al, 2008) and ASTM D1762-84 (ASTM, 2011). To determine the water soluble fraction of the sample, water extractives of filter cake sample (~2 g) were first determined using Soxhlet extractor for 24 hours with 4 to 5 siphon cycles per hour, following NREL protocol (Sluiter et al., 2008) with slight modification. The receiving flask containing the extract was cooled to room temperature. Contents of the flask was transferred into a 250 mL Erlenmeyer flask. The filter cake sample inside the thimble was thoroughly rinsed with distilled water, with the filtrate collected and added to the flask containing the extractives. The total mass of the collected extracts was recorded. The thimbles containing the extracted samples was then dried and the change in weigh of samples was taken to be the water extractives.

Soluble sugar recovered in the extractives were also analyzed using DNS (Miller, 1959) and phenol-sulfuric method (Dubois et al., 1956) for reducing sugar and total sugar, respectively. Residual base as dissolved in water extractives were also determined by titration with 0.01 N HCl until the pH meter indicated a neutral pH of 7 reading. Direct determination of residual base in CFC samples (~1 g) were also carried out by direct titration. Water of 50 mL volume was poured into an Erlenmeyer flask containing the sample and was heated to 100°C using a heating plate and stirred continuously with a magnetic stirrer for an hour. The mixture was then cooled, and a pH probe was immersed into the cooled mixture and titrated with 0.01 N standardized hydrochloric acid (HCl) solution until a pH of 7. Residual base were then expressed in equivalents of NaOH.

Total recoverable sugar of the samples was determined by employing modified NREL protocol (TP-510-42618) for algal biomass by Kostas et.al (Kostas et al., 2016), with some modifications. About 90 mg (weighed to the nearest 0.1 mg) of the sample was first hydrolyzed using 3 mL of 11 M sulfuric acid at 30°C for an hour. The mixture was then diluted with 30 mL distilled water to achieve a concentration of ~ 1 M sulfuric acid and incubated for two hours at 95°C in a water bath (Memmert One 10). Neutralization of the reaction mixture was done by adding sodium carbonate until effervescence ceased. The neutralized reaction mixture was filtered using ordinary filter paper into a 100-mL volumetric flask and diluted to the mark with distilled water. Hydrolysates obtained after filtration was then analyzed for its total sugar and total reducing sugar (Section 2.2.1).

2.2 Direct dilute acid hydrolysis

Hydrolysis of cellulosic filter cake was carried out using varied sulfuric acid concentration (5, 7.5, 10, 12.5, and 15% v/v) over different reaction times (5, 30, 60, 120, 180, 240, and 300 min) at constant temperature of 95˚C and solvent-to-solid ratio of 8 mL/g. About 10 g of sample was hydrolyzed with 80 mL of sulfuric acid solution in a 100-mL screw-capped media bottles, maintaining a solvent-to-solid ratio of 8 mL/g. Reaction bottles were incubated in a water bath at 95˚C and intermittently mixed every 30-minute interval. After the pre-determined hydrolysis time, the media bottles containing the hydrolysis mixture were immediately quenched in an ice bath. Vacuum filtration with Whatmann No.2 filter paper (8 m pore size) of the hydrolysis mixture was then carried out immediately to separate the liquid hydrolysates from the solid residues. The residues remaining on the filter paper, referred to as post-hydrolysis residue (PHR), were washed with 20 mL distilled water, transferred to an evaporating dish (120 mm in diameter), and dried at 100˚C to constant weight. Residual solids yield, Y_{RS} (g solids/g dry sample), was calculated using Equation 1, where m_{rd} (g) was the overall weight of dried filter paper, residue and evaporating dish, m_{dd} (g) the mass of dry evaporating dish, m_{df} (g) the mass of dry filter paper and M the moisture content of the sample being analyzed. Dried PHR were then stored in a sealable plastic for further proximate analysis (section 2.1.1) and total acid density determination (section 2.2.2).

$$Y_{RS} = \frac{m_{rd} - m_{dd} - m_{df}}{m_s \times (1 - M)} \quad (1)$$

The hydrolysate separated from the solid was pooled together with the washing and the resulting volume was measured using graduated cylinder. Hydrolysates collected as such were then analyzed for pH, transferred into a sample bottle, and stored at 4˚C for further analysis. The hydrolysate yield (Y_H) was calculated based on the volume V_h (mL) of collected hydrolysate and washing using Equation 2, where V_w (mL) was the volume used for washing and (mL) the volume of hydrolyzing medium.

$$Y_H = \frac{V_h}{V_w + V_{hm}} \quad (2)$$

2.2.1 Hydrolysate analysis

Hydrolysates were then analyzed for its total reducing sugar and total sugar content by employing DNS method (Miller, 1959) and phenol-sulfuric method (Dubois et al., 1956), respectively. Analysis of inhibitor concentration in the form of 5-hydroxymethyl furfural (5-HMF) in the hydrolysates was also done using high performance liquid chromatography (HPLC) following the protocol described by Ahmed et. al (Ahmed et al., 2013).

Total Reducing Sugar Analysis. DNS solution was prepared by dissolving about 10 g of dinitrosalicylic reagent and 2 g of crystalline phenol in 800 mL of 2% sodium hydroxide (NaOH) solution. Sodium sulphite solution was prepared separately by dissolving 5 g of sodium sulphite in 200 mL of 1% NaOH solution. Total reducing sugar content of the standard glucose solutions and hydrolysates was analyzed by pipetting about 2.4 mL of DNS (dinitrosalicylic acid) and 0.6 mL sodium sulfite into the sample (3 mL). After incubation of the vial containing the sample at 95°C in a water bath for 5 minutes, the vial was then removed from the water bath and 1 mL of 40% Rochelle salt solution was added into the vial to stabilize the color of the reaction

mixture. The vial was quenched in a water bath (~30°C) for 10 minutes, mixed thoroughly for 10 seconds using a vortex mixer, and analyzed using spectrophotometer (UV-1700, Shimadzu, Japan) at 540 nm. From the calibration curve, total reducing sugar concentration, C_{TRS} (mg/mL) was first determined, fractional yield, Y_{TRS} (g total reducing sugar/g dry filter cake sample), and the recovery (%), R_{TS} (g total reducing sugar/g total sugar content) in hydrolysate were calculated using Equation 3 and Equation 4.

