EFFECT OF HYDROTHERMAL PRETREATMENT ON THE ACIDIFICATION OF THICKENED WASTE ACTIVATED SLUDGE AND SOURCE SEPARATED ORGANICS

by
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The Effect of Hydrothermal Pretreatment on acidification of Thickened Waste Activated Sludge and Source Separated Organics

Master of Applied Science, 2019

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Abstract

The objective of this study was to investigate the impact of the hydrothermal pre-treatment (HTP) on solubilization and acidification of thickened waste activated sludge (TWAS) and source separated organics (SSO). The temperatures, retention times, and pressures used in this study ranges were 150-240°C, 5-30 min, and 69-488 psi, respectively. Mesophilic batch acidification tests were conducted for all pretreated and non-pretreated samples.

For the TWAS, the highest overall COD solubilization due to HTP and acidification of 64% was observed at "200°C-10 min" compared to 30% for raw TWAS. The highest VFAs yield of 2856 mg VFAs/g VSS added was observed at "190°C-10 min" compared to 1251 for raw TWAS.

For the SSO, the highest overall COD solubilization of 63% was observed at "210°C-20 min" compared to 17% for raw SSO. The highest VFAs yield of 1536 mg VFAs/g VSS added was observed at "210°C-20 min" compared to 768 for raw SSO.

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List of Abbreviations

AD	Anaerobic Digestion
BNR	Biological Nutrient Removal
BTU	British Thermal Unit
COD	Chemical Oxygen Demand
CSTR	Continued Stirred Tank Reactor
DF	Dark Fermentation
F/M	Food to Microorganism
FBR	Fluidized Bed Reactor
FW	Food Waste
HRT	Hydraulic Retention Time
HT	Holding time
HTP	Hydrothermal Pretreatment
MSW	Municipal Solid Waste
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
PBR	Packed Bed Reactor
PHA	Polyhydroxyalkanoate
PS	Primary Sludge
RT	Retention Time
SCOD	Soluble Oxygen Demand
SI	Severity Index
SSO	Source Separated Organics
TCOD	Total Chemical Oxygen Demand
TSS	Total Suspended Solids
TWAS	Thickened Waste Activated Sludge
UASB	Up flow Anaerobic Sludge Blanket
VFAs	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WWTP	Waste Water Treatment Plants

1 Introduction

Fulfilling the food and modern habitat demand for the growing population is one of the major challenges of the 21st century while reducing the adverse impact of the food waste, wood waste, yard and landscaping debris and paper fibers or source separated organics (SSO) production system on the environment (Grizzetti et al. 2013). Consequently, large amounts of SSO will be generated. On the other hand, increasing amount of sludge generated in waste water treatment plants (WWTP) inspired the scholars and engineers to dedicate more attention to specific aspects of its management, particularly to recycling and resource recovery. SSO and Sewage sludge is rich in organic carbon, so it can be utilised as a sustainable source for the production of value-added products such as Volatile Fatty Acids (VFAs). VFAs have variety of applications such as production of biodegradable plastics, generation of bioenergy, and utilization as a carbon source for biological nutrient removal (BNR) (Lee et al. 2014).

Volatile fatty acids including acetic acid, propionic acid, and butyric acid, are among the top-ranked organic chemicals which are mainly produced by chemical and petrochemical approaches that cause pollution and use none renewable raw materials (Huang et al. 2002). However, production of VFAs from waste through acidification process is considered to be a sustainable method for on-site application as a carbon source for BNR that uses renewable resources. Acidification process is part of the anaerobic digestion (AD) process. AD involves the conversion of complex organic matter to biogas (CO_2 and CH_4) through a multi- step process (Yesil et al, 2014). AD includes four steps which are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Abelleira-Pereira et al. 2015). If the acetogenesis, and methanogenesis steps are inhibited, the process is called dark fermentation or acidification and valuable products such as VFAs and hydrogen gas are generated (Lee et al. 2014). In the hydrolysis step, the complex organic polymers (such as carbohydrates, proteins, and lipids) in waste are broken down into simpler organic monomers (such as glucose, amino acids, and long chain fatty acids) by the enzymes excreted from the hydrolytic microorganisms. Successively, acidogens ferment these monomers into mainly VFAs and hydrogen (Weiland 2010).

Research have evidenced that waste pre-treatment is a viable strategy that promotes the conversion of waste to valuable product and accelerate the hydrolysis process which is the rate limiting step (Carrère et al. 2010). Different pre-treatment technologies such as thermal, chemical, biological,

and mechanical have been used to improve the hydrolysis step (Abe et al. 2013; Morgan-Sagastume et al. 2012; Carrère et al. 2010).

Most of organics existing in the sewage sludge are enclosed within the microbial cell wall and some pre-treatment mechanism such as thermal pre-treatment have been used to disrupt the microbial cells and release the extracellular polymeric substances (EPS) to the liquid to enhance the anaerobic digestion of sludge (Appels et al. 2010; Bougrier et al. 2006; Xue et al. 2015) and acidification process (Ben-yi & Liu. 2006; Liu et al. 2012; Zou & Li. 2016).

Various studies investigated the HTP of thickened waste activated sludge (TWAS) under different condition (Nazari et al. 2017; Barber 2016; Xue et al. 2015; Carlsson et al. 2012; Bougrier et al. 2007). The temperature range for HTP was as low as 60 ℃ (Xue et al. 2015) and as high as 295 ℃ (Shier & Purwono 1994).

The impact of HTP's temperature on AD performance have been studied concentrating mainly on biogas production as a key indicator. Nonetheless, numerous studies have investigated its effect on other parameters such as dewaterability and dissolved organic Nitrogen (Higgins et al. 2017). Majority of these findings confirmed the positive effect of thermal pre-treatment on different parameters. However, there are limited number of studies about the effect of HTP of TWAS on the dark fermentation process.

Several studies have applied the thermal pre-treatment on TWAS prior to the acidification process, the applied temperature ranged from 50 °C to 160 ℃ combined with the retention time of 30 to 60 minutes (Ben-yi & Liu 2006; Morgan-Sagastume et al. 2011; Xiao & Liu 2009; Zou & Li 2016). All of these Research confirmed the positive effect of thermal pretreatment on acidification process. For example, Zou & Li (2016) found that thermal pre-treatment at 70 ℃ for 30 minutes facilitates the recovery of carbon and phosphorus as well as hydrogen and VFA production. The total VFAs yield of raw sample was 80 mgCOD/gVSS and increased to 250 mgCOD/gVSS for thermally pretreated sludge after 3 days of fermentation. In another study, Ben-yi & Liu (2006) reported that optimal hydrothermal pre-treatment condition for Hydrogen production and energy saving was 100 ℃ - 30 min and the solubilization and VFAs concentration was increased by thermal pre-treatment from 1400 to 2300 mg/L. According to Morgan-Sagastume et al. (2011) findings, the highest VFAs yield and VFA production rate of 0.46 $gVFA_{COD}$/gTCOD and 9 $gVFA_{COD}$/L/d was achieved at 160 ℃ compared to 0.1 $gVFA_{COD}$/gTCOD and 1.7 $gVFA_{COD}$/L/d for the raw TWAS. Furthermore, (Xiao & Liu 2009) studied the effect of various pre-treatment on

dark fermentation of sewage sludge including hydrothermal pretreatment at 121 °C for 30 minutes. They reported that thermal pre-treatment demonstrated the maximum VFA concentration of 1142 mg/L compared to 287 mg/L for the raw sample.

Considering SSO, few scholars investigated the impact of HTP with temperature range of 100 ˚C to 220 ˚C specifically on fermentation of food waste and concluded that HTP promoted the solubilization of organic biomass and VFA production while in some case inhibited the hydrogen production (Ding et al. 2017; Li and Jin 2015; Menon et al. 2016; WYin et al. 2014). However, some studies in Korea and Canada revealed the enhancement of biohydrogen production by combining HTP with other pretreatments such as Ultrasonic and alkaline (Elbeshbishy et al. 2011; Kim et al. 2013; Li et al. 2014).

2 Research objectives

According to the mentioned studies and literature review, there is not enough Research about impact of HTP in a wide range of temperature. Therefore, the main objective of this study was to investigate the effect of HTP of TWAS and SSO at a wide range of temperature (150 – 240 °C) combining by different pre-treatment time (5 min – 30 min) on the solubilization of organic matters as well as production of VFAs in the acidification process.

The specific objectives were as follows:

- Investigate the effect of different pretreatment conditions on the TWAS and SSO characteristics with focus on the degree of solubilization and solids reduction. Fifteen different pretreatment conditions were applied to each substrate which were corresponding to five different severity indexes of 3, 3.5, 4.0, 4.5, and 5.0.
- Investigate the effect of pretreatment of TWAS and SSO on the acidification process with respect to the degree of solubilization and VFAs production.

3 Literature Review

3.1 Anaerobic Digestion

AD is a series of biological process that occurs in absence of oxygen. It uses a diverse population of bacteria to convert organic materials into value added products such as biomethane (Ribeiro et al. 2017). Also, the effluent of AD is a combination of solid and liquid which is called digestate (City of Toronto 2009). The end product biogas is composed of mainly CH_4 and CO_2 typically (60 – 70 % by volume) and (30 – 40 % by volume) respectively. H_2S and other trace gases can be found in a very small amount and rate as well. Biogas can be utilised in variety of applications such as combustion to generate electricity and heat or can be further processed into renewable natural gas and transportation fuel (Khalid et al. 2011).

AD takes place in four sequential steps by involvement of different group of micro-organisms. The four steps are hydrolysis, acedogenesis, acetogenesis and methanogenesis (Weiland 2010), see Figure 1. Each process substrate is the intermediate products from the previous steps.

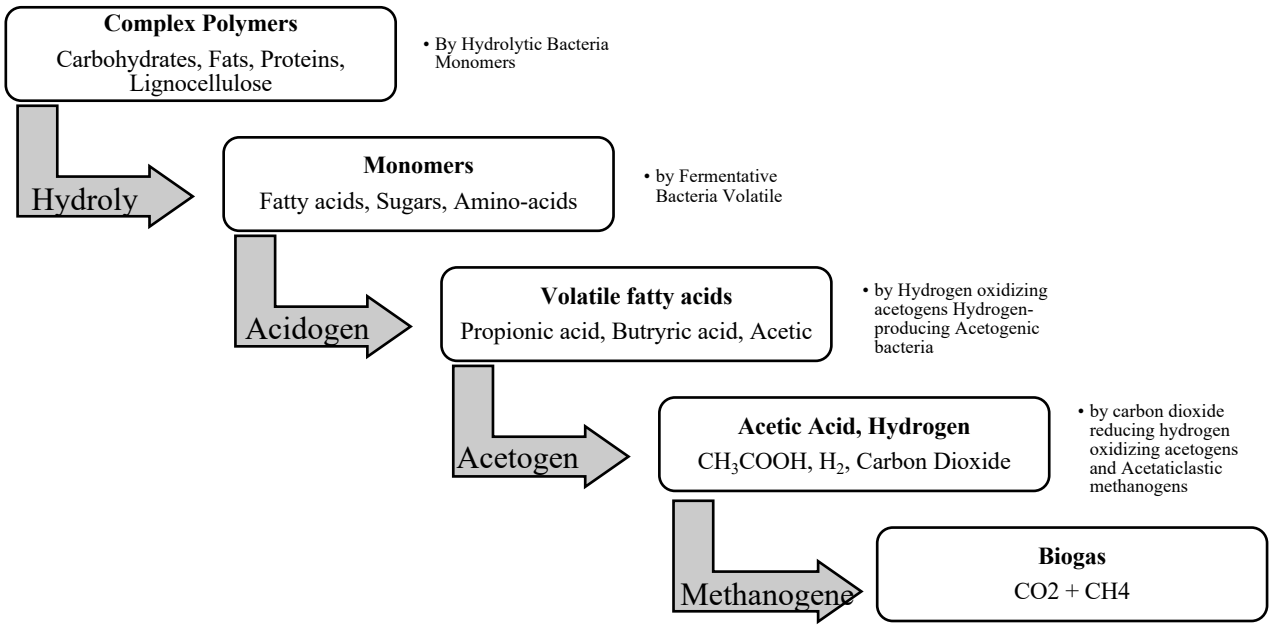


Figure 1. Schematic diagram of Anaerobic Digestion; Adopted from (Bajpai 2017)

Hydrolysis: When extracellular enzymes produced by hydrolytic microorganisms (for example, cellulase, amylase, protease, and lipase) decompose complex organic polymers into simple, soluble monomers, the first step, Hydrolysis occurs (Bajpai 2017). Proteins are broken down into amino acids, carbohydrates into sugars, lipids into long and short-chain fatty acids, and starch into glucose (Guilford and Chemistry 2017).

Acidogenesis: The simplified and degraded materials which are results of hydrolysis are converted by acidogens (fermentative bacteria) to a blend of VFAs such as acetic, propionic, and butyric acids and other minor products such as hydrogen, carbon dioxide, and alcohols (Lee et al. 2014). Acidogenesis is usually the fastest step in the anaerobic conversion of complex organic matter in liquid-phase digestion

Acitogenesis: The third step of AD is acetogenesis. Again, the products of the previous step (acidogenesis) is substrate for this step. acetogenic bacteria further convert the VFAs to acetate, CO_2, and hydrogen (Bajpai 2017).

Methanogenesis: The last step (fourth) of AD is methanogenesis, where the acetic acid and hydrogen produced from acetogenesis are converted to the CH_4 and CO_2 by acetoclastic and hydrogenotrophic methanogesis, respectively (Bajpai 2017). Some scholars also has recognized the methanogenesis to be the rate determining step in anaerobic digestion process when the substrate is soluble and has low solids content (Ariunbaatar et al. 2014).

3.2 Fermentation/Acidification

Fermentation or acidification is a process where bacteria takes the monomers and turn them into valuable products. The end products from fermentation process could be acetic, propionic, butyric acids, alcohols, hydrogen, and carbon dioxide. The type of products depends on the types of microorganisms and environmental conditions. Facultative anaerobic bacteria, strict anaerobic bacteria, or both (i.e. Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, and Streptococcus) are responsible for the acid production (Thompson 2008).

Fermentation process is widely used in industries to produce ethanol from sugar or starch-rich feedstock. However, the high cost of sugar and starch reach feedstock for dark fermentation makes it a costly technology. Therefore, the abundant amount of wastes generated, considering high costs of handling and disposal can be an alternative for the sugars and starches coming from eatable biomass. Municipal solid wastes such as food waste, kitchen waste, sludges generated from waste

water treatment facilities, agricultural waste, and dairy farms waste are potential substrates for production of H_2 and VFAs (Elbeshbishy et al. 2011; Ghimire et al. 2015; Yang et al. 2011).

3.3 Factors Affecting Fermentation Process

There are many factors that affect the performance of fermentation process such as the operational pH, retention time, temperature, organic loading rate (OLR), additives and food to micro-organism (F/M) ratio. These factors can affect the concentration, the yields, and types of VFA produced during fermentation process (Elbeshbishy et al. 2017). Many scholars have investigated the effect of each parameter individually (Ghimire et al. 2015) whereas, there are few studies on interaction of them (Lee et al. 2014).

pH: The most crucial parameter in fermentation process control is the pH. The pH affects the fermentation by shifting the by-product spectrum, microbial community structure and intracellular metabolic functions such as the hydrogenase activity (Chen et al. 2013; Elbeshbishy et al. 2017). For optimal growth of each microbial community involved in anaerobic degradation specific pH range is proposed. The optimal pH range for the hydrogen producing bacteria is 5.5 to 5.7 (Elbeshbishy 2011; Thompson 2008) while this range inhibit the activity of methanogens (Elbeshbishy et al. 2017). The range for methanogenic bacteria is 6.5 to 7.5 (Thompson 2008). In terms of VFA production, most of acidogens bacteria cannot survive in pH lower than 3 or higher than 12.

Temperature: Temperature is an important factor influencing the anaerobic process. Fermentation process has been carried out under different temperature ranges; psychrophilic (4-20°C), mesophilic (20-50°C), thermophilic (50- 60°C), and extreme-hyper thermophilic (60- 80°C) (Lee et al. 2014). Most often, fermentation processes operate in mesophilic and thermophilic conditions (Thompson 2008). The optimal temperature for acidogens bacteria ranges from 35 to 55 °C depending on the substrates and other parameters (Elbeshbishy 2011; Lee et al. 2014). Temperature can control the byproduct spectrum. Therefore, in order to shift the metabolic pathway towards acetate and butyrate production rather than solvent and alcohol production, optimization of the temperature is important (Elbeshbishy et al. 2017).

Retention time: Retention time is a crucial parameter in fermentation process as it determines the volume of the fermenter and consequently the capital cost of the technology. The retention time of the feedstock and mixed microbial cultures are called hydraulic retention time (HRT) and solid retention time (SRT), respectively (Lee et al. 2014). The HRT can affect the hydrolysis and

intermediate and end product of the dark fermentation. It also controls the methanogens activity (Ghimire et al. 2015). Enhancement of VFAs production is achievable by higher HRT as the bacteria will have more time to react with the substrate. However prolonged HRT (more than 6 days) leads to stagnant VFAs production (Lee et al. 2014) due to the methanogesis activities. On the other hand, SRT regulates the selection of predominant microbial species in the process. In case of using continuous stirred tank reactor (CSTR), SRT is equal to HRT (Ghimire et al. 2015; Lee et al. 2014).

3.4 Reactor configurations for dark fermentation

The two main technologies for the dark fermentation are attached growth and suspended growth (Lee et al. 2014). Consequently, different types of reactor configuration are developed based on these technologies. Packed Bed Reactor (PBR), see Figure 2 (a), is an attached growth process where porous packing materials, such as alumina-based ceramic cubes and granular activated carbon, are inserted in the reactor to allow the microorganisms to grow and attach on them.

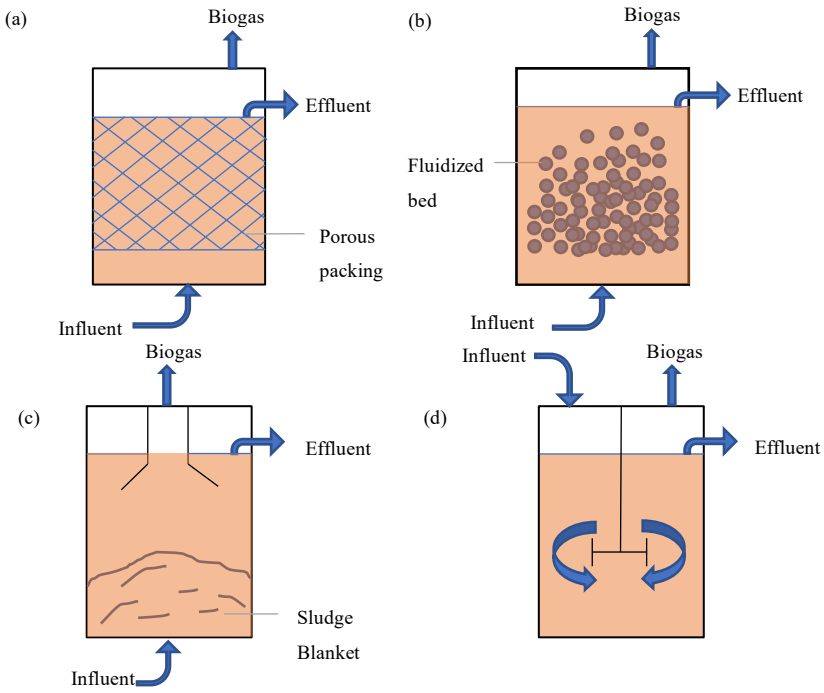


Figure 2. Types of reactor used for anaerobic production of VFA – (a) packed bed reactor, (b) fluidized bed reactor, (c) upflow anaerobic sludge blanket reactor (UASB) and (d) continuous stirred-tank reactor (CSTR) with recycling of biomass; Adopted from (Lee et al. 2014)

However, high concentrations of suspended solids in the feedstock cause clogging of the system. Fluidized Bed Reactor (FBR) is configured to avoid clogging, see Figure 2 (b). The biomass in this reactor grows on the solid mediums suspended in the liquid by the upward flowing motion. This solid medium is mostly sand (Henze et al. 2015). In contrast, biomass in suspended growth technologies can freely grow in suspension. Up-flow Anaerobic Sludge Blanket (UASB) reactor, see Figure 2 (c) and the CSTR, see Figure 2 (d).

The fermentation process can be classified based on the feed mode to batch, Figure 3(a), semi-continuous, Figure 3 (b), and continuous, Figure 3 (c). In the batch mode, the reactor filled once at the beginning of the process and stopped at the certain time. In the semi-continuous mode, the feedstock is adding and fermentate is withdrawing every certain time, typically every 24 hours. In the continuous mode, the feed and the fermentate are continuously added and withdrawn. Lab-scale studies on fermentation are typically performed using batch reactors because of the easy operation and flexibility of such systems. However in industrial scale, continuous mode reactors are utilized due to the practical reasons of waste management and economic considerations (Guo et al. 2010). The PBR, FBR, UASB, and CSTR can be operated in either continuous or batch mode.

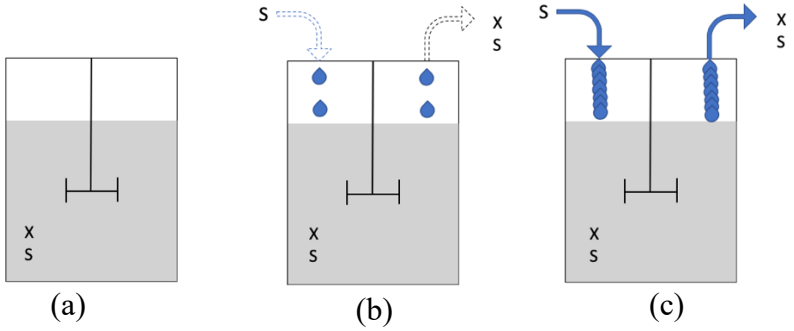


Figure 3: Anaerobic fermentation operation modes (a) Batch; (b) Fed-batch or Semi-continuous; (c) Continuous

3.5 Feedstocks for Fermentation Process

Variety of organic feedstocks have been employed for the dark fermentation. Simple sugars such as glucose or lactose as the carbon source are commonly utilised for the dark fermentation by many scholars (Bai, Anderson, and Moo-Young 2008). VFAs can be produced by utilizing organic rich wastes such as food waste, organic fracture of municipal waste (OFMSW), sludges from wastewater treatment plants and from the agricultural, dairy, pulp and paper industries (Bien et

al. 2004; Ennouri et al. 2016; Liu et al. 2012; Wang and Yin 2017; Xiao and Liu 2009; Yu et al. 2014; Zhang et al. 2014; Zou and Li 2016) have used Primary Sludge (PS).

Food waste or kitchen waste is commonly explored for VFAs production for two reasons: first, food waste is a dominant component (22–54%) in the huge volumes of municipal solid waste (MSW); and second, it has high TCOD in a range of 92,000–166,000 mg/L (Ariunbaatar et al. 2014). Nonetheless, efficient separation of food waste from MSW is one of the challenges to minimize the interference of other components in the production of VFAs (Wang et al. 2009). Source separation is a great approach for solving this problem, but the commitment of public is required which is not easily achievable. Another probable option is to establish a material recovery centre to separate the OFMSW as well as glass, plastics, aluminum cans, and ferrous metals (Giroux Environmental Consulting 2014). There are Research that have investigated the use of food waste, kitchen waste or OFMSW as a feedstock of fermentation (Ding et al. 2017; N. Liu et al. 2018; WYin et al. 2014; Zhou et al. 2013).

Despite the difficulties and slow rate of hydrolysis of lignocellulosic material due to the presence of higher lignin content, cellulosic, and lignocellulosic substances, efforts have been made to use this source of feedstock for an efficient fermentation process (Dahadha et al. 2017; Ravindran and Jaiswal 2016; de Carvalho et al. 2017; Eskicioglu et al. 2017; Yang et al. 2017) . Other potential feedstocks such as animal manure (Jia et al. 2013), algal biomass (Monlau et al. 2014; Nguyen et al. 2010), rice straw (He et al. 2014), and Olive husks (Pagliaccia et al. 2016) also have widely studied by scholars for VFAs production. However, the best type of substrate for VFAs production is not clear due to the difference in the operation conditions and VFAs production performance evaluation criteria. In general, wastes that are rich in organic matter with COD greater than 4000 mg/L considered to be a good substrate for VFAs production in a fermentation peocess (Ariunbaatar et al. 2014; Eskicioglu et al. 2017). This could serve as a preliminary guide for waste selection. However, to ensure stable and continuous waste supply for VFA production, in addition to the waste characteristics, the availability and the quantity of waste generated need to be considered (Chen 2013).

3.6 Application of Volatile Fatty Acids

VFAs are short-chain fatty acids consisting of six or fewer carbon atoms which can be distilled at atmospheric pressure (WYin et al. 2014). The three main types of VFAs produced during fermentation are acetic acid, butyric acid, and propionic acids (Morgan-Sagastume et al. 2011). Acetic acid is the most abundant acid produced by fermentation among all VFAs (Lee et al. 2014; Liu et al. 2018; Zhang et al. 2019a). "CH_3COOH" is the molecular formula of acetic acid. It is an important chemical reagent and industrial chemical. Butyric the second most abundant acid produced by fermentation. The molecular formula is, "$CH_3CH_2CH_2COOH$" with an unpleasant odor that arises in butter and animal fat as the glycerol ester. The acid is an oily, colorless liquid that is easily soluble in water, ethanol, and ether, and can be separated from an aqueous phase by saturation with salts such as calcium chloride. Propionic Acids is the third most abundant acid produced by fermentation. The molecular formula for propionic acid is, "CH_3CH_2COOH" from the Greek words protos, meaning "first", and pion, meaning "fat"; also known as propanoic acid is a naturally occurring carboxylic acid.

The VFAs produced by biological approaches has variety of applications. They can be utilised for the production of poly-hydroxyalkanoate (biodegradable plastics) (Mengmeng et al. 2009), generation of bioenergy such as electricity (Liu et al. 2004), hydrogen and biogas production, and biological nutrient removal (Lee et al. 2014). Figure 4 show the different application of the VFAs. For some of these applications, the VFAs produced from fermentation can be directly used. However, many processes need the VFAs to be further processed or treated to be used in that industry. Also, type of VFA produced during the fermentation affects the performance of the aforementioned applications (Lee et al. 2014).

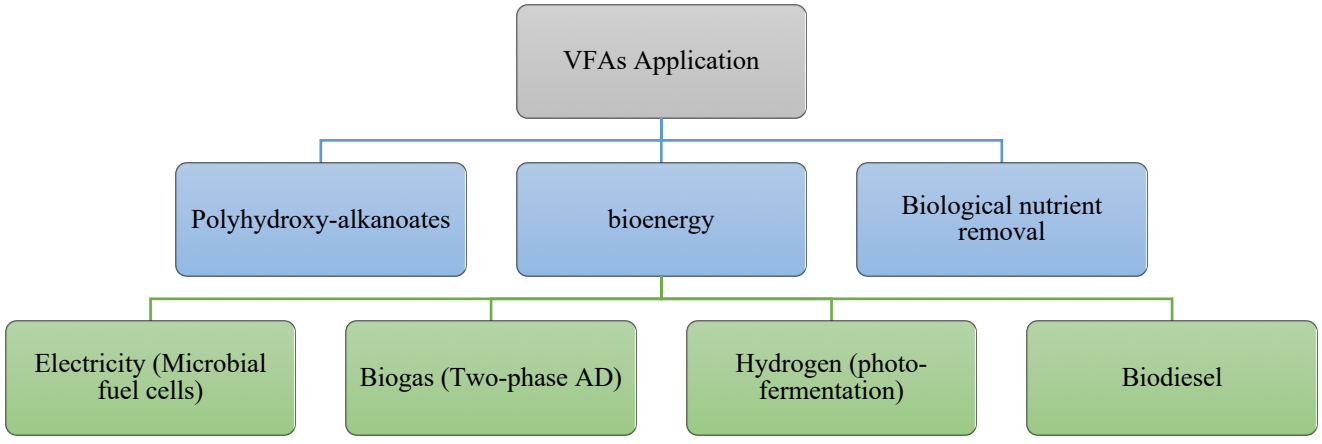


Figure 4 Application of waste derived VFAs

Polyhydroxyalkanoate: PHAs are biodegradable polymers (Lee et al. 2014). Substitutes for petroleum-based plastics can be produced by PHA. VFAs produced from fermentation of the wastes can be utilized as the carbon source for the biosynthesis of the PHAs (Chen et al. 2013). PHA being environmentally friendly, has a broad range of application in various industries. But the substitution of PHA over conventional petrochemical based plastic has been limited by the high production cost. Hence, waste-derived VFAs is a promising solution for the reduction of the production cost (Lee et al. 2014).

Bioenergy: considering the energy crisis, VFA is an inexpensive resource for production of different types of energies. Electricity, biogas, hydrogen and biodiesel are energy types that can be produced by employment of waste derived VFAs (Lee et al. 2014).

Electricity: Electricity can be generated directly from VFAs using microbial fuel cells (MFCs). MFC is a bio-electrochemical system. The electricity comes from usage of microorganisms to harness the chemical energy of the organic substrate (Lee et al. 2014; Wrenn et al. 2004). The supernatant fraction of digestates can be treated by MFC to degrade the residual suspended solid particles and produce electricity. However, the combination of MFC and fermentation processes is novel technology that few Research from 2013 to now has studies (Schievano et al. 2016).

Biogas: Biogas is another type of energy produced from VFAs which mainly contains methane (60-70 %) (Ding et al. 2017). Biogas is commonly used for the heat and power generation (Chen and Neibling n.d.). The production of biogas from VFAs can be achieved by AD of waste where the VFAs is the intermediate product. However, acidogens and methanogens favor different environmental conditions. Therefore, two-phases AD processes are developed for better function of two types of microbial communities (Khalid et al. 2011).

Hydrogen: Hydrogen can be produced as a by-product of the fermentation process. In addition, hydrogen is produced from VFAs by photo-fermentation, electro-hydrolysis, bio-catalyzed electrolysis, and microbial electrolysis cell (Ghimire et al. 2015; Lee et al. 2014).

Biodiesel: Biodiesel is a methyle esther of long-chain fatty acids that is produced through transesterification from lipids. The lipids used for the biodiesel production comes from edible materials such as rapeseed oil, palm oil and soybean oil that raises the concern of consuming food as fuel and is an high-priced source of substrate (Guo et al. 2010). The microbial lipid synthesized from waste-derived VFA by oleaginous microorganisms can be an alternative substrate which is more feasible and non-edible (Lee et al. 2014).

Biological nutrient removal: VFA is an important carbon source for removal of nitrogen and phosphorus from wastewater (Lee et al. 2014). The BNR can be done through aerobic nitrification and anoxic denitrification. Whereas, The phosphorus removal is done by enhanced biological phosphorus removal (EBPR) process (City of Toronto 2009). For a stable BNR process usually additional carbon substrates such as VFA is required as the carbon substrate in the wastewater is not sufficient. VFA produced through fermentation of wastes can be a cheaper source of carbon rather than synthetic VFA (Lee et al. 2014).

3.7 Pre-treatments before Dark Fermentation

In anaerobic digestion process in general, the hydrolysis step is the rate limiting step when particulate feedstock is used (Khalid et al. 2011). Whereas, for readily biodegradable substrates that contain low solids content, methanogenesis step is the rate limiting step (Ariunbaatar et al. 2014). Therefore, various studies have been conducted to accelerate the hydrolysis by pretreatment. The main gaols of pre-treating the feedstock are:

- Achieving better microbial interactions by improving surface properties
- Improving the hydrolysis rate kinetics for the particulate compounds
- Rising the availability of hardly accessible compounds

There are many factors to be considered when choosing the pretreatment technology for a particular biomass prior to be used as a feedstock for the fermentation. These factors include capital costs, operational costs, biomass costs, energy investments, overall efficiency, and feasibility and applicability over a broad range of substrates (Ravindran and Jaiswal 2016). Generally, four types of pretreatment technologies are employed for biomass cell disintegration which are physical or mechanical pretreatment, biological pretreatment, Chemical Pretreatment, and Thermal pretreatment. Figure 5 shows the main different types of pretreatment. Combination of the aforementioned pretreatment methods have been also studied prior to the fermentation (Li et al. 2016; Wang et al. 2012; Zinatizadeh et al. 2017).

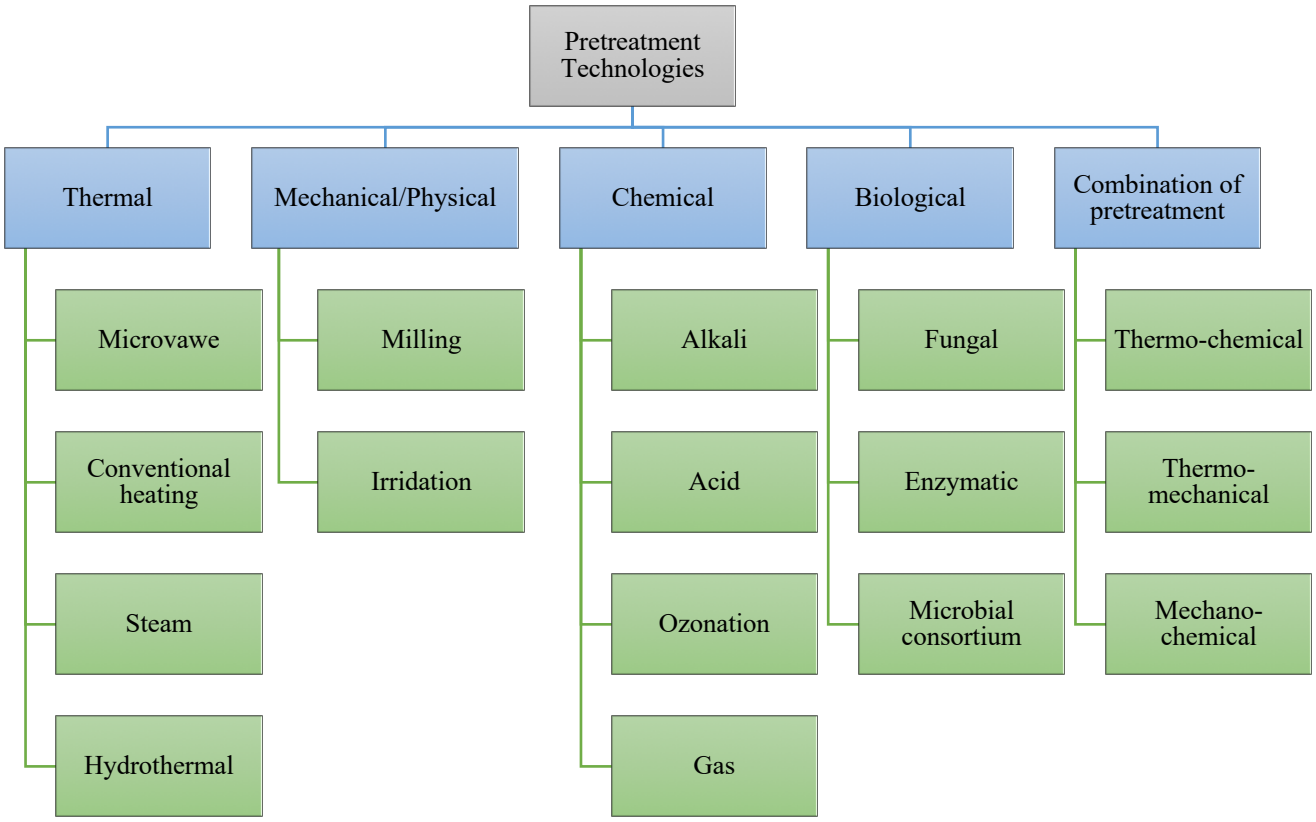


Figure 5: Classification of Pretreatment before fermentation

3.7.1 Mechanical pretreatment

Mechanical or physical pretreatment methods are expected to improve the physical properties of the substrates such as specific surface area. Better contact between substrate and anaerobic micro-organism will be provided by increase in specific surface area (Dhar et al. 2011) and hence, it facilitates shorter retention times for fermentation, but energy requirements are high (Motte et al. 2015). Milling, chopping, grinding, screw press, lysis-centrifugation, liquid shear collision, high pressure homogenization and ultrasonic homogenizer methods are employed as mechanical pretreatment methods to improve the physical properties of the biomass (Elbeshbishy 2011; Parthiba Karthikeyan et al. 2017).

3.7.2 Biological/Enzymatic pretreatment

Biological pretreatment can occur aerobically or anaerobically. The disintegration of the substrate can be achieved by adding specific enzymes such as peptidase, carbohydrolase, and lipase to AD (Ariunbaatar, Panico, Esposito, et al. 2014). The advantage of biological pretreatment is that unlike mechanical, chemical, and thermal pretreatment, it does not require high temperature or pressure,

or addition of any reactive additives. Biological pretreatment process is usually slow process thus time consuming and the control over the process is limited. However, some might employ this pretreatment due to the low capital cost and environmental advantages (Ravindran and Jaiswal 2016).

3.7.3 Chemical Pretreatment

Chemical pretreatment usually includes the addition of chemical compounds prior to AD or fermentation. Based on the reaction of chemical compounds with the cell wall and membrane to release organic matter from the cells, these chemicals can be categorized to the three main group of acids, alkalis, and oxidants (Okoye 2017). In terms of solubilisation of the carbohydrates, acid pretreatment is known to be more efficient (Hu and Chen. 2007). Though, solubilization of lignin, proteins, and lipids saponification is associated more by alkali pretreatment (Parthiba Karthikeyan et al. 2017). However, acids such as HCl, H_2SO_4 and others are corrosive and need non-corrosive coating for the equipment used for chemical pretreatment (Zinatizadeh et al. 2017). The alkali pretreatment requires longer reaction time, but the drawback is the formation of salts. Other chemical pretreatment such as ozonation (Ariunbaatar et al. 2014) and hydrogen peroxide addition (Abelleira et al. 2011) also have been investigated for the biomass cell wall disintegration. Ozone promotes osmosis through cell walls which compromises its integrity and releases intracellular material. Another type of advanced oxidation process is combination of O_3 and ultrasound which is a new technique. Enhancement of the mass transfer rate of ozone into the substrate is indication of efficiency of the ozonation by coupling with ultrasonication. Furthermore, the addition of free nitrous acid (FNA) to the sludge has been observed to improve the biodegradability of sludge and methane generation (Okoye 2017).

3.7.4 Thermal pretreatment

Thermal pretreatment is one of the most studied pretreatments prior to AD and FERMENTATION. It is a commercially developed technology successfully applied in industrial scale (Ariunbaatar et al. 2014). Thermal pretreatment disrupts the chemical bonds of the cell walls and the membrane, solubilize the components and release intracellular bound water (Elbeshbishy 2011; Wang and Yin 2017). Also, thermal pretreatment removes the pathogens, improves the dewatering performance, and reduces the viscosity of the digestate which enhance the digestate handling accordingly

(Ariunbaatar et al. 2014; Li et al. 2016; Ma et al. 2011; Wang et al. 2009). The temperature employed for thermal pretreatment range from 50 to 250°C for different substrates. Temperature of thermal pretreatment is the dominant factor of pretreatment which has direct relation with COD solubilization of the organic biomass. However, by combining lower temperatures with relatively longer retention time higher solubilization is was achievable (Jin et al. 2016; Y. Li et al. 2016; Qiao et al. 2011; Ribeiro et al. 2017). Though, according to the literature temperatures above 150 °C for many types of feedstocks such as food waste and lignocellulosic materials generate inhibitory compounds such as aromatic hydrocarbons, phenolic compounds, furfurals and hydroxymethylfurfural that are difficult to be biodegraded and in some cases inhibit the microbial activities (Bundhoo et al. 2015). Maillard reactions is one of the most known phenomena that occurs in results of reaction between carbohydrates and amino acids (Hauser et al. 2014). This phenomenon can occur either due to the elevated temperature or the combination of lower temperature with longer retention time (Hauser et al. 2014). In addition to the creation of inhibitory compounds in elevated temperatures, thermal pretreatment may also result in loss of volatile organics (Ariunbaatar et al. 2014). Hence, substrate type and thermal pretreatment's temperature controls the efficiency of the pretreatment. Thermal pretreatment can be done through use of electricity, steam, or microwave (Appels et al. 2013; Dwyer et al. 2008; Li et al. 2016).

