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Dairy Manure Digestate Age Increases Ultrasound Disintegration Efficiency at Low Specific Energies

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Abstract: Substantial insight into the effect of ultrasound disintegration on the changes in biochemical parameters of manure digestate and digestate age is needed to understand the potential of digestate treatment. To address this knowledge gap, in this study, the effect of digestate age on the efficiency of ultrasound (US) disintegration was investigated. In this scope, dairy manure digestate samples were incubated in an oven at 37 °C for a predetermined amount of time to obtain simulated digestate ages of 15, 22, 29, 36 and 43 days. The results showed that US disintegration efficiency significantly affected the initial biochemical characteristics of digestate and that the digestate age had a significant effect on the US disintegration efficiency. This effect diminished when the applied specific energy (SE) was higher than 3000 kJ/kg total solids (TS). A numerical partial least squares (PLS) model was constructed to investigate the relative influences of the initial biochemical parameters on the soluble chemical oxygen demand (sCOD) and soluble carbohydrates (sCARB) solubilization. The results of the high-quality ($R^2 = 0.8$) model indicated that the most influential parameters for the efficiency of US disintegration were the SE, the initial sCARB₀, the TS, the initial sCOD₀ and the volatile solids (VS).

Keywords: digestate; anaerobic digestion; pretreatment; digestate disintegration; manure: PLS modeling



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1. Introduction

Anaerobic digestion (AD) is generally regarded as a suitable technology to process and stabilize organic waste streams such as dairy manure. The use of dairy manure as an AD substrate is limited due to the presence of recalcitrant lignocellulosic matter [1,2]. Lignocellulosic matter is composed of lignin which tightly covers the cellulose and hemicellulose moieties, which creates a physical barrier for biodegradation [2–4]. Pretreatment methods such as ultrasonication have been extensively studied at both lab and industrial scales to achieve (i) disintegration of organic matter and delignification; (ii) enhanced hydrolysis of the (hemi)cellulose fractions and (iii) increased biogas production rates [3].

The main drawback of pretreatment methods is that the additional energy yield in the form of extra methane is often lower than the applied energy required for the disintegration, which impedes the potential implementation of pretreatment techniques [5–7]. The reason for the low energy yields is related to the substantial amount of readily biodegradable matter within substrates, which, in fact, does not require disintegration to increase its conversion efficiency [8]. Disintegration technologies such as ultrasonication are not selective and will, thus, direct the energy towards both the readily and non-biodegradable matter, decreasing the energy yield of these pretreatment technologies [8]. To address the energy problem of disintegration technologies, the existing technologies were applied on digestates (instead of the substrate) to increase the biodegradability of digestates for post-digestion [5,8–12]. Indeed, digestates contain a lower amount of organic matter (OM), as characterized by lower volatile solids (VS), total carbon (TC), lower total organic carbon

(TOC) content, lower total chemical oxygen demand (COD) and higher pH values compared to their respective feeds [13]—hence the application of disintegration technologies on digestates. This approach is called digestate disintegration, which is hypothesized to solubilize only the residual organic matter which cannot be effectively degraded during AD within reasonable hydraulic retention time (HRT) pretreatment. This is because digestate disintegration mainly focuses its energy on recalcitrant organic matter, since the readily degradable matter was already converted into biogas in the previous digestion step [8].

Ultrasound (US) disintegration is a pretreatment method which can be used for digestate disintegration [8]. US disintegration is the application of a cyclic sound pressure wave of frequencies over 20 kHz on a substrate. This alternative pressure wave creates a cavity in the liquid which, upon imposing, disintegrates the nearby organic matter [8,14,15]. Although there are some publications on the effect of US disintegration of manure digestate, little is known about the impact of the digestate age, i.e., the overall AD time of the substrate, on the efficiency of US digestate disintegration [9,14]. In a similar context, Cesaro et al. (2019) observed that the digestate age had a direct impact on the ozonation efficiency of two digestates with different digestate ages (i.e., 11 and 41 days), related to the abundance of the less accessible organic fraction that was not effectively solubilized during ozonation of the of 41-day-old digestate compared to the 11-day-old digestate [12].

This study investigates the relationship between the digestate age, the applied specific US energy and the COD solubilization. To the best of the authors' knowledge, this effect has not previously been quantified or proven for ultrasound digestate disintegration. The hypothesis of this study was that the digestate age significantly affected the US digestate disintegration efficiency. In this scope, the expected outcome would be an increase in US efficiency with increased digestate age.

