

Examining the mechanisms of short-term solubilization of ground food waste for high-rate anaerobic digestion



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ABSTRACT

Optimizing short-term solubilization is essential for high-rate anaerobic digestion of food waste. The purpose of this study was to measure the short-term kinetics of food waste solubilization and understand what mechanisms are responsible for driving food waste solubilization. Two solubilization assays were conducted to measure the solubilization of mechanically processed food waste. Three methods of mechanical grinding were utilized: an in-sink food disposer and a manual meat grinder with either small or medium size plate openings. A control assay was conducted to measure the release of endogenous soluble material into an aqueous medium. An enzyme assay was conducted to examine short-term food waste hydrolysis. The assays were diluted and buffered to prevent product and pH inhibition, respectively, and allow maximum solubilization to occur. Percent solubilization was calculated as the soluble chemical oxygen demand (COD) fraction of total COD. Data from the two assays were used to calculate the kinetics of endogenous solubilization and enzymatic hydrolysis. Regardless of grinding method, food waste was 27% solubilized within 1 h through endogenous solubilization. Enzymatic hydrolysis was responsible for an additional 29–31% solubilization by 4 h. This study showed that ground food waste was approximately 60% solubilized within 4 h under optimal conditions.

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1. Introduction

In the United States, food waste is a significant component of municipal solid waste and the vast majority of food waste is currently landfilled. In 2011, more than 36 million tons of food waste was disposed in the United States, or 21% of municipal solid waste, only 4% of which was diverted from landfills (USEPA, 2013). There are increasing efforts across the U.S. and Europe to divert food waste from landfills for beneficial use. One potential solution is anaerobic digestion, which creates biofuel and biofertilizer from food waste (Wilkie, 2008). Utilizing high-rate anaerobic digestion technology, such as fixed-film digestion, could improve the efficiency of anaerobic digestion of food waste. However, a significant hurdle to overcome in high-rate anaerobic digestion of food waste is optimizing the solubilization step (Wang et al., 2006; Liu et al., 2008). Various pretreatment methods, including thermal, freezing/thawing, enzymatic, and mechanical, are proposed as a

solution for increasing the solubilization of food waste and, therefore, the rate and extent of food waste anaerobic digestion. In order to examine the efficacy of pretreatment, it is first necessary to understand the mechanisms through which food waste solubilizes.

Organic matter in food waste can be represented as total chemical oxygen demand (TCOD), which can be divided into two primary fractions: water-soluble organic material and particulate organic material. Food waste contains endogenous intracellular and extracellular water-soluble organic material, as measured by soluble chemical oxygen demand (SCOD). In the present study, SCOD is any organic matter that can pass through a 0.45 µm filter. Particulate organic material, as measured by particulate chemical oxygen demand (PCOD), is any larger organic material that cannot pass through the filter. The relative proportions of SCOD and PCOD in food waste depend on the individual components of the food waste. Components with high sugar contents, such as fruits, generally have a higher proportion of SCOD, due to the ready solubility of simple sugars such as glucose and fructose. More fibrous components tend to have higher PCOD. In raw foods, SCOD is typically intracellular, such as cytoplasm contained within cell walls and cell membranes, while PCOD is comprised of the cell walls and cell membranes or larger intracellular structures, such as plastids. Some cooked or processed food waste components, such as bread and

Abbreviations: COD_{sf}, The soluble fraction of total chemical oxygen demand; COD_{esf}, COD_{sf} in the endogenous solubilization (control) assay; COD_{hsf}, COD_{sf} in the hydrolytic enzyme solubilization assay due only to enzymatic hydrolysis.

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cheese, are acellular as the cells have been disrupted and extruded during processing. In these processed foods, the SCOD is extracellular and is readily released when placed in water.

Solubilization is a critically important step in anaerobic digestion because the microbial consortia require organic matter in a soluble form for cellular assimilation. The rate at which particulate material is solubilized can determine the overall kinetics of anaerobic digestion and the overall vitality of the microbial consortia. Because anaerobic digestion necessitates acidogenesis of neutral solubilized compounds, there must be sufficient alkalinity in the digester to buffer the free organic acid intermediates. The rate and extent to which organic matter is solubilized and subsequently fermented into organic acids determines the alkalinity requirement of the digester. Thus, solubilization kinetics may dictate the required digester volume and the amount of base required for pH control. A pretreatment reactor for solubilization and/or acidification in a two-phase system may be beneficial in optimizing anaerobic digestion depending on the solubilization kinetics of the feedstock (Wang et al., 2006; Liu et al., 2008). Solubilization kinetics are also impacted when considering effluent recycling since the enzymes and microorganisms in the effluent may facilitate solubilization (Lü et al., 2008).