3.7.4.1 Microwave pretreatment

A modified version of thermal pretreatment is microwave pretreatment. Microwaves are electromagnetic waves with frequency range of 0.003 GHz to 300 GHz and wavelengths of 1mm to 1 meters in air (Appels et al. 2013; Bundhoo et al. 2015; Serrano et al. 2016; Xiao et al. 2017). Microwave pretreatment, opposed to the thermal pretreatment by steam, delivers the heat to the biomass directly in form of microwave radiations which transform to the thermal energy. The microwaves heat the entire volume from the inside by penetrating into the biomass. The microwave pretreatment process has following advantages (Ravindran and Jaiswal 2016):

- Low time requirement (rapid process)
- Uniformity in nature (uniform heating)
- Controlled temperature
- Good control over the whole process

Xiao et al. (2017) investigated the effect of microwave pretreatment on the acidogenic fermentation of sludge in a laboratory scale batch test. They found that the microwave pretreatment promotes the activity of the enzymes which consequently, increased the amount of VFAs by about 5 folds compare to the raw sludge. These results are in agreement with Appels et al. (2013) findings regard enhancement of dark fermentation when they used the microwave treatment in a pilot scale study.

3.7.4.2 Thermal pretreatment at low temperatures (<100)

Thermal pretreatment using low temperature (<100) promotes the dissolution of the flocculated macromolecules, but does not facilitate degradation of complex molecules (De los Cobos-Vasconcelos et al. 2015). Barjenbruch and Kopplow (2003) confirms this statement by applying thermal pretreatment at 90 °C. Their results indicated that filaments are not disintegrated, but they were only attacked with thermal pretreatment. Some studies reported that the overall performance of fermentation process can be negatively affected by thermal pretreatment at temperature less than 70 °C (Li and Jin 2015). However, as the thermal pretreatment, even in lower temperatures, can be efficient for pathogen removal, the EU Regulation EC1772/2002 requires organic wastes to be pretreated at least an hour at 70 °C (Ariunbaatar et al. 2014). Hence, many studies have been conducted on thermal pretreatment of the organic wastes under 70 °C HTP temperature (Ben-yi and Liu 2006; Li and Jin 2015; Zou and Li 2016).

3.7.4.3 Thermal pretreatment at high temperatures (>100)

Hydrothermal pretreatment using temperature higher than 100 °C improve the solubilization of organics and result in biodegradation of complex molecules (Ben-yi and Liu 2006; Ennouri et al. 2016; Liu et al. 2012). It can also reduce the viscosity and increase the SCOD, soluble sugars, soluble proteins, hydrogen production, VFAs production, and cumulative biogas production (Liu et al. 2012; Zhang et al. 2014; M. Li et al. 2014; WYin et al. 2014). However intensification of the HTP temperature to higher than 170 °C may result in formation of melanoidins (Gavala et al. 2003).

3.7.5 Thermal pretreatment of food waste

The effect of thermal pretreatment on dark fermentation and anaerobic digestion of food waste has been studied applying low and high hydrothermal pretreatment temperatures. Some of these studies are presented in Table 1. As shown in Table 1, increase of SCOD after thermal pretreatment was confirmed by majority of studies. Although the optimal hydrothermal condition in terms of organic dissolution varied from study to another depending on many factors such as characteristic and composition of food waste, operating condition, and etc. It was found that hydrothermal pretreatment reduced the concentrations of suspended solids by solubilizing the solid particles.

It is also reported that thermal pretreatment enhances the performance of the fermentation process (Ding et al. 2017; Li et al. 2014; Li and Jin 2015; WYin et al. 2014). Both VFAs and biohydrogen production was improved by utilizing thermally pretreated food waste as substrate for fermentation (WYin et al. 2014; Ding et al. 2017) . The optimal temperature for VFAs production range reported from listed studies fluctuates between 120 to 160 °C.

AD of food waste by application of thermal pretreatment was also promoted to some extend in several Research (Ariunbaatar et al. 2014; Jia et al. 2017; Y. Li et al. 2016). However, some scholars reported that thermal pretreatment adversely affected the AD process and subsequently methane production (Liu et al. 2012). Particularly, WYin et al. (2014) evaluated the effect of hydrothermal pretreatment on fermentation of food waste at temperatures of 120, 140, 160, 180, 200 and 220 °C for 30 minutes retention time. They found that highest COD solubilization after HTP occurred in samples pretreated at 180 °C. HTP temperatures higher than that leaded to mineralization of organic compounds. The concentrations of solid contents decreased at temperatures higher than 120 °C. The highest VS removal efficiency was observed at HTP temperature of 220 °C. The optimum HTP condition for VFAs production was 160 °C, the VFAs yield increased from 0.6 for raw sample to about 0.9 g/g $VS_{removal}$ for the pretreated sample.

Li and Jin (2015) studied the impact of hydrothermal pretreatment at different temperatures of 55, 70, 90, 120, 140 and 160 °C on acidification phase of two stage anaerobic digestion of kitchen waste. They observed that HTP condition of "120 °C-50 min" improved the acidification process by demonstrating about 50% degree of solubilization based on VS. The highest VFA concentration of 4,400 mg/L was achieved for sample pretreated at "120 °C-50 min" compared to 1,550 mg/L for the raw sample. Also, HTP influenced the distributions of VFA at different retention times and promoted the kitchen waste degradability and methane production.

Ding et al. (2017) investigated the effect of HTP of food waste for two stages AD, the temperatures of 100, 120, 140, 160, 180 and 200 °C were performed for RT of 20 min. In addition, one temperature set of 140 °C was performed for different RTs of 5, 10, 15, 25, and 30 min. They found that HTP promoted the solubilization of proteins while decreasing the solubilization of carbohydrates and enhancing maillard reactions at temperatures higher than 140 °C. The optimum HTP condition was "140°C -20min" which resulted in hydrogen and methane yields of 43 and 512 mL/g VS, respectively, compared to 35 and 388 mL/g VS for the raw sample.

Based on the aforementioned literature review, it was revealed that HTP is a promising pretreatment technology for enhancing the VFAs production. However, this impact is highly related to the severity of the HTP condition. Medium HTP temperatures 100 to 160 °C with different retention times which determines the severity of HTP is reported to be more efficient in terms of VFAs production.

Table 1 Studies on thermal pretreatment of Food waste prior to FERMENTATION and AD

Studies on thermal pretreatment of food waste following FERMENTATION					
Reference	Pre-treatment condition		Effect of Hydrothermal pre-treatment		
	Temperature (°C)	Retention time (min)	Increase in SCOD	Effect on Fermentation/AD	Solid Reduction
(Ding et al. 2017)	100-200	5-30	Highest COD solubilization 70 % at 180 °C	Highest VFAs production at 160°C-20, 85% higher compare to the raw	NA*
(Li and Jin 2015)	55-160	50-70	NA	Highest VFAs production at 120°C-50, 63% higher compare to the raw	Highest VS solubilization of 49% at 120 °C-50
(WYin et al. 2014)	100-220	30	43% more soluble COD than the control at 180 °C-30	Highest VFAs production at 160 °C-50, 35% higher compare to the raw	31% decrease in VS after HTP at 220 °C
(M. Li et al. 2014)	90 - 200	30	Highest COD solubilization of 26% at 150 °C	Highest hydrogen production at 200°C-30, of 55% higher than the raw	NA
Studies on thermal pretreatment of FW following AD					
(Jia et al. 2017)	90	30	NA	Methane increased by 29% compare to the raw	NA
(Y. Li et al. 2016)	55-160	15-120	NA	Highest methane production at 120°C-15% higher compare to raw	29% increase in VS proportion at 120°C -15
(Ariunbaatar et al. 2014)	70-150	30-60	NA	Highest methane production at 80°C-90% higher compare to raw	NA
(Liu et al. 2012)	175	60	SCOD increased significantly after HTP (No Numbers)	Methane decreased by 7.9% compare to raw at 175 °C-60	VSS solubilization ratio increased by 39%

**NA: Not Available*

3.7.6 Thermal pretreatment of Sludge

Impact of hydrothermal pretreatment on FERMENTATION and AD of municipal sludge waste, particularly WAS, has been investigated by many scholars. Some of these studies are reviewed in Table 2.

For FERMENTATION, low and medium temperatures were commonly utilised. All the studies reported the solubilization of organics after HTP. However, the percentage of organic solubilization was controlled by severity of HTP. Employing low to moderate (70–175 °C) temperatures increased the amount of VFAs and hydrogen production (Elbeshbishy et al. 2011; Morgan-Sagastume et al. 2011; Zhang et al. 2019b; Zinatizadeh et al. 2017; Zou and Li 2016). Furthermore, majority of studies stated that HTP enhanced the biogas production of municipal sludge (Choi et al. 2018a; Jeong et al. 2019; Xue et al. 2015), whilst few scholars observed that temperatures lower than 80 °C did not show significant impact on the ultimate methane production (Nazari et al. 2017).

TWAS hydrothermally pretreated at a full scale plant under 160 °C to evaluate the effect of HTP on FERMENTATION (Nazari et al. 2017). They reported that SCOD has been increased after HTP to 31 g/L compared to 5.4 g/L for the raw sample. Consequently, solubilization of particles occurred, they reported 20 to 30% decrease in suspended solids after the HTP. Hydrothermally pretreated WAS demonstrated 2–5 times increase in VFAs yield and 4–6 time increase in VFA production rate as compared to the raw sample.

Zinatizadeh et al. (2017) studied the impact of HTP (90 °C- 60 min) as well as chemical pretreatment on dark fermentation of granular sludge. The highest specific hydrogen production rate 31.4 mL H_2/g VSS. d was achieved from thermally pretreated sample compared to 18 mL H_2/g VSS for the raw sample. Furthermore, the results showed 76% increase in cumulative hydrogen production compare to the raw sludge.

Recently, Zhang et al. (2019b) pretreated mixture of primary and secondary sludge with temperatures of 155 to 175 °C for 30 min in a full scale high pressure thermal hydrolysis plant. This study was carried out by employing both raw and thermally pretreated samples in mesophilic and thermophilic acidification tests. The superior result in terms of VFAs production was observed at mesophilic condition, the highest VFAs yield of 0.22 gCOD/gVS was achieved, this yield was 45% higher than that produced from the raw sludge. After thermophilic acidification test, the VFAs yield for thermally pretreated sample was 0.19 gCOD/gVS which was still higher than that of raw

0.17. Consequently, thermally pretreated sludge at 55 °C produced 12.2% less amount of VFAs compare to the 35 °C.

Based on the above-mentioned studies on thermal hydrolysis of municipal sludge following by FERMENTATION, it was noticed that thermal pretreatment enhanced the organic dissolution and suspended solid removal efficiency. The medium HTP temperatures 150 – 180 °C was mainly used for the thermal pretreatment and proved to be effective in terms of improvement of VFAs production

Table 2 Studies on thermal pretreatment of Sludge prior to FERMENTATION and AD

Studies on thermal pretreatment of secondary sludge following FERMENTATION					
Reference	Pre-treatment condition		Effect of Hydrothermal pre-treatment		
	Temperature (°C)	Retention time (min)	Increase in SCOD	Effect on Fermentation/AD	Solid Reduction
(Zhang et al. 2019b)	155-175	30	increase of SCOD/TCOD from 2% to 27%	VFAs increased by 47% compare to the raw	decrease of TSS/TS from 93% to 73%
(Zinatizadeh et al. 2017)	90	60	NA*	Hydrogen production increased by 76% compared to the raw	NA
(Morgan-Sagastume et al. 2011)	160	NA	SCOD increased from 3.6 to 33 g/L	VFAs increased by 50 to 80% compare to the raw	20-30% decrease in VS
(Elbeshbishy et al. 2011)	70	30	20% increase in SCOD compare to raw	Hydrogen production increased by 40% compare to the raw	13% decrease in VS
Studies on thermal pretreatment of secondary sludge following AD					
(Jeong et al. 2019)	100-220	30	Increase in the SCOD/TCOD from 49 to 55% at 220 °C	Highest methane production at 180°C-30, 43- 46% higher compare to raw	Highest TS reduction of 7% at 220°C-30
(Choi et al. 2018b)	75-225	15-105	Maximum SCOD/TCOD of 30% higher than raw	Highest methane production at 180°C-76, 23% higher compare to raw	NA
(Higgins et al. 2017)	130-170	30	Highest Total COD reduction of 56% for 170 ℃ which was higher than raw	Highest methane production at 170°C-30, 5-6 % higher than other conditions	Highest VS reduction of 56% at 170°C-30
(Nazari et al. 2017)	40-80	1-3-5	COD solubilization increased by 18% compare to the raw	No significant effect on ultimate CH_4	VSS reduction of 28% compared to the raw
(Xue et al. 2015)	60-180	15-60	COD solubilization increased from 4.5% for raw to 53%	Highest methane production at 180 °C, 10% higher compare to raw	NA
(Bougrier et al. 2007)	135-190	35-50	COD solubilization increased by 18% compare to the raw	Highest methane production at 190 °C, 25% higher compare to raw	VS removal increased from 39% to 57%

4 Materials and Methods

5 Substrates and Inoculum

5.1 TWAS

The TWAS used in this study was collected from Ash-bridges Wastewater Treatment Plant (AWWTP). AWWTP located at Toronto's east end, Canada has a nominal capacity of 818000 m3/day and serves an equivalent population of 1,524,00. Wastewater treated in this facility undergo four major treatment stages which are preliminary treatment, primary treatment, secondary treatment (Activated Sludge), and disinfection (City of Toronto 2009). Raw wastewater flows in two preliminary treatment sections where grits and screenings are removed. Screened waste water enters to the settling tanks where solids particles (sludge) after settling are swept to the AD tanks. The secondary sludge (TWAS) is also directed to the AD tanks. These anaerobic digestion tanks operate under mesophilic temperature range (34-38 ℃) and sludge hydraulic retention time of 18 days (City of Toronto 2009). The sample for this study was taken from thickening section after secondary treatment. Table 3 summaries the main characteristics of the raw TWAS.

5.2 SSO

SSO is combination of food waste, wood waste, yard and landscaping debris and paper fibers collected from Toronto SSO green bin program collected from single and multi-family residential, various commercial and agencies departments. SSO samples were obtained from Disco Road Organic Processing Facility (DROPF) located at Toronto, Canada. In 2010, this facility was designed and built on CCI's technology platform, the BTA® Process. The design capacity is 75,000 metric tonnes of residential and commercial SSO per year. DROPF operates in three phases of pre-processing phase, conversion phase and utilization phase. During pre-processing phase, SSO is fed directly to the BTA® Hydromechanical Pre-treatment System, which is designed with 3 x BTA® Waste Pulpers and 3 x BTA® Grit Removal Systems where the organics will eventually turn into a liquid (slurry) pulp. Afterwards, pre-processed wastes undergo wet digestion process in

the mesophilic range using 2 x 5,300 m3 digesters that are continuously mixing using compressed biogas (Highlights 2013).

Sample for this study was obtained from system, after SSO passed through BTA® wet mechanical pre-treatment occurring within two core components, the BTA® waste pulper and the BTA® grit removal system and before feeding to anaerobic digester of organic fraction of mechanically pretreated wastes. Raw SSO is characterized in Table 3.

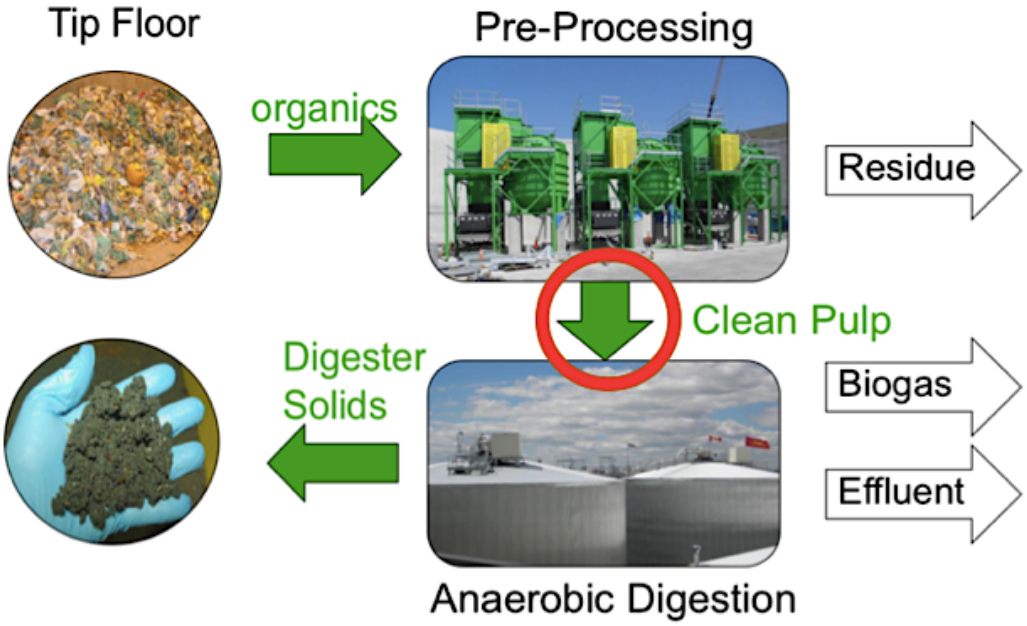


Figure 6 DISCO road facility schematic; Source: https://www.ccibioenergy.com

5.3 Inoculum

Inoculum used in this study was collected from a steady operating anaerobic digester at AWTP. The anaerobic digester is fed with mixture of primary sludge and TWAS and is operated at mesophilic temperature (34-38 °C). The average hydraulic retention time (HRT) of the anaerobic digester is about 18 days. The organic loading rate of the digesters averaged approximately 1.1 kg TVS per m^3 of digester capacity per day (City of Toronto 2009).

The inoculum was thermally pretreated for methanogenic inactivation (Ding et al. 2017). A stainless-steel pot containing 6 L of inoculum was heated gradually using a hot plate until the temperature reached 70 °C. Afterward the heated inoculum was incubated for 30 minutes in the incubator at 70 °C. It was then cooled to the room temperature and the pH was adjusted to 5 by adding HCl. The characteristics of raw inoculum are shown in Table 3.

Table 3 Raw TWAS, SSO and seed inoculum characteristics

Parameter	TWAS	SSO	Seed
TCOD (mg/L)	49600 ± 1539	144050 ±17254	26570 ± 709
SCOD (mg/L)	2580 ± 480	42167 ± 400	1270 ± 520
TSS (mg/L)	34000 ± 3400	66183 ± 860	18000 ± 300
VSS (mg/L)	22700 ± 2500	49250 ± 330	11600 ± 100
Total carbohydrates (mg/L)	3570 ± 430	11408 ± 1506	1175 ± 89
Soluble carbohydrates (mg/L)	112 ± 14	1209 ± 58	227 ± 9
T-Protein (mg/L)	921 ± 78	986 ± 113	ND[a]
S-Protein (mg/L)	703 ± 11	77 ± 9	ND
Ammonium nitrogen NH_3-N (mg/L)	252 ± 7	1716 ± 8	ND
Alkalinity (mg/L)	1063 ± 126	5183 ± 226	ND
PH	6.3 ± 0	5.9 ± 1	7.00 ±1

a ND: Not Determined

6 Hydrothermal pretreatment

To enhance the biodegradability of the employed substrates and facilitate faster hydrolysis, HTP was applied on raw substrates under five different severity indexes (SI) of 1, 2, 3, 4, and 5. Severity index is a parameter which is combination of temperature and retention time broadly applied by industries. SI is a useful parameter is commonly used to evaluate the impact of different conditions of temperature and retention time during hydrothermal pre-treatment. Severity index was calculated using equation (1).

$$SI = \log\ [t \times exp^{\left(\frac{T-100}{14.75}\right)}] \quad (1)$$

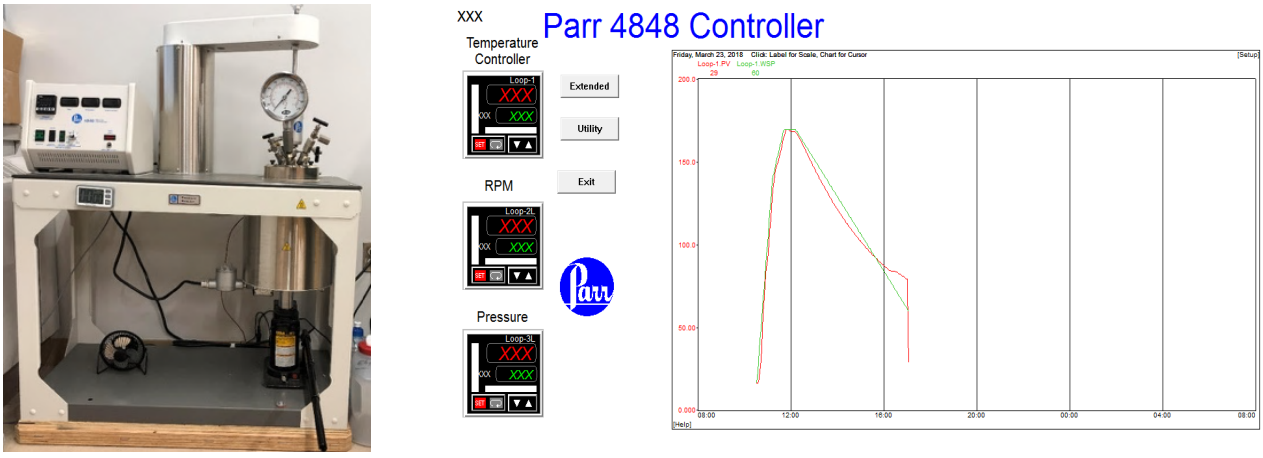
Where T is hydrothermal temperature (℃) and t is retention time (min).

HTP temperature of 150–240 ℃, retention time of 5-30 minutes, and pressure of 69-488 psi were used in the pre-treatment conditions. In each severity index, three different combination of temperature, pressure, and holding were considered to make three different scenarios in each severity index. Overall, 15 scenarios of HTP were designed, see Table 4.

Severity Index (SI)	3.0 ± 0.05			3.5 ± 0.05			4.0 ± 0.05			4.5 ± 0.05			5.0 ± 0.05		
Pre-treatment Parameters	(psi)	(°C)	(min)	(psi)	(°C)	(min)	(psi)	(°C)	(min)	(psi)	(°C)	(min)	(psi)	(°C)	(min)
Scenarios 1	69	150	30	114	170	30	181	190	20	227	210	20	337	220	30
Scenarios 2	89	160	20	145	180	15	225	200	10	337	220	10	407	230	15
Scenarios 3	114	170	10	181	190	10	277	210	5	407	230	5	488	240	8

Table 4 Hydrothermal pretreatment design

HTP was conducted by Parr 4848 Hydrothermal Reactor (Parr Instrument Company, IL, US) Figure 7(a) with a capacity of 2 L. The volume of the substrate for each pre-treatment in this study was 1 liter. The heating rate of materials initially were 3 ℃ per minute and then was reduced to 2 ℃ per minute until reaching the target temperature. Last cycle was the retention time of sludge. During the pre-treatment process, Substrate was constantly mixed and operated by specView Parr 4848 controller Figure 7 (b) equipped with proportional integral derivative (PID) programming with auto-tuning capabilities for accurate control of temperature, pressure, heating ramp, and soak (retention time). SpecView software was connected to the reactor controller to control parameters in eight different loops while providing real-time plotting.



(a) (b)

Figure 7 Hydrothermal pretreatment system; (a) Parr 4848 Hydrothermal reactor; (b)SpecView Parr 4848 controller

7 Solubilization Study

The degree of solubilization is often used as a performance indicator of the pre-treatment process (Higgins et al. 2017). In order to determine the effect of hydrothermal pre-treatment on solubilization of TWAS and SSO, soluble contents of the substrate such as soluble chemical oxygen demand (SCOD), soluble carbohydrates, and soluble protein were measured before and after HTP. The COD solubilization percentage (%) in this study was calculated using Equation (2):

$$Solubilization\ percentage\ (\%) = SCOD_{HTP} - SCOD_{Raw}/PCOD_{Raw} \times 100 \quad (2)$$

Where $SCOD_{HTP}$ and $SCOD_{Raw}$ are the concentrations of the SCOD of the pretreated and non-pretreated (raw) samples, respectively. $PCOD_{Raw}$ is the concentration of the particulate COD of the raw sample calculated using the Equation (3):

$$PCOD = TCOD_{Raw} - SCOD_{Raw} \quad (3)$$

Where $TCOD_{Raw}\ and\ SCOD_{Raw}$ denote the concentrations of total COD and soluble COD of the raw sample, respectively.

The solid reduction percentage "R" of the hydrothermally pretreated samples was calculated using Equation (4):

$$R\ (\%) = VSS_{Raw} - VSS_{HTP}/VSS_{Raw} \times 100 \quad (4)$$

Where VSS_{Raw} is the volatile suspended solids (VSS) concentration of the raw sample and VSS_{HTP} is the VSS concentration after the HTP pretreatment.

The percentage of COD solubilization during the acidification test was calculated using Equations (5), (6), (7) and (8).

$$Solubilization\ percentage\ (\%) = Mass\ of\ SCOD_{Produced}/PCOD_{in} \quad (5)$$

$$Mass\ of\ SCOD_{Produced} = Mass\ of\ SCOD_{final} - Mass\ of\ SCOD_{initial} \quad (6)$$

$$SCOD_{initial} = \frac{SCOD_{sub} \times V_{sub} + SCOD_{Seed} \times V_{seed}}{V_{Sub} + V_{Seed}} \quad (7)$$

$$PCOD = TCOD_{HTP} - SCOD_{HTP} \quad (8)$$

$$Mass\ of\ SCOD_{final} = SCOD_{final}\ x\ (V_{Sub} + V_{Seed}) - SCOD_{Seed} \times V_{seed} \quad (9)$$

Here $SCOD_{final}$ denotes the soluble COD at the end of the acidification test. $SCOD_{sub}$ represents soluble COD of the substrate, $SCOD_{seed}$ is soluble COD of the inoculum. V_{Sub} shows the volume of substrate added to the acidification reactor, V_{seed} is volume of inoculum added to each acidification reactor. $PCOD_{sub}$ is the particulate COD of the substrate before adding it to the

reactor. where $TCOD_{HTP}$ is the total COD concentration of the pretreated sample and $SCOD_{HTP}$ is the concentration of soluble COD of TWAS after hydrothermal pretreatment. Mass of $SCOD_{final}$ was calculated with an assumption that the SCOD in the seed didn't degraded or converted during the acidification process.

8 Acidification Experiment

The schematic diagram of the experimental setup of this study is shown in Figure 8. As seen the samples, first underdo hydrothermal pretreatments and then was mixed by the microbial cultures and. The raw samples were also mixed by seed to control and compare the results. All mixed samples the were then fermented. Acidification experiments were conducted in triplicates for each pretreated and raw sample. 45 mesophilic batches, each with total volume of 500 mL, were used to set up the experiment for the 15 scenarios of pre-treatment. In addition to the 45 reactors, three reactors for the raw substrate were run under same conditions as a control.

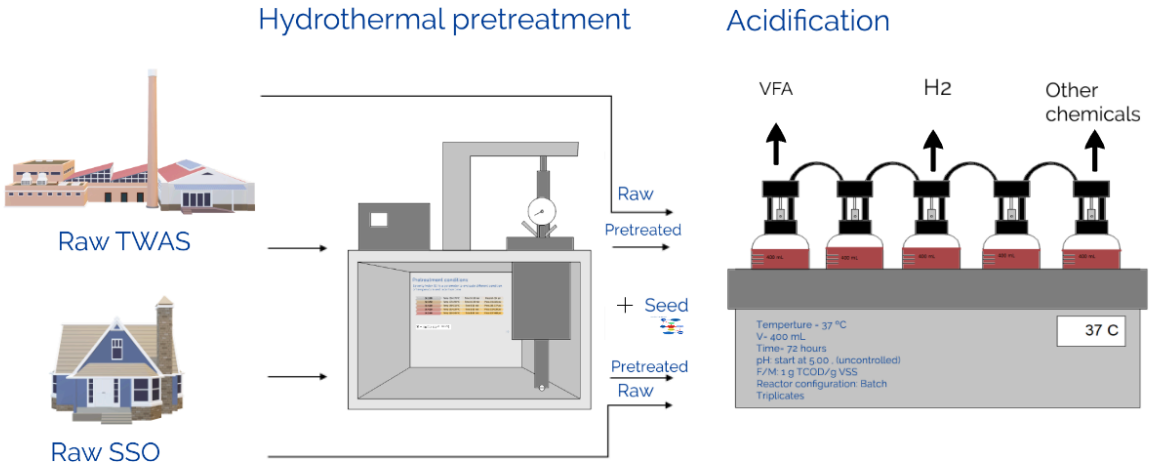


Figure 8 Schematic diagram of the pretreatment of TWAS and SSO following by acidification

The working volume of each reactor was 300 mL. Volumes of substrates and seed were calculated based on food to microorganism (F/M) ratio of 1 g-TCOD/g-VSS using Equation 10.

$$\frac{F}{M} = \frac{TCOD_{TWAS} \times V_1}{VSS_{seed} \times V_2} \quad (10)$$

Where V_1 and V_2 represent the volumes of substrate and seed, respectively. VSS_{seed} is the VSS of the seed and $TCOD_{TWAS}$ indicates the Total COD of the TWAS. After adding the substance and inoculum, the initial pH was adjusted to be 5.50 by using adequate 3.5 M HCl or NaOH. As the initial pH of the collected samples and seed was higher than 5.50 HCl was added mainly to drop the pH to the desired value. The reactors were then purged with nitrogen gas for 5 min to make sure the anaerobic condition is maintained and then the reactors were sealed. The acidification tests were carried out by Bioprocess AMPTS II, Automatic Methane Potential Test System shown in Figure 9. This system consists of two main components of the sample incubation unit (unit A) and gas volume measuring device (unit B). In the sample incubation unit, the sealed reactor was placed and incubated for 72 hours. The temperature was maintained at 37 °C and the mixer rotational speed was set at 120 rpm. In the gas volume measuring unit, the gas released from unit A was measured using a wet gas flow measuring device with a multi-flow cell arrangement. An integrated embedded data acquisition system was used to record, display and analyze the results.

The VFAs yield produced after acidification of pretreated and raw TWAS was calculated by equations (11), (12), and (13):

$$VFAs\ Yield = \frac{Mass\ of\ VFAs_{produced}}{Mass\ of\ VSS_{\ added\ in\ the\ Substrate}} \quad (11)$$

$$\text{Mass of VFAs produced} = VFAs_{final} \times (V_{sub} + V_{seed}) \quad (12)$$

$$\text{Mass of VSS added} = VSS_{sub} \times (V_{sub}) \quad (13)$$

Where $VFAs_{final}$ denotes the concentrations of VFAs at the end of the acidification test and VSS_{sub} is the concentrations of VSS of samples that was added to the reactor before the acidification test.

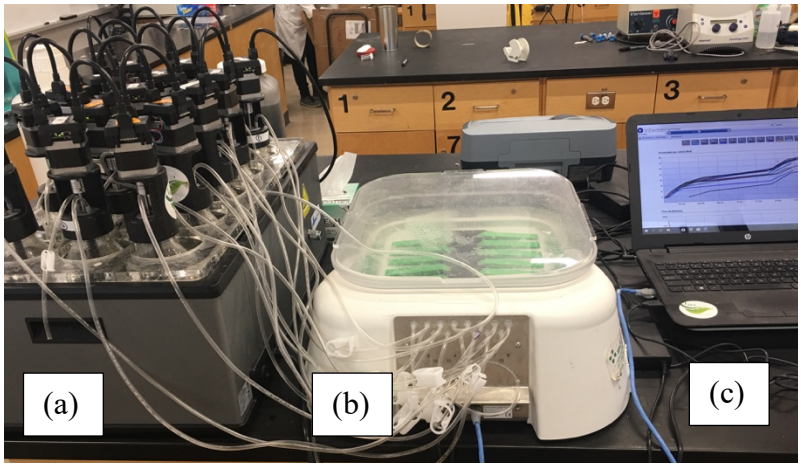


Figure 9 Dark Fermentation system; (a) Sample incubation unit, (b) Gas volume measuring device (c) Gas volume measuring device controller

9 Analytical methods

The soluble parameters were measured after filtering the samples through of 0.45 μm membrane filter paper (GN Metricel ® membrane disc filters). The soluble parameters include SCOD, soluble carbohydrates, soluble proteins, ammonium (NH_4-N) content, alkalinity, and VFAs.

The total suspended solids (TSS) and VSS were analyzed according to the Standard Methods (Environment 1999). The Total and soluble protein were determined via Coomassie Bradford assay (Dubois et al. 1956). Total and soluble carbohydrate were measured using phenol sulphuric acid method (Dubois et al. 1956). The particle size distribution (PSD) was determined via a laser diffraction particle size analyzer (model: LS 13 320, Beckman Coulter, Indianapolis, US). TVFAs, TCOD, and SCOD were measured using the reactor digestion method and salicylate methods (HACH 2003) . For the analysis of ammonia, and alkalinity the Hach spectrophotometer model 3900 was used to measure the absorbance at the wavelengths of 560 and 650 nm, respectively.

VFAs fractions were analyzed using Agilent 7820A gas chromatography equipped with a flame ionization detector (Agilent Technologies USA) and DB-wax column 15 m × 0.32 mm × 0.5 μm (Agilent Technologies USA). The oven temperature for VFA analysis was programed to initially hold at 80 °C for 1 min, then increase to 180 °C at a slope of 10 °C/min and maintained at 180 °C for 4 min. The hydrogen content in the produced gas was measured by Gas Chromatography method by Thermo-Scientific Trace 1310 GC after 16, 24, 48 and 72 hours under 100 °C detector temperature. The model of column used in the GC was TG-Bond Msieve 5A with 30 mm length and 0.53 mm diameter.

10 Statistical analysis

To evaluate the significance of the four variables, temperature, pressure, retention time, and severity index on the performance of the dark fermentation and sludge solubility, multifactor analysis of variance (ANOVA) was used. The main effect plot, interaction plot and Contour were created via Minitab and Matlab R2018a. Correlation between solubilization after HTP and fermentation for VFAs production was calculated using excel data functions. Confidence level for all analysis was chosen equal to 95%. The standard deviations of all measurements were calculated by Excel.

11 Results and discussion (TWAS)

12 Effect of hydrothermal pre-treatment on TWAS characteristics

12.1 COD solubilization of TWAS

Figure 10 (a) presents the concentration soluble COD of all hydrothermally pretreated and raw. The results of this study showed that the concentrations of SCOD after HTP for all the pretreatment conditions were higher than that the non-pretreated sample. The SCOD concentration of raw TWAS was 2580 ± 480 mg/L. The SCOD content of pretreated samples were as low as 15100 ± 350 mg/L at HTP condition of "150°C-30" and as high as 25400 ± 1440 mg/L at "200°C-10 min" which counts 6 to 10 times more than raw TWAS. This increase in SCOD is due to the release of intracellular organic matters because of the disruption of the cell wall and membrane by HTP. Polysaccharides, proteins, nucleic acids, and humic acids referring to extracellular polymeric substances (EPS) are released from the cell walls due to pretreatment (Appels et al. 2010). These results are in agreement with Choi et al. (2018), who reported an increase in the SCOD by 55% compared to the raw sample (Choi et al. 2018). They applied hydrothermal pre-treatment on sewage sludge at HTP temperature of 70 to 225 °C and retention time of 15 to 105 min, they found that the highest increase in the SCOD was at temperature of 180 and retention time of 76 min.

The COD solubilization of the hydrothermally pretreated samples were calculated using equation (2) and are illustrated via Figure 10 (b). The trend of this graph indicates that increasing the temperature of HTP resulted in enhancement of the COD solubilization which is in agreement with literature (Bougrier et al. 2006; Ennouri et al. 2016; Xue et al. 2015).

Moreover, the correlation coefficient between these two parameters was 0.89 demonstrating a strong positive correlation between the two variables (temperature and percentage increase in COD solubilization).

The organics solubilization continued to increase from 27% at lowest HTP condition of "150°C-30min" reaching its maximum value of 49% at "200°C-10 min" and it was almost constant afterwards.

The average COD solubilization values of 32, 39, 46, 45 and 46% were achieved for SIs of 3, 3.5, 4, 4.5 and 5 respectively. The COD solubilization values were almost similar for the high severity indexes of 4, 4.5, 5. By looking at the three scenarios of each SI separately, it was observed that the lower retention time combined with higher temperatures demonstrated higher solubilization

emphasizing that the temperature is the dominating parameter in the HTP process.