This study was divided into three sub-objectives: (i) Determining the effect of digestate age on biochemical properties in order to explain the effect of digestate age on changes in US efficiency; (ii) investigating the effects of US disintegration on the relevant biochemical parameters for each digestate age separately and elucidating correlations between the biochemical parameters; (iii) predicting the effect of digestate age on US disintegration. In this scope, a numerical model was constructed in order to evaluate the relative impact of the different biochemical parameters and the specific energy on the release of soluble COD.

2. Materials and Methods

2.1. Digestate Preparation

Dairy manure digestate was sampled from a pocket digester in Rumst, Flanders, which was fed daily with dairy manure. The digester had a volume of 100 m³, which operated at the day of sampling at an HRT of 15–17 days and a temperature of 37 °C. To obtain digestates with simulated prolonged digestate ages, the digestates were further incubated in an oven at 37 °C for 0, 7, 14, 21 and 28 days to obtain simulated digestate ages of 15, 22, 29, 36 and 43 days, respectively. These digestates will be called Dig15, Dig22, Dig29, Dig36 and Dig43, respectively. Due to the required volume of the digestates for incubation and US disintegration, the sampling occurred at different moments between February and May 2017.

2.2. Biochemical Parameters

Biochemical parameters such as pH, TS, VS, total alkalinity (TA), soluble chemical oxygen demand (sCOD) and total and soluble Kjeldahl nitrogen (sKJN and tKJN, respectively) were analyzed according to [16]. The total ammonia nitrogen (TAN) of the digestates was measured using Hach Lange test kits (LCK302). The soluble protein (in g N/L) was calculated according to Equation (1).

$$\text{sPROT} = 6.25 (\text{sKJN} - \text{TAN}) \text{ [g/L]} \quad (1)$$

The reducing sugar content was analyzed using the anthrone method [17]. Polysaccharides were hydrolyzed with 75% *w/w* sulfuric acid (Sigma Aldrich, Overijse, Belgium).

Afterwards, the sample was digested at 100 °C in the presence of anthrone (2 g/L) (Sigma Aldrich) for a period of 15 min. The reaction products were quantified by measuring the absorbance of the mixture at 578 nm with a Macherey-Nagel Nanocolor 500D Spectrophotometer.

2.3. Ultrasonication Experiment

The ultrasound experiments were conducted in a 2-L glass reactor with a 1.4-L active volume. The glass reactor was wrapped in flexible tubing through which cooling water circulated. The ultrasound device was a Sonopuls-497 US horn, with a maximum power of 150 W (Bandelin) and with a TT13 tip (Bandelin) which was placed at a depth of approx. 3 cm in the glass reactor. A frequency of 20 kHz and a power of 100 W were applied during the experiments, resulting in a sonication density and intensity of 71 W/L and 113 W/cm², respectively.

The specific energy was calculated according to Equation (2)

$$SE = \frac{P \times t}{m \times TS} \quad (2)$$

where P (W) is the US power, t (s) is the reaction time, m (kg) is the mass of the digestate and TS (%) is the total solid content. The temperature of the ultrasonicated digestate was measured to prevent thermal degradation. The content of the glass reactor was stirred with an overhead stirrer to ensure homogeneous ultrasonication. The selected target specific energies (SEs) ranged from 3000 to 18,000 kJ/kg TS, with increments of 3000 kJ/kg TS. Since the TS content of the digestates was measured prior to ultrasonication, slight variations in the applied SEs compared to the targeted SEs occurred, with a maximum variation of 11.1%. The applied SEs can be found in Table 1.

Table 1. Specific energy (SE) applied to the digestates per simulated digestate age.

Digestate Age (Days)	Applied Specific Energies (kJ/kg TS)						
15	0	3000	6000	9000	12,000	15,000	18,000
22	0	3000	6000	9000	12,000	15,000	18,000
29	0	3330	6670	10,000	13,340	16,670	20,000
36	0	3100	6204	9310	12,410	15,510	18,610
43	0	2770	5540	8310	11,080	13,860	16,630

2.4. Efficiency Calculation of US Disintegration

The efficiency of ultrasonication is defined as the amount of sCOD solubilized per applied SE and described via Equation (3).

$$\text{Efficiency} = \frac{BP_b - BP_a}{SE_b - SE_a} \quad (3)$$

where the efficiency is expressed in terms of an absolute change in the biochemical parameter (BP) per SE applied (e.g., g_{sCOD}/(kJ/kg TS)); BP_b is the value of the BP (e.g., sCOD) after disintegration with SE_b and BP_a is the value of the BP after disintegration with SE_a. In order to assess whether the digestate age had a significant influence on the slope of the disintegration per SE, the data were grouped in such a way that the factor of ANOVA was the digestate age.