$$Y_{TRS\ or\ TS} = \frac{C_{TRS\ or\ TSC} \times V_h}{m_S \times (1 - M)} \quad (3)$$

$$R_{TRS\ or\ TS} = \frac{Y_{TRS\ or\ TS}}{TSC\ or\ TRS} \times 100\% \quad (4)$$

Total Sugar Analysis. Phenol solution was prepared by dissolving approximately 50 g of phenol crystals with water and was diluted to 1 L. Total sugar concentration of the standard solutions and hydrolysate was determined by adding about 1 mL of phenol solution and 5 mL 96% sulfuric acid into the vial containing the sample (1 mL). The reaction mixture was allowed to stand for 10 minutes and mixed thoroughly using vortex mixer for 10 seconds. After incubation of the sample at 30°C for 20 minutes in a water bath, its total sugar content was then analyzed using a spectrophotometer (UV-1700 UV Visible Spectrophotometer Shimadzu) at a wavelength of 490 nm. The concentration of total sugar in the hydrolysate, C_{TS} (mg/mL), fractional yield, Y_{TS} (g total sugar/g dry filter cake sample), and the recovery (%) of total sugar, R_{TS} (g total sugar/r g total sugar content) in the hydrolysate were calculated using Equation 3 and Equation 4.

Analysis of inhibitor (5-HMF). The degradation product of glucose, 5-HMF, formed during acid hydrolysis was determined using HPLC equipped with C-18 Reverse-phase Column (ODS-3V, Inertsil, Europe), LC 10AT high pressure pump, CTO-10A oven and UV-VIS detector, SPD-10AV (Shimadzu, Japan). A mixture of acetonitrile, water and acetic acid at 11:88:1 v/v/v proportion was used as the mobile phase. Hydrolysate samples were filtered using sterilized membrane filter (0.45 µm) and injected (20 µL injection volume) into the HPLC system at 30°C, with elution at constant flowrate of 1 mL/min, and absorbance measured at 276 nm wavelength. The inhibitor concentrations (g/L) were calculated from the calibration curve (peak area vs. mass of analyte) obtained from the response detection of the standard 5-HMF solutions.

2.2.2 Yields of proximate components and acid sites in PHR

To understand the mass gain or loss of the post-hydrolysis residue (PHR), yields of proximate components were calculated using the data obtained from proximate analysis of the PHR and residual solids yield. Post hydrolsate residue samples were also analyzed for its total acid density adopting the procedure employed by Boehm et.al (Boehm et al., 1964). About 0.2 g of the sample was added with 50 mL (V_B) standardized 0.05 M NaOH solution and mixed thoroughly for 24 h at 200 rpm in an incubator shaker. The solids were then separated from the solution by filtration using Whatman filter paper (8 m pore size) and the volume of the filtrate (V_s) was measured using a graduated cylinder. An aliquot of about 10 mL (V_a) of the filtered solution is pipetted and added with 25 mL (V_A) standardized 0.035 M HCl solution. The acidified solution was added with three drops of phenolphthalein indicator and titrated with the standard 0.05 M NaOH solution using a buret until the color of the solution changed from colorless to pink. Volume of the titrant dispensed (V_T) was calculated by taking the difference of the final and initial buret reading. Total acid density of the residual solids in mmol H^+/g dry residue (ρ_{AD}) was then calculated using Equation 5, where C_B (mmol/mL), C_A (mmol/mL), and C_T (mmol/mL) were the concentrations of NaOH, HCl, and the titrant (NaOH) used, respectively.

$$\rho_{\mathrm{AD}} = V_B \left\{ \frac{C_B - \frac{[C_A V_A - C_T V_T]}{V_a}}{m_{RS}} \right\} \quad (5)$$

3. RESULTS AND DISCUSSION

Carrageenan filter cake (CFC) samples as received had a moisture content of 89.22±1.00% w/w (wet basis). Characteristics of dried and milled CFC are summarized in Table 1. Dry filter cakes contain ~75% w/w ash and ~25%w/w organics based on the amount of volatile matter and fixed carbon present. Considering that majority of what is left as residue during the production of refined carrageenan is cellulose, the organic fraction was expected to be purely composed of sugars. Upon determination of the total recoverable sugar content, it was found to contain 26.78± 5.80% w/w, with 15% of that fraction determined to be soluble sugar. This is consistent with the estimated organic fraction based on the proximate analysis confirming that the organic fraction of CFC is primarily cellulose.

The amount of cellulose as determined is much lower than those reported in literature about residues from carrageenan extraction. In a study by Tan and Lee (2015) wherein they performed laboratory-scale carrageenan extraction,

TABLE 1: Characteristics of the collected carrageenan filter cakes (CFC).

Proximate Constituents	Composition (g /100g) [a]
Moisture content	3.83 ± 0.10
Volatile matter	22.18 ± 0.70
Fixed Carbon	3.44 ± 0.20
Ash	74.38 ± 0.30
Total recoverable sugar	
Total sugar	26.78 ± 5.80
Total reducing sugar	24.52 ± 0.25
Water extractives	18.85 ± 1.00
Soluble sugar	
Total sugar	4.39 ± 0.70
Total reducing sugar	1.07 ± 0.00
Residual base	0.17± 0.04[b]/ 0.09 ± 0.01[c]

[a] *relative to filter cake in dry basis*
[b] *1g of sample suspended in 50 mL hot water while being titrated*
[c] *titration of water extractives*

the resulting residue, referred to as macroalgae carrageenan residue (MCR), was found to contain ~68%w/w cellulose. However, in their work, perlite or filter aids were not added during the separation of the extracted carrageenan; this explains why MCR only had approximately ~32%w/w ash. In a separate study by Masarin et.al (2016), residues (23 to 28 g residue per 100 g seaweed) left after laboratory-scale carrageenan extraction were reported to contain 59 to 62%w/w sugars and only 10 to 15%w/w ash. Given that perlite is added as filter aid in industrial carrageenan extraction at a minimum of 500 kg per 1700 kg of seaweeds processed, the total ash in CFC may add up to at least 56 to 67%w/w, which explains the high ash content of the CFC obtained in this work. The large fraction of ash in CFC is mainly contributed by the presence of perlite added during the filtration process, which is not incorporated in laboratory-scale carrageenan extraction. Other sources of ash include the inherent minerals found in the algal biomass and the residual base.