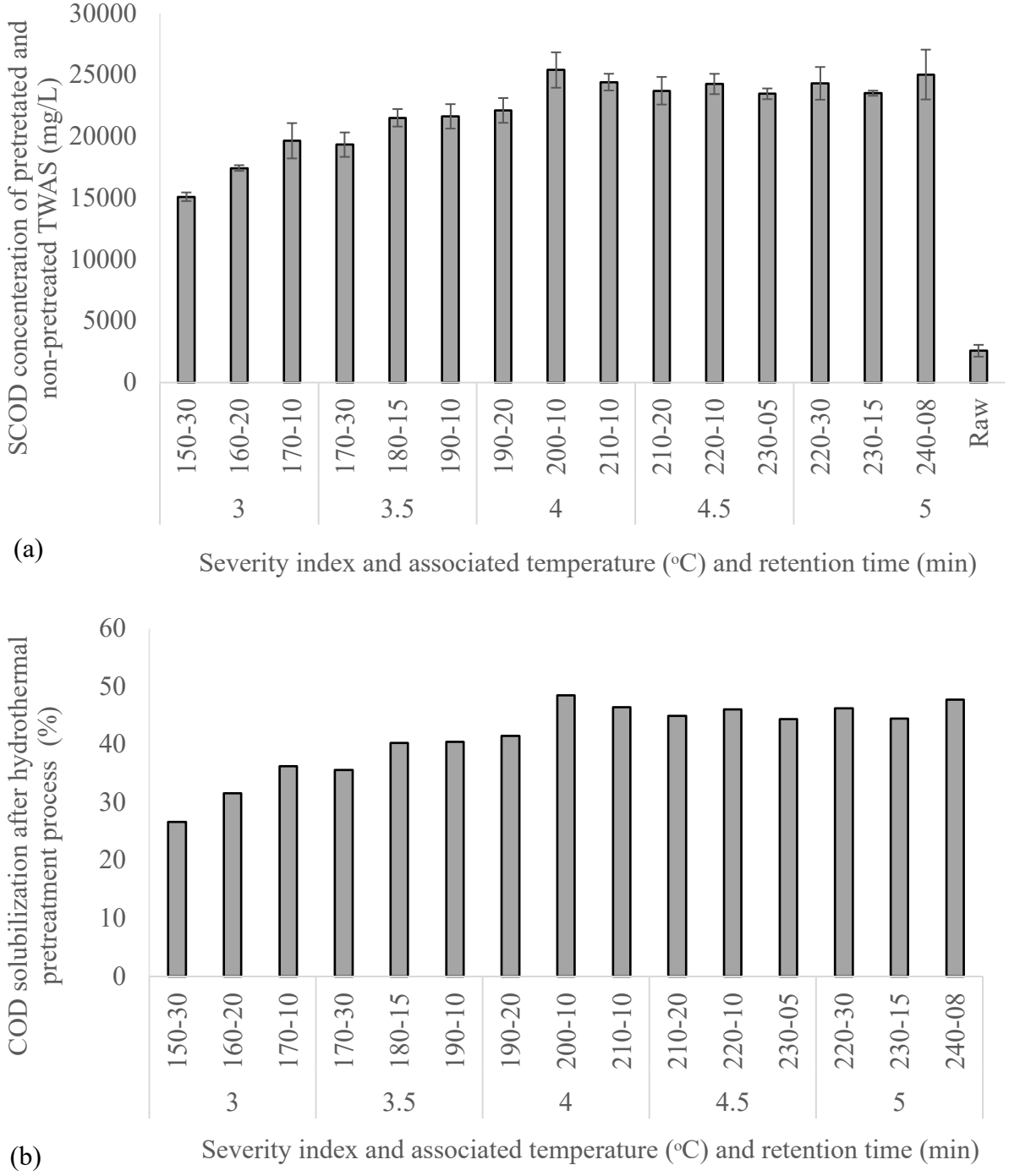


Figure 10 Effect of HTP on organic dissolution (a) Concentration of Soluble COD after hydrothermal pre-treatment; (b) COD solubilization due to HTP

Overall, the effect of both hydrothermal pre-treatment temperature and RT on sludge solubilization was statistically significant ($p<0.005$) Table 5. To illustrate the relationship between COD

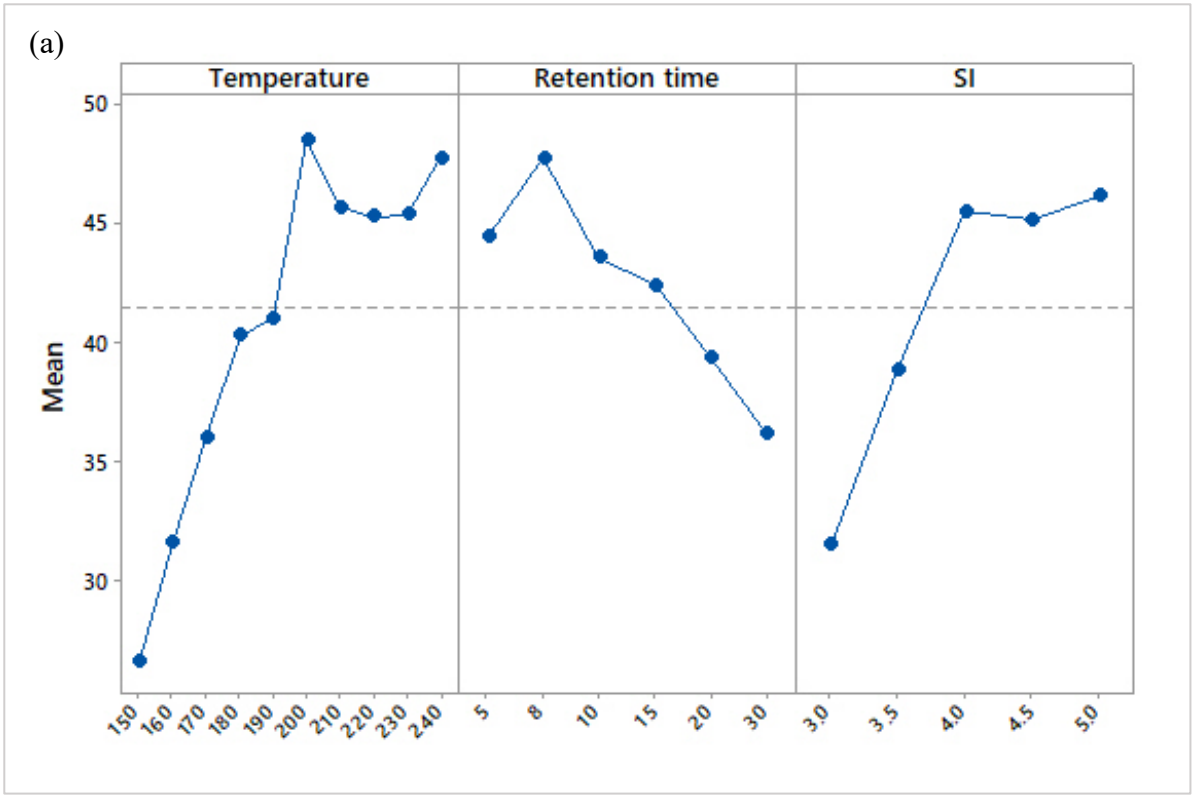
solubilization percentage and three main variables, the main effect plot of COD solubilization percentage vs. HTP severity index, temperature, and retention time is showed by Figure 11 (a). Besides, the interactions between the four main variables (temperature, pressure, time, and SI) for the COD solubilization after hydrothermal pre-treatments are represented by Figure 11 (b).

Table 5 ANOVA for COD solubilization after HTP considering the effect of HTP temperature

	df	SS	MS	F	Significance F
Regression	1	455	455	52	7.01E-06
Residual	13	114	9		
Total	14	570			

After pre-treatment, the soluble portion of both carbohydrates and protein were higher than that of raw indicating the positive influence of HTP on sludge wall disintegration as indicated in Figure 12 (a), (b). The highest concentrations of soluble carbohydrates and protein were 2040 ± 28 and 2100 ± 16 mg/L at pre-treatment conditions of "160°C-20min" and "150°C-30min", respectively. Having the highest concentration of soluble carbohydrates and proteins in the lowest HTP temperature and its drop by increase in HTP temperature, it can be revealed that hydrothermal pre-treatment temperature rise demonstrated a significant impact on dissolution of carbohydrates and proteins ($p<0.05$). The correlation coefficient of both parameters was -0.9 indicating negative correlation between soluble protein and carbohydrates concentration and temperature. This negative effect due to the intensification of temperature which might be because of the reactions between soluble carbohydrates and themselves or soluble carbohydrates and proteins (Appels et al. 2010). The total carbohydrates content also decreased by increase in temperature detonating the solubilization and degradation of carbohydrates.

It can be concluded that lower severity indexes (3.00 and 3.5) demonstrates higher solubilization yield for both carbohydrates and proteins, whereas, thermal pre-treatment at higher severity indexes (4.00, 4.5 and 5.00) expresses reduction of these parameters.



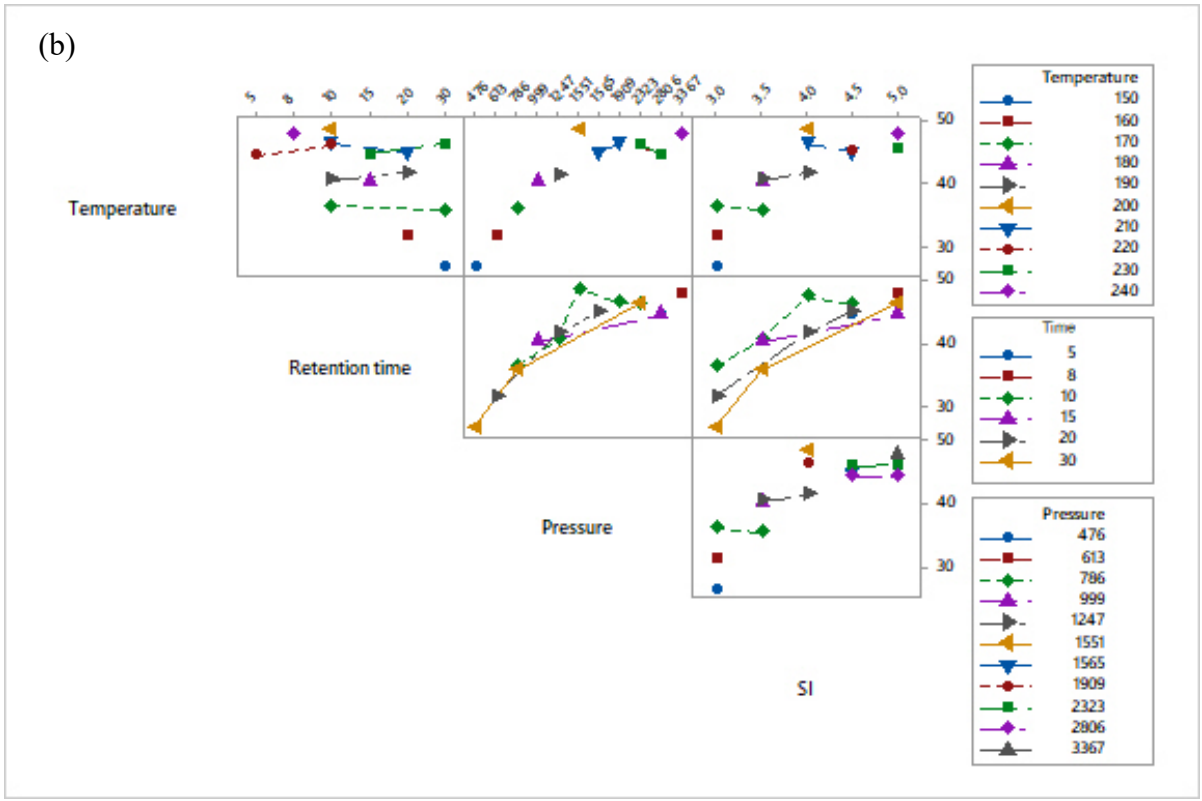
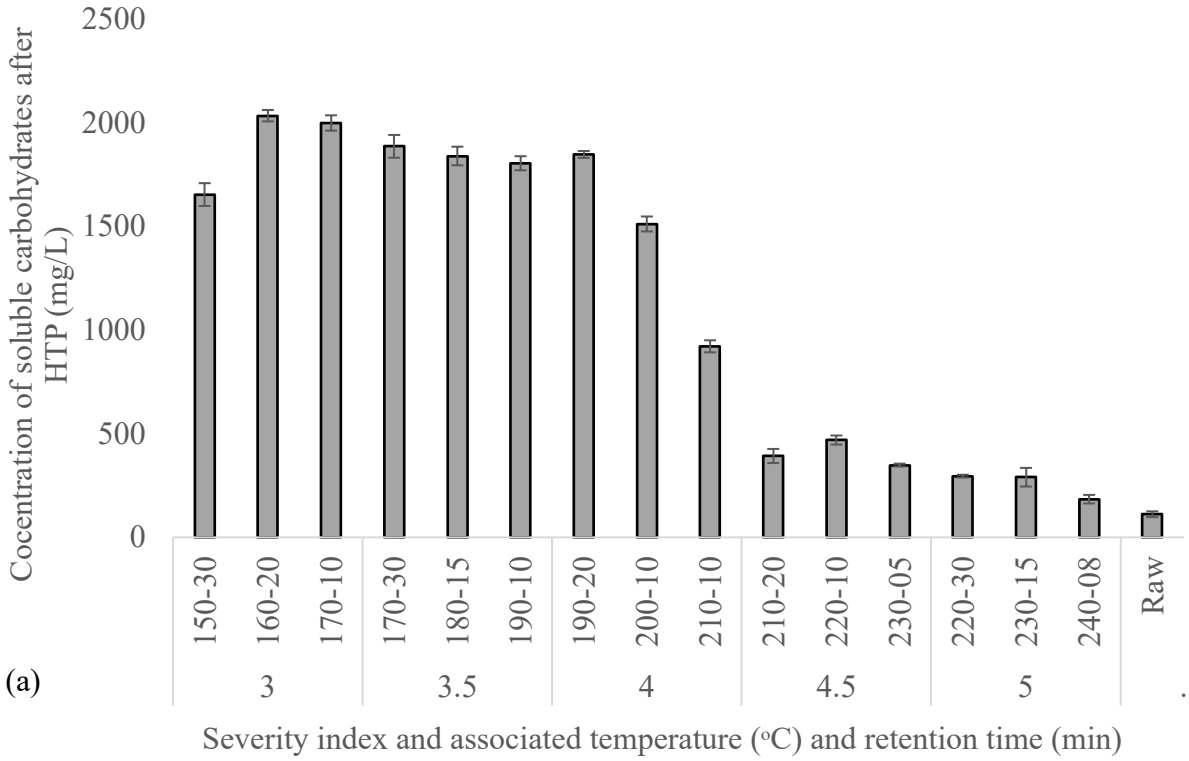


Figure 11 (a) The main effect plots of COD solubilization percentage vs. HTP severity index, temperature and retention time ; (b) The interaction plots of temperature, time, pressure and severity index for the COD solubilization due to hydrothermal pretreatment



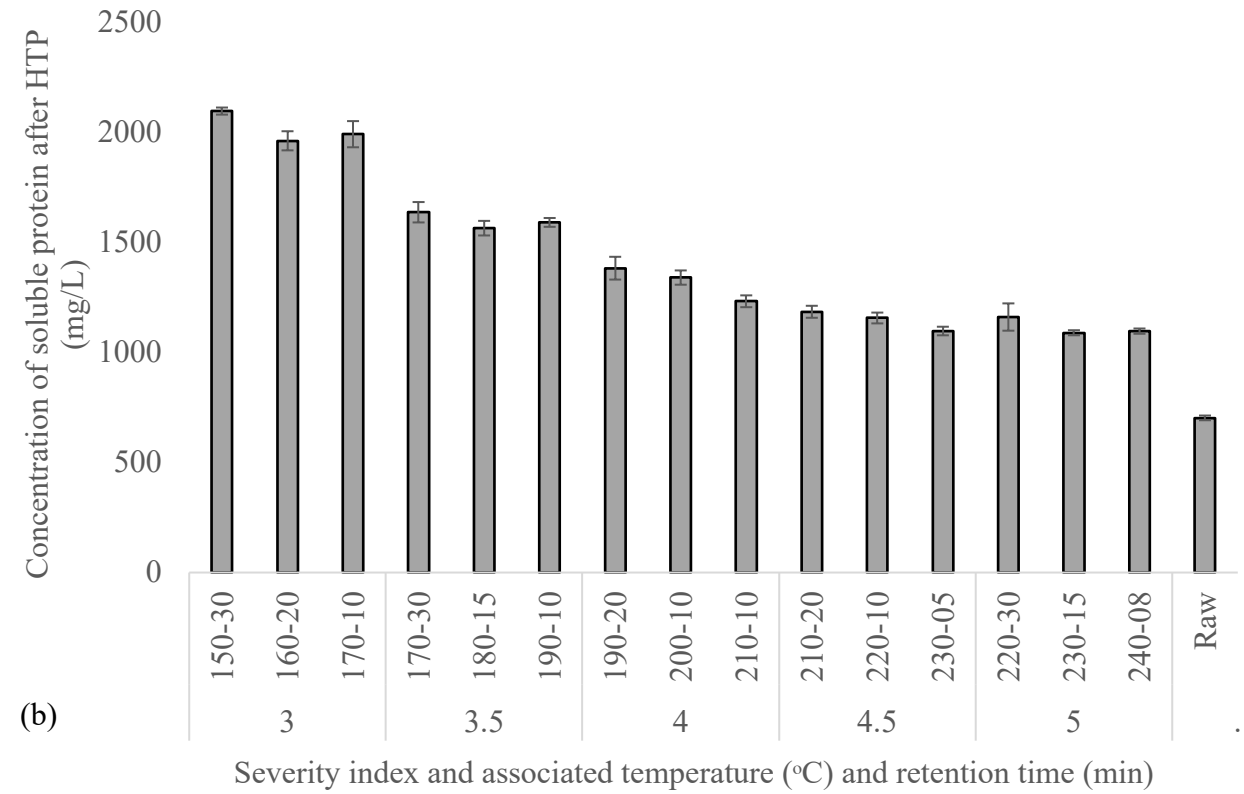


Figure 12 The effect of HTP on soluble content of carbohydrates and proteins; (a) concentration of soluble carbohydrates after HTP; (b) concentration of soluble protein after HTP

12.2 Solid reduction

After hydrothermal pre-treatment of TWAS, the suspended solid concentrations of all pretreated samples were lower than that of raw sample. The concentrations of total and volatile suspended solids of pretreated and non-pretreated samples are presented in Figure 13 (a). The lowest concentrations of TSS and VSS of 17.1 ± 2.5 and 10.2 ± 1.2 g/L were observed at pre-treatment condition of "210°C-20 min". Ennouri et al. (2016) observed decrease in TSS and VSS concentrations after thermally pretreating municipal waste activated sludge under temperature range of 60 to 120 °C for 30 minutes. Ennouri et al. reported 20% to 40% of VS reduction while in current study, we observed higher VSS reduction ranged between 21% to 55%, that might be due to the higher temperature that we applied and the nature of the waste.

Similar to COD solubilization, the solid reduction exhibited an increase with increasing the temperature ($p<0.05$). As shown in Figure 13 (b), the solid reduction increased with increasing the temperatures until 210 °C and then it begun to decline afterwards. This declination in TSS and VSS reduction could be due to the formation of insoluble macromolecular polymers (hybrid polymer) as a result of polymerization of inorganic compounds with organic that occurs in intensified HTP temperatures.

The highest percentage reduction in VSS of 55% was achieved at pretreatment condition of "210 °C-20 min". These findings were in agreement with Abe et al. (2013) who employed hydrothermal pretreatment temperatures ranging from 120 to 200 °C for one hour on TWAS. Abe et al. reported highest VSS reduction of 70 % at hydrothermal pretreatment condition of "200 °C-60 min", which has the same severity for "210 °C-20 min" (HTP condition of highest VSS destruction efficiency in this study).

The influence of retention time inspected in each severity index indicated that at distinctive severity indexes, the impact of retention time is different. The following conditions demonstrated higher solid removal efficiency than other conditions among three scenarios of each SI: (a) SI of 3.5 lower temperature with higher RT, (b) Severity indexes of 3 and 4.5, higher temperatures with lower RTs, (c) SI of 4 and 5, moderate temperature and moderate RT.

Consequently, it can be concluded that lower severity indexes are not an optimum condition due to the low solids reduction and didn't disintegrate all the possible higher molecular components. Furthermore, the results of this study revealed that SI higher than 4.5 was not favourable for TWAS solubilization.

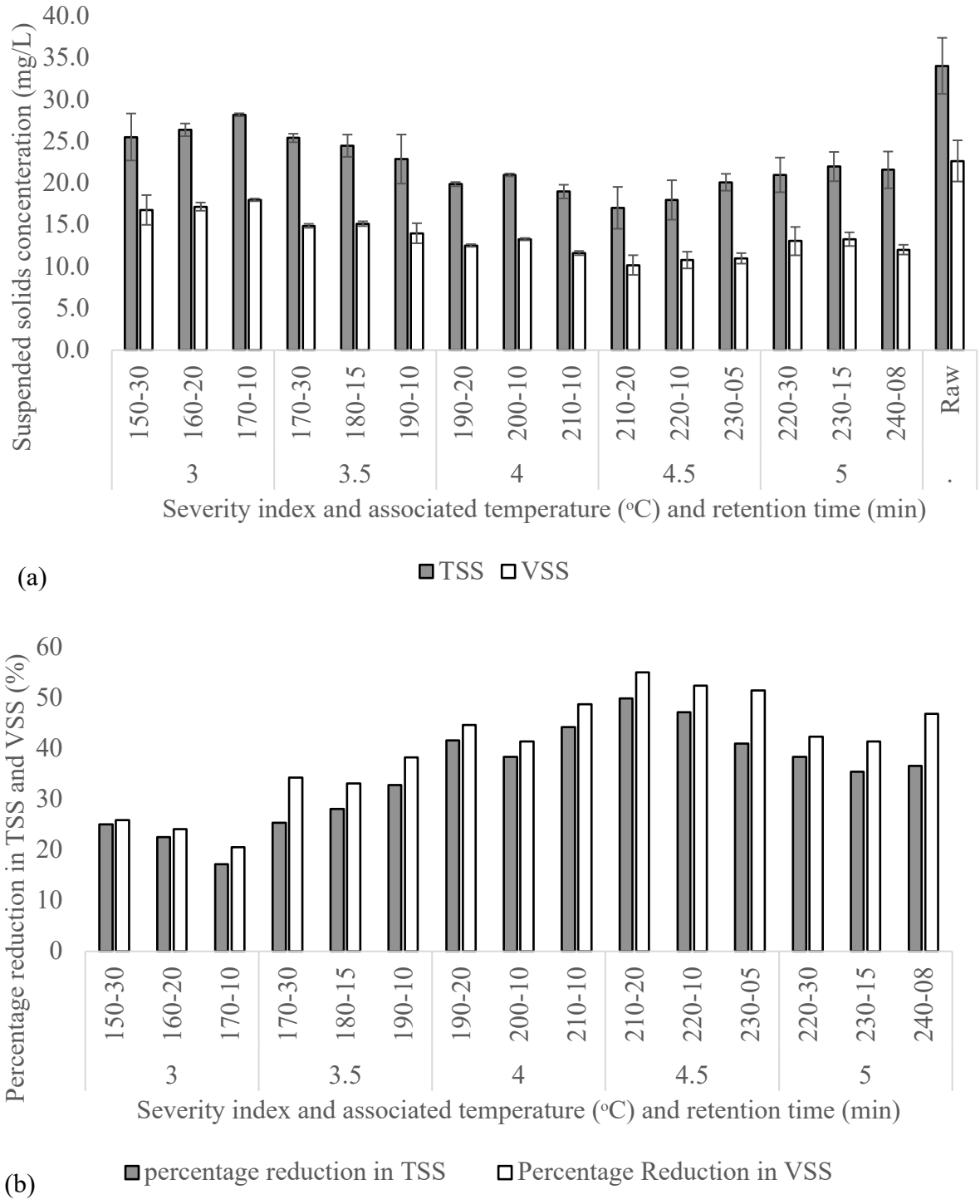


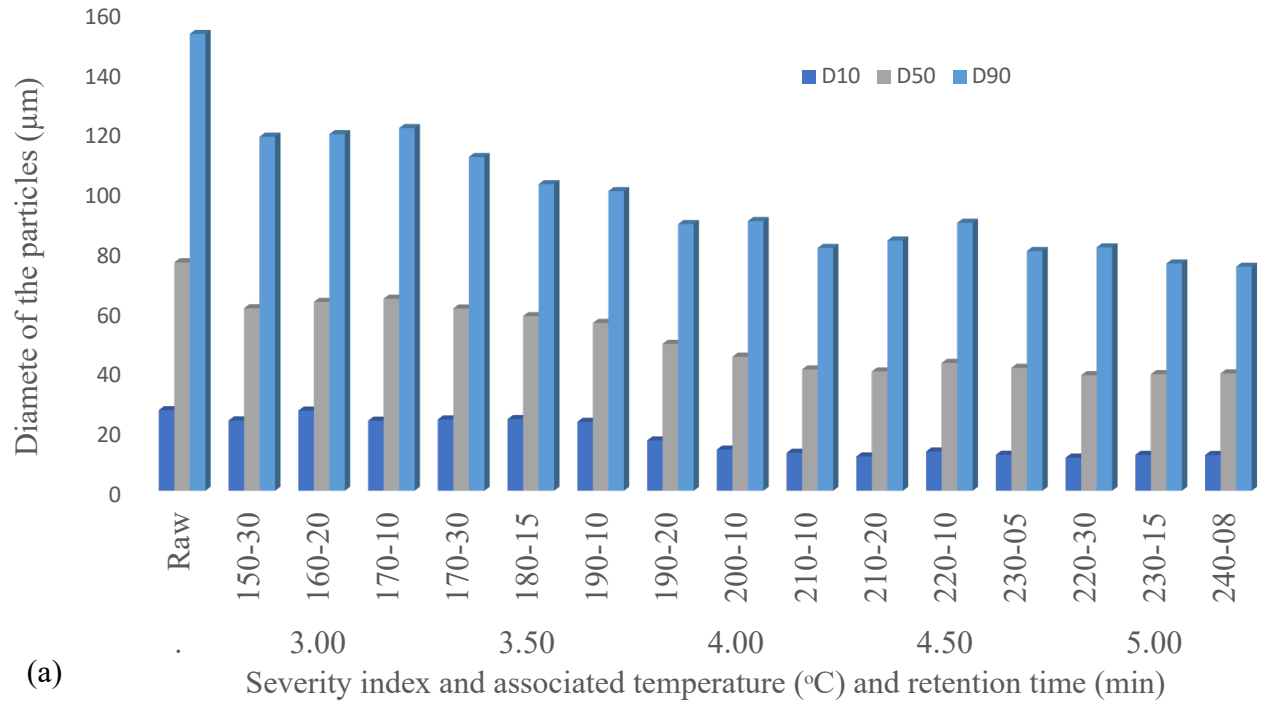
Figure 13 The effect of HTP on solid reduction; (a) Solid concentration before and after HTP; (b) solid reduction percentage of the TWAS samples after and before HTP

12.3 Particle size distribution

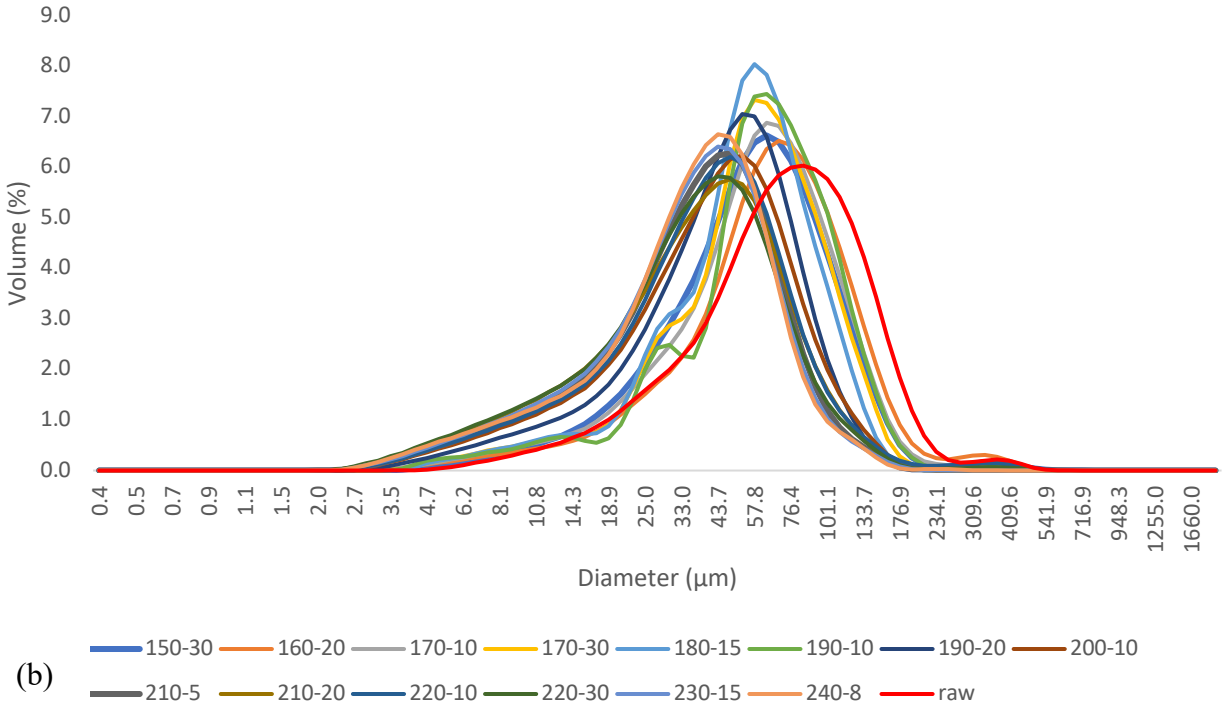
Hydrolysis is the rate limiting step of fermentation of particulate matter and highly influenced by particle size. Higher degradation efficiency can be achieved by smaller particle size and lower concentrations of the particles (Elbeshbishy, 2010). According to the literature, particle size reduction could be achieved by application of thermal pre-treatment that subsequently improve the entire anaerobic process (Abudi et al. 2016; Li et al. 2016).

The particle size distribution of all hydrothermally pretreated and raw samples is shown in Figure 14 (a). As shown in the Figure, the particle size of all pretreated samples was lower than that of the raw sample. The particle sizes range decreased from 27 to 152 μm for the raw sample to 11 to 121 μm for the pretreated samples. It was observed that the intensification of the HTP temperature resulted in continuously reduction in the particle size of the sludge ($P<0.05$). The highest decrease in particle size was observed for samples pretreated at higher severity indexes of 4, 4.5 and 5 counting approximately two times less than the raw sludge. For the pretreatment conditions of "240°C-08min" resulted in the lowest particle sizes. The d_{10}, d_{50}, and d_{90} of the pretreated sample were 12, 39, and 75 μm, respectively, which counted for 44%, 51%, and 49 % less than that of the raw sample. These results were in agreement with Elbeshbishy et al., (2010) findings who observed a reduction in the mean particle size diameter (d_{50}) from 59 μm for raw sample of hog manor to the 21 μm after ultrasonic pretreatment at specific energy of 30,000 kJ/kg.TS. In this study, the d_{50} decreased from 76 μm to the minimum of 39 μm at HTP conditions of "240°C-08min" which counts for approximately 50% reduction in mean particle size.

Also, From Figure 14 (b) it can be observed that after pretreatment the particle size of all samples dropped compared to the raw. A shift in particle size from the biggest particle size to the lowest for the sample hydrothermally pretreated at HTP condition of "240 °C-08 min" is spotted. The volume of the samples containing lower particle size, also increased by application of hydrothermal pretreatment. Comparing hydrothermally pretreated samples with respect to the hydrothermal pretreatment temperature, the shift from larger particle size to the lower was spotted.



(a)



(b)

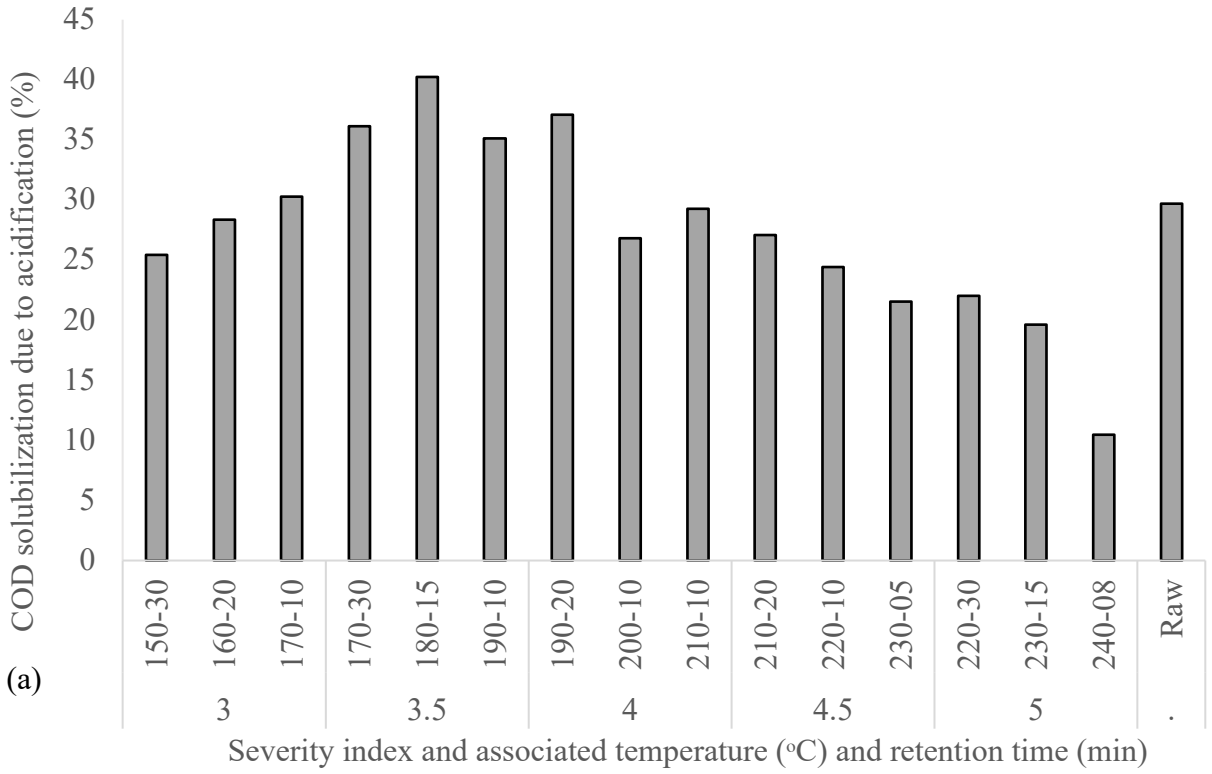
Figure 14 (a) The change in the value of D10 , D50* and D90* after hydrothermal pretreatment (Particle Size Distribution); (b) The change in the particle size of the raw and pretreated samples; * The D10, D50 and D90 (µm) is the diameter at which 10%, 50% and 90% of the sample particles are with a diameter less than this value*

13 Effect of hydrothermal pre-treatment on fermentation of TWAS

13.1 Acidification of organics

During the acidification process, the insoluble organic substances are converted to the soluble matters resulting in an increase in SCOD. The percentage of COD solubilization due to acidification of all pretreated and raw samples are illustrated in Figure 15 (a). The percentage of COD solubilization during the acidification process was calculated based on the mass increase in SCOD divided by the mass of particulate COD added in the substrate.

The COD solubilization percentage for hydrothermally pretreated samples fluctuated from 10% to 40%. Some of the hydrothermally pretreated samples had higher solubilization percentage than raw sample, while, others had lower and some were equal to the raw. Hydrothermal pretreatment temperatures of 170 °C and 190 °C performed like a boundary presenting the same COD solubilization percentage as raw. Where, pre-treatment conditions having temperatures lower than 170 °C or higher than 190 °C showed lower solubilization compare the raw. The highest solubilization percentage of 40% was achieved for the sample pretreated at 180 °C for 15 minutes (SI of 3.5) where the raw sample demonstrated an acidification percentage of 30%, which resulted in 33% increase in solubilization compare to the raw. The highest solubilization, during the acidification process, of 37% was achieved at SI of 3.5 compared to 30% for the raw sample, which resulted in a 23% increase in the solubilization due to the HTP. For the SI of 4, there was no change in the solubilization compared to raw sample. Interestingly, the solubilization, during the acidification process, for the pretreated samples at SIs of 3, 4.5, and 5 was lower than the raw sample. The lowest solubilization of 17% was observed for severity index of 5 which was about 43% lower than the raw sample. The decrease in solubilization after the HTP was reported in the literature and the reason might be due to the mallard reaction occurring due to the degradation of carbohydrates at elevated temperature and formation of toxic materials (inhibitory to COD solubilization) called melanoid (Li et al. 2014).



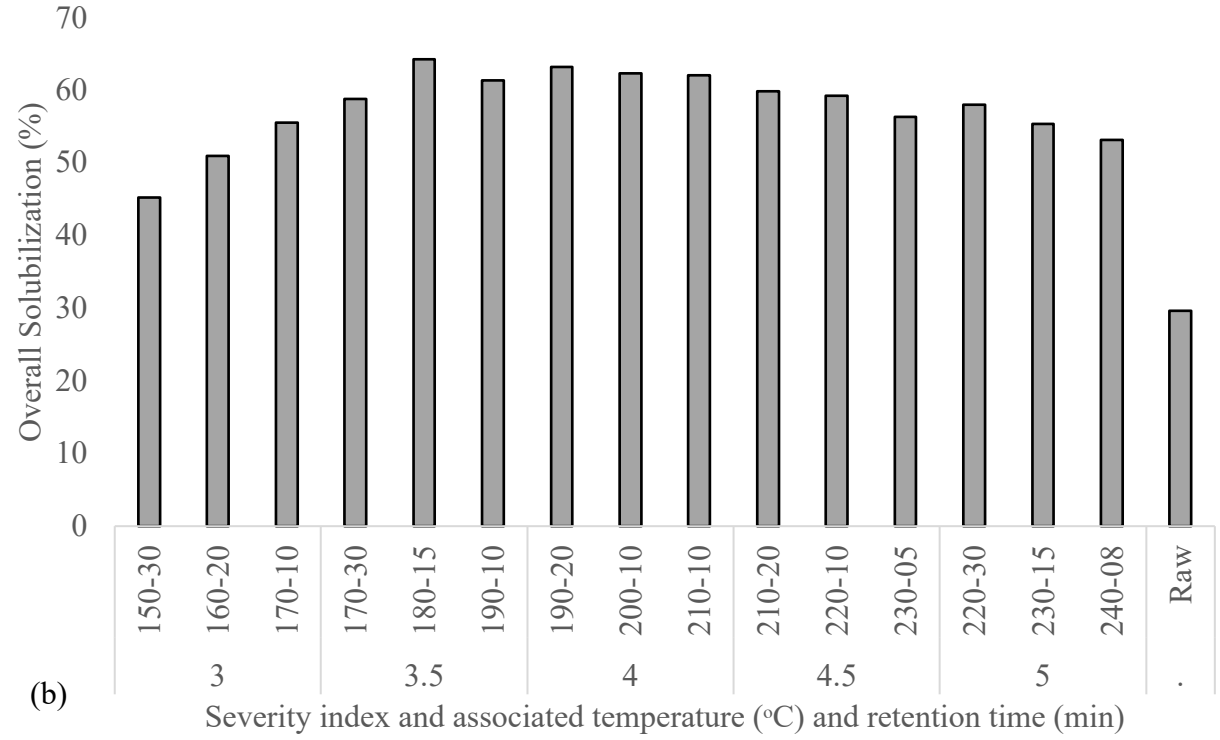


Figure 15 the effect of HTP on soluble organics after acidification (a) COD solubilization after acidification; (b) Sequential COD solubilization due to hydrothermal pretreatment and acidification test

This findings is in accordance with (Morgan-Sagastume et al. 2011) observation about acidogenic fermentation of sewage sludge pretreated under only one condition 160 °C and 6 bar. In the mentioned study both SCOD and TCOD of the influent and effluent was the same indicating that HTP did not promoted the solubilization under this HTP temperature.

In other hand Zhang et al. (2019) reported that hydrothermally pretreated sludge at 155 – 175 °C for 30 minutes after fermentation demonstrated higher SCOD concentrations of 19.2 g/L which was two times more than the SCOD concentration of effluent from raw sludge which agrees this study's results.

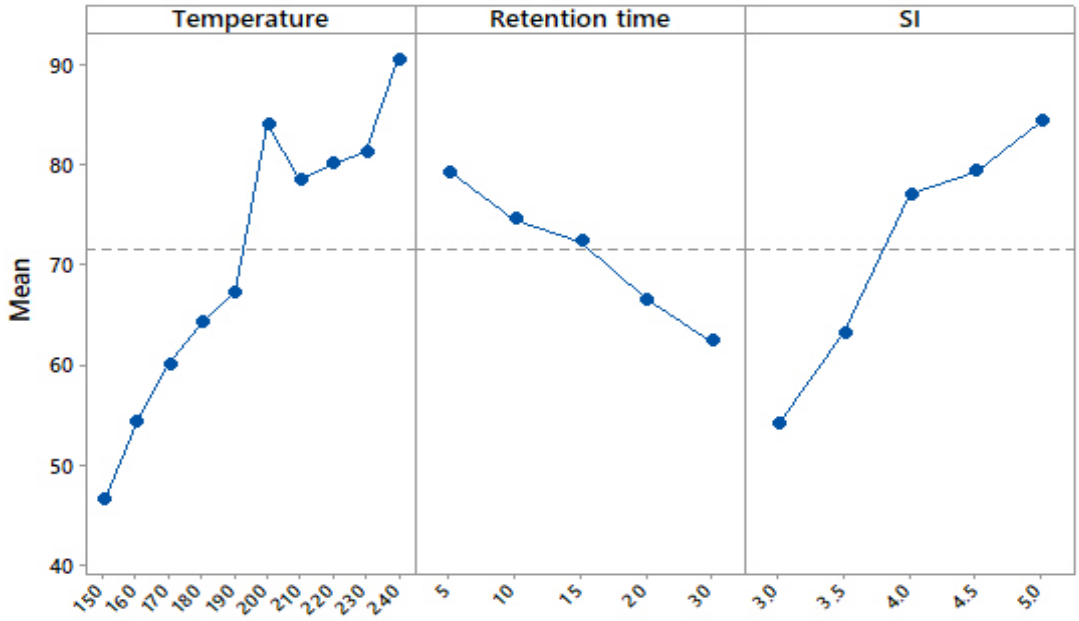


Figure 16 Main effect plot for overall COD solubilization

Furthermore, analyzing the data for each severity index revealed that: (a) at lower SI (3), the best scenario is combining higher temperature with lower retention time, (b) at moderate SI (3.5 and 4), the best scenario is combining moderate temperature with moderate retention time, and (c) at high SI (4.5 and 5), the best scenario is combining lower temperature with long retention time.