For particulate feedstocks, such as food waste, solubilization has been considered the rate-limiting step of the entire digestion process (Eastman and Ferguson, 1981; Palmowski and Müller, 2003; Wang et al., 2006; Izumi et al., 2010). The ratio between SCOD and PCOD endogenous to the feedstock is critical for the overall anaerobic digestion kinetics (Prashanth et al., 2006). While SCOD can be readily assimilated by the microbial consortia, PCOD must be hydrolyzed by extracellular hydrolytic enzymes produced by the microbial consortia. There are many factors that limit enzymatic hydrolysis rates. Four principal factors that limit enzymatic hydrolysis are adequate enzyme levels, substrate characteristics (particulate surface area and resistance to degradation), pH inhibition of enzyme activity, and product inhibition of enzyme kinetics. The characteristics of particulate material present in food waste significantly impact the kinetics of enzymatic hydrolysis (Neves et al., 2008). More recalcitrant materials, such as cellulose, structural proteins, and other oligomers of lipids, proteins and/or carbohydrates, have slower hydrolysis kinetics than more easily degradable materials, such as starch. Disruption of the substrate cells and tissues can increase the substrate availability to hydrolytic enzymes (Izumi et al., 2010). Enzymatic hydrolysis can also be limited by pH or product inhibition (Veeken et al., 2000; He et al., 2006). Because acidogenesis tends to lower pH, it is important that acids are either buffered or rapidly converted to CH₄ and CO₂ to maximize hydrolysis and anaerobic digestion kinetics. Although acidification in

anaerobic digestion is typically more detrimental to acetogenesis and methanogenesis, an excessively low pH can also reduce enzymatic hydrolysis due to reduced enzyme activity outside of the enzymes' pH optima. This can be potentially problematic in a two-phased anaerobic digestion system with a separate hydrolytic/acidogenic reactor, which tends to operate at a lower pH than the methanogenic reactor. Accumulation of hydrolysis end-products, which can result if acidogenesis is reduced, may also inhibit enzymatic hydrolysis and consequently limit acetogenesis because there are insufficient acid intermediates available to the acetogens. Product inhibition can be overcome with robust microbial consortia that rapidly consume these end-products or by dilution with an aqueous phase, which requires a larger digester volume. Active microbial consortia can also reduce pH inhibition by consuming the products of acidogenesis and preventing acidification.

Optimal anaerobic digestion of food waste relies on balanced microbial consortia. The vitality of the consortia depends on a supply of organic intermediates consistently available supply of organic intermediates for microbial assimilation. The short-term solubilization kinetics determine the amount of soluble organic material that is rapidly available to the acidifying microorganisms and subsequently to the acetogenic and methanogenic microorganisms. Kim et al. (2012) demonstrated that in a thermophilic anaerobic digester fed with food waste and sewage sludge, higher rates of acetogenesis and methanogenesis are supported by higher hydrolytic enzyme activity compared to mesophilic conditions; hence, the rate of hydrolysis can be the driving factor in overall anaerobic digestion kinetics.

The purpose of the present study was to examine the short-term solubilization kinetics of ground food waste and to understand what mechanisms are responsible for driving and limiting short-term solubilization when pH and product inhibition are excluded. Mechanically ground food waste was used in this study to maximize solubilization by rupturing cells and disrupting tissues. In order to measure the relative contribution of endogenous solubilization and enzymatic hydrolysis, two different solubilization assays were conducted. A control assay measured the release of endogenous SCOD by diluting mechanically ground food waste with a buffered aqueous solution. An enzyme assay measured enzymatic hydrolysis of mechanically ground food waste in the presence of excess hydrolytic enzymes in a buffered aqueous medium, such that enzymes were not limiting and the maximum rate of hydrolysis could be achieved. By using identical food waste under identical assay conditions, the kinetics of both endogenous SCOD release and enzymatic hydrolysis could be determined.

2. Materials and methods

2.1. Standard food waste

Due to the general heterogeneity of food waste, a standardized food waste was developed for the present study to allow repeatable experiments and directly comparable data. The standard food waste consisted of fresh food waste constituents representing the major macromolecule groups of food waste: carbohydrates, proteins, and fats (Table 1). The constituents were proportioned to simulate actual food waste analogous to that generated from local food service establishments (i.e. restaurants and schools). Fresh food waste was used because it would allow the immediate and short-term solubilization to be measured, unlike actual food waste collected from a food service area, which may have already begun the solubilization process. To ensure that the standard food waste was representative of food waste generated in the community, the physicochemical properties of the standard food waste were compared to the physicochemical parameters of food waste

Table 1
Composition of standard food waste.