Considering CFC was obtained from alkali carrageenan extraction, the amount of residual base expressed as NaOH equivalent were also determined as this could neutralize the acid and interfere in the hydrolysis process. Analysis of the water extractives revealed that only a small amount of residual base (~0.09%w/w NaOH equivalent) was present in the CFC. However, perlite, primarily made of alumina and silica, can possibly react with the acid. Thus, a second approach of determining the equivalent base (–OH) through direct titration of the solids suspension with hydrochloric acid was carried out. About 0.17%w/w of NaOH equivalent was determined using this method, which is twice than the amount of base dissolved in the water extract. Nevertheless, this amount of residual base does not significantly reduce the actual concentration of the acid during the determination of total recoverable sugar and is considered not to cause interference during the analysis.

3.1 Acid hydrolysis of carrageenan filter cake

The effect of acid concentration (5% v/v to 15% v/v) and hydrolysis time (5 min to 300 min) on the hydrolysis of CFC at a temperature of 95°C and solvent-to-solid ratio (SSR) of 8 mL/g were investigated in this study. The responses that were determined to evaluate the effect of the variables were the amount of total sugar and total reducing sugar in terms of yield and concentration. Generally, it can be observed that at all acid concentrations (5% v/v to 15% v/v) used, the total sugar yield is greater than the total reducing sugar at any time (Figure 1). This indicates that not

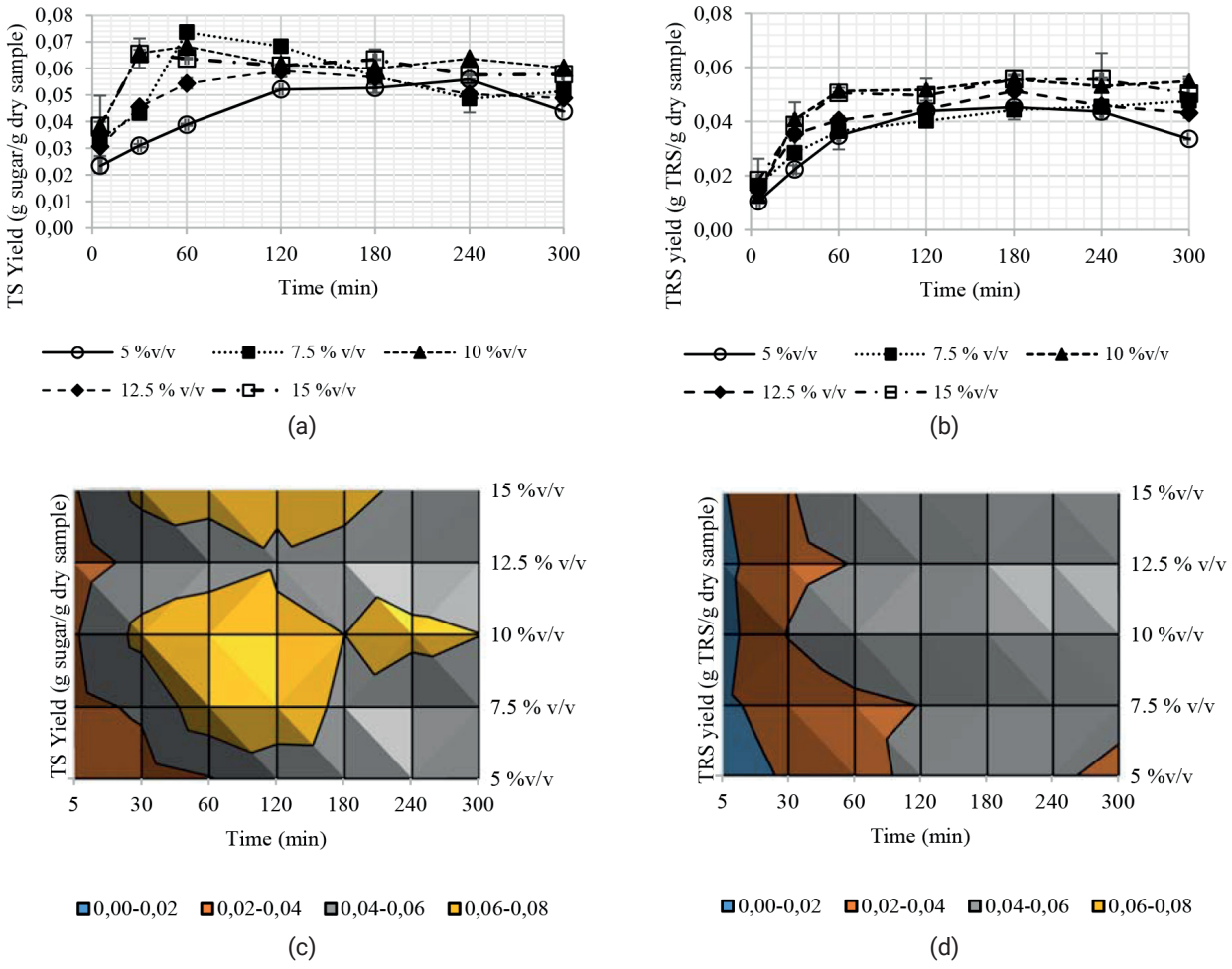

FIGURE 1: Sugar yields during hydrolysis at 95°C and SSR of 8 mL/g (a and c) total sugar (TS) yield versus time (b and d) total reducing sugar (TRS) versus time (This figure is to be printed in black and white in the printed version but colored in the online version).

all sugars released from the cellulosic structure are in its monomeric form.