2.5. Statistical Methods

A one-way analysis of variance (ANOVA) was used to determine the significance of the difference of multiple means in groups. If the assumptions of the ANOVA were not fulfilled, a non-parametric variant of ANOVA, i.e., the Kruskal–Wallis test, was used. Statistical significance was established at the $p < 0.05$ level. A Tukey post-hoc analysis

was performed to identify significant differences in means whenever the ANOVA or the Kruskal–Wallis yielded significant results. Principal component analysis (PCA) was used to determine the covariance of variables within the data and performed in RStudio© version 1.2.5033 using the package FactoMineR [18].

Partial least squares (PLS) regression was used to derive a linear numerical model (Equation (4)) describing the relationship between the input parameters x_i ($i = 1 \dots N$), denoted as the X matrix, and the output parameters y_j ($j = 1$), denoted as the Y matrix. The PLS methodology was chosen instead of ordinary linear regression because PLS is more robust than multiple linear regression (MLR) when the sample size is small, and the X matrix has collinearity [19]. The collinearity was determined via PCA in RStudio© version 1.2.5033 using the package FactoMineR [18].

This numerical model (Equation (4)) predicts the sCOD after ultrasonication as a function of the applied SE and the initial biochemical parameters.

$$\hat{y}_j = b_0 + b_1x_1 + \dots + b_Nx_N \quad (4)$$

where \hat{y}_j is the predicted output, b_0 is the intercept and b_i ($i = 1 \dots N$) are the regression coefficients. To accomplish this, PLS deconstructs the X and Y matrices into a set of “ A ” orthogonal latent variables (LVs) according to Equations (5) and (6). ([1]).

$$X = \sum_{k=1}^A t_k p_k^T + E_x \quad (5)$$

$$Y = \sum_{k=1}^A u_k q_k^T + E_y \quad (6)$$

where A is the number of LVs; t_p and u_j the score vectors for the input and output matrices; p_j and q_j are the loading vectors for the input and output matrices and E_x and E_y are the residual terms.

The data were auto-scaled before decomposition so that the mean of all variables was zero and the standard deviation was one. Cross-validation by means of the leave-one-out (LOO) methodology was used during decomposition. The PLS model was constructed in RStudio© version 1.2.5033 using the package PLSdepot ([20]). The model robustness was assessed using following parameters:

- The root mean square error of calibration (RMSEC) was used to determine the accuracy of the calibration of the model.
- The coefficient of determination, R^2 [0, 1]. A model with $R^2 > 0.7$ can be considered as a good fit [21].
- Q^2 cum $[-\infty, 1]$ or the cumulative percentage of the variation in the Y matrix that could be explained by the variation on the X matrix of the partial dataset used during the validation part of cross-validation. A model with Q^2 cum > 0.5 is considered to have good predictivity [22].

The digestate age itself was not included as an input variable in the numerical model. The reasoning is as follows: (1) the digestate age is highly correlated with the biochemical parameters, and thus, the digestate age can be considered as a proxy variable for the biochemical parameters. As the digestate age changes, the biochemical parameters will change, and thus, the information of the digestate age is incorporated in the biochemical parameters; (2) To obtain a model that can be used outside the framework of this specific research and, thus, to be applied on other digestates with biochemical parameters within the appropriate ranges, the digestate age should not be required as essential information. As an example, it might be true that more efficient digesters obtain higher VS removal efficiencies than this study for equal digestate ages. Hence, this would create contradictory information within the numerical model, i.e., digestate age does not correspond with

expected biochemical parameters. Therefore, this would result in large deviations between observed and predicted data.

3. Results

3.1. Effect of Digestate Age on Biochemical Parameters

In order to assess the effect of digestate age on the efficiency of US digestate disintegration, the influence of the digestate age on the biochemical parameters needs to be quantified first. This is necessary to explain the effect of digestate age on changes in US efficiency. The results can be found in Table 1.