Components	Percent composition (% ww ^a)	Percent composition (% of TCOD ^b)	Representative organic macromolecule
Apple, Red Delicious	24	11.9	Carbohydrate (sugar, pectin)
Potato, Russet	24	13.7	Carbohydrate (starch)
Beans, red kidney (canned, drained and rinsed)	20	21.2	Protein
Broccoli (florets)	12	4.3	Carbohydrate (cellulose)
Bread, white hamburger bun	12	29.7	Carbohydrate (starch)
Cheese, Sharp Cheddar	8	19.2	Protein, lipid

^a ww = wet weight.

^b TCOD = Total chemical oxygen demand.

collected from local schools and restaurants (Table 2). Thirty samples of daily food waste generated from three local schools and fifty samples of daily food waste generated from three local restaurants were analyzed in triplicate (Table 2). The standard food waste was within the mean \pm one standard deviation range for each property measured on the school and restaurant food waste.

2.2. Hydrolytic enzymes

A commercial digestive enzyme formulation (Digest[®], Enzymedica Inc., Venice, FL) was applied in excess in order to measure the maximum short-term hydrolysis of ground food waste. The formulation contained a variety of hydrolytic enzymes including cellulases, amylases, proteases and lipases, *inter alia*, obtained from *Aspergillus niger* and *Aspergillus oryzae*. Preliminary studies indicated that the enzymes were not limiting at a dosage rate of 1 g per 500 g of food waste. Therefore, to ensure the enzymes were applied in excess, a dosage rate of 1 g per 10 g of food waste was used in the enzyme assay.

2.3. Mechanical grinding methods

The standard food waste in this study was ground using three different methods: an in-sink food disposer (Badger 9 model, InSinkErator, Racine, WI) with a strainer hole size of 0.75 cm, and a manual meat grinder with two different extrusion plates that had either medium (1.0 cm) or small (0.5 cm) plate openings (Grizzly H7778 No 32, Grizzly Industrial, Inc., Bellingham, WA). The strainer hole and plate opening dimensions only determined the maximum particle size that could pass through the disposer or grinder, but did not determine the overall particle size reduction of the ground food waste. The primary driver in particle size reduction in the meat grinder was the shear action of the helical impeller against the wall of the grinder. These grinding methods were selected as bench-scale analogues of commercial-scale food waste grinding equipment to represent different mechanical maceration techniques.

To ensure representativeness, 500 g of the standard food waste was formulated and ground separately by each grinding method. For the grinder treatment, 500 g of food waste was coarsely chopped and then fed through the grinder. Ground food waste was mixed by hand and 10 g (wet weight (ww)) representative samples were obtained for use in the assays. For the disposer treatment, 500 g of food waste was flushed through the disposer using 500 mL of deionized (DI) water, resulting in a 1:1 mixture of food waste and water from which 20 g (ww) representative samples were obtained for use in the assays. To account for any variation in the food waste, TCOD was measured in triplicate on each formulation (i.e. for each grinding method in each assay) and was used to normalize the solubilization data as a percent of TCOD.

Table 2

Physicochemical properties of standard food waste and food waste collected from local schools and restaurants.

Property	Standard food waste (mean \pm SD)	School/Restaurant food waste (mean \pm SD)
Total solids (TS) (%)	29.94 \pm 0.20	28.8 \pm 13.5
Volatile solids (% TS)	95.41 \pm 0.001	91.0 \pm 5.8
Total COD (g kg ⁻¹ ww ^a)	348.89 \pm 22.87	376.9 \pm 184.3
Soluble COD (g kg ⁻¹ ww)	87.9 \pm 2.7	–
Conductivity (mS cm ⁻¹)	4.37 \pm 0.16	–
Alkalinity (mg CaCO ₃ eq. kg ⁻¹ ww)	1344.8 \pm 3.67	–
Total nitrogen (% TS)	3.08 \pm 0.12	3.03 \pm 0.812
Total phosphorus (% TS)	0.32 \pm 0.0008	0.41 \pm 0.18

^a ww = wet weight.