It can be observed that across the different acid concentrations used, a maximum sugar yield of ~0.065 w/w was achieved, corresponding to a total sugar recovery of ~20% to 24%, respectively. Use of higher acid concentrations apparently results in faster rate of release of sugars from CFC as evident by a steeper curve during the earlier part (5 to 30 minutes) of the hydrolysis. For instance, the rate of release of structural sugars at 7.5 to 15% v/v H_2SO_4 ranges from 0.006 to 0.007 g/g.min, whereas a rate of 0.004 g/g.min at 5% v/v was obtained. At lower acid concentrations (5%v/v) used, the maximum sugar yield was achieved at 240 minutes while at higher acid concentrations (7.5%v/v to 15% v/v) used, maximum was achieved at 30 to 120 minutes (Figure 1a); using 10%v/v H_2SO_4 for 60 to 120 minutes appears as a good combination for acid concentration and hydrolysis time as this can achieve a sugar yield of ~ 0.06 w/w. However, this yield translates to relatively low sugar recovery of ~24%. The low recovery may be due to the crystallinity of cellulose, which made degradation into its monomeric constituents difficult. It is possible that the major fraction of the sugar hydrolyzed were the soluble sugars (3.5%w/w) originally present in the filter cake. The additional sugar recovered could be from the hydrolysis and breakdown of amorphous cellulose. Cellulose structure has amorphous and crystalline region. Crystalline cellulose is more stable due to its stable structure making it highly recalcitrant towards acid hydrolysis (O'Sullivan, 1997). In addition, no traces of 5-HMF were found during the analysis of inhibitor which suggests that the hydrolysis conditions employed may not be severe enough to further degrade the reducing sugars to form furans or perhaps the furans have already been further degraded to other compounds which were not detectable in the analytical conditions employed.

Several studies on the hydrolysis of algal biomass (Table 2) have been conducted. A study of Meinita et al. (2012) on the hydrolysis of K. alvarezii using 0.2 M (1% v/v) sulfuric acid at a temperature and SSR of 130°C and 5 mL/g, respectively, for 15 minutes achieved a sugar yield of ~20%. Hydrolysis of other algal biomass such as Gracilaria tenuistipitata, Gracilalriopsis chorda and Gelidium amansii, at the same hydrolysis conditions achieved a sugar yield of 27%, 23%, and 29%, respectively (Dyah et al., 2014; Meinita et al., 2013). Higher sugar yield was expected for the hydrolysis of raw seaweeds considering that its structure contains hemicellulose and no lignin unlike carrageenan filter cakes which are predominantly composed of cellulose (Daroch et al., 2013; Tan and Lee, 2014). Hemicellulose are easily hydrolyzed into its monomeric sugars in contrast to cellulose which have high degree of crystallinity (Kumar et al., 2009).

Enzymatic hydrolysis of CFC has been studied by Masarin et.al (2016) and Tan & Lee (2014) who reported a sugar yield of 64% to 68% w/w which corresponds to a sugar recovery of >99% (Table 2). This sugar recovery was achieved at mild conditions (50°C and 4.8 to 5.4 pH) but

TABLE 2: Hydrolysis of different algal biomass.

Substrate	Type of catalyst	Catalyst concentration	Solvent to solid ratio (ml/g)	Temp. (°C)	Reaction Time (h)	Sugar Yield (% w/w) / Sugar Recovery (%)	Total Sugar (g/L)	Furan (g/L)	Ref.
CFC	H_2SO_4	2.7 M	8	95	0.5	~6/25	7.3	Not Detected	This study
Kappa Alvarezii	H_2SO_4	0.2 M	10	130	0.4	8.9[a]/17[a]	~8.9	nd	(Maria Dyah Nur Meinita et al., 2010)
	H_2SO_4	0.2 M	5	130	0.4	20.2[a] /51.6[a]	40.4	5.9	(Meinita et al., 2012)
	H_2SO_4	0.2 M	10	130	0.4	-	38.5	3.6	(Dyah and Meinita, 2012)
Gracilaria tenuistipitata	H_2SO_4	0.2 M	10	130	0.4	26.6[a]/65.1[a]	26.6	1.2	(Meinita et al., 2013)
Gracilariopsis chorda	H_2SO_4	0.2 M	10	130	0.4	23.4[a]/68.0[a]	23.4	2.8	
Gelidium amansii	H_2SO_4	0.2 M	10	130	0.4	29.2[a]/48.7[a]	29.2	4.8	
Gelidium latofolium	H_2SO_4	0.2 M	20	130	0.4	22.2[a]/37.0[a]	11.1	3.5	(Dyah et al., 2014)
MCR	Enzyme[b]	0.09 % v/v	50	45	72	68.5[a]/100[a]	13.7	None	(Masarin et al., 2016)
MCR	Enzyme[c]	5.8 % v/v	50	50	54	63.9/99.8	12.8	None	(Tan and Lee, 2014)
MCR[d]	Solid catalyst (Dowex ™ Gr-D8)	4 % w/v	10	120	0.5	nd	nd	nd	(Tan and Lee, 2015)
	Enzyme [c]	0.2 %v/v	50	50	30	67.8/99.8	13.6	none	

Note: nd-no data; [a] *calculated value based on available data; 0.2 M is equal to ~1%v/v; 2.7 M is equal to ~15 %v/v;* [b] *92 FPU/ml (Cellic Ctec II) & 1800 UI/ml (β-glucosidase);* [c] *82.08 FPU/ml (Celluclast 1.5L) & 326.12 CBU/ml (Novozyme 188); dtwo-step hydrolysis*

a relatively longer reaction time (54 to 72 hours) was required. To hasten the enzymatic hydrolysis, pre-treatment of the biomass was done to reduce the crystallinity of cellulose. Tan & Lee (Tan and Lee, 2015) pre-treated MCR using a solid-acid catalyst (Dowex Gr-D8). This reduced the reaction time for enzymatic hydrolysis from 72 hours to 30 hours. Sugar concentration in hydrolysates achieved from enzymatic hydrolysis of MCR was ~13 g/L. In this study, a maximum sugar concentration of ~7.2 g/L was achieved at an acid concentration of 10% v/v (Figure 2). Considering that the achieved substrate concentrations range from 2 to 7 g/L, the sugar-rich hydrolysate recovered in this study may serve as potential substrate for fermentative processes, specifically in the pre-cultivation stages, such as the production of ethanol using Saccharomyces cerevisiae (ATCC 24860) (which requires 1.6 g total sugar/L substrate concentration, fermented at 3.7 pH, 30°C for 24 hours) (Ergun and Ferda Mutlu, 2000) and succinic acid production using Corynebacterium glutamicum (which requires 3.6 g glucose/L substrate concentration fermented at 30°C for 24 hours) (Okino et al., 2008).