The digestate age significantly altered sCOD, TS, VS, TAN, TA and pH (Table 2). Initially, the sCOD significantly decreased with increased digestate age from 17.0 ± 0.5 g/L in Dig15 to 12.0 ± 0.1 g/L in Dig29 ($p = 0.000$). The TS content also significantly decreased with increasing digestate age from 7.5% in Dig15 to 6.2% in Dig36. Conversely, a slight increase to 6.7% TS was observed in Dig43 (Table 1). The decreasing trends in sCOD, TS and VS were a result of the microbial degradation of organic matter, while the increase in TS and VS from Dig29 to Dig43 may have been the result of water evaporation (i.e., thickening). Figure 1 shows the PCA biplot of the biochemical parameters for each digestate age. The digestate age was negatively correlated with sCOD, TS and VS, while these three latter parameters were positively correlated with each other. The total carbohydrate (tCARB) and soluble carbohydrate (sCARB) contents were not significantly changed, although a 32% decrease in tCARB from Dig15 to Dig43 was observed (Table 3). The pH increased from 7.5 (Dig15) to 8.4 (Dig43). A decrease in sCOD, TS and VS and an increase in pH with increasing digestate age were also reported by Li et al. (2018) during the digestion of corn stalks [23].

However, the decrease in TAN by 25% and in TA by 10% with increasing digestate age from 15 to 43 days was unexpected. The decrease in TA was strongly and significantly correlated with the decrease in TAN (Table 1 and Figure 1). The decrease in TAN during an incubation period of over 27 days was most probably the result of a higher rate of TAN volatilization or, possibly, struvite settling rather than TAN production rate due to digestion [13]. Struvite settling increases with pH and is most optimal when the pH is between 9 and 10.7. If the decrease of 0.6 g N/L were completely due to struvite settling, this would require 1.0 g/L magnesium and 3.6 g/L phosphate. The elemental analysis of dairy manure digestate, taken from the same farm-scale reactor, conducted by Somers et al. (2020) revealed that the magnesium concentration was 13.9 g/kg TS or, at a TS concentration of 7.5%, 1.0 g/L, which is close to sufficient. However, the phosphorous concentration was 11.6 g P/kg TS or 2.4 g PO_4 /L, which is insufficient to account 100% of the TAN removal to struvite settling [16]. Hence, at least in part, the TAN decrease must be attributed to TAN volatilization. However, struvite formation was observed in a wider pH range, from 7 to 11 [24]. The soluble fractions of the organic matter such as the sKJN, soluble protein content (sPROT) and sCARB varied in the ranges of 2.6–3.3 g-N/L, 1.7–6.4 g/L and 0.5–1.7 g/L, respectively, but the changes were insignificant (Table 1). These results were consistent with the assumption that the hydrolysis of organic matter is the rate-limiting step during lignocellulosic biomass digestion [25]. Overall, these results indicated that the digestate age significantly changed digestate characteristics.

3.2. Effect of US Disintegration on the Biochemical Parameters

The different digestates (ages 15 to 43 days) were subjected to ultrasound disintegration with SEs ranging from 3000 to 20,000 kJ/kg TS. The biochemical parameters, such as sCOD and sCARB, increased in all digestates (Table 4).

Table 2. Influence of digestate age (in days) on biochemical digestate parameters: soluble chemical oxygen demand (sCOD), total solids (TS), volatile solids (VS), total ammonia nitrogen (TAN), soluble Kjeldahl nitrogen (sKJN), soluble proteins (sPROT), total carbohydrates (tCARB), soluble carbohydrates (sCARB), total alkalinity (TA) and pH.

Age (Days)	sCOD (g/L)	TS (%)	VS (%)	TAN (g-N/L)	sKJN (g-N/L)	sPROT (g/L)	tCARB (g/l)	sCARB (g/L)	TA (g HCO ₃ ⁻ /L)	pH
15	17.0 ± 0.5	7.5 ± 0.0	5.6 ± 0.0	2.4 ± 0.0	3.3 ± 0.1	5.3 ± 0.6	7.7 ± 0.7	1.2 ± 0.0	9.0 ± 0.1	7.5 ± 0.0
22	15.1 ± 0.8	7.1 ± 0.1	5.3 ± 0.1	2.3 ± 0.0	2.6 ± 0.2	1.7 ± 1.5	5.1 ± 0.3	1.7 ± 0.2	8.2 ± 0.2	7.9 ± 0.0
29	12.0 ± 0.1	6.4 ± 0.1	4.5 ± 0.0	2.2 ± 0.1	2.9 ± 0.0	4.6 ± 0.7	5.7 ± 0.6	0.5 ± 0.1	8.7 ± 0.3	7.8 ± 0.0
36	12.3 ± 0.1	6.2 ± 0.0	4.5 ± 0.0	2.2 ± 0.1	2.8 ± 0.4	3.5 ± 2.7	5.5 ± 0.3	1.2 ± 0.3	8.7 ± 0.1	7.9 ± 0.0
43	12.3 ± 0.1	6.7 ± 0.1	4.5 ± 0.1	1.8 ± 0.1	2.8 ± 0.0	6.4 ± 0.8	5.2 ± 0.1	1.0 ± 0.1	8.1 ± 0.2	8.4 ± 0.1