Although HTP did not promoted the COD solubilization of all hydrothermally pretreated samples but its impact on the sequential COD solubilization due to HTP and due to acidification (Overall solubilization) was significant see Figure 15 (b). According to the ANOVA test Table 6, the effect of HTP temperature is highly significant ($p<0.05$) and there is a strong positive correlation between hydrothermal pre-treatment temperature and overall solubilization whereas retention time and

overall COD solubilization is negatively correlated. Figure 16 indicates the effect of each parameter of temperature, SI and RT on the overall solubilization. According to this graph the HTP temperature and SI promoted the overall COD solubilization whereas RT adversely affected the overall solubilization.

Although the optimum HTP condition for the solubilization after pretreatment was HTP condition of "200°C-10 min" but this condition did not demonstrate superior results after acidification. Consequently, the highest overall COD solubilization of 64% was detected for the sample pretreated at HTP condition of "180°C-10 min", the HTP condition that had the highest result of solubilization after acidification. This percentage was approximately 2 times higher than the overall solubilization of the raw sample.

Table 6 ANOVA for overall COD solubilization due to HTP and dark fermentation

	df	SS	MS	F	Significance F
Regression	1	2034	2034	126	4.6E-08
Residual	13	209	16		
Total	14	2244			

On the other hand, HTP demonstrated a significant impact on carbohydrates and protein dissolution. The concentrations of soluble carbohydrates Figure 17 (a) and protein of all hydrothermally pretreated samples were higher than that of raw sample. Meanwhile, HTP temperature showed a negative correlation with soluble carbohydrates and protein concentrations revealing that HTP temperatures higher than 200 °C (considering COD solubilization) adversely affects the process. Ben-yi and Liu (2006) also observed the decline of soluble proteins and carbohydrates after acidification when applied the HTP to the TWAS with temperatures range of 50 °C to 121 °C.

Despite the production of VFAs, the pH in all the tests did not drop, they increased slightly from initial adjusted pH of 5.7 to 6.0 Figure 17 (b). This raise in PH might be due to the decomposition of proteins resulting in production of ammonia which could neutralize the VFAs and stabilize the pH of sludge. Xiao Ben-yi, (2006) also observed an elevation of pH after fermentation of hydrothermally pretreated sludge.

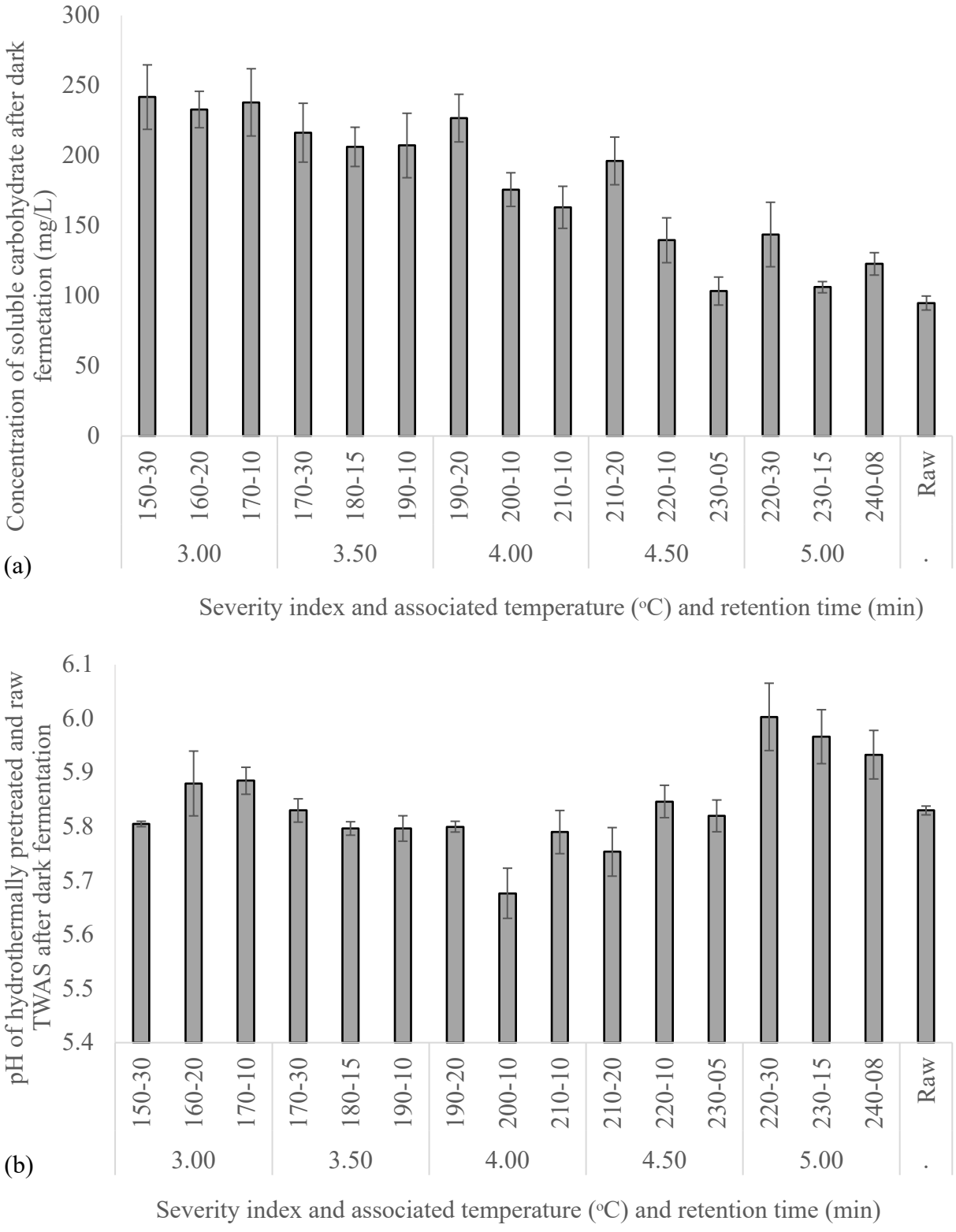


Figure 17 (a) Concentration of soluble carbohydrates after acidification; (b) pH of hydrothermally pretreated and raw TWAS after dark fermentation

13.2 VFA production

The total VFAs (TVFAs) produced due to the acidification of hydrothermally pretreated and raw samples are exhibited in Figure 19 (a). The TVFAs concentrations of all hydrothermally pretreated samples were higher than that of raw sample except the one that pretreated at "240°C-08min" indicating that the HTP enhanced the production of TVFAs. The TVFAs concentrations of raw sample was 1.8 g/L and the highest TVFAs produced was 5.4 g/L at HTP conditions of "190°C-10min" which is 3 times more than the TVFA produced from the raw sample. On the other hand, there was no difference in TVFAs production between the raw sample and the sample that pretreated at "240°C-08min". Similar observations were reported in the literature (Morgan-Sagastume et al. 2011; Xiao Ben-yi 2006). Two types of WAS with different solid content percentage pretreated in full scale hydrothermal pretreatment plant under 160 °C and 6 bar was fermented by (Morgan-Sagastume et al. 2011). Morgan's results revealed that the VFAs yield of thermally pretreated WAS increased by 2-5 times compared to the raw. Xue et al. (2015) Used different combinations of hydrothermal pretreatment temperatures from 50 to 121 °C and retention time of 30 to 60 minutes on TWAS to evaluate its impact on dark fermentation. It was found that HTP promoted the VFA production. In mentioned study the highest amount of VFA was produced (2.2 g/L) from sample pretreated at HTP condition of "121°C-60min".

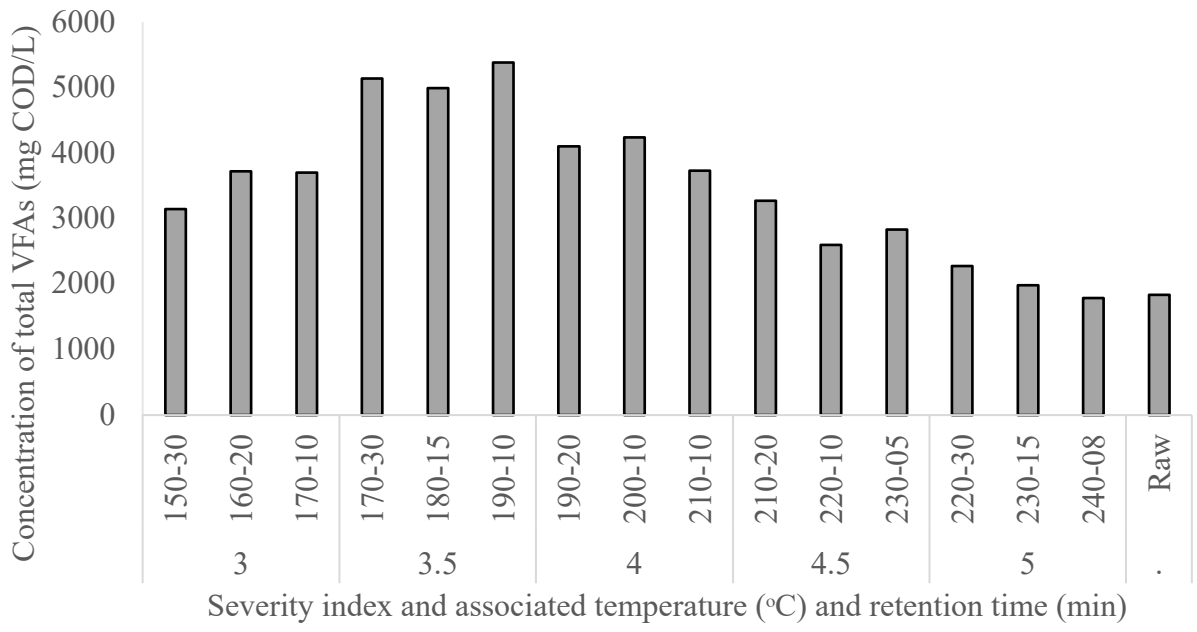


Figure 18 Total VFA production after acidification

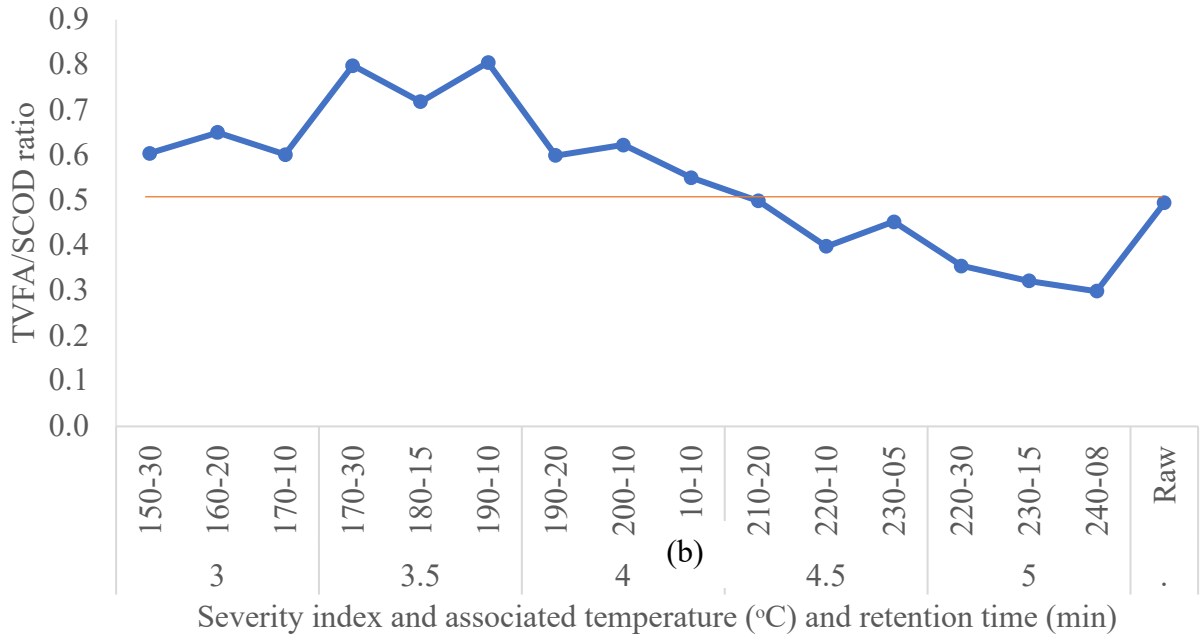


Figure 19 Total VFAs to SCOD ratio

The TVFAs to SCOD ratio after acidification are presented in Figure 19 (b). The VFAs to SCOD ratio of 49% was observed for the raw sample. The VFA/SCOD ratio of the hydrothermally pretreated samples varied compared to the raw being higher, lower or equal. The HTP condition of "210 °C -20min" showed similar VFA/SCOD percentage. HTP conditions using temperatures higher than 210 °C had lower percentage of VFA over SCOD, while HTP temperature lower than 210 °C demonstrated higher values. Therefore, it is revealed that lower SIs (3, 3.5, 4) was more efficient in terms of converting the SCODs to VFA compared to the higher SIs (4.5, 5). The highest VFAs to SCOD ratio of 81% was observed for the pretreated sample at "190 °C-10min", while the lowest TVFAs to SCOD ratio of 30% was observed for the sample that pretreated at "240°C-08min". Thus, the high amount of SCOD production from the samples that pretreated at temperatures higher than the 190 °C did not necessarily resulted in higher VFAs production. The reason behind this phenomenon might be the production of some toxic materials such as melanoidins limiting the acid forming bacteria generation and sludge biodegradation (WYin et al. 2014). These observations was in align with (Morgan-Sagastume et al. 2011) who compared the VFA/SCOD of raw sludge and hydrothermally pretreated sludge under 160 °C after undergoing

fermentation. Morgan observed that VFA/SCOD ratio increased from 18% for the raw sample to the 52% for the hydrothermally pretreated sample.

The COD solubilization demonstrated a positive correlation to the TVFAs from hydrothermal pretreatment temperature of 150-190 °C. However, after HTP temperature of 190 °C, the scenario was the opposite. Besides, the COD solubilization after fermentation test followed the same trend as the VFA production. The highest VFA production was observed for the samples which demonstrated a range of 35 to 40% COD solubilization after pretreatment and after acidification. Figure 20 The surface plot of the TVFAs vs COD solubilization due to HTP and acidification illustrates the afore mentioned correlation.

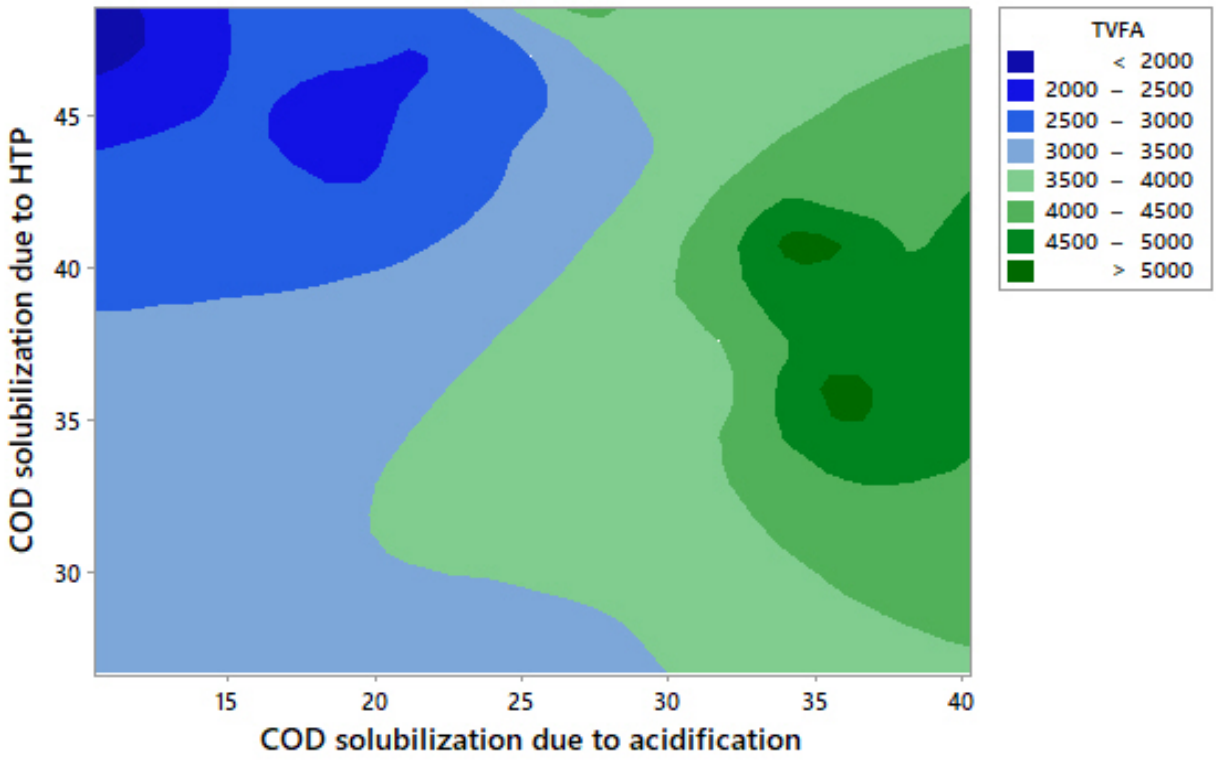


Figure 20 The contour plot of total VFAs vs COD solubilisation after hydrothermal pretreatment and acidification

13.2.1 Effect of HTP temperature and retention time

The TVFAs concentration increased by increasing the hydrothermal pretreatment temperature reaching to its peak at “190°C-10min” and then began to drop by elevating the HTP temperature. The difference between these results was due to the release of organic matters and screening of diverse micro-organisms during each pre-treatment (Ben-yi and Liu 2006). These results were in

agreement with (Kim et al. 2005; Wilson & Novak 2009; Xiao Ben-yi 2006; Xue et al. 2015) findings regarding the increase of VFAs by elevated HTP temperature after acidification.

The VFAs yield considering the effect of hydrothermal retention time was statistically significant too ($p<0.05$). However, the RT performed contrarily studying each severity index. Comparing three HTP condition in each SI, at lower severity indexes (3 and 3.5), shorter RT demonstrated higher VFAs yield. Ben-yi and Liu (2006) after application of hydrothermal pretreatment of 50 to 121 °C for 30 and 60 minutes also reported that shorter HTP retention time (30 in that case) resulted in higher VFAs production. Whereas, at higher severity indexes (4, 4.5 and 5), higher VFAs was observed at longer RT. Donoso-Bravo et al. (2011) evaluated the effect of HTP retention time on the macromolecular composition and biodegradability of sewage sludge. Six HTP conditions under fixed HTP temperature of 170 °C for 5, 10, 15, 20, 25 and 30 minutes was examined. Bravo reported an increase in VFAs production from 100 mg/L to 350 mg/L when retention time increased from 5 minutes to 30 minutes with constant temperature of 170 °C. The variation in performance of RT might be different because temperature was the dominant parameter during the hydrothermal pre-treatment.

13.2.2 Product spectrum

The variation of all VFAs for pretreated and raw samples after acidification are shown in Figure 21. Six VFAs including acetic acid, propionic acid, iso-butyric acid, butyric acid, Iso-valeric acid and valeric acid were detected in all samples. Corresponding to the TVFAs graph trend, all types of VFAs production increased by elevated temperature reaching to the HTP condition of "190°C-10min" and began to decline after that which conveys the significance of the HTP temperature on VFAs type.

The most abundant type of VFAs was acetic acid followed by propionic acid, iso-butyric acid, and iso-valeric acid for all hydrothermally pretreated samples. The amount of acetic acid in reactor containing raw sample was 522 mg COD/L. While, the amount of acetic acid for hydrothermally pretreated sludge ranged from 389 to 2225 mg COD/L. Except HTP condition of "150°C-30min" (at SI of 3) all hydrothermally pretreated sample had higher concentrations of acetic acid compared to the raw. The proportion of acetic acid to the TVFA was between 12 to 41% for all hydrothermally pretreated samples. Acetic acid contributed as 28% of TVFA produced from raw sample that again is a lower fraction compare to all pretreated samples except "150°C-30min".

The ratio of acetic acid to propionic acid ranged from 0.8 to 5.2 for all pretreated samples. Similarly, the proportion of acetic acid to the iso-butyric, acid butyric acid, iso-valeric acid and valeric acid were high-pitched. This indicates the higher concentration of acetic acid over other VFAs. WAS hydrothermally pretreated during studies under 160 °C HTP temperature also validated the abundance of Acetic acid in the pretreated samples compared to the raw and other acids (Morgan-Sagastume et al., 2011). The proportion of acetic acid to the TVFA in Morgan's study was from 35 to 40% which is in the same range as the current study's result.

The next most abundant VFA after acetic acid, propionic acid had the lowest concentrations of 288 mg COD/L at HTP condition of "170°C-10min" (at SI of 3) and similar to the acetic acid the lowest concentration was in lower pretreatment temperatures. The highest amount of propionic acid 1275 mg COD/L was observed at "190 °C-10min". Raw sample having 892 mg COD/L of propionic acid had higher amount of propionic acid compared to the SIs of 3 and 5, and lower amount compared to the SI of 3.5,4 and 4.5. In contrary to the acetic acid, propionic acid was the most abundance VFA produced from raw TWAS having 48% propionic/TVFA share. All thermally pretreated samples demonstrated lower ratios compared to the raw by ranging from 8 to 35%.

The amount of iso-butyric and butyric acids ranged from 330 to 1089 mg COD/L and 201 to 1312 mg COD/L, respectively. The amount of both mentioned acids was 298 and 187 mg COD/L, respectively, which were lower than the pretreated samples. Consequently, the proportion of iso-butyric/TVFA (11%) and butyric/TVFA (8%) for raw sample were lower than that of pretreated. The iso-butyric/TVFA ratio for hydrothermally pretreated samples consist of 11 to 33% where the highest percentage was observed to belong to the HTP condition of "150°C-30min" keeping in mind that this condition had the lowest concentrations of acetic acid. The butyric acid ratio for pretreated sludge varied from 9 to 26% over the total VFAs. Again, the lowest butyric acid proportion was detected in HTP condition of "150°C-30min". Both iso-butyric and butyric acid produced from TWAS being hydrothermally pretreated followed the same trend as the TVFA.

The concentrations of both iso-valeric and valeric acids were higher than that of raw TWAS (50 and 22 mg COD/L, respectively). The amount of these acids was negatively affected by the HTP temperature. The HTP condition of "150°C-30min" had the highest amount of 786 and 312 mg COD/L for iso-valeric and valeric acids, respectively. Generally, the SI of 3 (lowest SI) presented the highest yield for these acids, which can be translated as iso-valeric acid and valeric acid favours

lower HTP temperatures. Only 3 and 1% of the total VFAs was detected to be iso-valeric and valeric acid. Also, for thermally pretreated TWAS the ratio of these two acids were lower than others ranging from 1 to 25% for iso-valeric acid and 2 to 10% for valeric acid.

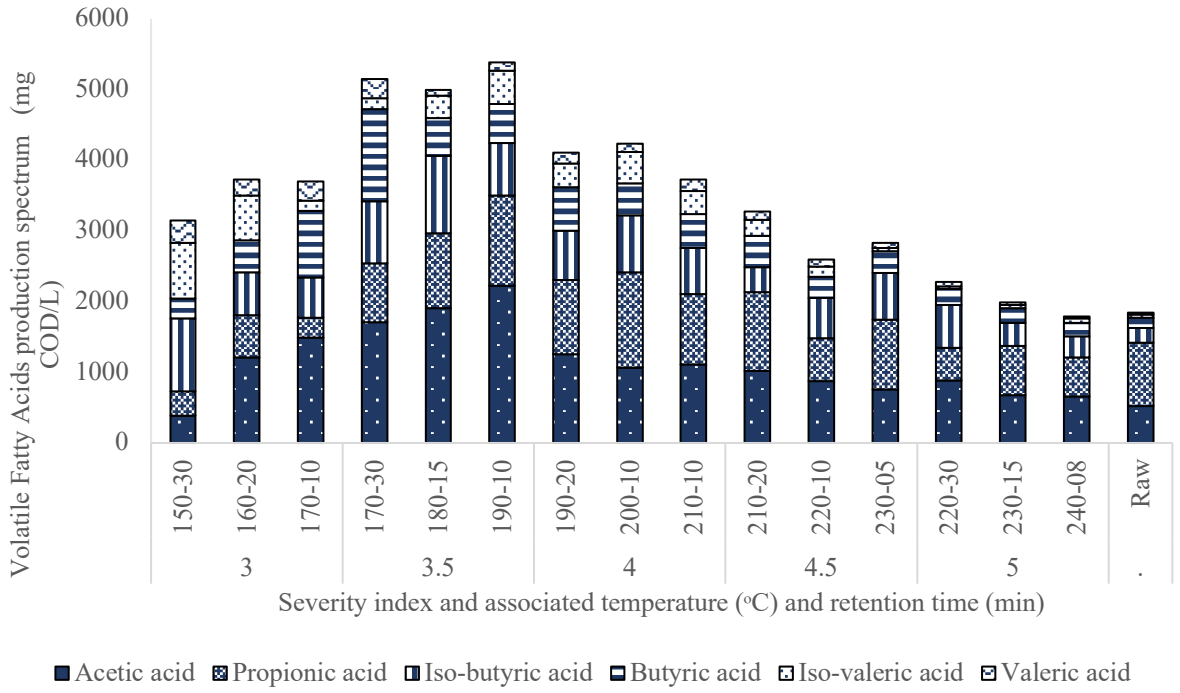


Figure 21 VFAs production spectrum after acidification test

14 Results and discussions (SSO)

15 Effect of hydrothermal pre-treatment on source separated organics

15.1 COD Solubilization of SSO

SSO samples were hydrothermally pretreated at fifteen different scenarios with temperature range of 150 ˚C to 240 ˚C and retention time of 5 to 30 minutes. After hydrothermal pre-treatment, soluble COD of all pretreated substrates were higher than that of non-pretreated which implies that HTP promoted the solubilization of solid organics in the SSO, see Figure 22 (a). As hydrothermal temperature was elevated from 150 ˚C to 220 ˚C, the COD solubilization percentage was enhanced from 14% to 34% and the ANOVA analysis ($p<0.05$), see Table 7, confirmed that the effect of temperature on organic dissolution was significant. In spite of this, when the temperature increased to 240 ˚C, the solubilization percentage dropped to 27%, see Figure 22 (b). Likewise, Ding et al. (2017) reported that at higher temperatures the COD solubilization percentage begun to shrink. The reason behind this phenomenon is the formation of insoluble high-carbon hydrochar which is result of intensified carbonization of SSO by high temperature (Liu et al. 2013).

Table 7 ANOVA for COD solubilization after hydrothermal pretreatment

	df	SS	MS	F	Significance F
Regression	1	509	509	53	5.9E-06
Residual	13	124	10		
Total	14	633			

Looking into each severity index to evaluate the effect of retention time on dissolution of the SSO, it was found that at lower severity indexes (3.00 to 4.5), higher temperature with lower retention time demonstrate higher COD solubilization Whereas at higher severity indexes (5.00), lower temperature with higher retention time had superior results. Hence, longer retention time did not have significant effect on substrates cell disintegration as its p-value was higher than 0.05 demonstrating moderate evidence on RT significance. Ultimately, Severity index of 4.5 was found to be the optimal HTP condition in terms of COD dissolution. To illustrate the relationship between COD solubilization percentage and three main variables, the main effect plot of COD

solubilization percentage vs. HTP severity index, temperature, and retention time is showed in Figure 23 (a). Besides, the interactions between the four main variables (temperature, pressure, time, and SI) for the COD solubilization after hydrothermal pre-treatments are represented in Figure 23 (b).

In this experiment, the highest percentage of solubilization occurred at pretreatment conditions of "220 ˚C-10 min" and "230 ˚C-05 min" with maximum solubilization percentage of 34%. Menon et al. (2016) applied HTP on food waste at different temperatures of 80, 105 and 130 ˚C, they found that the highest COD solubilization percentage of 43% was achieved at 130 ˚C for 30 minutes which contradict with current study's result. In another study, Ding et al. (2017) reported the highest peak of COD solubilization of 70% was achieved at 180 ˚C temperature for 20 minutes when they pretreated food waste with temperature range of 100 to 200 ˚C. This dissimilarity for the optimal HTP condition might be due to the nature of substrates, as SSO contains more lignocellulosic material which need higher temperature to be degraded (Ravindran and Jaiswal 2016). After HTP of SSO, pH of all pretreated substrates decreased compare to the raw SSO, demonstrating the generation of organic acids at high temperatures (WYin et al. 2014). Consequently, we can conclude that the HTP promotes the COD solubilization of SSO and the optimal condition for the highest COD solubilization would be at SI of 4.5.

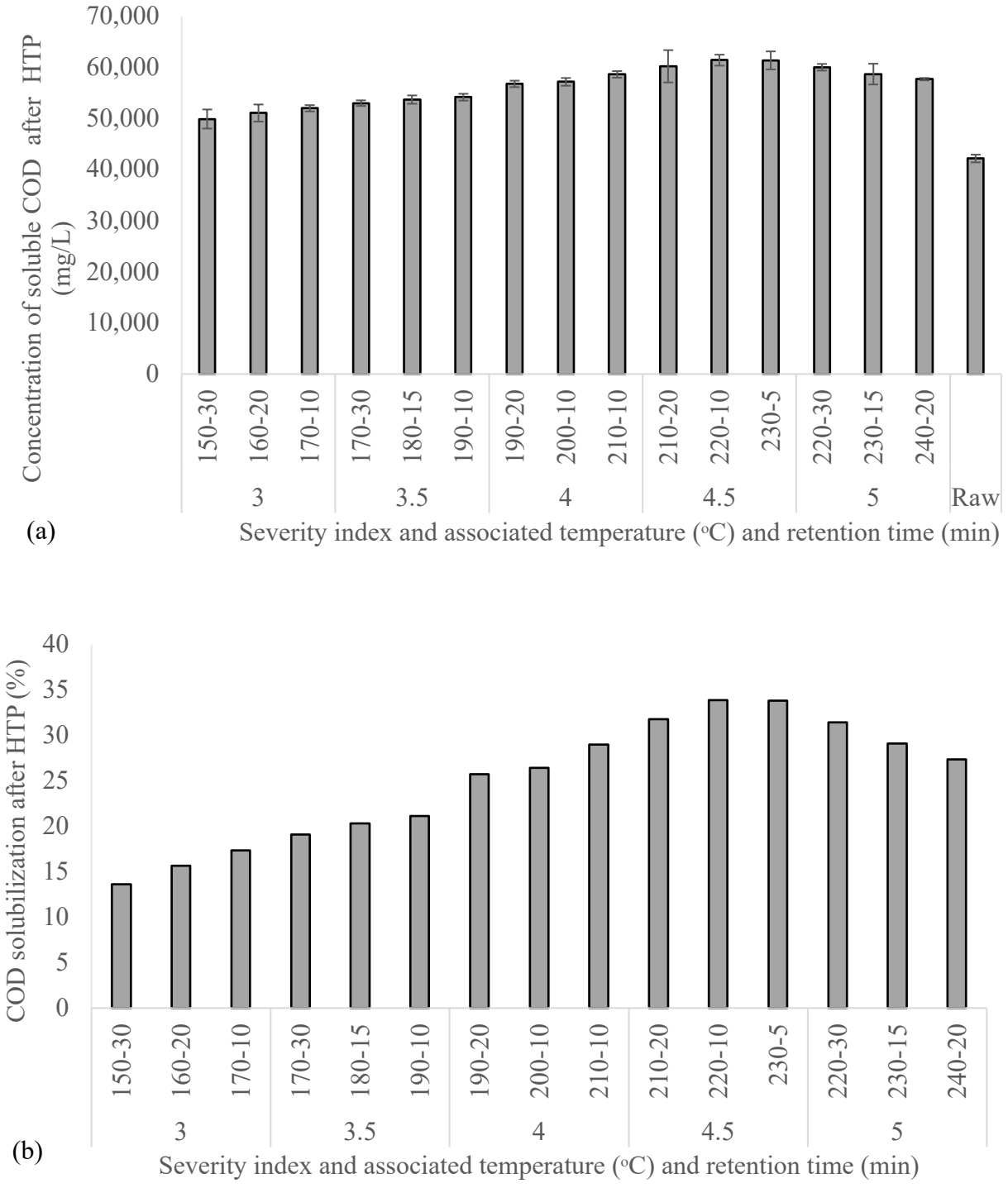


Figure 22 Effect of HTP on soluble content of SSO (a) Concentration of Soluble COD after HTP, (b) COD solubilization after HTP

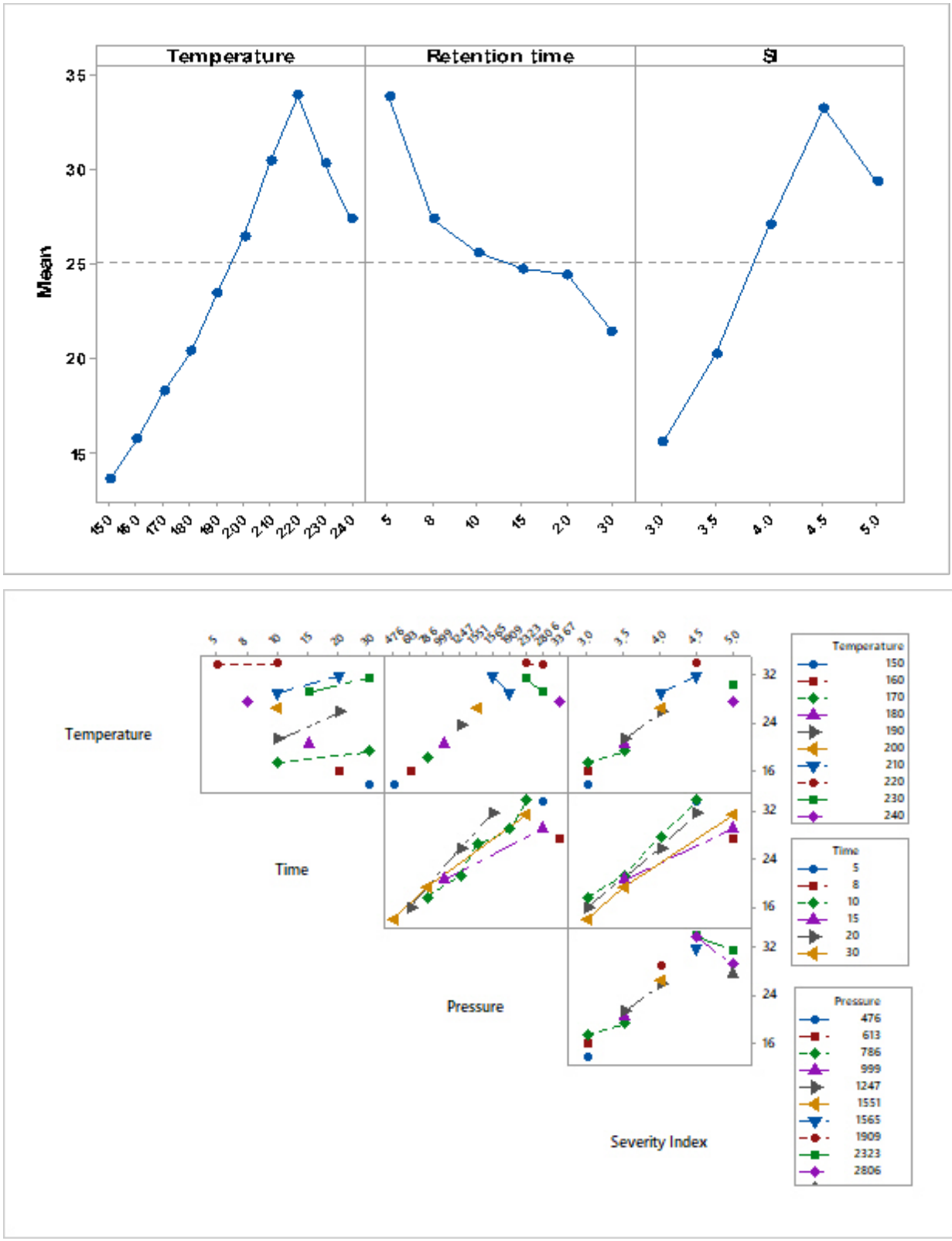


Figure 23 (a) Interaction plots of temperature, time, pressure and severity index for the COD solubilization after HTP; (b) Main effect plot for solubilization due to HTP

15.1.1 Carbohydrates and Proteins

Increase in temperature of HTP demonstrated considerable effect on both total and soluble carbohydrates of hydrothermally pretreated SSO. With increasing temperature, the soluble carbohydrates increased to highest concentration of 1828 mg/L at temperature of 200 ˚C, after which, it started to drop by increase in the temperature, see Figure 24 (a). Whereas, the concentration of total carbohydrates was negatively correlated with raising temperature.

Enhancement of soluble carbohydrates at the lower temperature of 150 ˚C to 200 ˚C was because when lignocellulosic martials were subject to HTP, the large-molecular-weight carbohydrate polymers (e.g., starch, cellulose, and hemicellulose) were hydrolyzed into small-molecular- weight oligosaccharides and monosaccharides (e.g., glucose and xylose) directing to the release of soluble sugar from solid carbohydrates in SSO (Li et al. 2016). However, some soluble sugars, such as hemicellulose derivatives, were further degraded into short-chain VFAs, such as acetic acid hence minimizing total carbohydrates (Monlau et al. 2014). Though, the reduction of the soluble carbohydrates in intensified HTP temperature is due to formation of amadori like compound which are by-products of melanoidins (Ariunbaatar et al. 2014). Melanoidins are formed by reaction between soluble carbohydrates with themselves or proteins (Li et al. 2014). Ding et al. (2017) employing 100-200 ˚C HTP temperature on WAS also found that increase in HTP temperature resulted in enhancement of soluble carbohydrates solubilization from 51% to 74% when the temperature reached 180 ˚C and then dropped to the 54% when the temperature increased to 200 ˚C. On the other hand, as the HTP temperature was increased from 150 ˚C to 170 ˚C, the concentration of soluble proteins was enhanced from 330 to 420 mg/L (the highest soluble protein content) following by a significant drop up to 131 mg/L at “240 ˚C-8 min”, see Figure 24 (b).

The protein and ammonia results indicate that the solubilization of protein was dramatically promoted after HTP whereas the degradation of protein was not remarkable. This observation is in accordance with two similar studies (Ding et al. 2017; WYin et al. 2014). The highest temperature used for HTP of food waste in these Research was 200 ˚C. It was found that increase in hydrothermal pretreatment temperature increases the protein solubilization.