Hydrolysate yield is also an important parameter to be looked into considering that the hydrolysate obtained in this study can be a potential substrate for subsequent fermentation process. The amount of hydrolysate available for fermentation is a useful information in later process design. The average hydrolysate yield from CFC was found to range from 65 to 78% of the total liquid used in the process. The lower hydrolysate yield (65%) observed when using 5%v/v H_2SO_4 (Figure 3a) was possibly due to the absorption of hydrolysate by CFC. Apart from the quantity of recoverable hydrolysate and sugar content, inhibitory substances in the hydrolysate should also be accounted. Considering that cellulose is made up of cross-linked glucose sugars, hydrolysates may contain degradation products of glucose such as 5-HMF (Kanchanalai et al., 2016). However, analysis of inhibitor using HPLC showed that no traces of 5-hydroxymethyl furfural in the hydrolysates, which suggests that the hydrolysis conditions may not be severe enough for the reducing sugars to be degraded to form 5-HMF. The presence of 5-HMF in the acid hydrolysates is undesirable in subsequent fermentation process as it in-

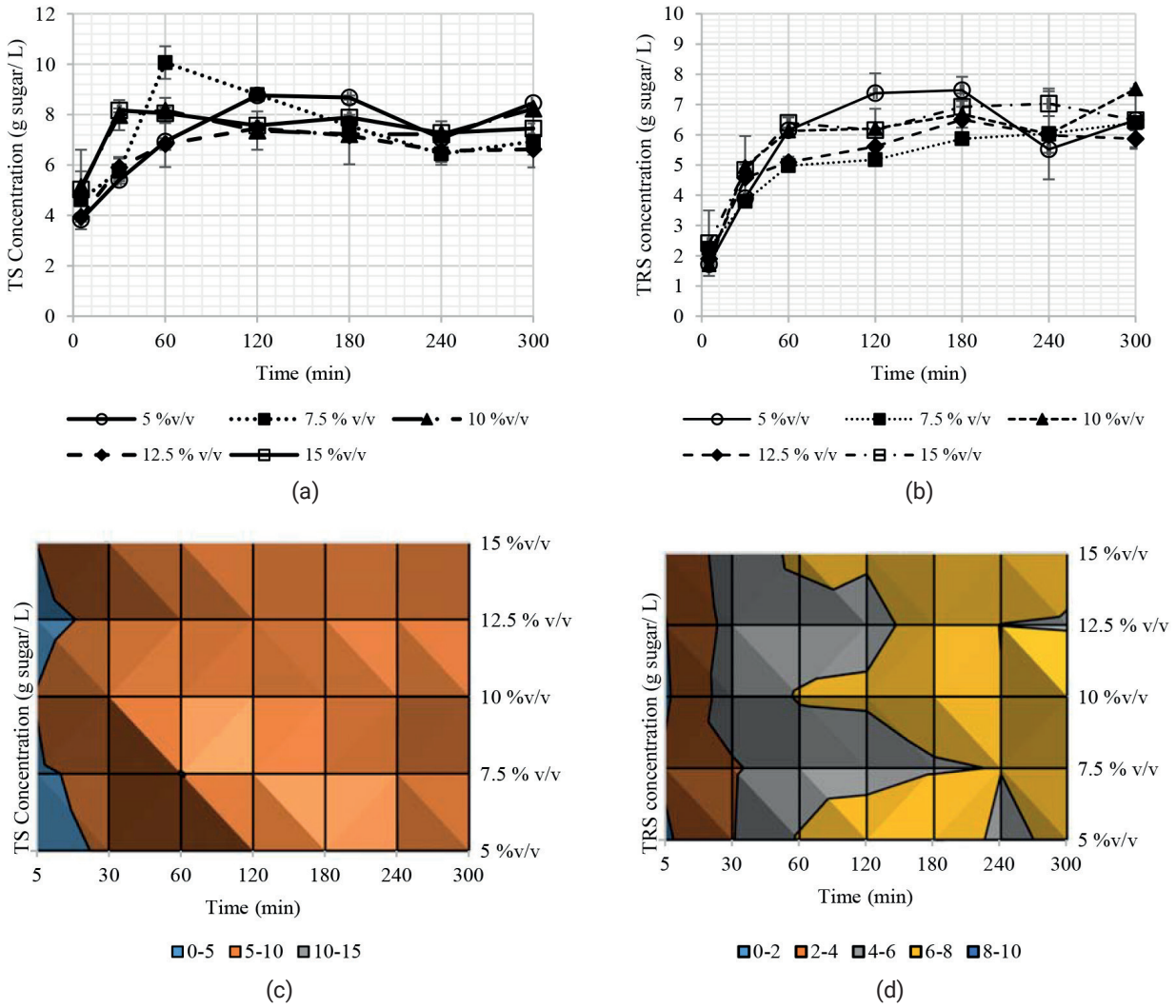

FIGURE 2: Sugar yields during hydrolysis at 95°C and SSR of 8 mL/g (a and c) total sugar (TS) yield versus time (b and d) total reducing sugar (TRS) versus time (This figure is to be printed in black and white in the printed version but colored in the online version).

hibits growth of cells, and prolongs the lag phase, thereby decreasing the productivity of microorganisms (Almeida et al., 2007). Inhibitory effects of 5-HMF to oleaginous yeast (Cryptococcus curvatus) is significant for concentrations over 3 g/L (Yu et al., 2011). Meanwhile, for Rhodosporidium toruloides yeast strain, complete inhibition was found even at 0.1 g/L 5-HMF. Another consideration is the presence of high sulfate ions present owing to the amount of sulfuric acid used. The presence of sulfate groups leads to osmotic stress to the fermentation bacteria. Nonetheless, its inhibitory effects on glucose consumption of S. cerevisiae is found significant beyond concentrations of 0.2 M concentration (Casey et al., 2013). which could be addressed during the neutralization of the hydrolysate prior to fermentation as addition of lime would result in the precipitation of the sulfate ions in the form of calcium sulfate.