2.4. Solubilization assays

Two solubilization assays were conducted for 8 h to measure the short-term solubilization kinetics of food waste. A control assay was conducted by adding freshly ground food waste to buffered water to measure the maximum short-term release of endogenous SCOD from food waste. An enzyme assay was conducted by adding freshly ground food waste and excess hydrolytic enzymes to buffered water to measure the maximum short-term solubilization due to enzymatic hydrolysis. Each assay was conducted in triplicate for each type of grinding method. The complete experimental regime is shown in Table 3. Assays were conducted in 1 L glass beakers maintained at 35 °C in a water bath (Versa-Bath S Model 236, Thermo Fisher Scientific, Waltham, MA) under static conditions. Beakers were covered with plastic film (Glad[®] Cling Wrap) held with a rubber band to reduce evaporation and potential contamination. To prevent pH inhibition, the assay was buffered with a phosphate buffer (0.5 M NaOH titrated into 0.5 M KH₂PO₄ to pH 6.5). To prevent product inhibition, food waste was diluted to 3.5 g COD L⁻¹ in buffered DI water. Twenty mL aliquots were obtained from each replicate to measure SCOD at 0.1, 1, 2, 4, 6, and 8 h. Replicates were thoroughly mixed with a magnetic stir bar while sampling. The SCOD measured in each assay was normalized as a percent of the TCOD of that specific formulation of the standard food waste to calculate the soluble fraction of chemical oxygen demand (COD_{sf}). The COD_{sf} of the control assay represented the release of soluble endogenous material (COD_{esf}). The COD_{sf} of the enzyme assay represented both the soluble organic matter produced through enzymatic hydrolysis (COD_{hsf}) and the COD_{esf}. Thus, the COD_{sf} of the enzyme assay minus the COD_{sf} of the control assay (COD_{esf}) represents the COD_{hsf}. In the enzyme assay, SCOD of the enzyme cocktail itself was measured in triplicate and the average value was subtracted from all measurements.

2.5. Statistical analysis

First-order kinetics were assumed for regression analysis of endogenous solubilization and enzymatic hydrolysis as follows: $COD_{sf_t} = COD_{sf_f} * (1 - e^{-kt})$, where COD_{sf_t} was the SCOD fraction of TCOD at time t , COD_{sf_f} was the maximum SCOD fraction of TCOD, and k was the kinetic rate constant (h⁻¹). The solver add-in in Microsoft Excel 2007 was utilized to solve for COD_{sf_f} and k by minimizing the residual sum of squares of triplicate data points. The standard error of each parameter estimate was calculated using the square root of the variance calculated from the change in residual sum of squares around the optimal parameter estimate. A Student's t -test ($\alpha = 0.01$) was used to determine significant differences between means of parameter estimates.

Table 3

Experimental regime for solubilization assays of ground food waste.

	Control assay	Enzyme assay
Food waste	10 g (ww ^a)	10 g (ww)
Phosphate buffer	250 mL	250 mL
Commercial enzyme	N/A	1 g
Total volume	1 L (640 mL deionized water)	1 L (640 mL deionized water)
Loading rate	3.5 g COD L ⁻¹	3.5 g COD L ⁻¹
Grinding methods ^b	Grinder (small plate opening)	Grinder (small plate opening)
	Grinder (medium plate opening)	Grinder (medium plate opening)
	Disposer	Disposer

^a ww = wet weight.

^b Assays were conducted in triplicate for each grinding method.

2.6. Analytical methods

Total solids (TS), volatile solids (VS), TCOD, SCOD, conductivity, alkalinity, total nitrogen (TN), and total phosphorous (TP) were measured on the standard food waste to characterize the substrate. Soluble COD, organic acids, and sugars were measured on aliquots taken throughout the assay, and pH was measured at the time of sampling to confirm the assay did not vary beyond pH 6.4–6.5 (data not shown). Total solids and VS were measured according to standard methods (APHA, 2005) by drying 100 g (ww) samples at 103 °C for 24 h and ashing at 550 °C for 2 h. Total COD and SCOD were measured using Hach COD reagent tubes (HR 20–1500 mg COD/L) according to standard methods (APHA, 2005). For TCOD, samples were fully homogenized using a 360 mL stainless steel blender for 1 min on high speed prior to sampling for analysis. For SCOD, fully homogenized samples of the standard food waste and aliquots from the assay were placed in a refrigerated water bath at 4 °C to quench metabolic activity and subsequently centrifuged at 12,000 rpm for 30 min in a refrigerated centrifuge at 4 °C. Supernatant was then filtered through a 0.45 µm nylon syringe filter. Conductivity was measured on an Accumet Model 30 conductivity meter (Thermo Fisher Scientific, Waltham, MA) after diluting food waste 1:1 in DI water. Alkalinity was measured according to standard methods (APHA, 2005) after diluting food waste 1:1 in DI water. To measure TN and TP, samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). The catalyst used was 1.5 g of 9:1 K₂SO₄:CuSO₄, and digestion was conducted for at least 4 h at 375 °C using 6 mL of H₂SO₄ and 2 mL H₂O₂. Nitrogen and phosphorus in the digestate were measured by semiautomated colorimetry (Hambleton, 1977). Organic acids and sugars were measured by high-pressure liquid chromatography (HPLC) using an HP 1090 Series II chromatograph (Hewlett Packard, Palo Alto, CA) equipped with a Bio-Rad Aminex HPX-87H ion exclusion column (45 °C; solvent phase of 4 mM H₂SO₄; flow rate of 0.5 mL min⁻¹; injection volume of 10 µL) and dual detectors (refractive index detector and UV detector set at 210 nm). One mL of each aliquot from SCOD analysis was filtered using a 0.2 µm nylon syringe filter. Ten µL of 0.1 N sulfuric acid were added to 1 mL samples. Samples were analyzed with standards for glucose, xylose, lactic acid, succinic acid, formic acid, acetic acid, propionic acid, butyric acid, and ethanol. The pH of the assays was measured using an Accumet Model 10 pH meter with a Ross Sure Flow combination electrode.