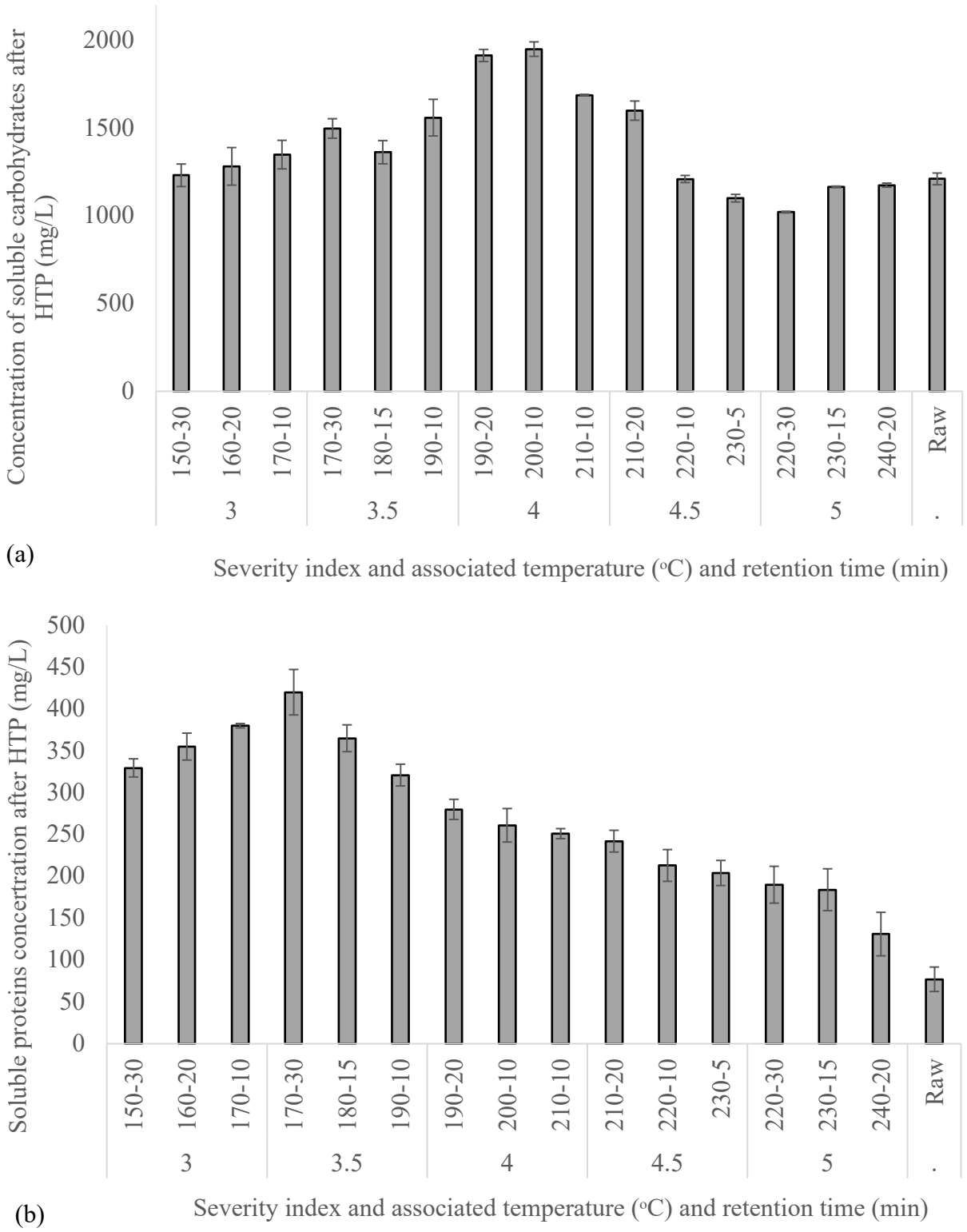


Figure 24 Effect of HTP on soluble content of carbohydrates and proteins; (a) Concentration of soluble carbohydrates of SSO after HTP; (b) Concentration of soluble proteins of SSO after HTP

15.2 Solid reduction

The concentration of TSS and VSS of all hydrothermally pretreated and raw substrates are shown in Figure 25 (a). According to this graph, the solid concentration of all hydrothermally pretreated substrates decreased compared to the raw SSO. It was determined that the percentage of solid reduction had a straight relation with increasing temperature until SI of 4.5 and then it started to decrease. This indicated that at higher temperatures (SI of 4.5), VSS mainly hemicellulose and cellulose, was decomposed to lower molecular organics, such as monosaccharides, furans, and organic acids (Takata et al. 2013). The highest percentage reduction of 51% and 55% for TSS and VSS, respectively, were observed at HTP condition of "220 ˚C-10 min" or SI of 4.5, see Figure 25 (b).

For TSS and VSS reduction due to HTP, comparing different combination of temperature and retention time inside each severity index, it was observed that from SI of 3.00 to 4.5, higher temperature with lower retention time showed higher percentage reduction of both TSS and VSS. Whereas, at high SI of 5.00, lower temperature with longer retention time had higher solid reduction efficiency. This is in accordance with what Ding et al. (2017) observed about retention time when they pretreated food waste at different retention times of 5 to 30 minutes with constant temperature of 140 ˚C. Based on the above mentioned results, it can be concluded that longer retention times are not necessarily enhance the solid reduction considerably for SSO and therefore it is moderate evidence for RT to be a significant factor (p-value <0.05).

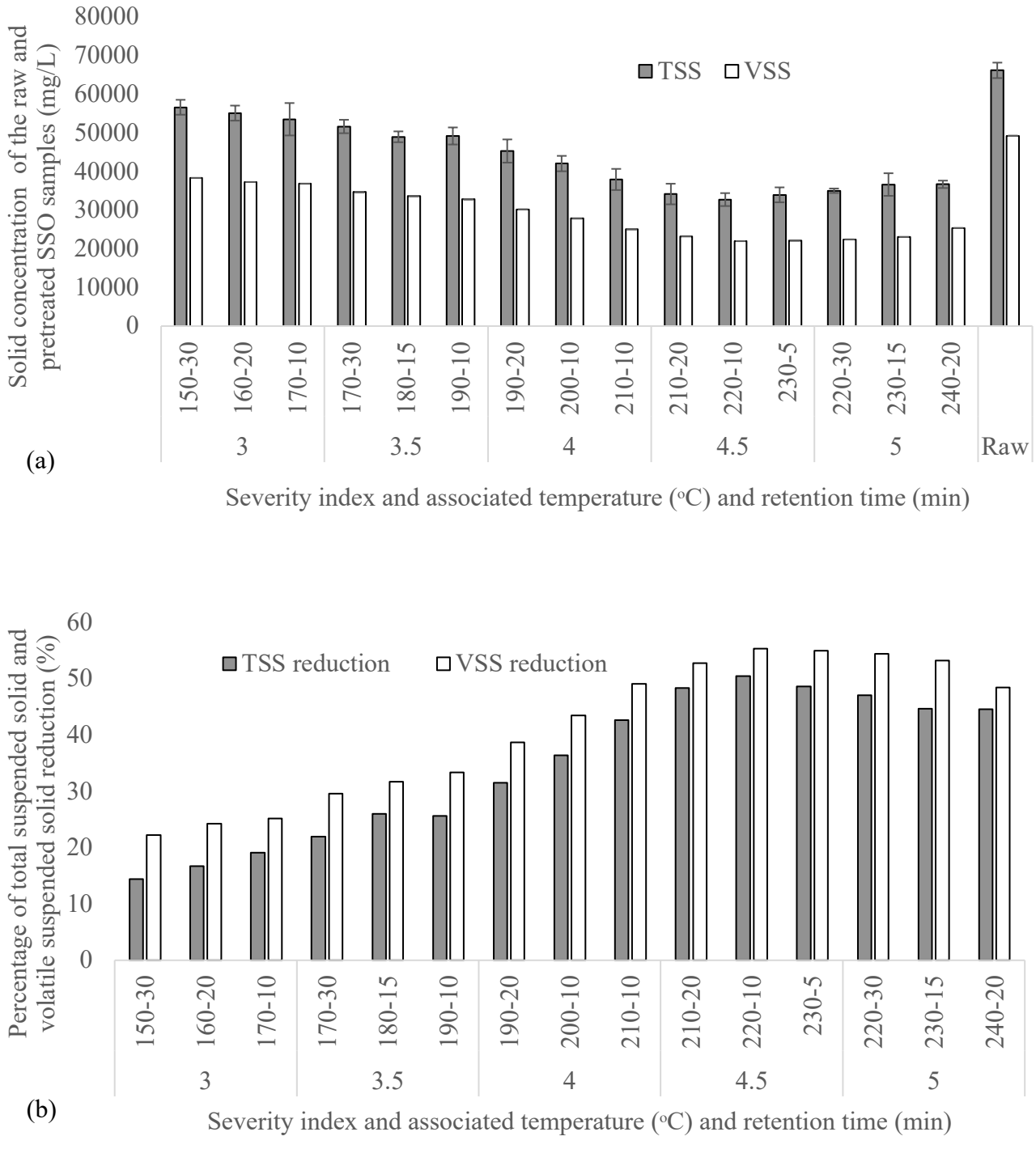


Figure 25 Effect. of HTP on suspended solids; (a) Solid concentration before and after HTP, (b) solid reduction percentage of the SSO samples after and before HTP

15.3 Viscosity

Viscosity, providing clue about the mode of agitation and energy consumption of the bioreactors, becomes a significant and useful parameter for designing and monitoring biological processes (Liu et al. 2012). Visually, after HTP, the SSO samples were transformed to a more fluid slurry mass. The viscosities of SSO samples after and before hydrothermal pre-treatment are shown in Figure 26. Results revealed that after hydrothermal pre-treatment, the viscosity of all samples decreased significantly. The lowest viscosity of 45-50 centi-point was observed at SI of 5 which is approximately 76% lower than the viscosity of raw SSO. Viscosity and HTP temperature demonstrated very strong negative correlation where increase in temperature resulted in decrease of viscosity. These observations were in accordance with the Xue et al. (2015) results. Xue et al. (2015) evaluated the effect of low and high temperature HTP ranging from 60 to 180 ˚C by combining with wide range of retention time (15 to 180 minutes). The viscosity of the sludge dropped from 4480 cP to the lowest value of 1.4 cP at 180 ˚C.

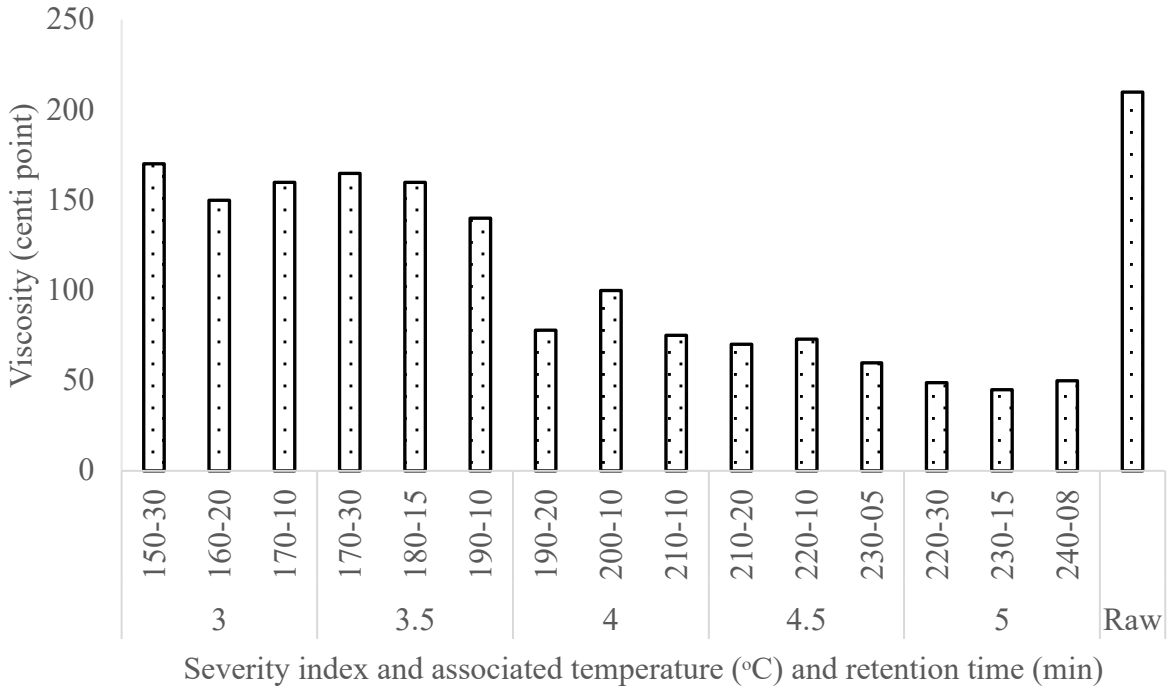


Figure 26 Viscosity of raw and hydrothermally pretreated samples

16 Effect of hydrothermal pre-treatment on acidification of source separated organics

16.1 Acidification of organics

Hydrothermally pretreated and raw SSO were used for running the dark fermentation (acidification) experiment. The percentage of COD solubilization after acidification is shown in Figure 27 (a). Increase in hydrothermal temperature from 150 to 190 ˚C resulted in increase in COD solubilization and maintained a positive correlation up to the mentioned temperature. Hence, point of transition for COD solubilization where it begun to drop was HTP condition of "190 ˚C-10min". The COD solubilization continue to decrease up to HTP temperature of 240 ˚C (the highest employed HTP temperature). The optimal HTP condition in terms of COD solubilization was "190 ˚C-10 min" by 54% COD solubilization which was 31% higher than that of raw.

Comparing the COD solubilization of each pretreated sample to the raw SSO, it was observed that some of the samples had superior results than raw, some was equal, and some was lower than the raw sample. HTP condition of "230 ˚C-15min" demonstrated lower COD solubilization percentage of 32% compared to that of raw (37%). The HTP condition of "240 ˚C-8min" had similar COD solubilization as the raw. All the rest of samples exhibited higher COD solubilization percentage. By evaluating the COD solubilization of SSO after acidification in each severity index, it was discovered that the percentages of solubilization for samples pretreated at severity indexes of 3, 3.5, 4 and 4.5 were higher than that of raw sample. Whereas, higher severity index of 5 had lower or equal solubilization percentages than raw. The average COD solubilization at severity indexes of 3, 3.5, 4 and 4.5 were 50, 51, 43, and 40%, respectively. These values were 8 to 26% higher compared to the acidification of the raw SSO. Whilst at severity index of 5, average solubilization of all HTP scenarios was 36% presenting slightly lower solubilization compared to the raw sample. WYin et al, (2014) also reported the increase of COD solubilization after HTP of food waste and its positive correlation with HTP temperature by employing HTP temperature of 100 to 200 ˚C and RT of 30 minutes for all samples. They found that HTP condition of "180 ˚C-30min" had the highest SCOD concentration of (127.50 ± 1.55 g/kg) after fermentation compared to the raw and other HTP conditions.

Considering three lower SIs of this study (3.00, 3.5, and 4.00), which indicated higher solubilization percentage), it was observed that substrates pretreated at higher temperature with

lower retention time demonstrate higher solubilization percentage than those with lower temperature and higher RT emphasizing that the HTP temperature was the dominant factor.

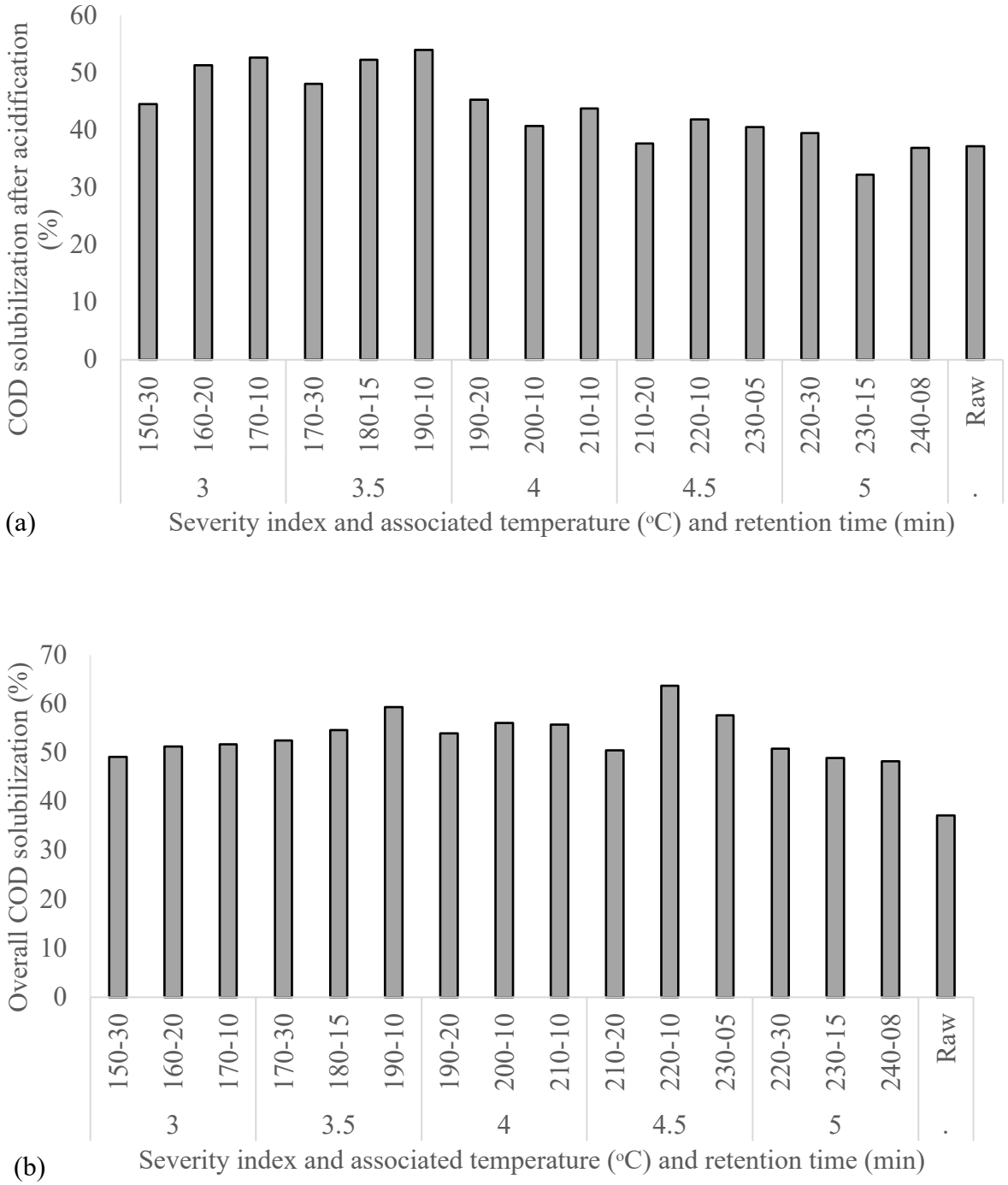


Figure 27. Effect of HTP on COD after acidification (a) COD solubilization after acidification, (b) Overall COD solubilization due to sequential HTP and acidification

As shown in Figure 27 (b), the overall COD solubilization due to sequential HTP and acidification also increased by increasing the HTP temperature from 150 ˚C to 220 ˚C and dropped sharply afterward. By evaluating the overall COD solubilization, it was revealed that despite the lower COD solubilization during the acidification step of the sample that pretreated with SI of 5 compared to the raw sample, the overall COD solubilization of all hydrothermally pretreated samples were higher than that of raw sample. This implies that in general the hydrothermal pretreatment at all temperatures promoted the overall COD solubilization. The highest sequential COD due to HTP and acidification was 64% which was almost two times higher than that of the raw sample.

As shown in Figure 28 (a), the soluble carbohydrates concentration after acidification declined by increasing HTP temperature. This phenomenon might be due to the formation of some toxic and none biodegradable products which might raise the stress of fermenting microorganisms (Matsakas et al. 2014). For instance, maillard reactions that occurs between proteins and carbohydrates in the raw materials at higher temperature (Li et al. 2014) and the by-products that have been reported to be antimicrobial agents (Hauser et al. 2014). The final soluble carbohydrates concentrations at lowest severity index (3.00), specifically at 170 ˚C were higher than other scenarios. These results were in agreement with WYin et al. (2014) who observed a carbohydrates solubilization inhibition at elevated temperature of 200 ˚C (the highest HTP temperature in their study).

After 72 hours of fermentation, concentrations of soluble protein and ammonia in reactors containing hydrothermally pretreated substrates were lower than reactors with raw SSO. Although, with increasing the temperature of HTP, the soluble proteins concentrations after HTP increased, however, the abundance dissolution of the proteins after HTP did not lead to the higher degradation, see Figure 28 (b). This results was in line with WYin et al. (2014) who found that the concentrations of soluble proteins were almost constant after 2 days fermentation and the degradation of proteins were limited.

Alike soluble protein, concentrations of ammonia for hydrothermally pretreated samples were lower than that for the raw SSO. Although the trend of this reduction was not corresponding to the raising HTP temperature. The amount of ammonia in all reactors was approximately equivalent, demonstrating the peak of 707 mg/L NH_3-N in reactor containing samples hydrothermally pretreated at HTP condition of “210 ˚C-20 min”. Overall, HTP effect was significant in terms of

organic cell integration and solubilization, however it was not very efficient in terms of protein degradation.

The stability of a digester medium can be described by an indicator such as pH since it is dependent on the buffering capacity of the digester itself (Li and Jin 2015). The initial pH of the digesters was adjusted to 5.00 and the final pH of all the reactors did not change significantly, the final pH values varied from 5:00 to 5.6.

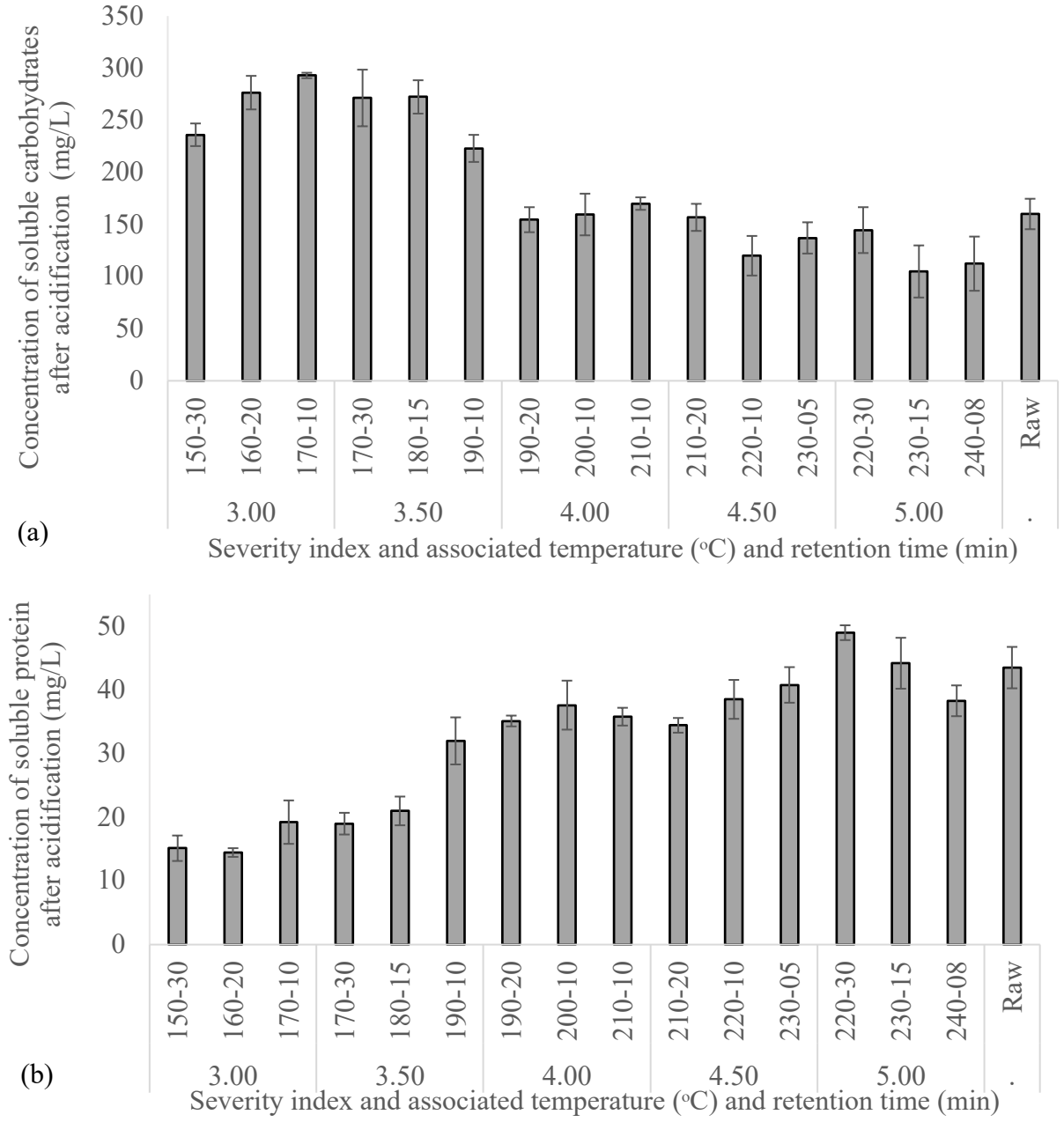


Figure 28. Soluble carbohydrates and proteins content after acidification; (a) concentration of soluble carbohydrates; (b) Concentration of soluble proteins

16.2 VFA production

The enhancement of VFAs production from MSW via application of hydrothermal pretreatment were investigated and confirmed by several studies (Ding et al. 2017; Elbeshbishy et al. 2011; Ozkan et al. 2011; WYin et al. 2014). Figure 29 (a) presents the VFAs yields per mass of VSS added after acidification of pretreated and raw SSO samples. As shown in the Figure, the VFAs yields from all pretreated samples were higher than that of raw SSO representing the positive impact of HTP on VFA production. The highest VFAs yield of 1,536 mg VFAs/g VSS added was achieved for the pretreated sample at HTP condition of "210 ˚C- 10 min" compared to 768 mg VFAs/g VSS added for the raw sample which is corresponding to about 50% enhancement.

Ding et al. (2017) reported that HTP enhanced the production of VFAs from kitchen waste by using pretreatment temperature ranging from 100 to 160 ˚C. The highest VFAs yield achieved in Ding's study was 1248 mg VFAs/g VSS_{added} which was 16% more than the VFAs yield (1051 mg VFAs/ VSS_{added}) from the raw sample. Whereas, WYin et al. (2014) applied HTP temperatures ranging from 140 to 200 ˚C under RT of 30 minutes on food waste and found that the highest VFAs concentration of 908 mg/g VSS_{added} was for food waste sample anaerobically fermented at HTP temperature of 160 ˚C for 30 minutes and 586 mg/g VSS_{added} for the raw sample. The noticeable contrast between the optimum HTP condition of different studies can be due to the difference in the nature of the substrates, as SSO contains more slowly biodegradable materials and less fat and organic substance (lower TCOD) compared to food waste.

Consequently, the impact of SI on VFAs production was statistically significant ($P<0.05$). Increase in SI resulted in increase in VFAs production up to SI of 4 and it started to decrease afterward.

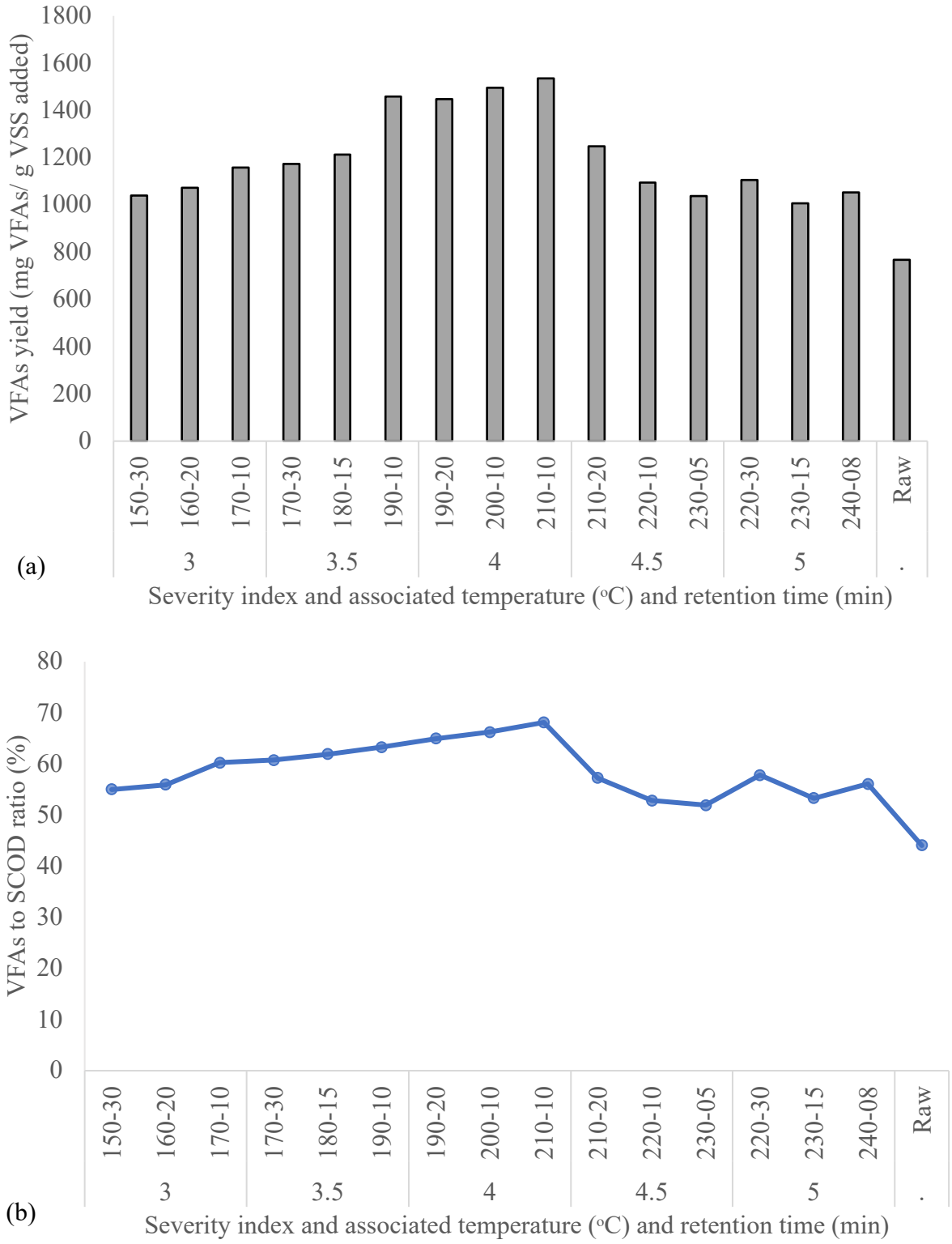


Figure 29. Volatile fatty acids yield after acidification process

The VFAs/SCOD ratio of the fermented SSOs are illustrated in Figure 30 (b). The VFAs/SCOD ratio of all hydrothermally pretreated samples ranged from 52 to 68% which were higher than that of raw SSO (44%). Hereby, it can be concluded that the hydrothermal pretreatment promoted the VFas protion of the SCOD for all pretreated samples compared to the raw. The highest VFAs/SCOD ratio of 68% was observed for the sample hydrothermally pretreated at HTP condition of "210 ˚C-10min". Whereas, for raw sample, the VFAs/SCOD ratio was 44%. WYin et al. (2014) reported that the highest VFAs to SCOD proportion of 32% for the food waste was for the sample hydrothermally pretreated at "160 ˚C-30 min". WYin et al. (2014) observations regarding the VFAs/SCOD ratio was somewhat off from current studies results since, his optimal condition was at lower HTP temperatures with a lower ratio. The reason for this contradiction could be the nature of substrates used.

The effect of solubilization after pretreatment and COD solubilization after fermentation on the VFAs production is illustrated by Figure 30 as a contour plot. From this graph, it can be observed that the higher the COD solubilization after HTP and fermentation, the higher the VFAs produced. Also, increasing the COD solubilization resulted in an increase in VFAs production making the COD solubilization has significant effect on VFA production ($p<0.05$).

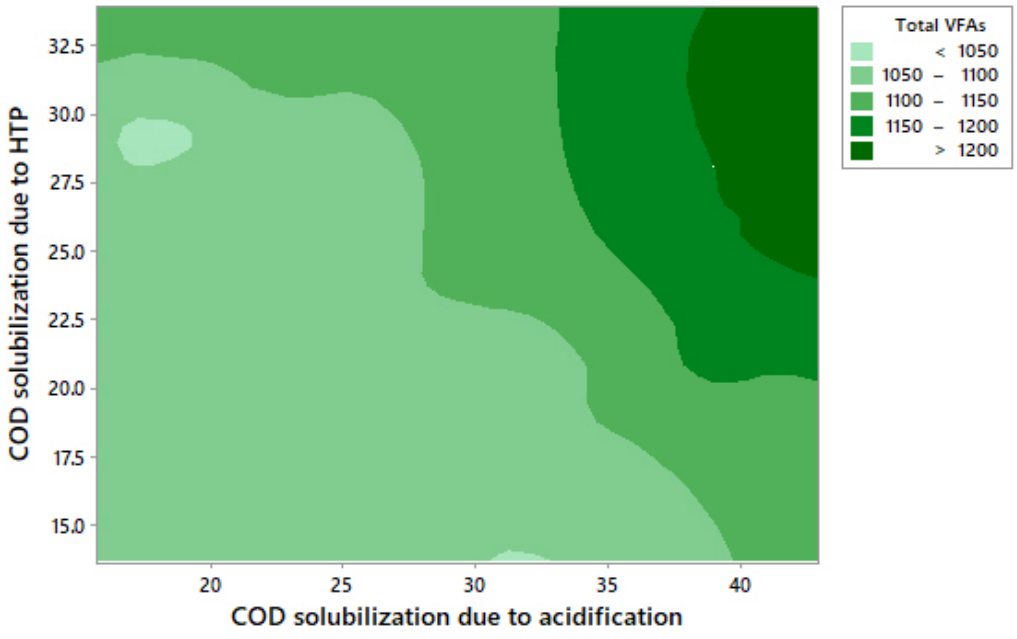


Figure 30. The contour plot of total VFAs vs COD solubilisation after hydrothermal pretreatment and acidification

16.2.1 Effect of HTP temperature and retention time

Generally, the effect of hydrothermal temperature and SI on VFAs production was statistically significant ($P<0.05$). The VFAs concentrations increased by intensification of HTP temperature reaching to the HTP condition of "210 ˚C- 10 min" and then dropped. The release of organic matters and screening of diverse micro-organisms during each pre-treatment can be the reason behind this trend. Ding et al. (2017) used food waste and applied hydrothermal pretreatment for 20 min at 100, 120, 140, 160, 180, and 200 °C. They found that HTP temperature exceeding 160 ˚C resulted in sharp decrease in VFAs. However, in our experiment the point where VFAs concentrations begun to decline was 210 ˚C. Therefore, it can be concluded that HTP under HTP temperatures higher than 210 ˚C does not necessarily lead to higher VFAs production as many inhibitors such as formation of melanoid might have affect the process (Elbeshbishy et al. 2017).

The effect of retention time on VFAs production differed according to the severity indexes. At lower SIs of 3.00, 3.5 and 4.00, the shorter the retention time, the higher the VFAs yield and vice versa for higher SIs of 4.5 and 5.00. Thereby, it is revealed that the dominant parameter of HTP in terms of VFAs production was HTP temperature.

16.2.2 Product spectrum

The concentration of all types of VFAs produced for hydrothermally pretreated and raw samples after acidification is illustrated by the Figure 31. The detected VFAs included acetic acid, propionic acid, iso-butyric acid, butyric acid, Iso-valeric acid and valeric acid. All types of VFAs, corresponding to the VFAs production graph trend, increased by elevation of the HTP temperature reaching to the HTP condition of "210 ˚C-10min" and begun to decline by intensification of the temperature. The VFAs concentration of all types of VFAs were higher than that of raw for most of the HTP conditions.

Acetic acid was the most abundant VFAs among all types of detected VFAs. All hydrothermally pretreated samples showed higher concentration of acetic acid compared to the raw SSO. The concentration of acetic acid increased by increasing the HTP temperature up to 190 ˚C and dropped afterwards. Although the highest amount of total VFAs was observed at HTP condition of "210 ˚C-10min" but in terms of acetic acid, the optimum HTP condition was "190 ˚C-10min" with a concentration of (1,980 mg COD/L). The concentration of acetic acid in raw sample was (723 mg COD/L) which was approximately 3 times less than that of the optimal condition. The acetic acid

to VFAs ratio of all hydrothermally pretreated samples ranged from 24 to 50% while the raw sample had a ratio of 30%. Hence, the hydrothermally pretreated sample had higher, lower and equal acetic acid/VFAs ration compared to the raw. Mainly, SIs of 4.5 and 5 showed lower ratios compared to the raw, whereas, lower SIs of 3 to 4 demonstrated higher percentages. Ding et al. 2017 comparing the VFAs produced after fermentation of the food wastes samples thermally pretreated at 100 to 200 ˚C for 20 minutes, with the raw sample also reported that acetic acid was the most abundant VFAs compared to other type of VFAs. In addition, Ding's results indicated that the concentration of acetic acid for all thermally pretreated samples were higher than that of raw, which is in agreement with the findings of this study. In Ding's study the concentration of acetic for the optimum thermal temperature was 11.43 g/L compared to the 5.6 g/L for the raw sample.

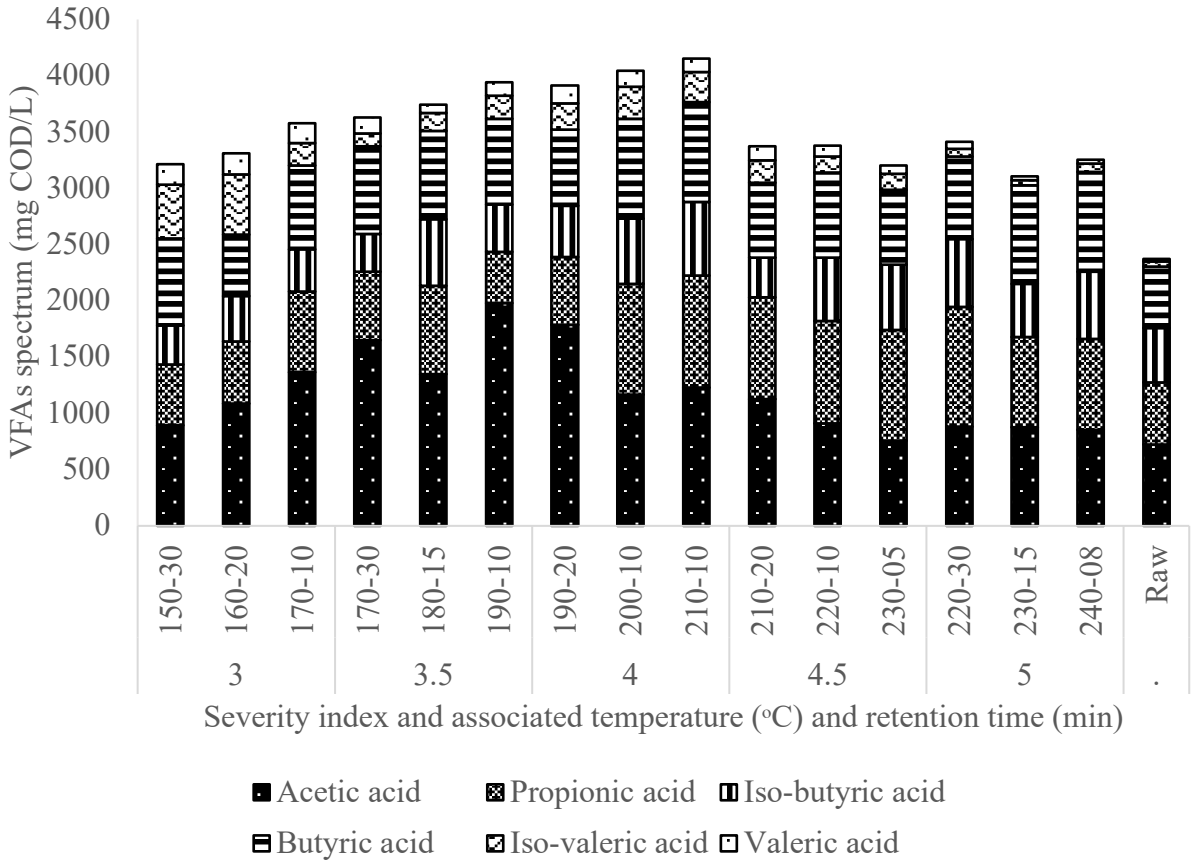


Figure 31 Concentration of VFAs variations

Propionic acid ranked as the second type of VFAs considering its concentration compare to other types of VFAs. The concertation of propionic acid ranged from the lowest amount of 456 to 1059 mg COD/L for all hydrothermally pretreated samples. The lowest amount of propionic acid was achieved at HTP condition of "190 ˚C-10min" which was the optimum condition for acetic acid. While, the highest amount of propionic acid was detected at HTP condition of "220 ˚C-30min". The concentration of propionic acid for raw sample was 550 mg COD/L which was lower than all of hydrothermally pretreated samples except sample pretreated at HTP condition of "190 ˚C-10min".