The hydrolysates obtained when using acid concentrations of 5% v/v to 15% v/v have a pH ranging from 0.02 to 0.58 (Figure 3b). The pH of the hydrolysate is higher than the pH of stock solution which ranges from -0.34 to 0.22. The increase in pH can be attributed to the loss of hydronium ions due to its consumption during hydrolysis and possible absorption of the acid in the matrix of the biomass. Nevertheless, the acid concentrations remained low enough (pH < 1) and is not completely neutralized, which eliminates the possibility that hydrolysis did not proceed owing to neutralization of the available acids. In addition, the pH of the resulting hydrolysates decreases with prolonged hydrolysis time. This may be owing to the adsorption of the ions on the solid matrix and was not recovered during the washing step after filtration. As could be observed, the hydrolysate yields were also lower at shorter hydrolysis time, indicating part of the hydrolysates being trapped in the post hydrolysis residues and was later easy released and recovered at longer hydrolysis times.

3.2 Proximate constituents of PHR

Aside from hydrolysate yield, PHR solids had to be examined to validate the extent of hydrolysis. The residual solids left after hydrolysis of carrageenan filter cake using sulfuric acid (5% v/v to 15% v/v) at reaction time of 5 to 300 min, and at constant temperature and SSR of 95°C and 8 mL/g, respectively, had moisture contents of ~ 0.20 to 0.30 w/w. The PHR yields has a range of 84% to ~117%. Considering that carrageenan filter cake has a total sugar content of ~0.25 w/w, the PHR yield being above 75% could mean that there were still sugars left unhydrolyzed. At increasing acid concentration, at any time, PHR yield was observed to increase (Figure 4), which is contrary to what is expected because the mass of residual solids should decrease since part of the solid is broken down and solubilized during hydrolysis. This increase in PHR yield may be due to the residual sulfuric acid that remained with the PHR after the filtration step. When the PHR was dried at 105°C, the residual sulfuric acid did not volatize but instead may have sulfonated the residue and added up to the dry solid mass. At higher acid concentrations, more acids were available to sulfonate the solid, further increasing the residual solids yield.

From the proximate analysis of PHR, it was further found that volatile matter and ash yields increased, whereas fixed carbon yield decreased with the increase in acid concentration used (Figure 5). Volatile matter is comprised of hydrocarbons and some sulfur components that are removed upon heating at elevated temperature (950°C). The increase in volatile matter can be due to the attachment of sulfonic groups (-SO_3H) on the residues, thereby increasing volatile O, S and H elements. At higher acid concentration, more sulfonic groups are available for sulfonation, which also corresponds to the increase in the available volatile matter. The decrease of the fixed carbon

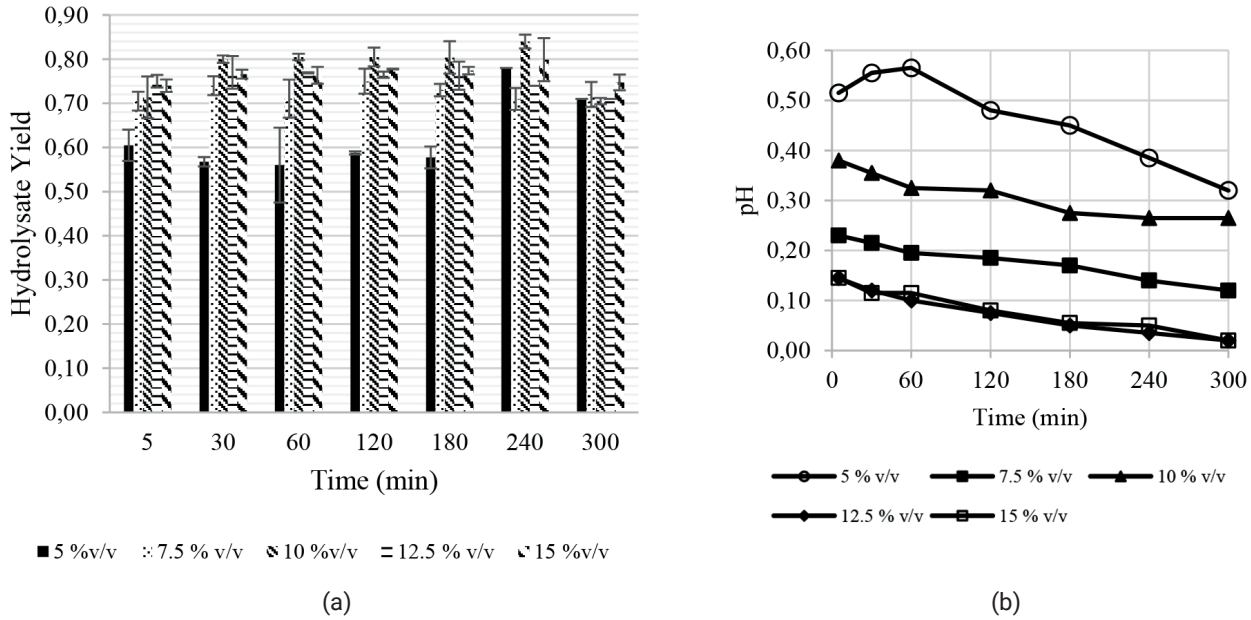

FIGURE 3: Hydrolysate yield (a) and pH (b)of hydrolysates at constant temperature and SSR of 95° C and 8 mL/g, respectively, and varying acid concentration (5 % v/v to 15 % v/v) and reaction time (0 to 300 min).