3. Results

3.1. Control assay

Fig. 1 shows the SCOD, glucose, and xylose released from freshly ground food waste into aqueous solution (control assay). The SCOD measurements for all three grinding methods were similar (data not shown); therefore, data is shown for the grinder (small plate) as a representative grinding method. Immediately after grinding (0.1 h), food waste ground with the grinder (small plate) reached 834.0 ± 45.2 mg SCOD L⁻¹ and within 1 h reached 879.3 ± 21.4 mg SCOD L⁻¹. After this initial solubilization, SCOD remained constant throughout the remainder of the 8 h in the control assay. Glucose levels reached 149.4 ± 13.1 mg COD L⁻¹ (140.2 ± 12.3 mg glucose L⁻¹) immediately after grinding and remained relatively constant throughout the remainder of the assay, averaging 15% of the SCOD. Xylose levels reached 16.2 ± 0.7 mg COD L⁻¹ (15.2 ± 0.7 mg xylose L⁻¹) immediately after grinding and remained constant throughout the remainder of the assay, averaging 1.7% of the SCOD. This indicated that the soluble sugars, as a constituent of the SCOD, were released into solution

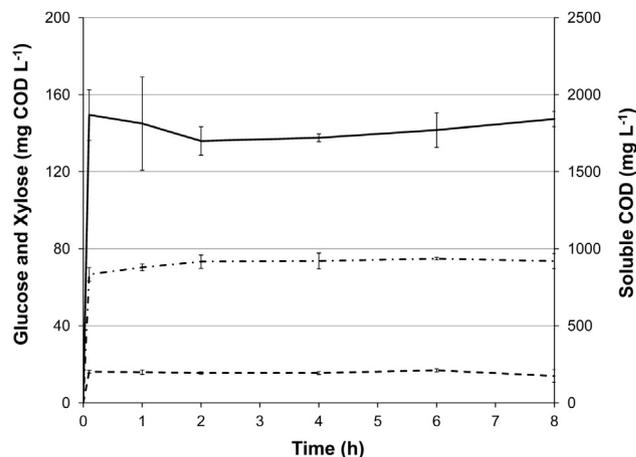


Fig. 1. Soluble COD, glucose and xylose analysis of ground food waste in aqueous solution. Data shown is for the grinder (small plate): Soluble COD (—●—); Glucose (—); Xylose (---).

immediately. No organic acids were detected in the 8 h of the assay, which indicated that the solubilized organic matter was not fermented through acidogenesis during the assay.

The SCOD measurements were normalized as the fraction of TCOD solubilized through endogenous solubilization in the control assay (*CODesf*). Fig. 2 shows *CODesf* of the three grinding methods at 0, 0.1, 1, 2, 4, 6, and 8 h (data points). Food waste ground with the grinder (small plate) was $25.4 \pm 1.4\%$ solubilized immediately following grinding (0.1 h), while food waste ground with the grinder (medium plate) and disposer was $19.5 \pm 1.1\%$ and $21.1 \pm 0.4\%$ solubilized at 0.1 h, respectively. By 1 h, food waste solubilization plateaued at $27.4 \pm 1.5\%$ for all three grinding methods for the remainder of the assay. The *CODesf* data were used for regression analysis to estimate the first-order rate constant (*k*) and the maximum *CODesf* (*CODesf_f*) (Table 4). The regressions are also shown in Fig. 2. The grinder (small plate) resulted in a significantly higher first-order rate constant (24.3 ± 2.6 h⁻¹) than the grinder (medium plate) (12.7 ± 1.4 h⁻¹) and the disposer (14.9 ± 0.9 h⁻¹). For all three grinding methods, *CODesf_f* was approximately 27%.