The propionic acid to VFAs ratio of all hydrothermally pretreated sample was in the range of 11 to 31%. This percentage was 23% for the raw sample. In contrast to the acetic acid, the lower SI of 3 and 3.5 had lower proportion propionic acid compared to the raw. The SI of 4 had similar and SIs of 4.5 and 5 had higher propionic to VFAs ratio compared to the raw. Ding et al. 2017 also reported that propionc acid was the second most abundant VFAs after acetic acid, and the concentration of propionic acid for hydrothermally pretreated samples were higher than that of raw. The highest concentration of propioninc acid was 2.27 g/L at 160 ˚C comparing to the 1.38 g/L for the raw.

The concentration of iso-butyric acid and butyric acid ranged from 336 to 654 mg COD/L and 543 to 889 mg COD/L, respectively. The concentration of iso-butyric acid and butyric acid for the raw samples was 485 and 543 mg COD/L, respectively. The amount of iso-butyric acid for hydrothermally pretreated samples was higher, lower or equal to the raw. Higher SIs had greater amount of iso-butyric acid compared to the raw. The highest amount of iso-butyric acid and butyric acid was 654 and 889 mg COD/L for sample pretreated at "210 ˚C -10min" which was roughly ½ time higher than that of raw. Most conditions of HTP in lower SIs demonstrated lower amount of iso-butyric acid compared to the raw. However, the concentration of butyric acid for all hydrothermally pretreated samples except sample pretreated at HTP condition of "160 ˚C -20min" showed higher amount of butyric acid compared to the raw.These observations were in agreement with the Ding et al. 2017 finding regarding the decrease of iso-butyric acid after HTP compared to the raw but contradict the butyric acid results. The concentrations of the iso-butyric and butyric acid for raw food waste and the optimal HTP temperature of 160 ˚C in Ding's study, was (0.07 and 0.42 g/L) and (0.05 and 0.35 g/L), respectively. As observed the concenteration of both mentioned acids were lower than that of raw in that study.

The iso-butyric acid to VFAs and butyric acid to VFAs ratio of the thermally pretreated samples ranged from (9-18%) and (16-28%), respectively. Where, these percentages were 20 and 23% for the raw sample. The proportion of iso-butyric acid to VFAs for all hydrothermally pretreated samples were lower than that of raw. Similarly the butyric to VFAs ratio of all hydrothermally pretreated SSO except HTP conditions of "230 ˚C -15min" and "240 ˚C-08min" was also lower than that of raw.

The concentration of iso-valeric and valeric acids was as low as (50 and 33 mg COD/L) and as high as (536 and 189 mg COD/L), respectively. The HTP temperature had a negative correlation with the concentration of both iso-valeric and valeric acids. The higher the temperature the lower the concentration of the two mentioned acids. The highest amount of iso-valeric and valeric acids 536 and 189 mg COD/L, respectively, was produced from sample hydrothermally pretreated at HTP conditions of "160 ˚C-20min". The concentration of iso-valeric and valeric acids in raw sample was 48 and 22 mg COD/L, respectively. These percentages are almost 10 times for the sample pretreated at HTP condition of "160 ˚C-20min". (Ding et al. 2017) also confirmed the enhancement of the iso-valeric and valeric acids by applying thermal pretreatment compared to the raw.

The iso-valeric and valeric acids to VFAs ratio of all hydrothermally pretreated samples were higher than that of raw ranging from 2-16% for iso-valeric acid and 1-6% for valeric acid. The iso-valeric and valeric acids to VFAs ratio of raw SSO was 2 and 1%, respectively. The highest iso-valeric and valeric acids to VFAs percentage of 16 and 6% was observed in SI of 3.

In general, HTP affected the amount and percentage of different types of VFAs produced after fermentation process. The HTP temperature demonstrated different correlations with each type of VFAs. Each type of VFAs had a different optimum HTP condition in terms of VFAs production. Acetic acid favoured medium temperatures and SI (3.5 and 4). Propionic acid, iso-butyric and butyric acid had preferred higher HTP temperature and SI (5). Iso-valeric and valeic acid had the maximum VFAs production under the lowest HTP temperature and SI (3).

17 Conclusions and future work

18 Conclusion

The results of current study revealed that hydrothermal pretreatment promoted all factors of interest such as COD solubilization, solid reduction efficiency, and VFAs production. The HTP temperature was the dominant factor compare to the RT. Generally, at lower SIs, the shorter RT combined by higher temperatures demonstrated superior results. SI of 5 adversely affected the process for both substrates which might be due to generation of the toxic substances, therefore it is not a recommended condition. Summary of the optimal HTP conditions and their corresponding results is shown in Figure 32. It was observed that HTP highly affected the solubilization of TWAS compared to the SSO after hydrothermal pretreatment. Although, this was vice versa for the solubilization after acidification as the SSO demonstrated higher efficiency in terms of COD solubilization after acidification compared to the TWAS. Nonetheless, the overall solubilization of both substrates were quite similar (64% for both substrates). These results were observed at lower HTP temperature for the TWAS compared to the SSO. The optimal temperature for COD solubilization for TWAS and SSO were 180 and 220 °C, respectively. These results revealed that SSO required higher temperatures compared to the TWAS for the optimal results due to the nature of the substrate, mainly presence of lignocellulosic materials which need higher temperature to be solubilized.

The solid reduction efficiency due to HTP process for TWAS was higher than that of SSO, although the temperatures were almost similar. In terms of VFAs yields, TWAS produced slightly higher amount of VFAs compared to the SSO. The maximum VFAs production from the SSO was achieved at a higher temperature compared to TWAS.

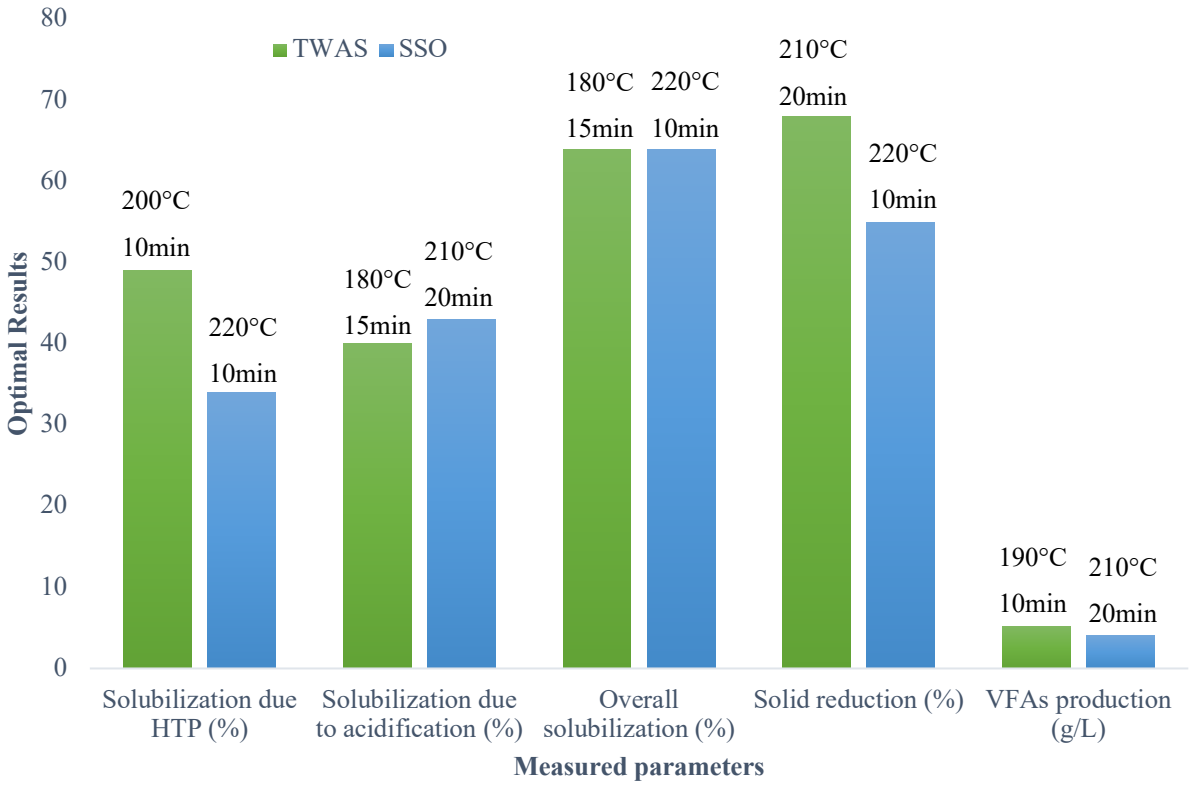


Figure 32 Comparison of the optimum results of the TWAS and SSO

Current research results regarding effect of hydrothermal pretreatment on acidification of TWAS revealed that the HTP increased the potential of VFAs production and facilitated more solubilization, but elevated HTP temperatures higher than 200 °C did not correspond to the higher acidification and VFA production.

- The maximum solubilization of 49% after HTP was observed for TWAS sample hydrothermally pretreated at "200°C-10min".
- After acidification, the highest solubilization percentage of 40% was achieved for the sample pretreated at "180°C-15min" whereas, it was 30% for the raw.
- The maximum overall solubilization of 64% was for the sample hydrothermally pretreated at HTP condition of "180°C-15min" which was 2 times higher than the raw.
- The highest TSS and VSS reduction efficiency of 50 and 55% was achieved at the HTP condition of "210°C-20min", respectively.

- The maximum VFA yield of 2856 mg VFAs/g VSS_{added} after acidification was for sample pretreated at HTP condition of "190°C-10min" compared to 1251 for raw TWAS.

Considering the impact of HTP on acidification of SSO it was found that although HTP promoted the solubility of SSO but HTP temperatures greater than 230 °C caused the production of inhibitory substances and decrease of microbial hydrolytic enzymes.

Based on the SSO results, the following points can be concluded:

- The highest COD solubilization of 34% after hydrothermal pretreatment was observed at HTP condition of "220°C-10min".
- After acidification, the highest solubilization percentage of 43% was achieved for the sample pretreated at "210°C-20min" whereas, it was 27% for the raw.
- The maximum overall solubilization of 64% was observed at HTP condition of "220°C-10min" whereas it was 37% for the raw sample.
- The solid reduction efficiency of 51 and 55% based on TSS and VSS, respectively, was achieved for the sample hydrothermally pretreated at HTP condition of "220°C-10min".
- VFAs production yield after acidification was enhanced to the 1248 mg VFAs/g VSS_{added} at "210°C-20 min" compared to 915 for raw SSO.

19 Future work and recommendations

Based on the current research findings following works for future work is recommended:

- investigating the effect of the acidification process parameters such as food to micro-organism ratio, pH, HRT, and other parameter to evaluate the effect of each factor as well as interaction of these parameters.
- Employment of two-stage anaerobic digestion for the same substrates and hydrothermal pretreatment conditions to determine the effect of HTP subsequent biomethane production and the quality of the digestate.
- Undertake a preliminary economic analysis of the cost of making changes to the SSO processing facility by applying hydrothermal pretreatment plant.

20 Appendices

21 TWAS pretreatment

Figure A 1 Concentration of Ammonia after HTP

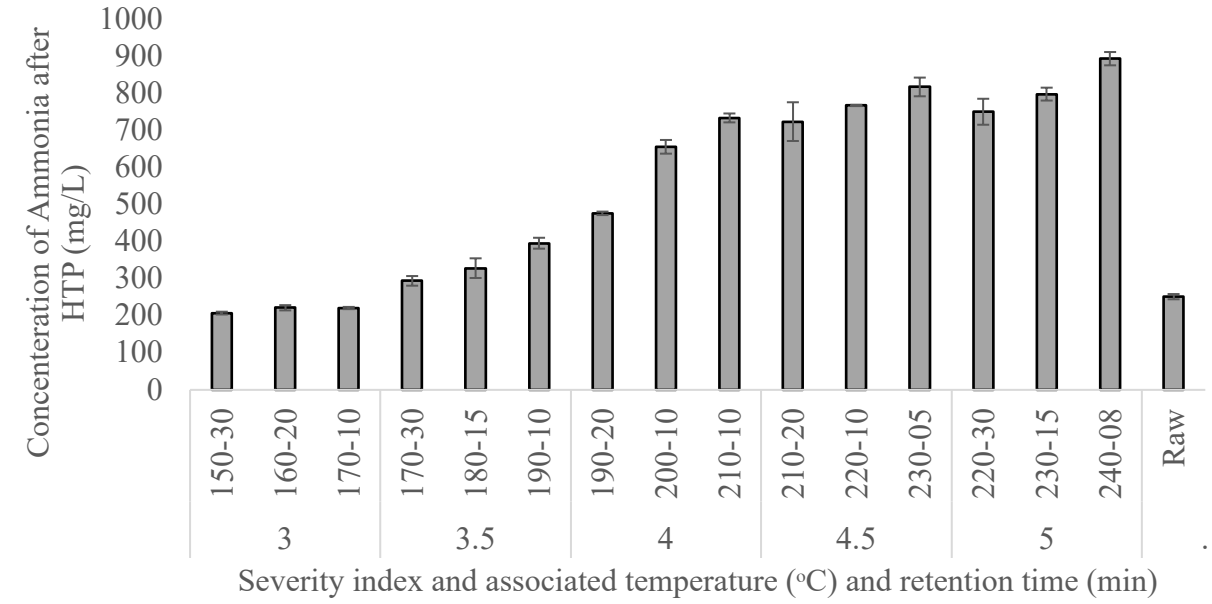


Figure A 2 Concentration of alkalinity after HTP

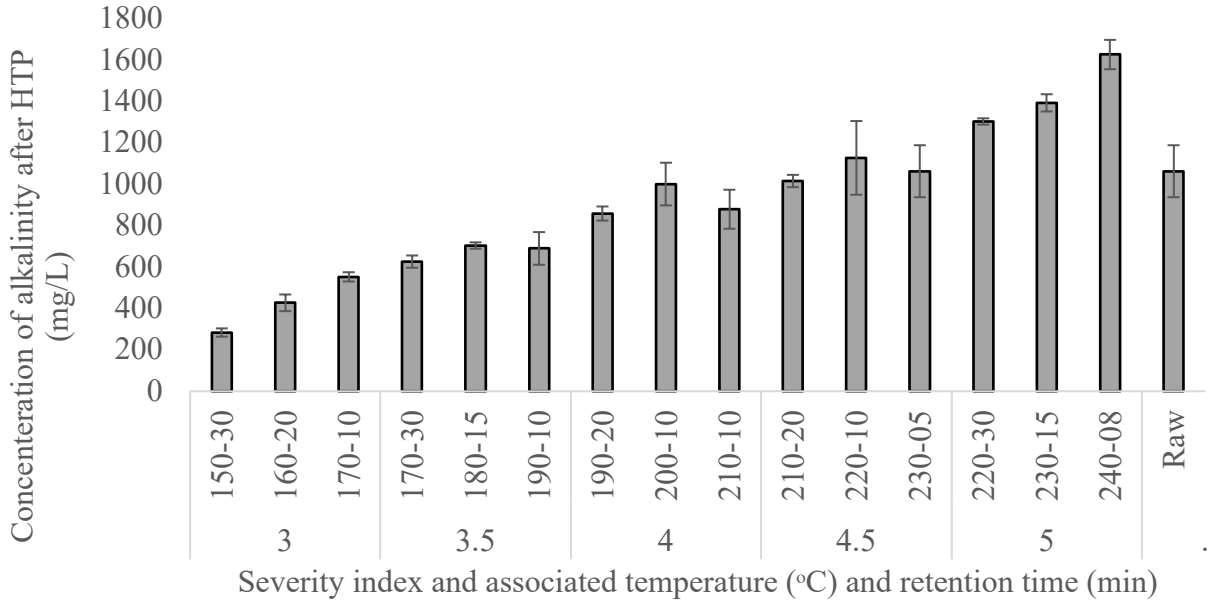


Figure A 3 Concentration of total carbohydrates after HTP

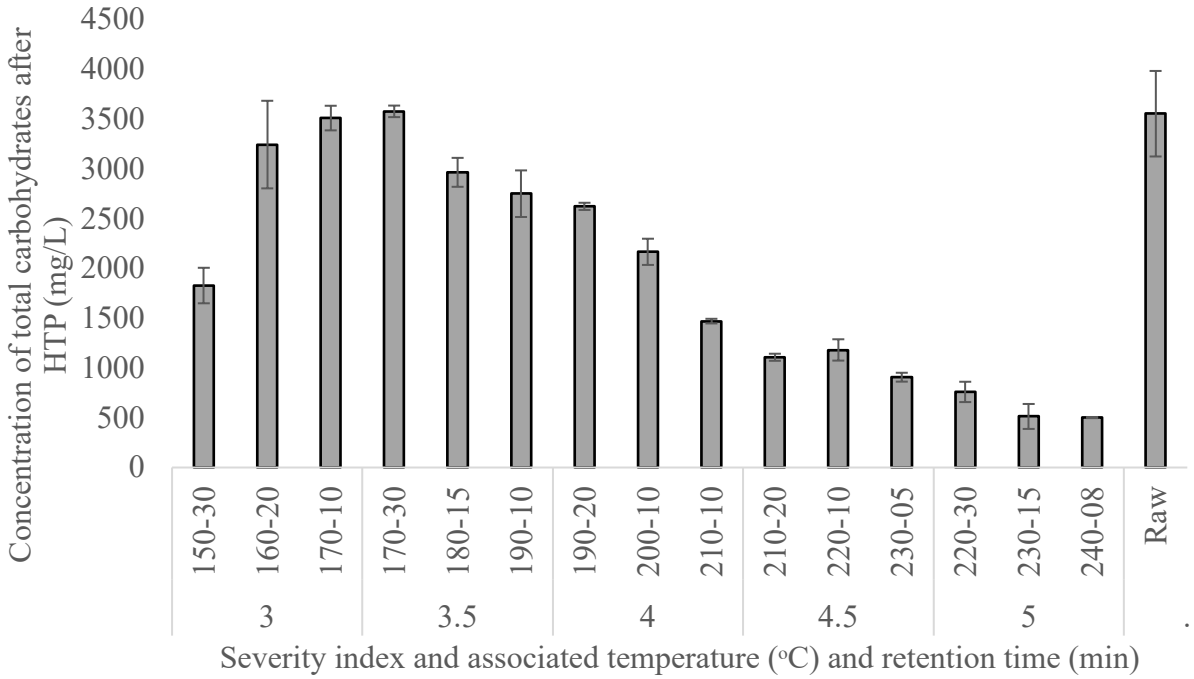
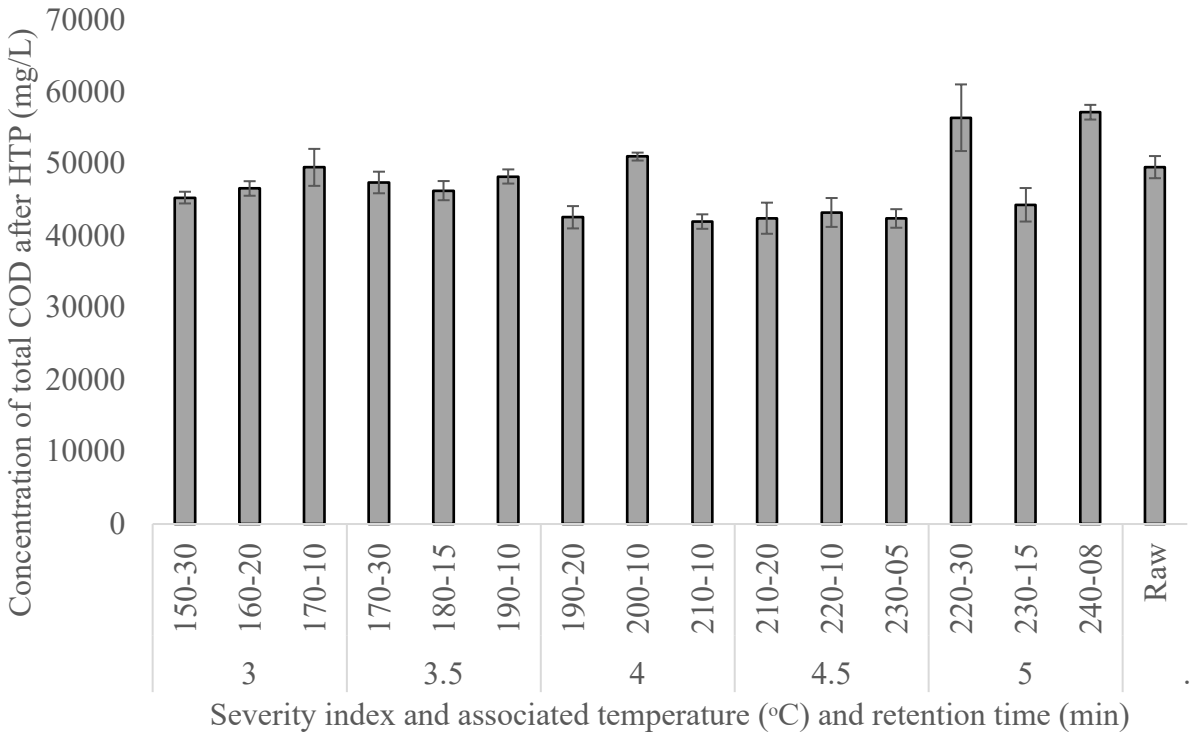


Figure A 4 Concentration of total COD after HTP



Selected sample of hydrothermal pretreatment heating and cooling rate records

Green line presents the adjusted condition of heating and cooling rate whereas, red line presents the actual heating and cooling rate.

Figure A 5 "160℃-20min-89psi" (SI:3)

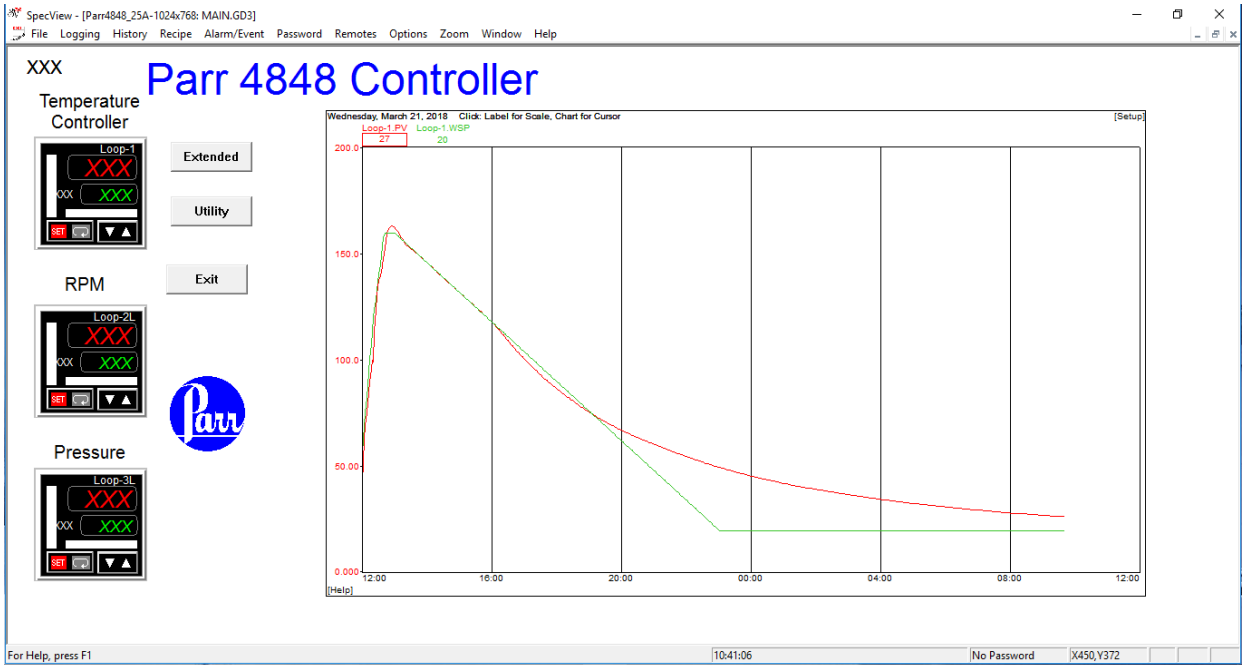


Figure A 6 "170℃ -30min-114psi" (SI:3.5)

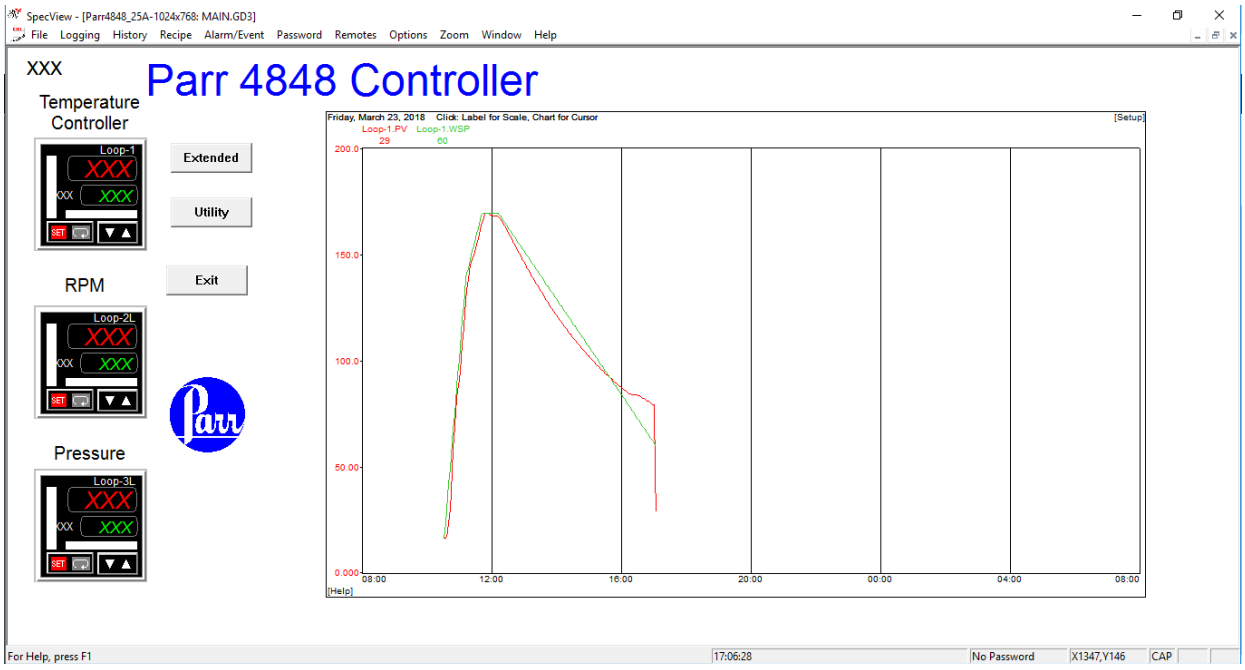


Figure A 7 "190℃ -20min-181psi" (SI:4)

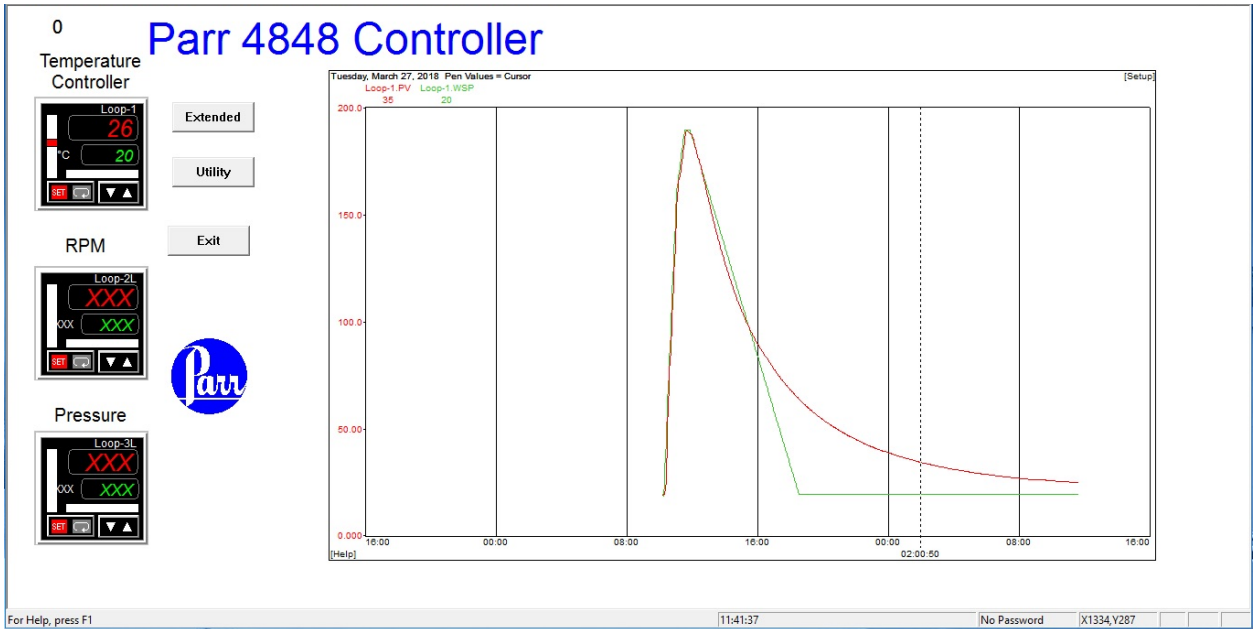


Figure A 8 "210℃ -20min-227psi" (SI:4.5)

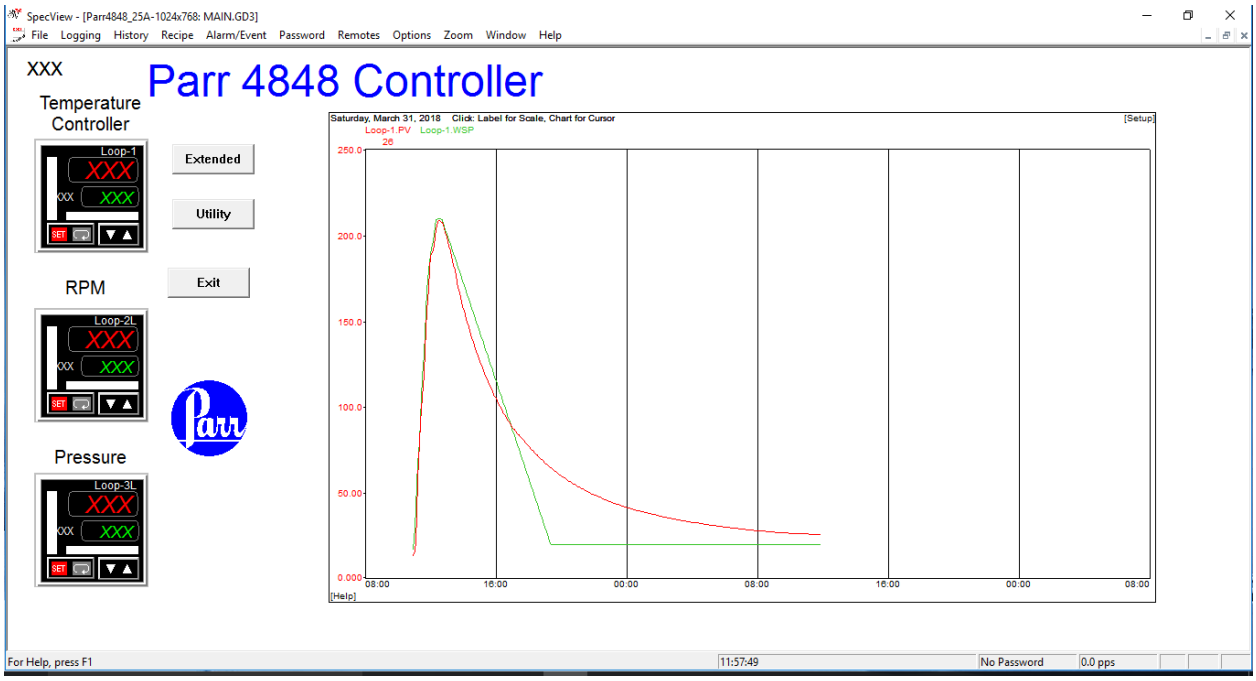


Figure A 9 "220℃ -30min-337psi" (SI:5)

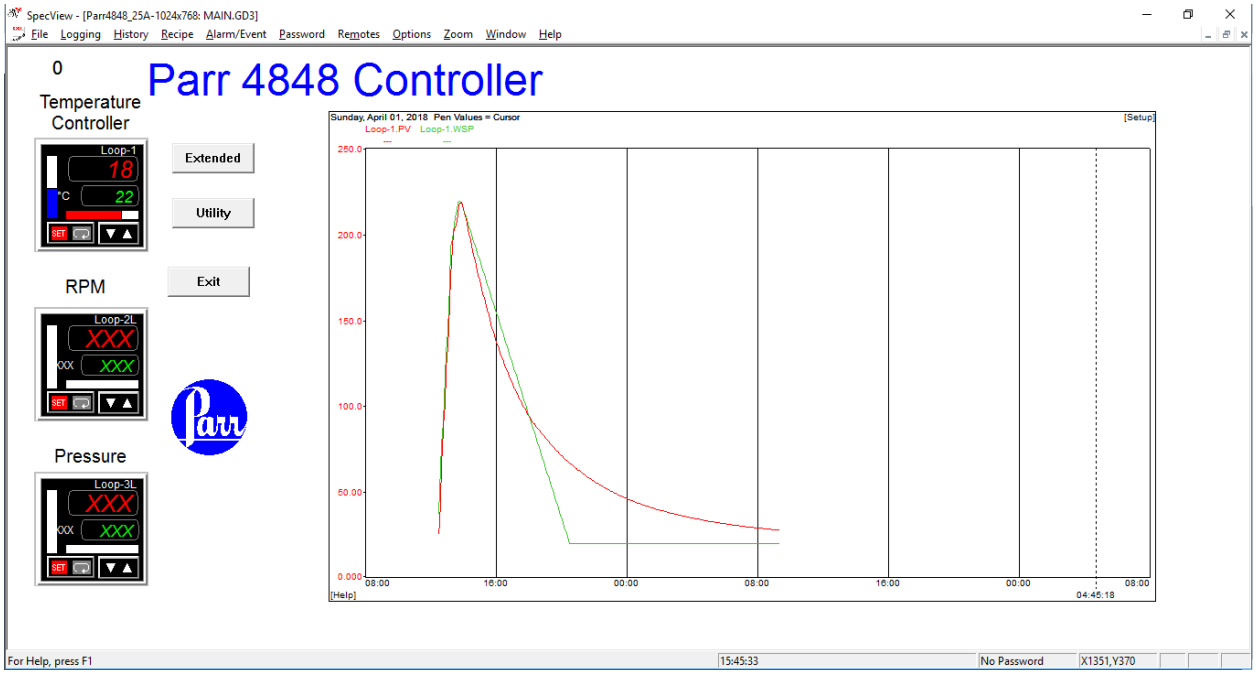


Table A 1 Particle size distribution of TWAS after and before HTP

SI	HTP condition: Temp-Time-Pressure	D10	SD	D50	SD	D90	SD
	(°C)(min)(psi)	μm		μm		μm	
3	150-30-69	23.5	2.5	60.9	7.6	118.2	18.3
3	160-20-89	26.7	4.2	63.0	64.0	119.0	120.0
3	170-10114	23.4	3.3	64.1	9.6	121.0	23.1
3.5	170-30-114	23.8	2.1	60.8	6.4	111.4	14.5
3.5	180-15-145	23.9	2.6	58.3	6.7	102.3	14.5
3.5	190-10-181	23.0	24.0	56.0	57.0	100.0	100.0
4	190-20-181	16.8	1.3	49.0	4.2	89.0	89.0
4	200-10-225	13.7	0.7	44.7	2.9	90.0	9.1
4	210-10-277	12.7	0.6	40.5	2.5	81.0	7.0
4.5	210-20-227	11.5	0.5	39.8	2.7	83.5	9.0
4.5	220-10-337	13.1	1.0	42.7	3.7	89.4	17.7
4.5	230-05-407	12.0	13.0	41.0	42.0	80.0	81.0
5	220-30-337	11.0	0.4	38.5	1.7	81.2	5.6
5	230-15-407	12.0	0.4	38.9	1.9	75.8	5.4
5	240-08-488	11.9	0.5	39.2	2.0	74.8	5.7
0	Raw	26.9	0.9	76.2	3.1	152.5	6.0