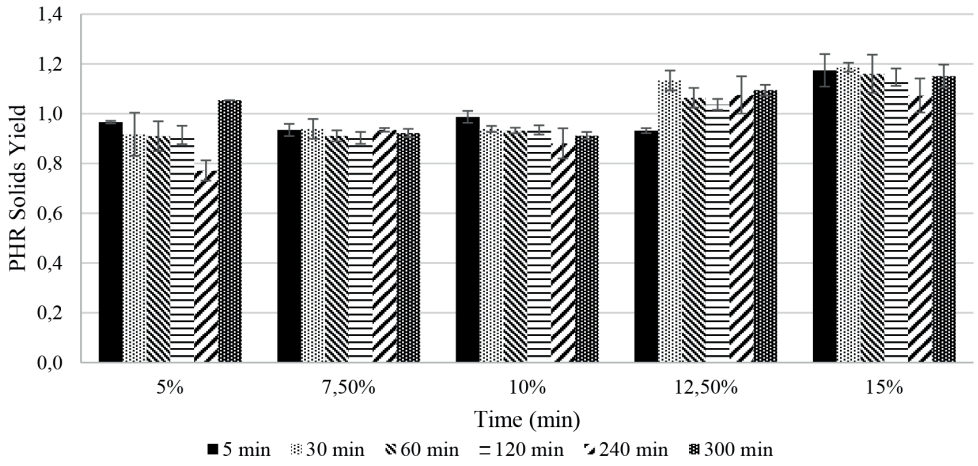

FIGURE 4: PHR solids yield obtained from the hydrolysis of carrageenan filter cake at 95 °C, 8 mL/g solvent-to-solid ratio, 5 to 300 min reaction time, and 5 to 15 % v/v acid concentration.

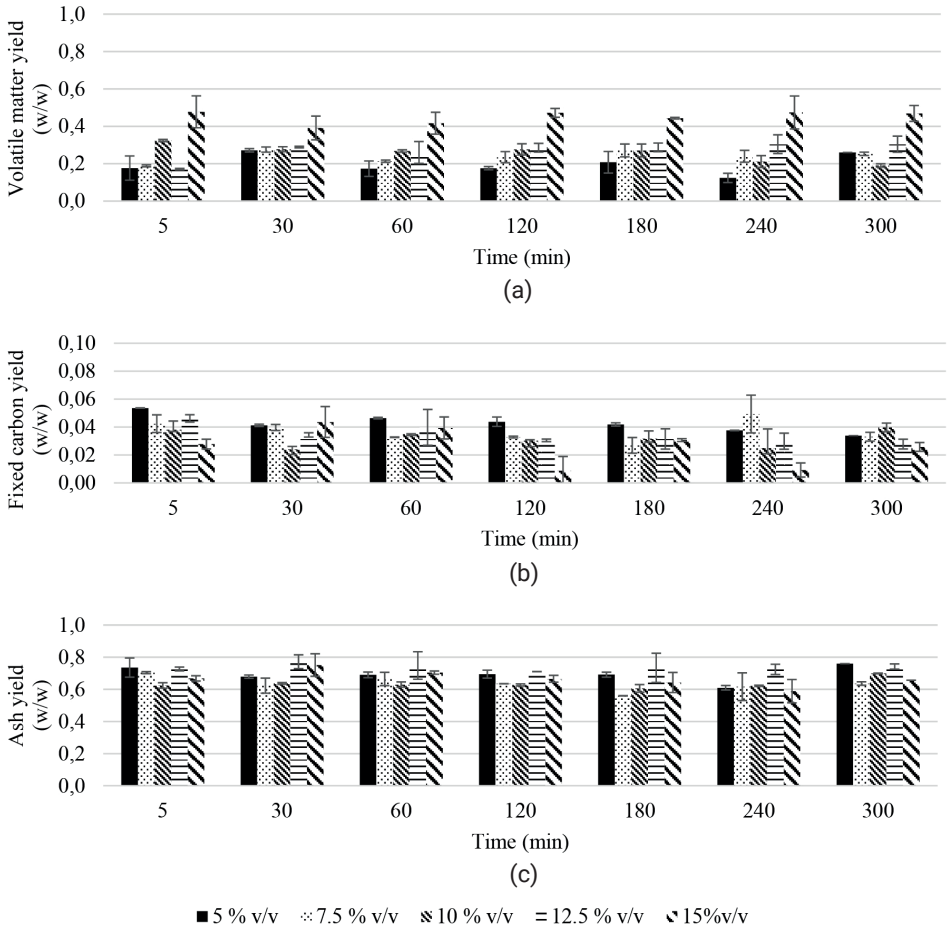

FIGURE 5: Proximate yields of the PHR (a) volatile matter (b) fixed carbon (c) ash at 95C, 8 mL/g solvent-to-solid ratio, 5 to 300 min reaction time, and 5 to 15 % v/v acid concentration.

yield is due to the fact that some of the solids were hydrolyzed during the hydrolysis process, and also possibly due to further degradation of the residue during drying at 105°C. The PHR ash yield, ranging from 0.6 to 0.8 w/w with an average of 0.67 w/w, is very similar to the initial ash content of the CFC, indicating the possibility of recovering perlite as the bound sulfonic groups can be easily removed during the calcination of perlite, which is carried out at elevated temperatures of 800 to 850°C (Samar and Saxena, Shweta, 2016).

In a study by Al-Dulaimi et.al (2015), hydrolysis of cellulose using concentrated acid (58%w/w) at constant solvent-to solid ratio of 12 mL/g and temperature of 45°C, resulted to an increase in molecular weight due to the en-

hancement of sulfonic group on the surface of the cellulose. Although the reactions in this study was carried out under lower acid concentration (5 to 15% v/v), it is still possible for sulfonation to have occurred considering that higher reaction temperature (95°C) was employed. Moreover, sulfonation by residual acid could have been further promoted during drying of the wet PHR at 105°C.

Sulfonation and carbonization of the PHR during drying is also supported by its appearance. As can be seen in Figure 6b, after filtration, the sample is still wet due to the retention of some acid hydrolysates. After drying at 105°C for 24 hours, PHR turned black (Figure 6c) probably due to the dehydration of the cellulosic structure and other organics in the presence of sulfuric acid. Sulfuric acid is a dehydrating agent that removes oxygen and hydrogen molecules in the cellulose structure in the form of water and leaves behind a carbon backbone as can be indicated by its black appearance (Woishnis and Ebnesajjad, 2012).