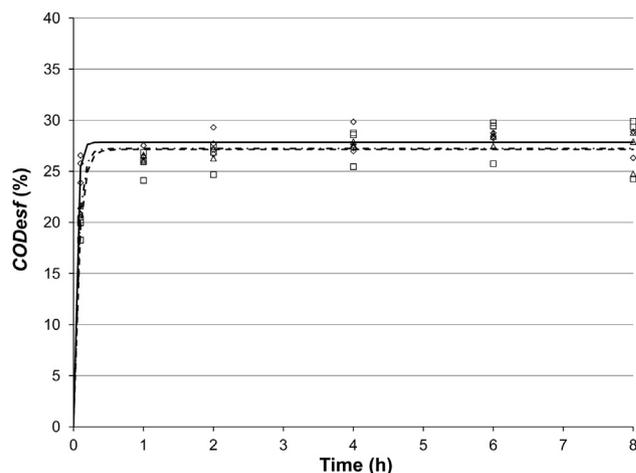


Fig. 2. Endogenous solubilization of ground food waste (triplicate data points with regressions): Grinder, small plate (—◇— regression, ◇ data); Grinder, medium plate (---□--- regression, □ data); Disposer (—●— regression, △ data).

Table 4

Regression parameter estimates for endogenous solubilization and estimated 4 h endogenous solubilization of ground food waste.

	k (h^{-1})	$\text{COD}_{\text{hsf}} (\%)^{\text{a}}$	$\text{COD}_{\text{hsf}_4} (\%)^{\text{b}}$
Grinder (small plate opening)	24.3 ± 2.6 x	27.8 ± 0.3 x	27.8
Grinder (medium plate opening)	12.7 ± 1.4 y	27.1 ± 0.4 x	27.1
Disposer	14.9 ± 0.9 y	27.2 ± 0.2 x	27.2

Different letters (x, y) in the same column represent significant difference ($\alpha = 0.01$).

^a COD_{hsf} = maximum fraction of TCOD solubilized through endogenous solubilization.

^b $\text{COD}_{\text{hsf}_4}$ = fraction of TCOD solubilized through endogenous solubilization after 4 h.

3.2. Enzyme assay

Fig. 3 shows the SCOD, glucose, and xylose released from freshly ground food waste into aqueous solution with excess hydrolytic enzymes (enzyme assay). The SCOD measurements for all three grinding methods were similar (data not shown); therefore, data is shown for the grinder (small plate) as a representative grinding method. Immediately after grinding (0.1 h), food waste ground with the grinder (small plate) reached 928.0 ± 22.0 mg SCOD L^{-1} and by 4 h reached 1897.3 ± 76.0 mg SCOD L^{-1} . After 4 h, solubilization remained relatively constant. Glucose levels reached 886.2 ± 44.5 mg COD L^{-1} (831.3 ± 41.7 mg glucose L^{-1}) by 4 h and remained relatively constant throughout the remainder of the assay, averaging 47% of the SCOD. The higher amount of glucose as a percent of SCOD compared to the control assay indicated that the hydrolytic enzymes were rapidly hydrolyzing the food waste into glucose monomers. Xylose levels did not increase along with the glucose and SCOD and were similar to levels in the control assay, reaching 17.7 ± 2.2 mg COD L^{-1} (16.6 ± 2.1 mg xylose L^{-1}) immediately after grinding and remaining constant throughout the remainder of the assay, averaging 1.0% of the SCOD. This indicated that the relatively limited amount of xylose in the substrate was released immediately and no further xylose monomers were produced by hydrolysis. No organic acids were detected in the 8 h of the assay, which indicated that the solubilized organic matter was not fermented through acidogenesis during the assay.

Because the food waste, grinding methods, and assay conditions were identical in both assays, it was assumed that the endogenous solubilization that occurred in the control assay also occurred in the enzyme assay. Therefore, the SCOD measured in the control assay (normalized as COD_{esf}) was subtracted from the SCOD measured in

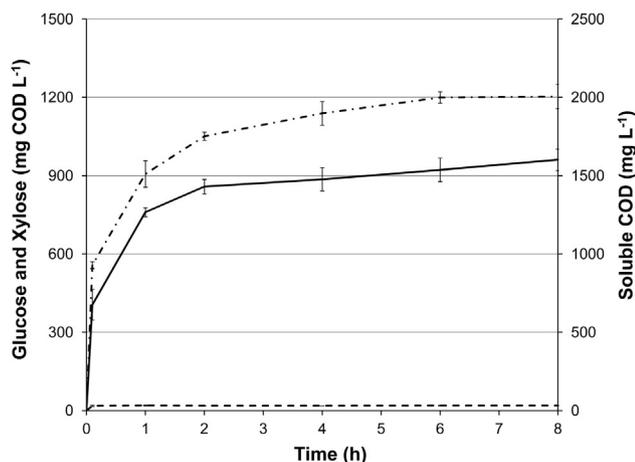


Fig. 3. Soluble COD, glucose and xylose analysis of ground food waste in aqueous solution with excess hydrolytic enzymes. Data shown is for the grinder (small plate): Soluble COD (—●—●—); Glucose (—); Xylose (---).