22 TWAS acidification

Figure A 10 Concentration of TSS after acidification of TWAS

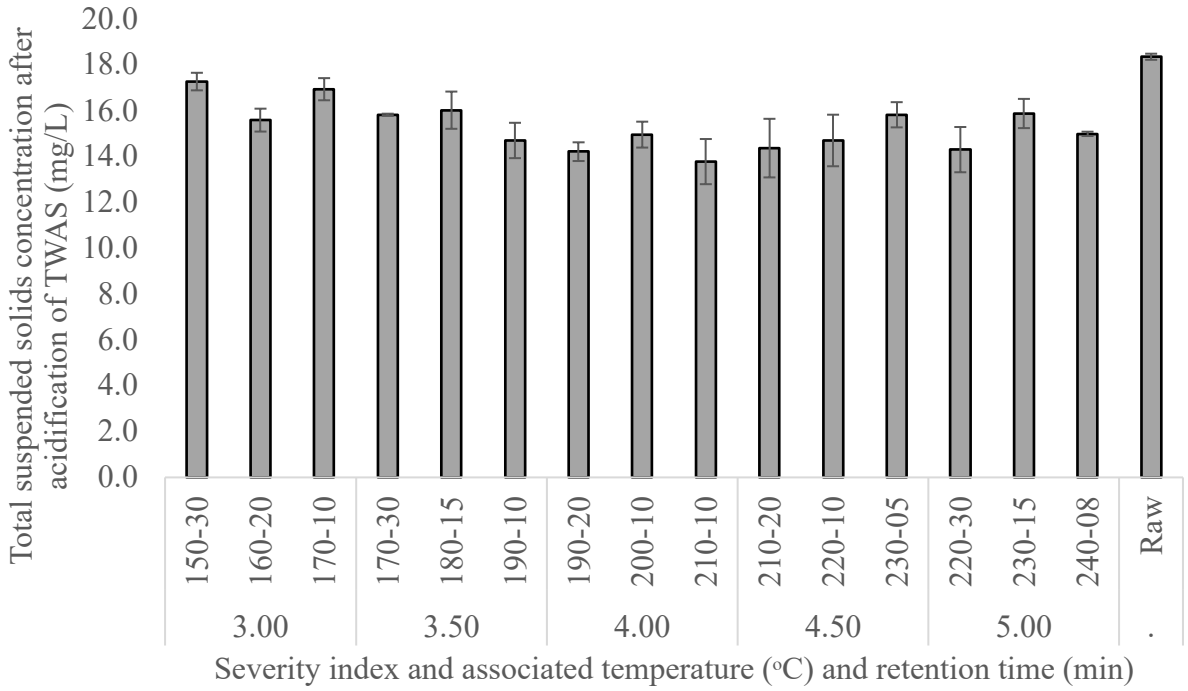


Figure A 11 Concentration of VSS after acidification of TWAS

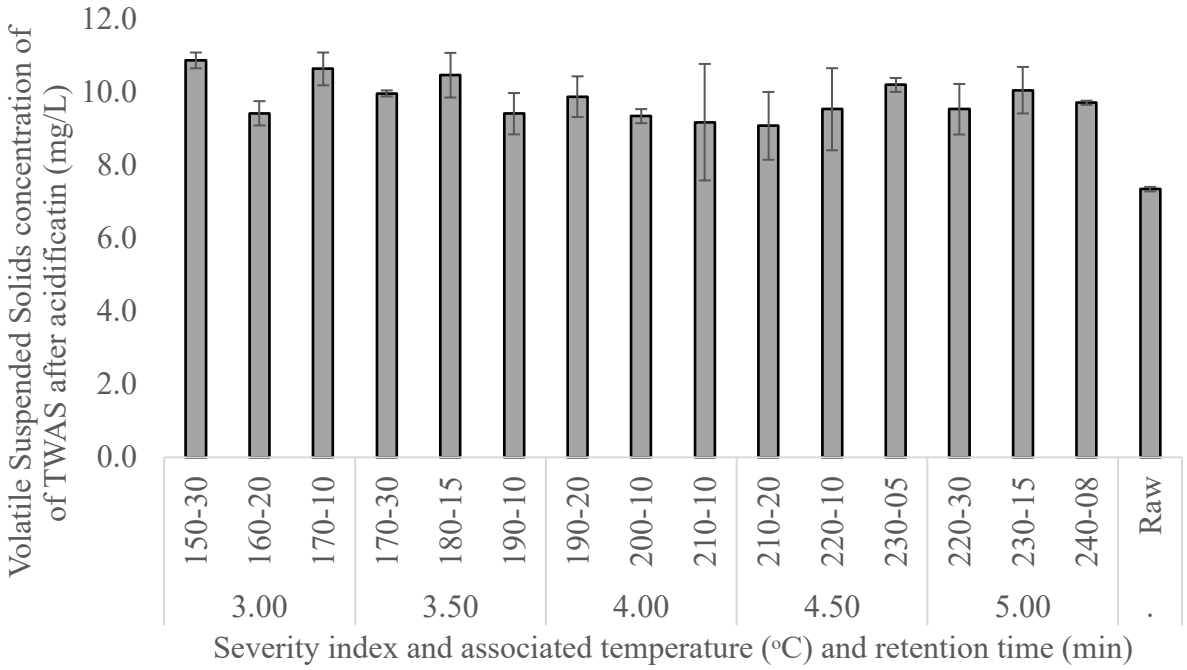


Figure A 12 TCOD concentration after acidification of TWAS

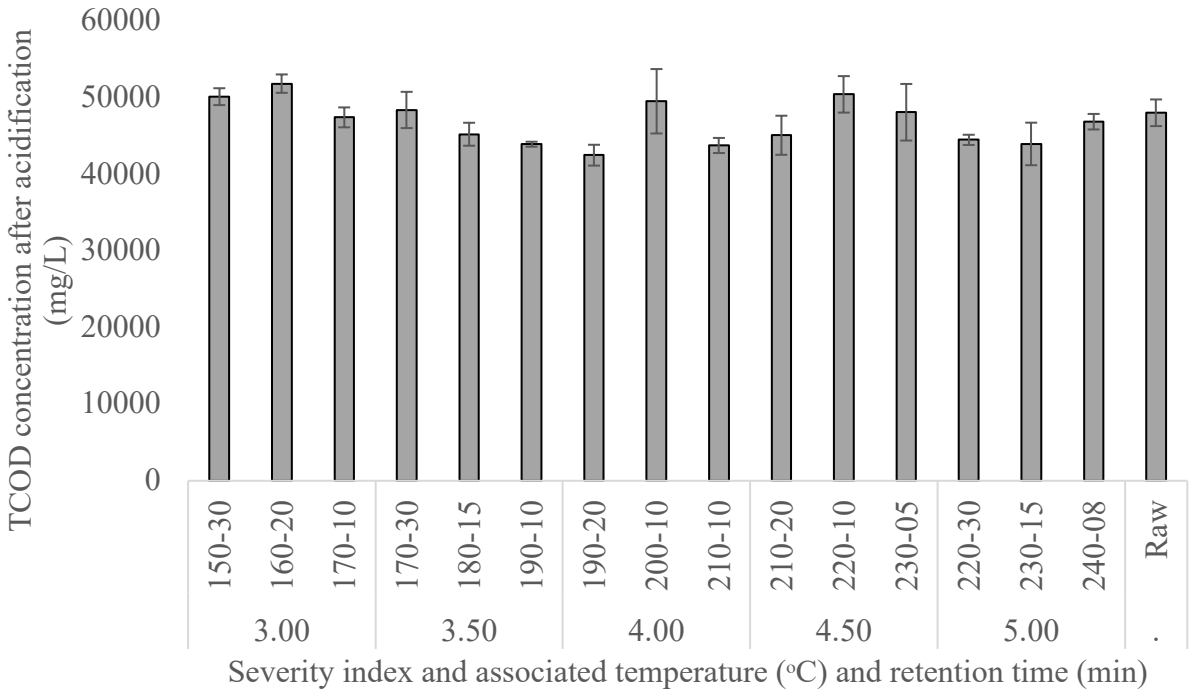


Figure A 13 Total carbohydrates concentration after acidification of TWAS

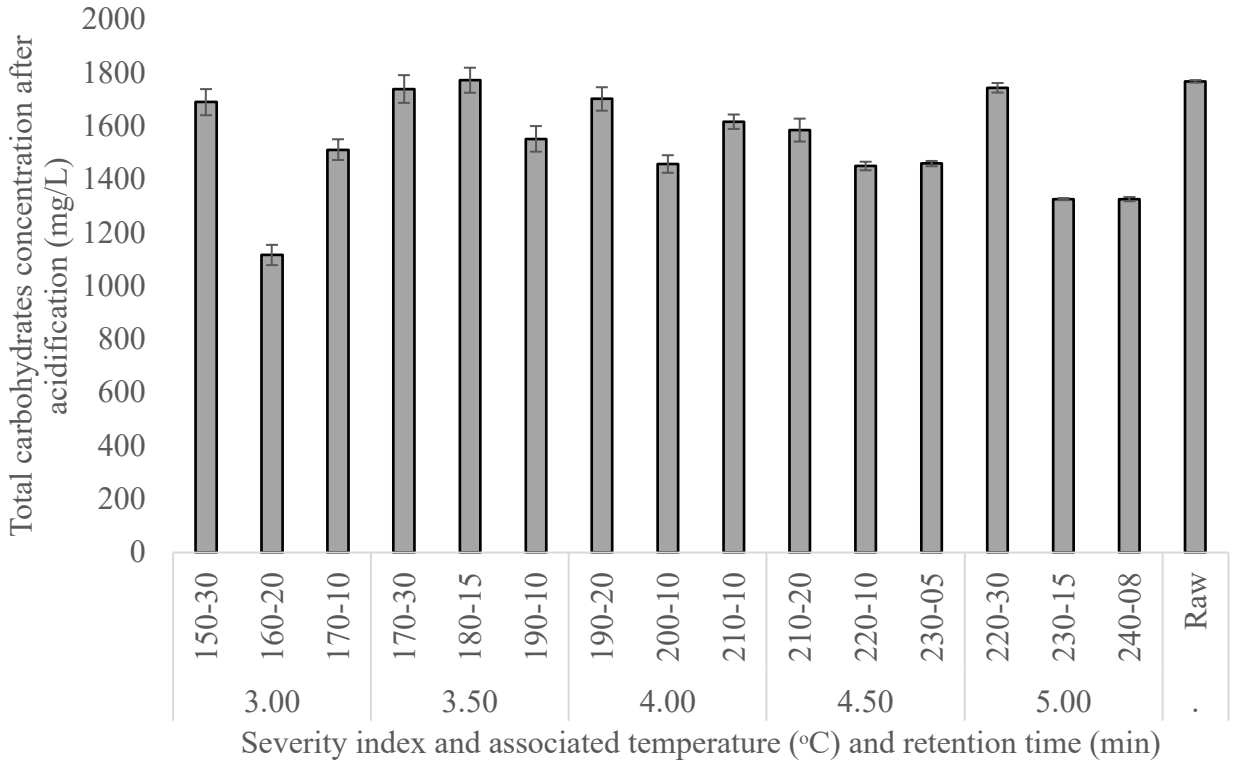


Figure A 14 Main effects plots for solubilization due to acidification

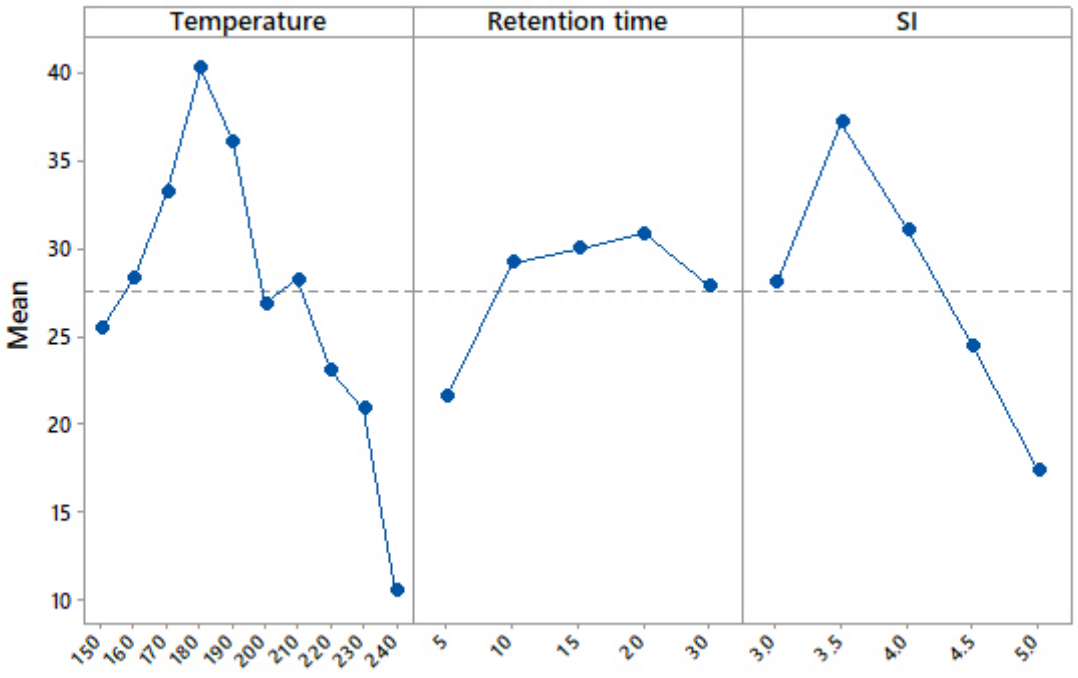


Figure A 15 Main effects plots for overall solubilization

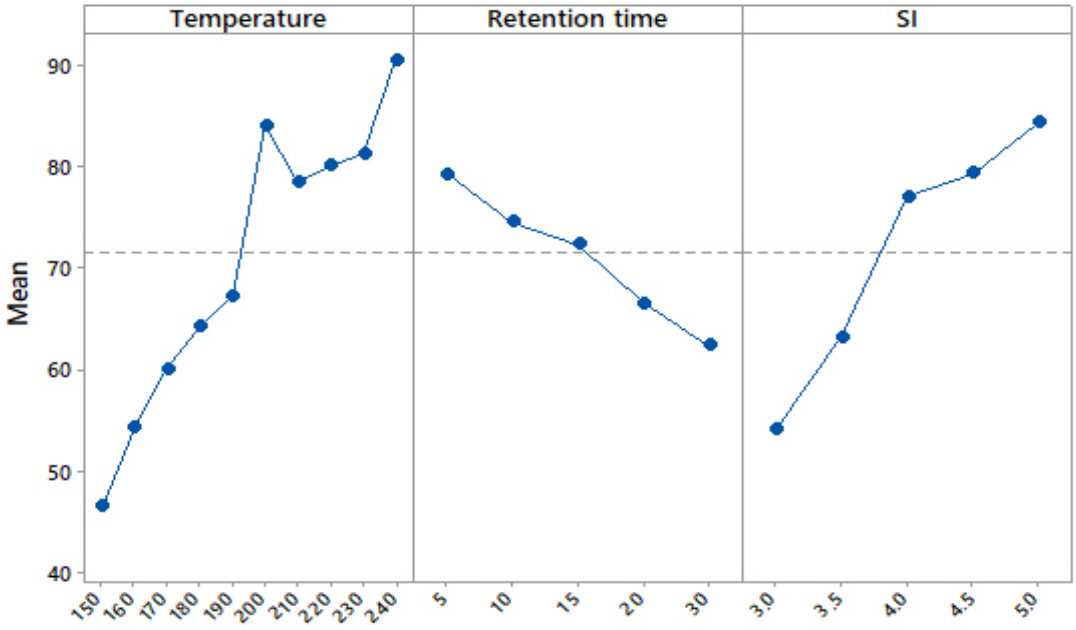


Figure A 16 Main effects plots for Total VFAs produced due to acidification

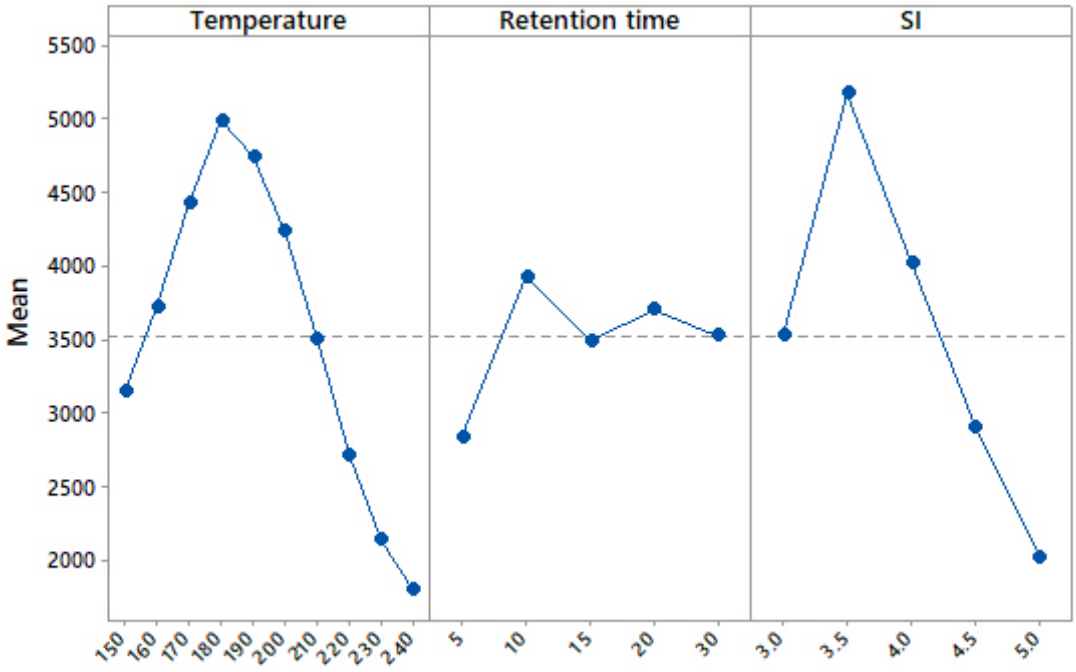


Figure A 17 The interaction plots for total VFAs

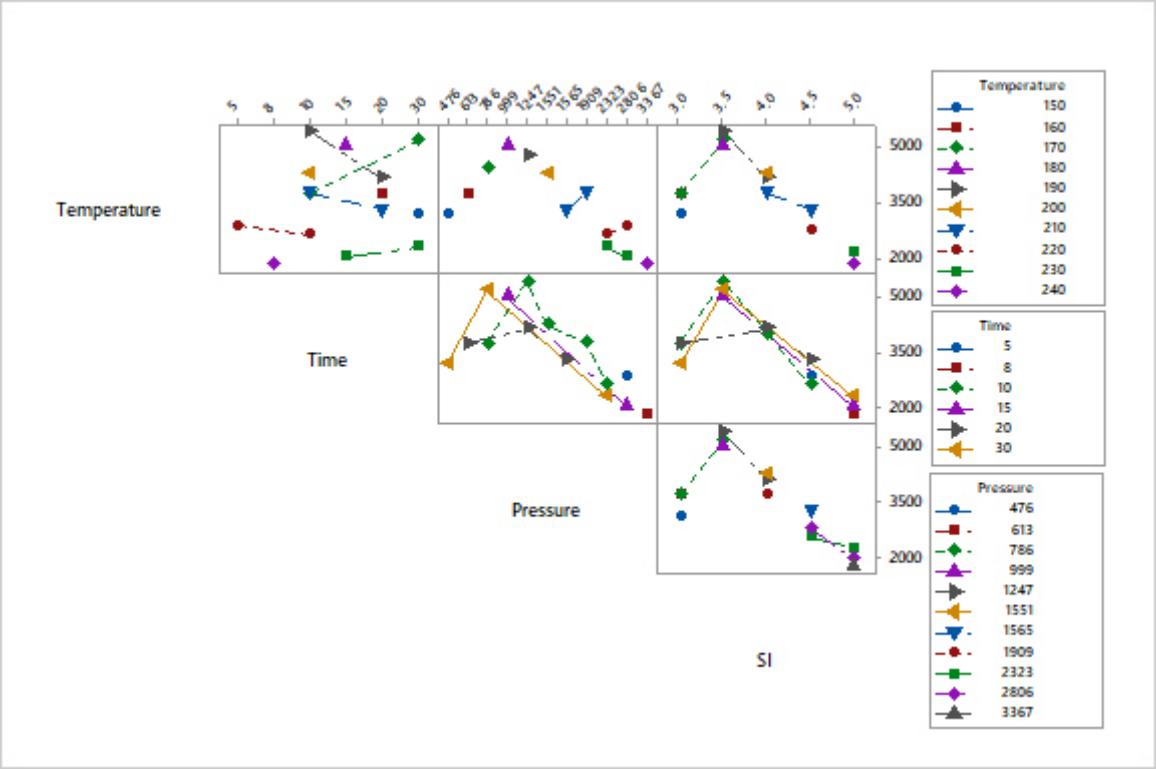


Table A 2 Correlation between three responses and SI

	SI	Solubilization due to HTP	Solubilization due to acidification	VFA production
SI	1			
Solubilization due to HTP	0.79	1		
Solubilization due to acidification	-0.71	-0.41	1	
VFA production	-0.74	-0.39	0.99	1

Table A 3 Correlation between three responses and temperature

	Temperature	Solubilization due to HTP	Solubilization due to acidification	VFA production
Temperature	1			
Solubilization due to HTP	0.89	1		
Solubilization due to acidification	-0.61	-0.3	1	
VFA production	-0.64	-0.29	0.91	1

Table A 4 Correlation between three responses and Retention time at lower SI

	RT	Solubilization due to HTP	Solubilization due to acidification	VFA production
RT	1			
Solubilization due to HTP	-0.99	1		
Solubilization due to acidification	-0.99	0.99	1	
VFA production	-0.85	0.85	0.91	1

Table A 5 Correlation between three responses and RT at High SI

	RT	Solubilization due to HTP	Solubilization due to acidification	VFA production
RT	1			
Solubilization due to HTP	-0.28	1		
Solubilization due to acidification	0.86	-0.73	1	
VFA production	0.99	-0.37	0.91	1

Table A 6 Main responses after HTP and fermentation

SI	HTP condition: Temp-Time-Pressure	Solubilization due to HTP	solubilization acidification	Overall solubilization	TVFA	TVFA Yield
	(°C) (min) (psi)	(%)	(%)	(%)	(mg COD/L)	(mg VFA/g VSS added)
3	150-30-69	27	25	46	3144	628.9
3	160-20-89	32	28	54	3723	744.6
3	170-10114	36	30	62	3700	739.9
3.5	170-30-114	36	36	58	5145	1028.9
3.5	180-15-145	40	40	64	4989	997.9
3.5	190-10-181	41	35	67	5383	1076.6
4	190-20-181	42	37	68	4107	821.4
4	200-10-225	49	27	84	4237	847.3
4	210-10-277	46	29	79	3728	745.6
4.5	210-20-227	45	27	78	3276	655.3
4.5	220-10-337	46	24	81	2595	519.0
4.5	230-05-407	44	22	79	2832	566.4
5	220-30-337	46	22	82	2277	455.4
5	230-15-407	45	20	80	1988	397.5

5	240-08-488	48	10	91	1792	358.4
0	Raw	0	30	30	1840	368.0

Table A 7 Variation of VFAs

SI	HTP condition: Temp-Time-Pressure	Acetic acid	Propionic acid	Iso-butyric acid	Butyric acid	Iso-valeric acid	Valeric acid
	(°C)(min)(psi)	(mg COD/L)					
3	150-30-69	389	340	1031	286	786	312
3	160-20-89	1210	593	606	457	632	225
3	170-10114	1487	288	570	936	147	272
3.5	170-30-114	1709	835	870	1312	147	272
3.5	180-15-145	1904	1065	1089	538	316	77
3.5	190-10-181	2225	1275	740	558	463	122
4	190-20-181	1258	1043	701	612	336	157
4	200-10-225	1065	1345	805	456	440	126
4	210-10-277	1112	992	658	478	325	163
4.5	210-20-227	1023	1107	355	443	223	125
4.5	220-10-337	872	613	565	302	145	98
4.5	230-05-407	756	985	666	304	48	73
5	220-30-337	884	459	606	234	31	63
5	230-15-407	679	693	330	201	50	34
5	240-08-488	658	553	298	187	66	30
0	Raw	522	892	209	145	50	22

SI	HTP condition: Temp-Time-Pressure	Acitic /TVFA	propionic/ TVFA	Iso-butyric/TVFA	butyric/TVFA	Iso-valeric/TVFA	valeric/TVFA
	(°C)(min)(psi)	(%)					
3	150-30-69	12	11	33	9	25	10
3	160-20-89	33	16	16	12	17	6
3	170-10114	40	8	15	25	4	7
3.5	170-30-114	33	16	17	26	3	5
3.5	180-15-145	38	21	22	11	6	2
3.5	190-10-181	41	24	14	10	9	2
4	190-20-181	31	25	17	15	8	4
4	200-10-225	25	32	19	11	10	3
4	210-10-277	30	27	18	13	9	4
4.5	210-20-227	31	34	11	14	7	4
4.5	220-10-337	34	24	22	12	6	4
4.5	230-05-407	27	35	24	11	2	3
5	220-30-337	39	20	27	10	1	3
5	230-15-407	34	35	17	10	3	2
5	240-08-488	37	31	17	10	4	2
0	Raw	28	48	11	8	3	1

Table A 8 Ratio of VFA variation to TVFAs

Table A 9 VFAs variation at HTP condition of 150-30

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	1.7	60	101	101	1.07	108.6
Propionic acid	1.3	74	93	114	1.51	139.8
Iso-butyric acid	6.43	88.11	567	831	1.82	1031.1
Butyric acid	1.49	88.11	131	193	2.18	286.2
Iso-valeric acid	8.53	102.1317	871	1482	2.05	1785.9
Valeric acid	1.49	102.13	152	259	2.05	312.0
iso-caproic acid	1.9	116.1583	221	427	2.21	487.7
Hexanoic acid	1.84	116.1583	214	413	2.21	472.3
n-heptanoic acid	1.92	130.1849	250	542	2.58	644.9

	Total (as mg AA/L)	4362	5269

Table A 10 VFAs variation at HTP condition of 160-20

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	9.7	60	581	581	1.07	621.3
Propionic acid	1.7	74	128	157	1.51	192.6
Iso-butyric acid	0.412	88.11	36	53	1.82	66.1
Butyric acid	0.815	88.11	72	105	2.18	156.5
Iso-valeric acid	0.63	102.1317	64	109	2.05	131.9
Valeric acid	1.15	102.13	117	200	2.05	240.8
iso-caproic acid	1.62	116.1583	188	364	2.21	415.9
Hexanoic acid	1.6	116.1583	186	360	2.21	410.7

n-heptanoic acid	1.82	130.1849	237	514	2.58	611.3
	19.4		Total (as mg AA/L)	2443		2847

Table A 11 VFAs variation at HTP condition of 170-10

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	52.7	60	3166	3166	1.07	3387.4
Propionic acid	2.1	74	156	192	1.51	234.9
Iso-butyric acid	0.439	88.11	39	57	1.82	70.4
Butyric acid	10.08	88.11	888	1303	2.18	1936.2
Iso-valeric acid	0.7	102.1317	71	122	2.05	146.6
Valeric acid	1.3	102.13	133	226	2.05	272.2
iso-caproic acid	0.53	116.1583	62	119	2.21	136.1
Hexanoic acid	12.26	116.1583	1424	2755	2.21	3147.3
n-heptanoic acid	2.44	130.1849	318	689	2.58	819.5
	82.6		Total (as mg AA/L)	8628		10151

Table A 12 VFAs variation at HTP condition of 170-30

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	57.7	60	3466	3466	1.07	3708.7
Propionic acid	2.1	74	156	192	1.51	234.9
Iso-butyric acid	0.439	88.11	39	57	1.82	70.4
Butyric acid	13.08	88.11	1152	1691	2.18	2512.4
Iso-valeric acid	0.7	102.1317	71	122	2.05	146.6
Valeric acid	1.3	102.13	133	226	2.05	272.2
iso-caproic acid	0.53	116.1583	62	119	2.21	136.1
Hexanoic acid	15.26	116.1583	1773	3429	2.21	3917.4
n-heptanoic acid	2.44	130.1849	318	689	2.58	819.5
	93.6		Total (as mg AA/L)	9990		11818

Table A 13 VFAs variation at HTP condition of 180-15

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	20.3	60	1219	1219	1.07	1304.3
Propionic acid	0.6	74	43	53	1.51	64.9
Iso-butyric acid	36.1	88.11	3181	4667	1.82	5789.0
Butyric acid	2.8	88.11	247	362	2.18	537.8
Iso-valeric acid	1.51	102.1317	154	262	2.05	316.1
Valeric acid	0.37	102.13	38	64	2.05	77.5
Iso-caproic acid	0.6	116.1583	70	135	2.21	154.0
Hexanoic acid	5.6	116.1583	650	1258	2.21	1437.6
n-heptanoic acid	0.82	130.1849	107	231	2.58	275.4
	68.7		Total (as mg AA/L)	8252		9957

Table A 14 VFAs variation at HTP condition of 190-10

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	3.5	60	210	210	1.07	224.9
Propionic acid	11.4	74	845	1042	1.51	1275.2
Iso-butyric acid	0	88.11	0	0	1.82	0.0
Butyric acid	0.3	88.11	26	39	2.18	57.6
Iso-valeric acid	0.24	102.1317	25	42	2.05	50.2
Valeric acid	0	102.13	0	0	2.05	0.0
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0.7	116.1583	81	157	2.21	179.7
n-heptanoic acid	0	130.1849	0	0	2.58	0.0

	16.1	Total (as mg AA/L)	1490		1788

Table A 15 VFAs variation at HTP condition of 190-30

VFAs	Concentration (mM) (mM/L)	Molecular weight (MW)-mg/mM	Concentration (mg/L)	Concentration as Acetic Acid (mg/L)	TCOD (g/g)	Concentration as COD (mg/L)
Acetic acid	1.7	60	101	101	1.07	107.9
Propionic acid	4.0	74	293	362	1.51	443.0
Iso-butyric acid	0.63	88.11	56	81	1.82	101.0
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.17	102.1317	17	30	2.05	35.6
Valeric acid	0.27	102.13	28	47	2.05	56.5
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0

n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	6.7		Total (as mg AA/L)	621		744

Table A 16 VFAs variation at HTP condition of 200-10

VFAs	**Concentration**	**Molecular weight**	**Concentration**	**Concentration as Acetic Acid**	**TCOD**	**Concentration as COD**
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	0.0	60	0	0	1.07	0.0
Propionic acid	0.0	74	0	0	1.51	0.0
Iso-butyric acid	0	88.11	0	0	1.82	0.0
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0	102.1317	0	0	2.05	0.0
Valeric acid	0.6	102.13	61	104	2.05	125.6
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0

n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	0.6		Total (as mg AA/L)	104		126

Table A 17 VFAs variation at HTP condition of 210-10

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	0.0	60	0	0	1.07	0.0
Propionic acid	2.1	74	156	192	1.51	234.9
Iso-butyric acid	0.36	88.11	32	47	1.82	57.7
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.12	102.1317	12	21	2.05	25.1
Valeric acid	0.3	102.13	31	52	2.05	62.8
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0
n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	2.9		Total (as mg AA/L)	311		381

Table A 18 VFAs variation at HTP condition of 210-20

VFAs	Concentration	Molecular weight	Concentration	Concentration as Acetic Acid	TCOD	Concentration as COD
	(mM/L)	(mg/mM)	(mg/L)	(mg/L)	(g/g)	(mg/L)
Acetic acid	0.0	60	0	0	1.07	0.0
Propionic acid	9.9	74	733	905	1.51	1107.4
Iso-butyric acid	1.9	88.11	167	246	1.82	304.7
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.2	102.1317	20	35	2.05	41.9
Valeric acid	0	102.13	0	0	2.05	0.0
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0
n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	12.0		Total (as mg AA/L)	1185		1454

Table A 19 VFAs variation at HTP condition of 220-10

VFAs	Concentration (mM) (mM/L)	Molecular weight (MW)-mg/mM	Concentration (mg/L)	Concentration as Acetic Acid (mg/L)	TCOD (g/g)	Concentration as COD (mg/L)
Acetic acid	0.0	60	0	0	1.07	0.0
Propionic acid	1.9	74	141	174	1.51	212.5
Iso-butyric acid	0.4	88.11	35	52	1.82	64.1
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0	102.1317	0	0	2.05	0.0
Valeric acid	0.3	102.13	31	52	2.05	62.8
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0
n-heptanoic acid	0	130.1849	0	0	2.58	0.0

2.6	Total (as mg AA/L)	277	339

Table A 20 VFAs variation at HTP condition of 230-05

VFAs	Concentration (mM) (mM/L)	Molecular weight (MW)-mg/mM	Concentration (mg/L)	Concentration as Acetic Acid (mg/L)	TCOD (g/g)	Concentration as COD (mg/L)
Acetic acid	1.1	60	64	64	1.07	68.1
Propionic acid	1.7	74	122	151	1.51	184.6
Iso-butyric acid	0.41	88.11	36	53	1.82	65.7
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.23	102.131	23	40	2.05	48.2
Valeric acid	0	102.13	0	0	2.05	0.0
iso-caproic acid	0	116.158	0	0	2.21	0.0
Hexanoic acid	0	116.158	0	0	2.21	0.0
n-heptanoic acid	0	130.184	0	0	2.58	0.0
	3.4		Total (as mg AA/L)	307		367

Table A 21 VFAs variation at HTP condition of 220-30

VFAs	Concentration (mM) (mM/L)	Molecular weight (MW)-mg/mM	Concentration (mg/L)	Concentration as Acetic Acid (mg/L)	TCOD (g/g)	Concentration as COD (mg/L)
Acetic acid	1.3	60	78	78	1.07	83.5
Propionic acid	4.1	74	304	375	1.51	458.6
Iso-butyric acid	0.66	88.11	58	85	1.82	105.8
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.15	102.1317	15	26	2.05	31.4
Valeric acid	0	102.13	0	0	2.05	0.0
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0
n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	6.2		Total (as mg AA/L)	564		679

Table A 22 VFAs variation at HTP condition of 230-15

VFAs	Concentration (mM) (mM/L)	Molecular weight (MW)-mg/mM	Concentration (mg/L)	Concentration as Acetic Acid (mg/L)	TCOD (g/g)	Concentration as COD (mg/L)
Acetic acid	1.2	60	74	74	1.07	79.0
Propionic acid	8.9	74	658	812	1.51	993.3
Iso-butyric acid	2.06	88.11	182	266	1.82	330.3
Butyric acid	0	88.11	0	0	2.18	0.0
Iso-valeric acid	0.24	102.1317	25	42	2.05	50.2
Valeric acid	0	102.13	0	0	2.05	0.0
iso-caproic acid	0	116.1583	0	0	2.21	0.0
Hexanoic acid	0	116.1583	0	0	2.21	0.0
n-heptanoic acid	0	130.1849	0	0	2.58	0.0
	12.4		Total (as mg AA/L)	1193		1453

23 SSO pretreatment

Figure A 18 Concentration of ammonia after HTP

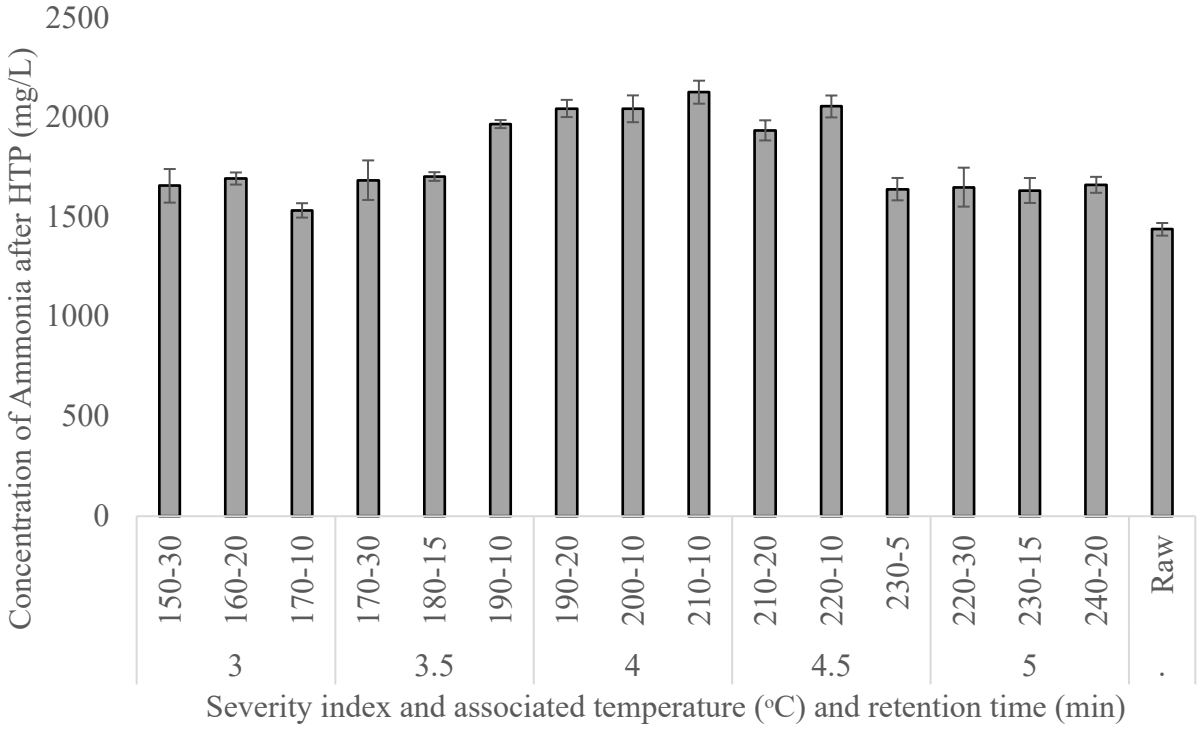


Figure A 19 Concentration of alkalinity after HTP

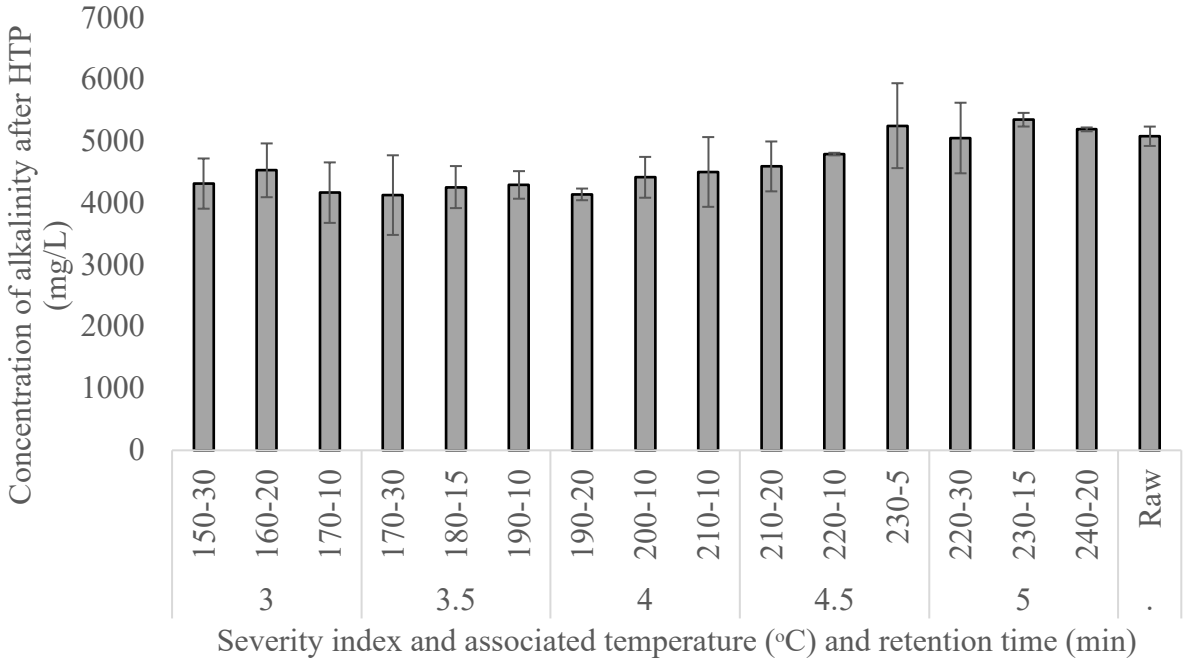
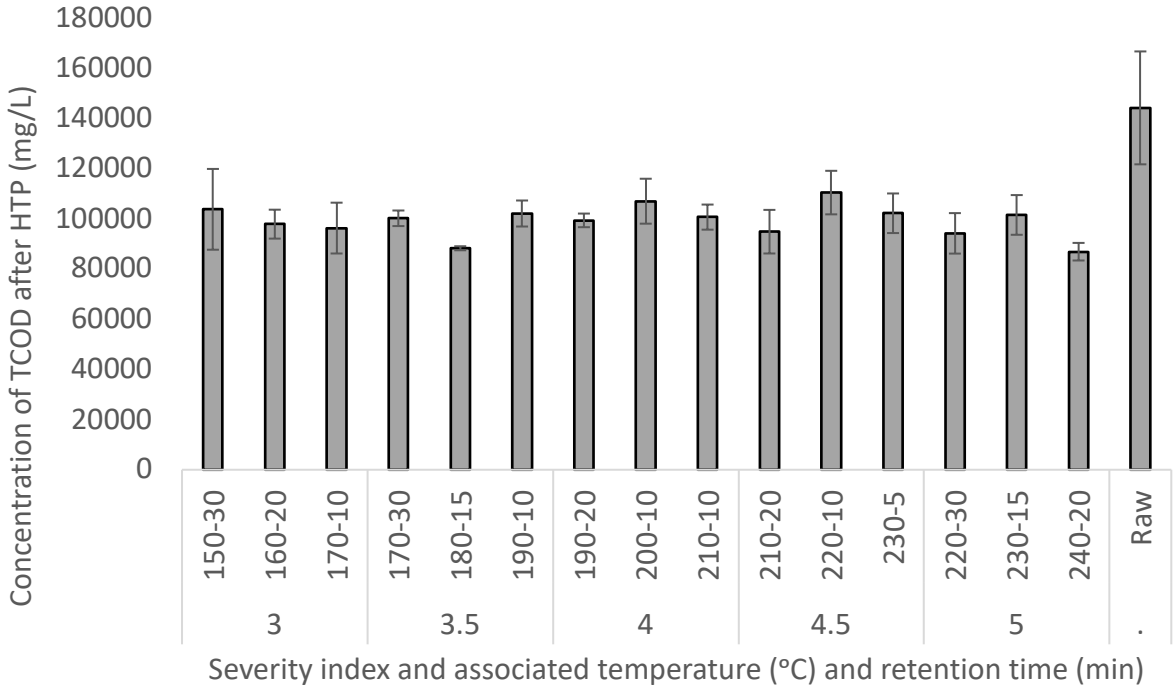


Figure A 20 Concentration of TCOD after HTP



Selected sample of hydrothermal pretreatment heating and cooling rate records

Figure A 21 "150℃-30min-69psi" (SI:3)