To further validate the possible sulfonation of the PHR, total acid density was determined. It was found that a total acid density ranging from 4 to 7 mmol H^+ for every gram of sample was obtained at an acid concentration of 5% v/v to 15% v/v during hydrolysis for 120 minutes at 95°C and solvent-to-solid ratio of 8 mL/g. As presented in Figure 7, there is an increasing trend of total acid density at increasing acid concentration (5% v/v to 15% v/v). This is understandable because at higher acid concentration, more sulfonic groups from sulfuric acid is available for sulfonation. During hydrolysis and drying of the residue, dehydration and sulfonation possibly occurred simultaneously. It is observed that the actual total acid density determined is within the range of the theoretical amount of acid (in moles based on the amount of hydrolysates left in the residue after filtration) in the solid residue after hydrolysis (Figure 7). Considering that there are two hydronium ions in one mole of sulfuric acid, one may have facilitated the dehydration of the residue leaving behind the black carbon back bone while the other hydronium ion remained as part of the sulfonic group (SO_3H) formed during the sulfonation process.

Several studies on the use of partially carbonized and sulfonated cellulose as a solid acid catalyst has been published in literature. The total acid density of the dried

FIGURE 6: Appearance of carrageenan filter cake (a) before hydrolysis (b) after filtration (c) after drying at 105°C for 24 hours.

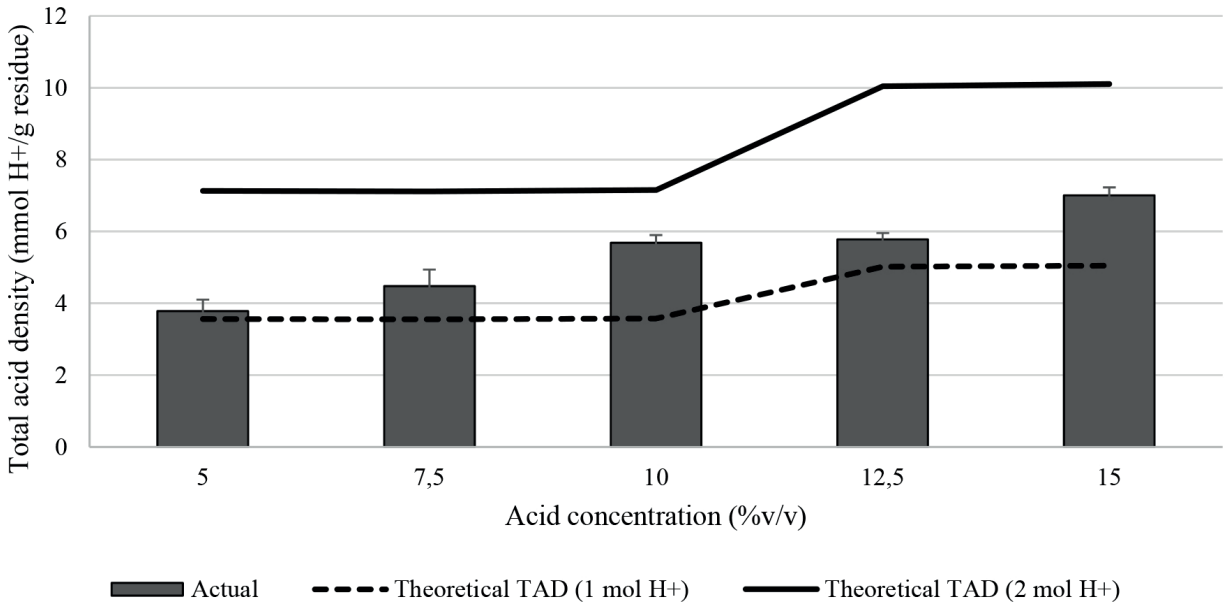

FIGURE 7: Total acid density (TAD) of PHR obtained at 5 to 15 % v/v acid concentration, 8 mL/g SSR, 95°C for 120 minutes (theoretical TAD was calculated based on the amount of hydrolysates left in the residue).

residues obtained after hydrolysis is comparable to those which have been successfully employed in the synthesis of biodiesel and hydrolysis of cellobiose. Carbon-based solid acid catalyst derived from sugars like glucose (Lokman et al., 2015), sucrose, and starch (Lokman et al., 2016) which had a total acid density of 4.2, 7.0, and 12.5 mmol H^+/g, respectively, and were successfully used in the synthesis of fatty acid methyl ester having high biodiesel yields of 89 to 94%. Suganuma et.al (Suganuma et al., 2010) also synthesized solid acid catalyst, but using microcrystalline cellulose as the raw material, which resulted in a catalyst with a TAD of 7.3 mmol H^+/g and was successfully used in the complete hydrolysis of cellobiose. Hence, the potential of the PHR from this study as a bio-based solid acid catalyst for biofuel (biodiesel and bioethanol) production may also be looked into in the future.

4. CONCLUSIONS AND RECOMMENDATION

Cellulosic filter cake obtained from carrageenan processing industry still contains sugar of as much as 25%w/w, with the remaining fraction being ash, which is primarily comprised of perlite. Hydrolysis of CFC can be carried out as means of recovering the sugars and at the same time prepare the residues after hydrolysis for subsequent sulfonation to produce a carbon-based solid acid catalyst. An increase in acid concentration during hydrolysis resulted to faster rate of recovery of sugars from cellulosic structure. A maximum sugar yield of ~0.05 to 0.07 w/w was obtained which corresponds to a sugar recovery of ~20 to 25%, respectively. Moreover, a maximum reducing sugar concentration of ~7 g/L was obtained at all acid concentrations with no traces of inhibitors (5-HMF) present in the hydrolysate, making it a potential substrate in subsequent fermentation processes. Drying of residues from the hydrolysis not only removed the remaining water but also resulted in the sulfonation of the PHR. The total acid density of PHR ranged from 4 to 7 mmol H^+/g and was found to increase as acid concentration during hydrolysis was increased from 5% v/v to 15% v/v, respectively. While hydrolysates can be used as substrate in subsequent fermentation process to produce high-value platform chemicals and biofuels, hydrolyzed residue can be further processed to produce heterogenous acid catalyst. Furthermore, perlite may potentially be recovered by simply subjecting the dried residue at high temperatures during calcination.

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