the enzyme assay. The difference was normalized as the fraction of TCOD solubilized through enzymatic hydrolysis (COD_{hsf}). Fig. 4 shows COD_{hsf} of the three grinding methods at 0, 0.1, 1, 2, 4, 6, and 8 h (data points). Immediately after grinding, hydrolysis was responsible for $6.8 \pm 3.1\%$ solubilization for all three grinding methods. However, by 4 h, hydrolysis plateaued, at which point $30.7 \pm 2.7\%$ of ground food waste was solubilized through enzymatic hydrolysis alone for all three grinding methods. The COD_{hsf} data were used for regression analysis to estimate the first-order rate constant (k) and the maximum COD_{hsf} ($\text{COD}_{\text{hsf}_4}$) (Table 5). The regressions are also shown in Fig. 4. All three grinding methods had statistically similar rate constants, at approximately 1 h^{-1} , while the grinder (small plate) had a significantly higher $\text{COD}_{\text{hsf}_4}$. However, for all grinding methods, enzymatic hydrolysis was responsible for 29–31% solubilization of ground food waste by 4 h in addition to the 27% solubilization due to endogenous solubilization.

4. Discussion

The two assays in this study were used to elucidate the mechanisms for short-term solubilization of ground food waste with and without excess hydrolytic enzymes. From these assays, it was possible to determine the kinetics of both endogenous solubilization and enzymatic hydrolysis and to quantify the relative contribution of each process to the overall short-term solubilization of ground food waste. Solubilization in the control assay represented the release of endogenous SCOD (i.e. endogenous solubilization) in the absence of hydrolytic enzymes. The SCOD of the substrate was measured at 87.9 ± 2.7 g SCOD kg^{-1} food waste (ww), or approximately 25% of TCOD (Table 2). Therefore, the entire complement of SCOD from the 10 g of food waste was released into solution within 1 h via endogenous solubilization.

The additional solubilization in the enzyme assay over the control assay was due to enzymatic hydrolysis. Because the hydrolytic enzymes were added in excess, this represents the maximum short-term hydrolysis of ground food waste. Since endogenous solubilization only accounted for 27% solubilization of TCOD, enzymatic hydrolysis by exogenous hydrolytic enzymes is critical to maximizing food waste solubilization. Presumably, hydrolytic enzymes in a well-balanced and active anaerobic digester would behave similarly to the hydrolytic enzymes in the present study. The enzyme assay indicated that, even in the short term (4 h), enzymatic hydrolysis was responsible for the majority of

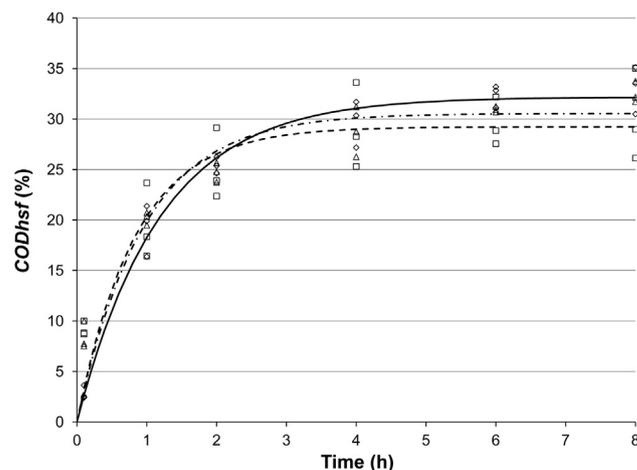


Fig. 4. Enzymatic hydrolysis of ground food waste (triplicate data points with regressions): Grinder, small plate (—◇—); Grinder, medium plate (---□---); Disposer (---△---).

Table 5
Regression parameter estimates for enzymatic hydrolysis and estimated 4 h enzymatic hydrolysis of ground food waste.

	k (h^{-1})	CODhsf_f (%) ^a	CODhsf_4 (%) ^b
Grinder (small plate opening)	$0.83 \pm 0.05 x$	$32.2 \pm 0.5 x$	31.1
Grinder (medium plate opening)	$1.19 \pm 0.22 x$	$29.2 \pm 1.0 y$	29.0
Disposer	$1.05 \pm 0.13 x$	$30.5 \pm 0.8 y$	30.1

Different letters (x, y) in the same column represent significant difference ($\alpha = 0.01$).

^a CODhsf_f = maximum fraction of TCOD solubilized through enzymatic hydrolysis.

^b CODhsf_4 = fraction of TCOD solubilized through enzymatic hydrolysis after 4 h.

solubilization (~29–31%), although enzymatic hydrolysis occurred at a slower rate than endogenous solubilization. Mechanically grinding the food waste prior to enzyme addition likely exposed greater surface area of the food waste, which facilitated enzyme–substrate interactions.