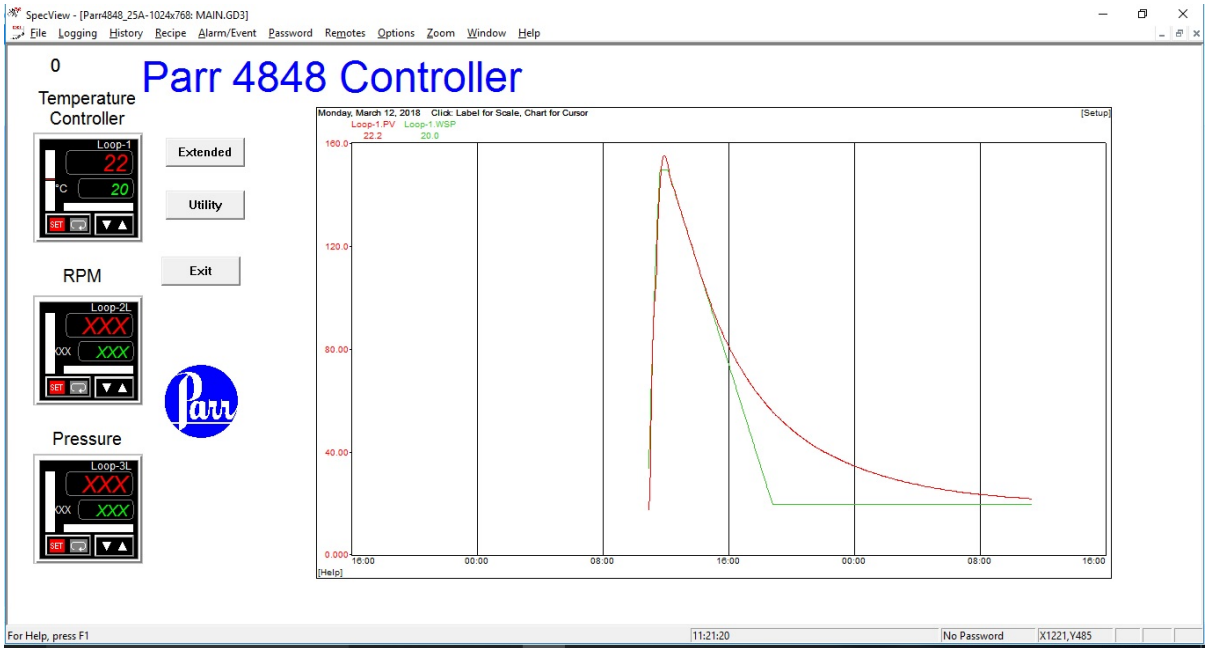


Figure A 22 "180℃ -15min-145psi" (SI:3.5)

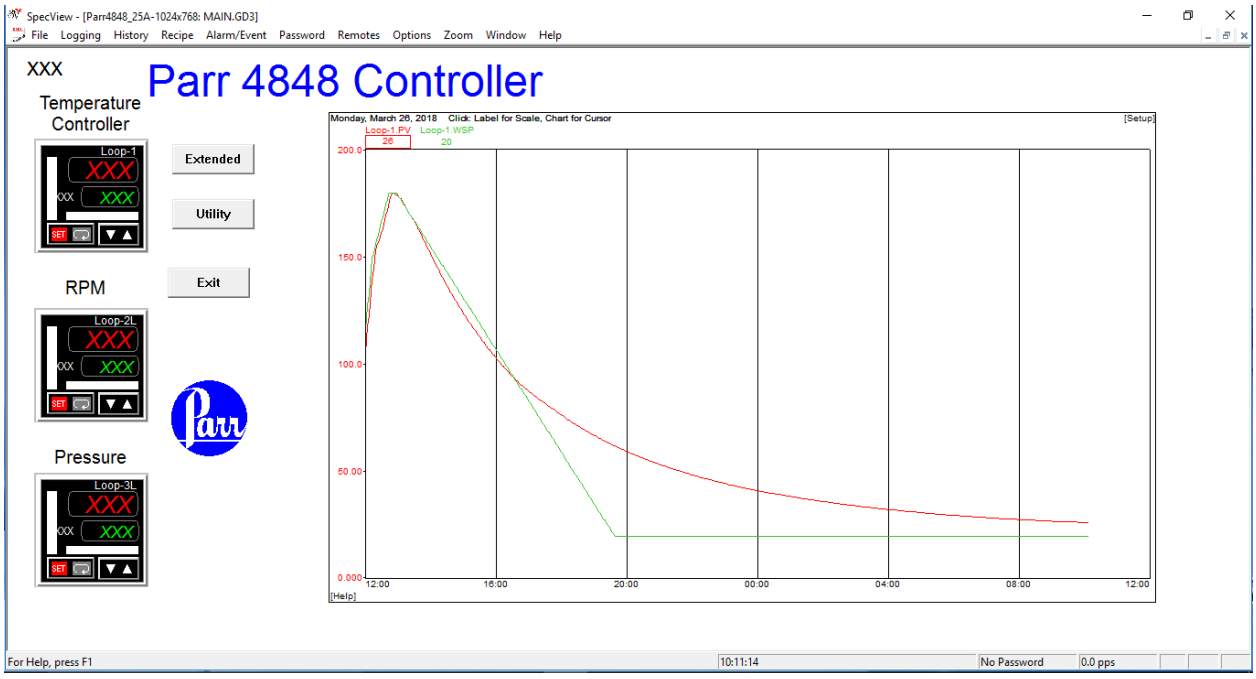


Figure A 23 "200℃ -10min-225psi" (SI:4)

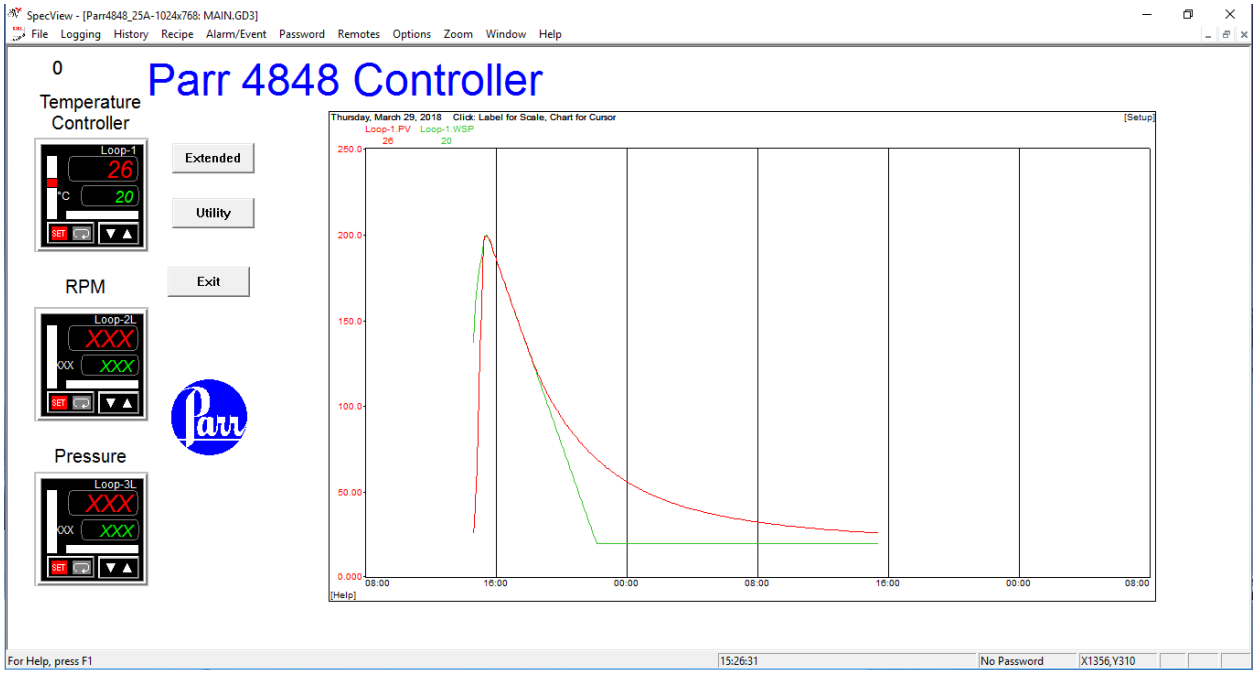


Figure A 24 "220℃ -10min-337psi" (SI:4.5)

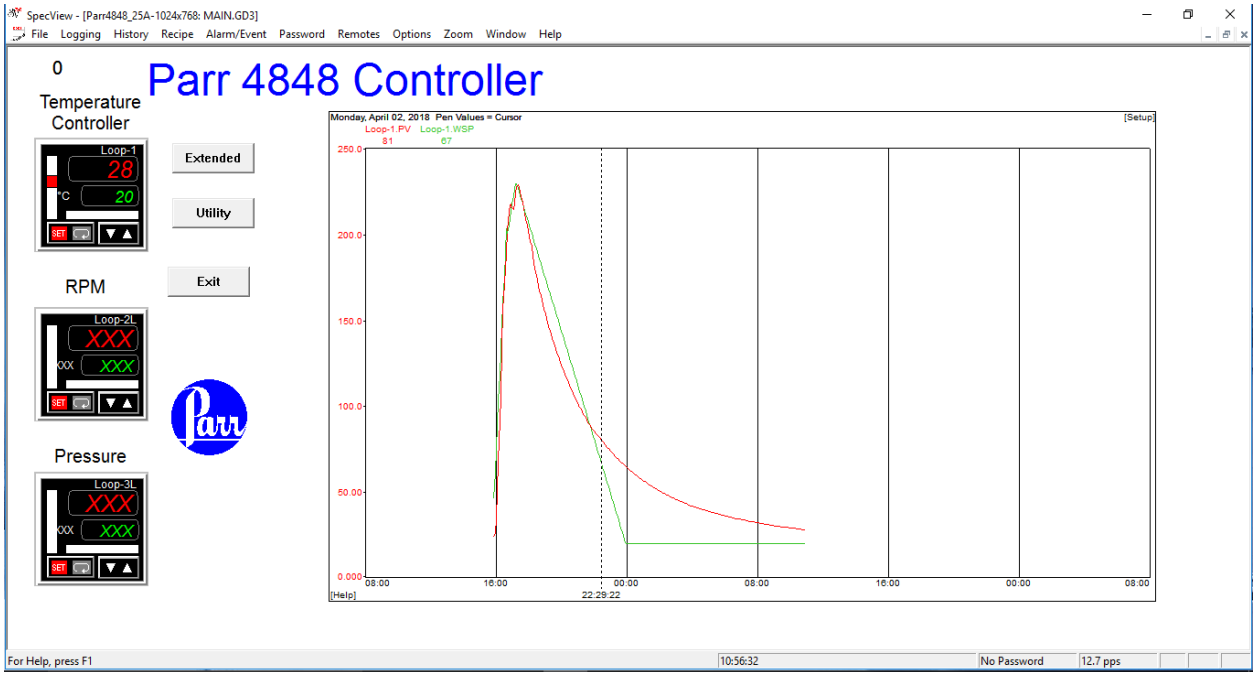
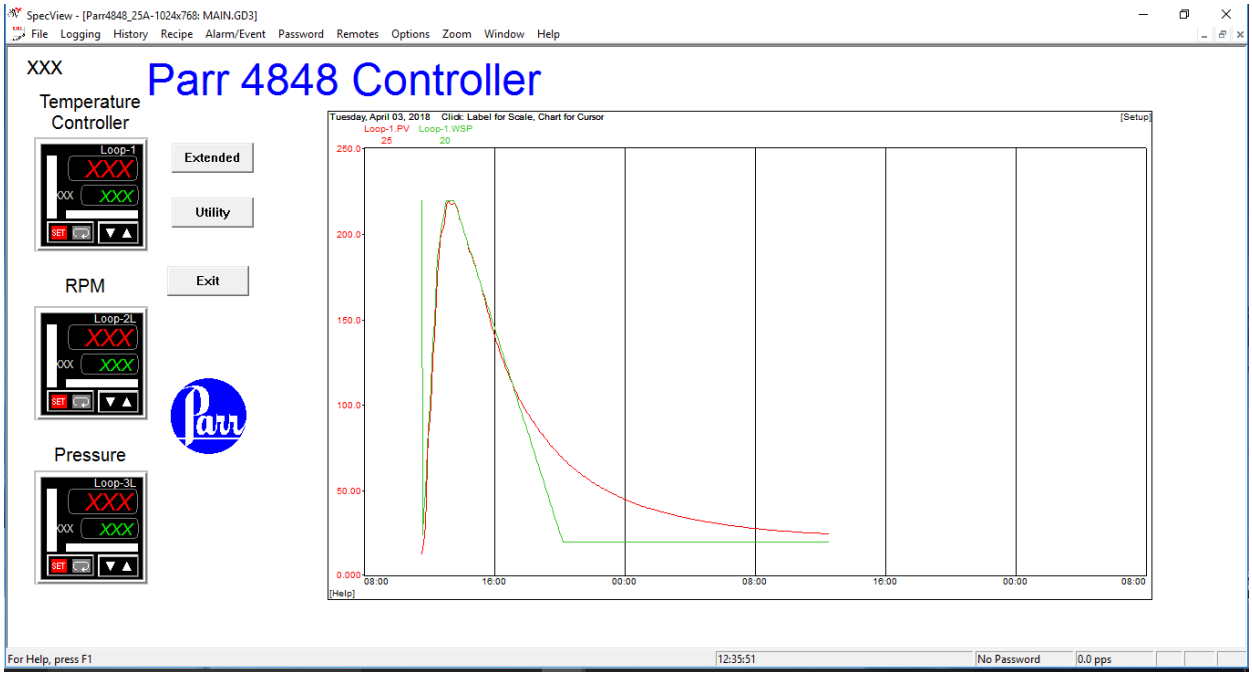


Figure A 25 "230℃ -15min-407psi" (SI:5)



24 SSO acidification

Figure A 26 Concentration of TSS after HTP

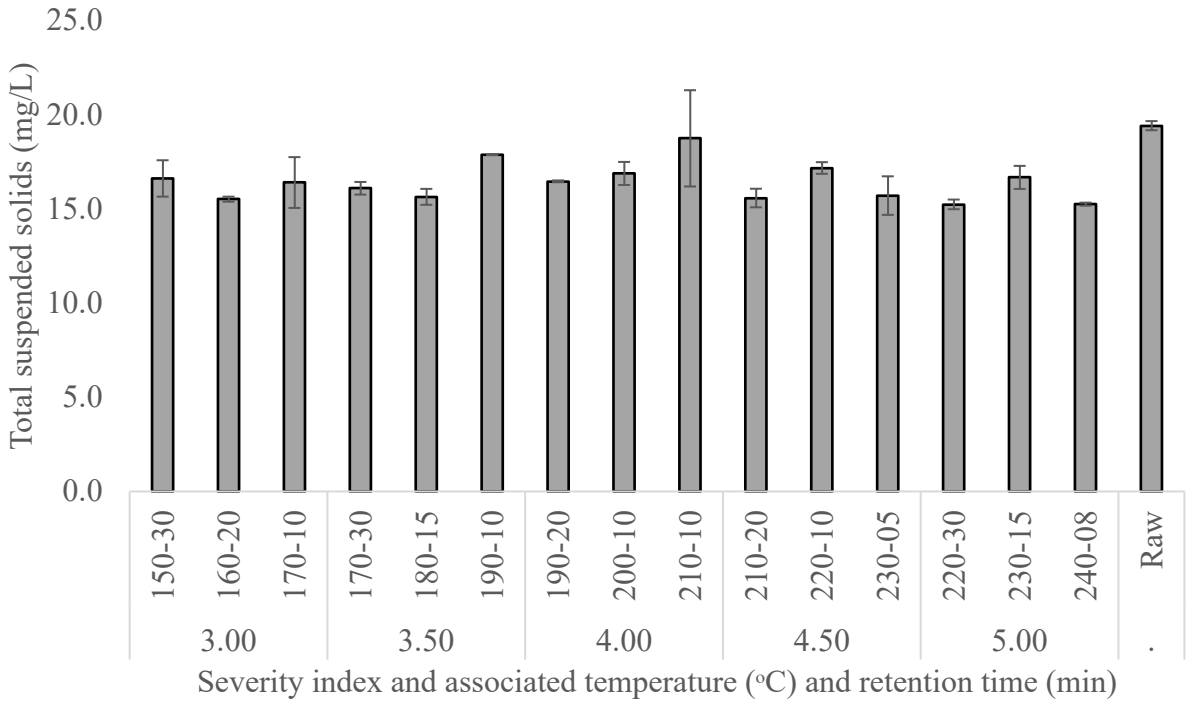


Figure A 27 Concentration of VSS after HTP

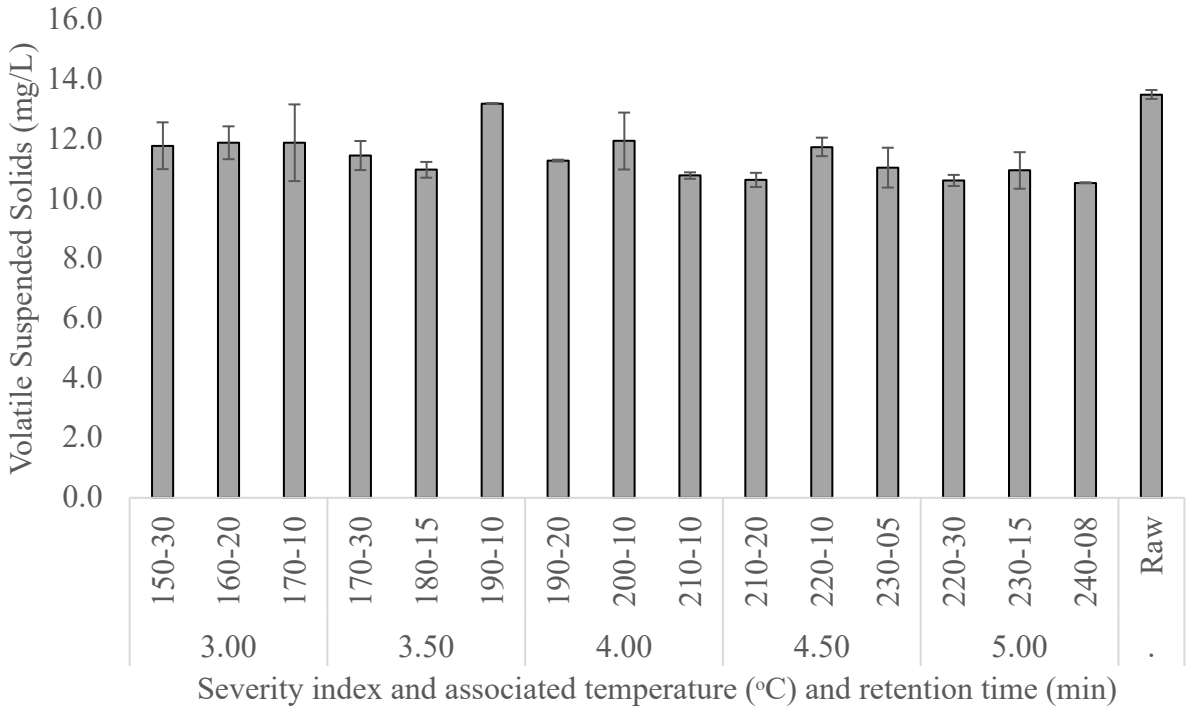


Figure A 28 Concentration of alkalinity after HTP

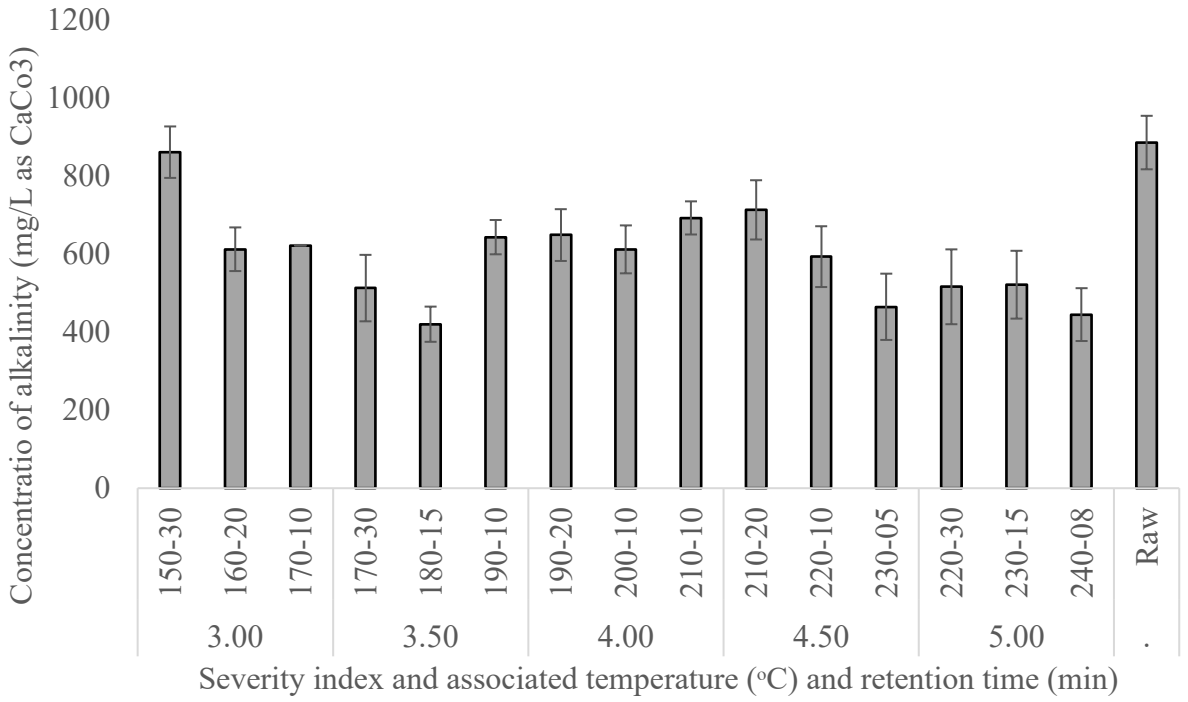


Figure A 29 Concentration of ammonia after HTP

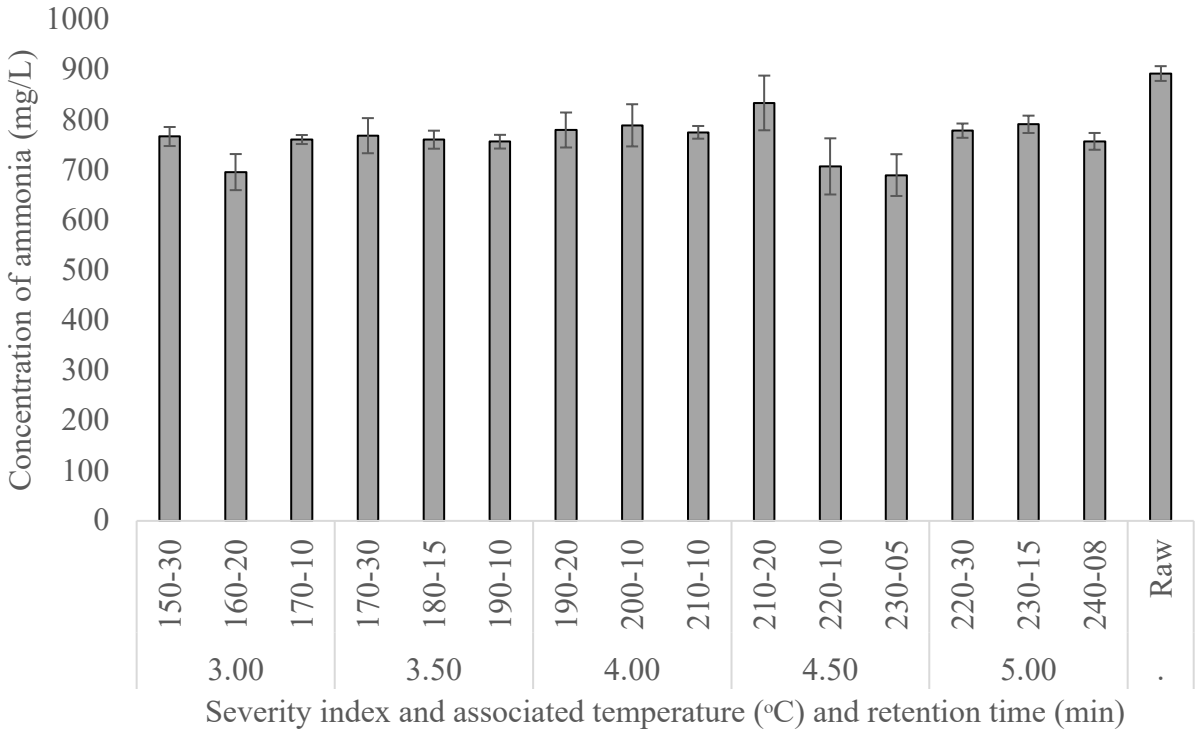


Figure A 30 Concentration of total carbohydrates after HTP

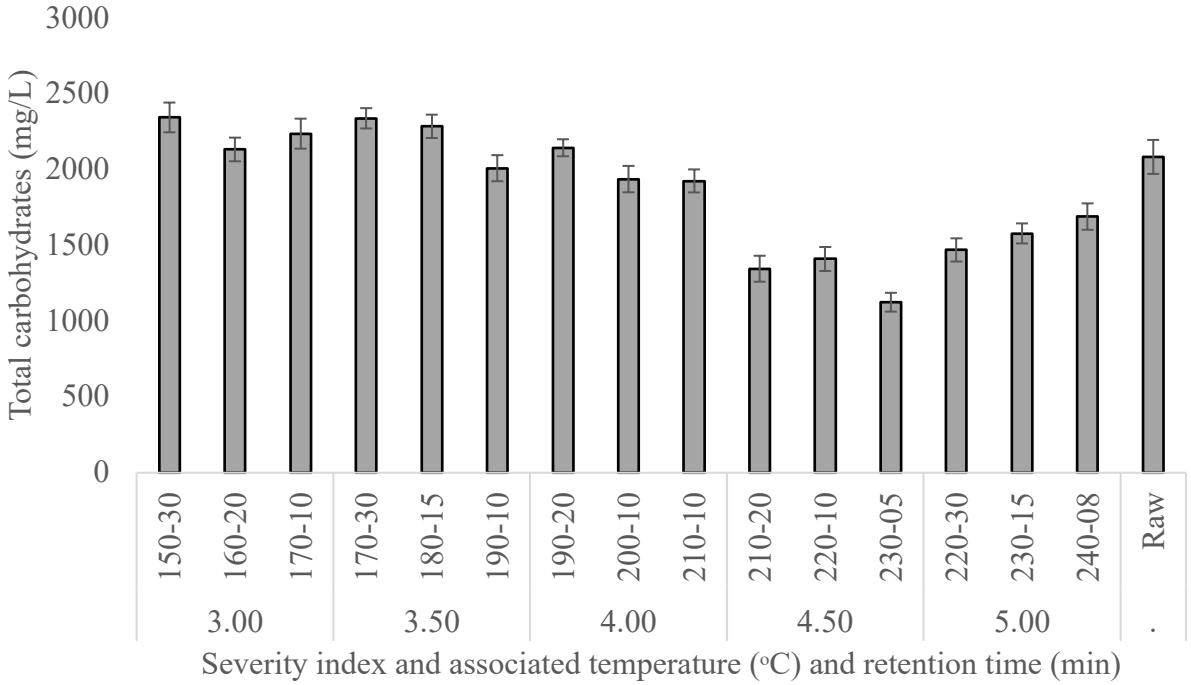


Figure A 31 Concentration of total proteins after HTP

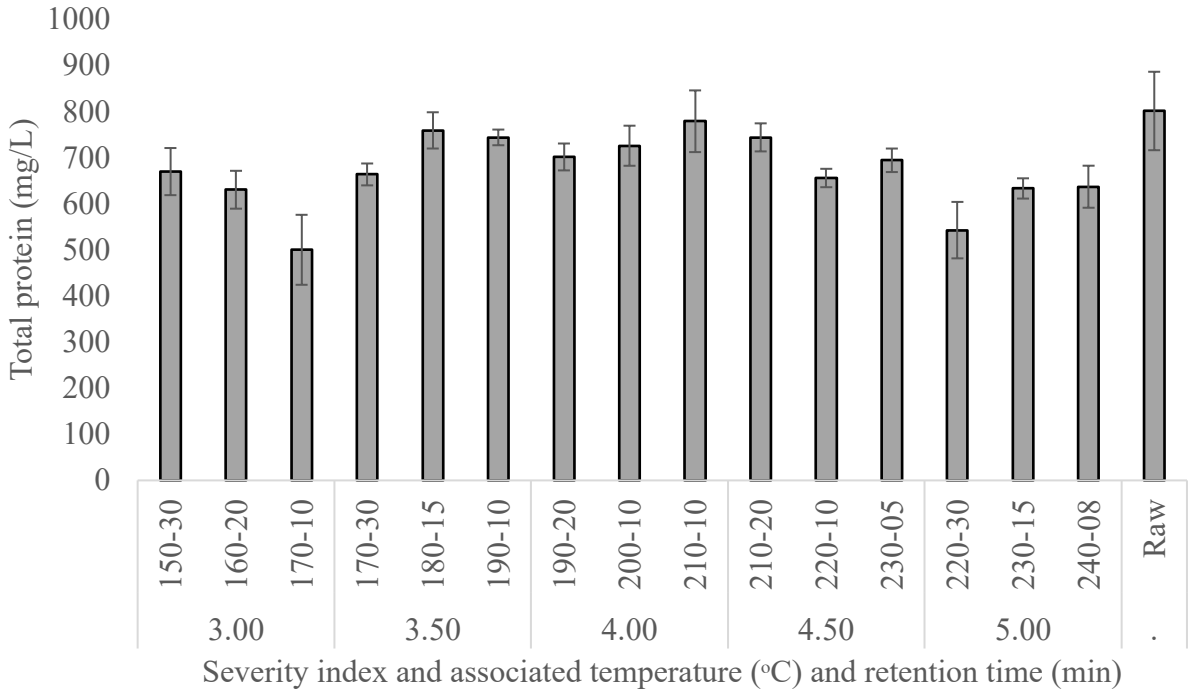


Figure A 32 Main effect plot for overall solubilization

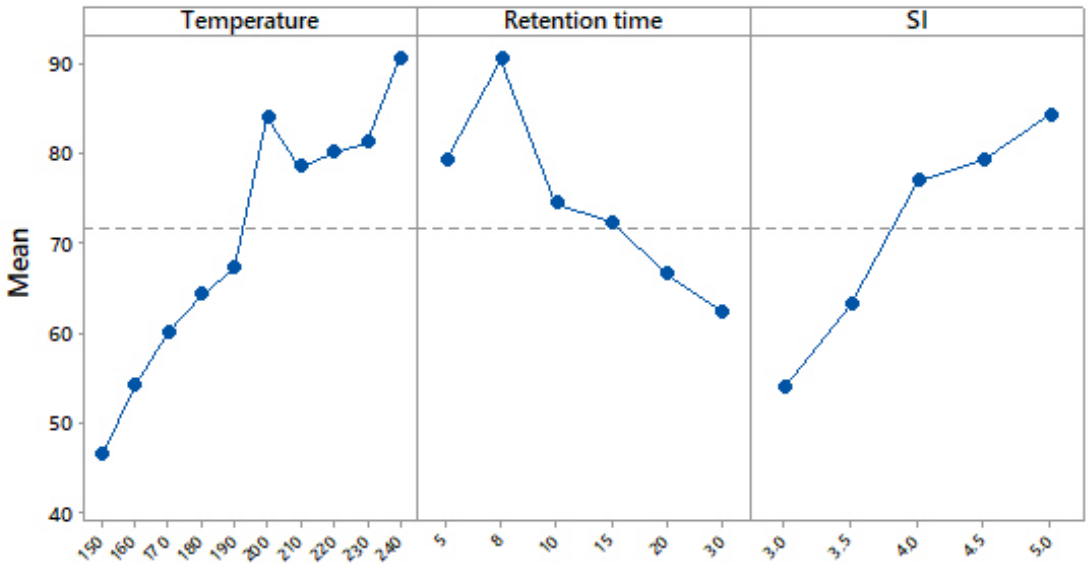


Figure A 33 Main effect plot for total VFA production

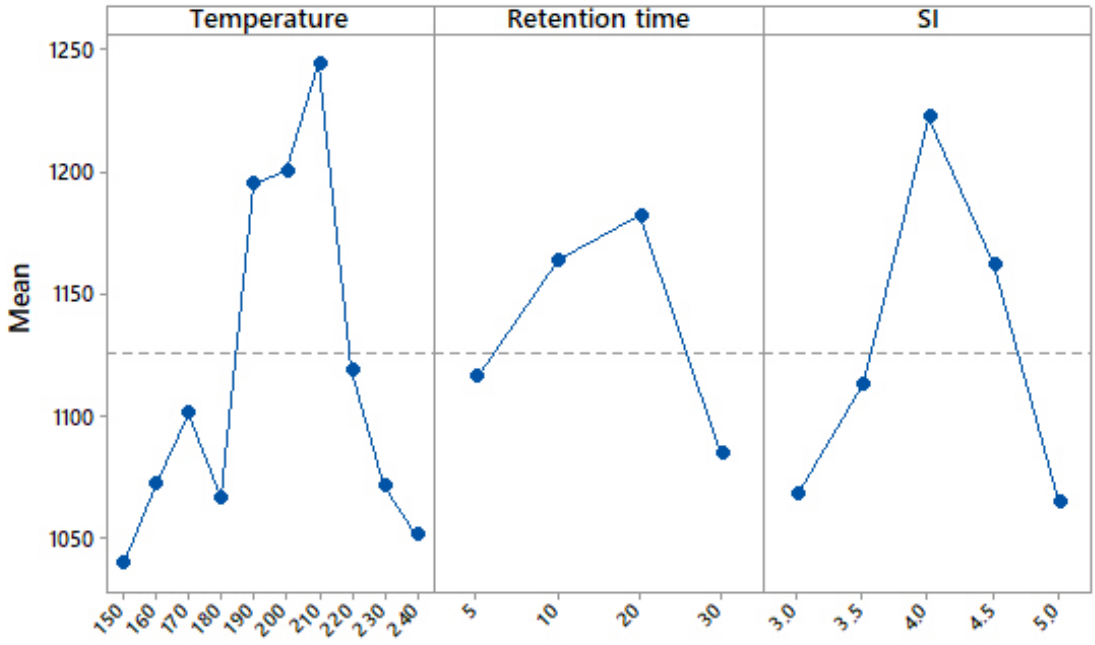


Figure A 34 Interaction plots of temperature, time, pressure and severity index for the VFAs production

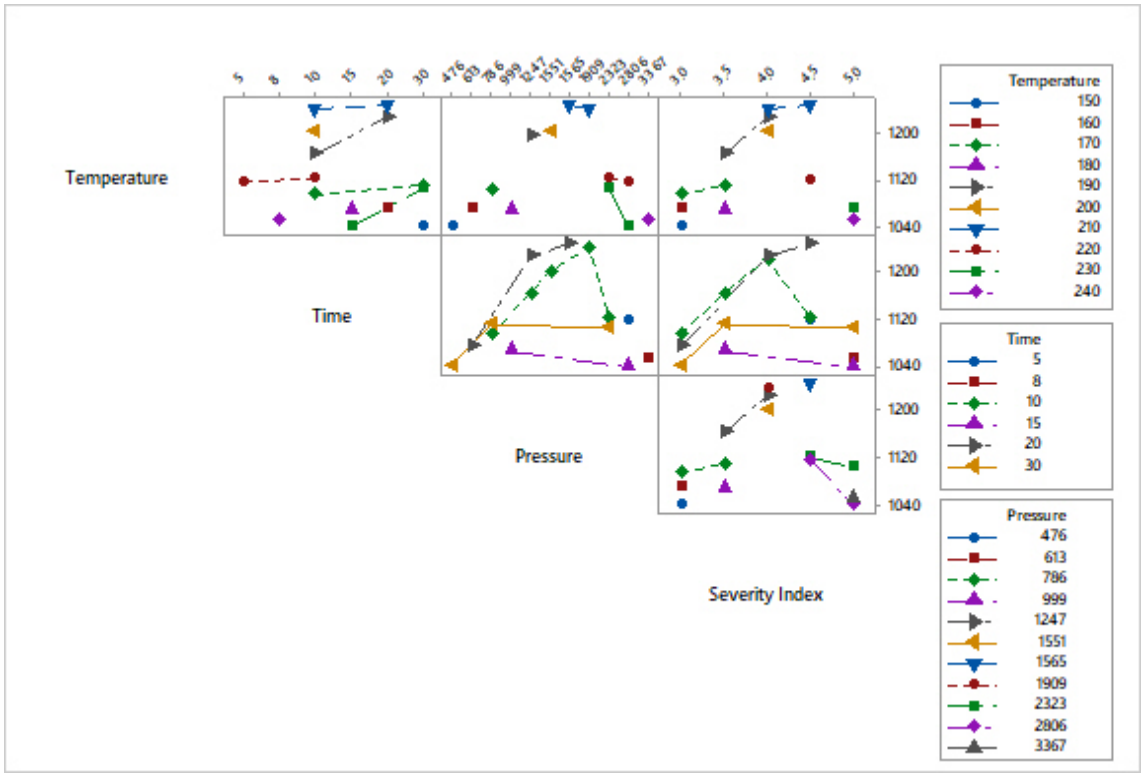

Temperature
Time
Pressure
Severity Index
5 8 10 15 20 30
476 613 786 999 1247 1551 1565 1909 2323 2806 3367
3.0 3.5 4.0 4.5 5.0
1200
1120
1040
Temperature
150
160
170
180
190
200
210
220
230
240
Time
5
8
10
15
20
30
Pressure
476
613
786
999
1247
1551
1565
1909
2323
2806
3367

Figure A 35 Correlation between three main responses and SI

	SI	Solubilization due to HTP	Solubilization due to acidification	VFA production
SI	1			
Solubilization due to HTP	0.91	1		
Solubilization due to acidification	-0.66	-0.45	1	
VFA production	0.86	0.75	-0.79	1

Figure A 36 Correlation between three main responses and HTP temperature

	Temp	Solubilization due to HTP	Solubilization due to acidification	VFA production
Temperature	1			
Solubilization due to HTP	0.91	1		
Solubilization due to acidification	-0.48	-0.35	1	
VFA production	0.28	0.19	-0.11	1

Figure A 37 Correlation between three main responses and Hydrothermal RT at lower SI

	RT	Solubilization due to HTP	Solubilization due to acidification	VFA production
RT	1			
Solubilization due to HTP	-0.99	1		
Solubilization due to acidification	-0.99	0.99	1	
VFA production	-0.99	0.99	0.99	1

Figure A 38 Correlation between three main responses and Hydrothermal RT at higher SI

	RT	Solubilization due to HTP	Solubilization due to acidification	VFA production
RT	1			
Solubilization due to HTP	0.99	1		
Solubilization due to acidification	0.14	0.02	1	
VFA production	0.47	0.35	0.94	1

Table A 23 Main responses after HTP and fermentation

SI	HTP condition: Temp-Time-Pressure	Solubilization due to HTP	solubilization due to acidification	Overall solubilization	TVFA (mg/L)
	(°C) (min) (psi)	(%)	(%)	(%)	Mg/L
3	150-30-69	14	32	46	3212
3	160-20-89	16	34	54	3311
3	170-10114	17	35	62	3377
3.5	170-30-114	19	36	58	3427
3.5	180-15-145	20	32	64	3295
3.5	190-10-181	21	39	67	3144
4	190-20-181	26	43	68	3313
4	200-10-225	26	40	84	3243
4	210-10-277	29	43	79	3351
4.5	210-20-227	32	43	78	3372
4.5	220-10-337	34	21	81	3381
4.5	230-05-407	34	16	79	3203
5	220-30-337	31	23	82	3412
5	230-15-407	29	18	80	3208
5	240-08-488	27	23	91	3355
0	Raw	0	27	27	3071

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