Fig. 5 shows ground food waste solubilization through the release of endogenous SCOD and enzymatic hydrolysis of PCOD as a percent of the TCOD of the standard food waste. Nearly all of the SCOD was released through endogenous solubilization by 0.1 h and 41% of the PCOD, or 30.7% of the TCOD, was hydrolyzed by 4 h. Because pH and product inhibition were prevented and the hydrolytic enzymes were applied in excess, this study demonstrated that approximately 60% of the standard food waste used was able to be solubilized within 4 h under optimal conditions. Since solubilization is the precursor to acidogenesis, which is often the fastest step in anaerobic digestion, it can be assumed that the solubilized fraction of the food waste will be rapidly acidified. This is important in regards to the alkalinity requirement for a digester in that it must be sufficient to buffer 60% of the food waste, but alkalinity is not required to buffer 100% of the food waste TCOD. Food waste does not have a high content of recalcitrant lignocellulosic material. Therefore, it can be assumed that most of the remaining 40% TCOD is slower degrading particulate material and would eventually be hydrolyzed with further enzymatic hydrolysis. However, this material does not contribute to the rapid dynamics of initial substrate solubilization that impacts digester stability.

The present study measured the short-term kinetics of endogenous solubilization and enzymatic hydrolysis under optimum conditions. In a similar study, Cekmecelioglu and Uncu (2013) added carbohydrases to ground food waste as a means of increasing glucose production. The authors found that glucose

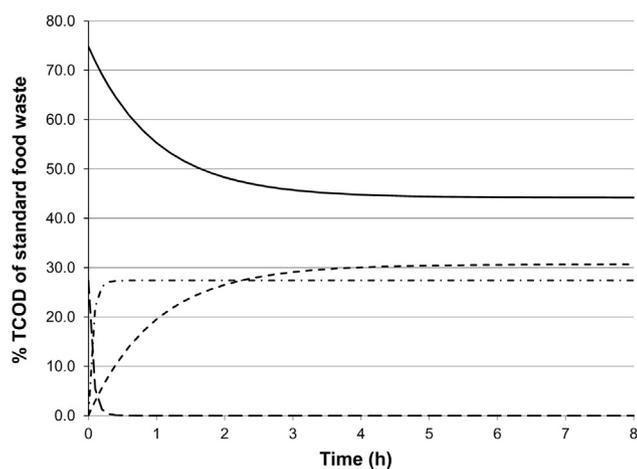


Fig. 5. Solubilization of ground food waste from endogenous solubilization and enzymatic hydrolysis. Data is based on the regression analysis for the grinder (small plate): Particulate COD (—); Soluble COD-Enzymatic hydrolysis (---); Soluble COD-Endogenous solubilization (-•-•-); Soluble COD-Unreleased endogenous (- - -).

concentration plateaued at approximately 6 h, food waste was approximately 70% solubilized to glucose within 6 h, and solubilization occurred at a first-order kinetic rate of 0.644 h^{-1} . Kim et al. (2005) utilized a hydrolytic enzyme cocktail as a means of increasing food waste solubilization for volatile fatty acid generation from food waste. They applied the enzyme cocktail at a rate of $0.4\% (\text{v v}^{-1})$ to blended, screened (1.41 mm) food waste diluted to 20 g COD L^{-1} and buffered to pH 6.5. After 12 h, an untreated control was 37.5% solubilized, while the enzymatically treated ground food waste was 62.0% solubilized. Ground food waste reached a maximum solubilization of 68.5% after 4 days. Their study found similar total solubilization after 12 h with enzyme treatment as the present study's maximum of 60% after only 4 h. However, unlike the present study, Kim et al. (2005) used food waste that had been in storage and the food waste was already partially acidified prior to the experiments. In their study, solubilization in the untreated control cannot be attributed solely to endogenous solubilization due to the abundance of spoilage microorganisms in their food waste. Some solubilization had already occurred prior to any measured SCOD and, therefore, their results for the untreated control may be artificially high and the relative contributions from endogenous solubilization and enzymatic hydrolysis could not be determined. The present study, however, was able to elucidate endogenous solubilization and enzymatic hydrolysis kinetics by using a freshly ground, standard food waste with and without excess hydrolytic enzymes.

5. Conclusions

This study identified the relative contribution of endogenous solubilization and enzymatic hydrolysis to the solubilization of food waste and demonstrated that ground food waste can be a rapidly degradable substrate, with the majority of the material solubilized within 4 h. With mechanical grinding, 27% of the TCOD was available almost immediately through the release of endogenous soluble organic material, and an additional 30% of food waste TCOD was enzymatically hydrolyzed by 4 h. This rapid solubilization can facilitate the use of high-rate anaerobic digesters for food waste digestion because solubilized material is readily available for microbial uptake, which allows for the establishment and maintenance of robust microbial consortia.

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