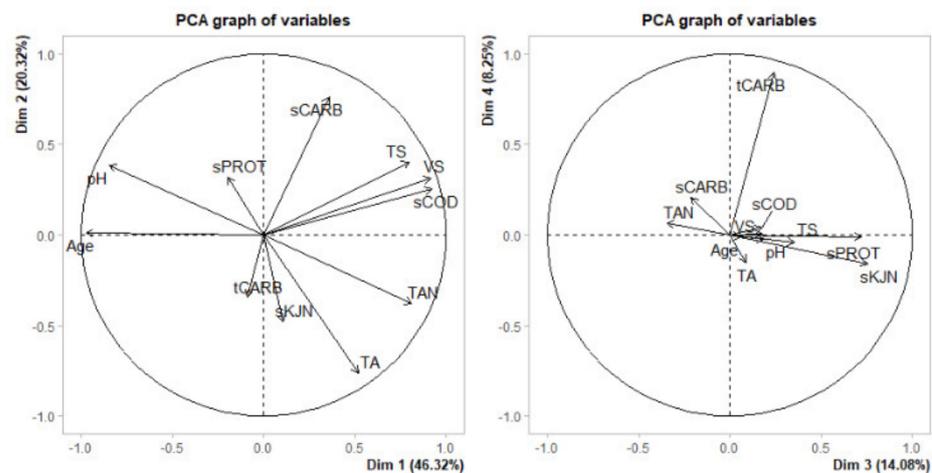


Figure 1. Principal component analysis (PCA) plot of the effect of digestate age on biochemical parameters.

Table 3. Spearman correlation (top right) coefficients of the effect of age and the biochemical parameters and their interdependencies with blue and red representing strong positive (+1) and negative correlations (−1), respectively. The bottom left depicts the respective *p*-values with significant *p*-values in bold.

	Age	sCOD	TS	VS	TAN	sKJN	sPROT	tCARB	sCARB	TA	pH
Age		−0.611	−0.687	−0.840	−0.958	−0.081	0.482	0.005	−0.349	−0.557	0.826
sCOD	0.016		0.854	0.721	0.495	0.505	0.159	−0.141	0.675	0.080	−0.472
TS	0.005	0.000		0.932	0.588	0.368	0.110	−0.182	0.443	0.054	−0.458
VS	0.000	0.003	0.000		0.753	0.426	−0.110	−0.055	0.321	0.243	−0.622
TAN	0.000	0.089	0.038	0.004		0.104	−0.440	0.190	0.248	0.674	−0.889
sKJN	0.792	0.081	0.217	0.242	0.737		0.791	0.424	0.038	0.382	−0.374
sPROT	0.095	0.604	0.723	0.723	0.135	0.002		0.165	−0.187	−0.121	0.467
tCARB	0.985	0.616	0.516	0.845	0.535	0.149	0.590		−0.384	0.428	−0.307
sCARB	0.202	0.007	0.100	0.242	0.415	0.906	0.541	0.157		−0.277	−0.213
TA	0.031	0.776	0.850	0.383	0.012	0.197	0.694	0.111	0.318		−0.213
pH	0.000	0.076	0.086	0.013	0.000	0.208	0.222	0.266	0.447	0.020	

Table 4. Results of the ANOVA of the biochemical parameters factored per age. Bold *p*-values are significant.

Age	sCOD	TAN	sKJN	sPROT	sCARB	pH
15	0.002	0.013	0.041	0.096	0.001	* 0.018
22	* 0.009	* 0.188	0.000	0.613	0.000	0.004
29	0.000	0.075	0.678	0.201	0.002	* 0.010
36	* 0.006	0.007	0.134	0.098	0.007	0.000
43	0.000	0.018	0.567	* 0.446	0.000	0.002

* *p*-value obtained with the Kruskal–Wallis test.

Azman et al. (2020) conducted a similar study in which they used US disintegration on manure digestate (age = 20 days). They reported a 17% increase in sCOD after disintegration with an SE of 3000 kJ/kg TS [14]. This result was significantly lower than that obtained in the present study, in which a 41% increase in sCOD for Dig22 was observed (Figure 2). The differences may be explained by the different set-up of the experiments. In this study, batch digestion was used to artificially obtain a digestate with a higher digestate age. In the study of Azman et al., the obtained digestate was acclimated by continuous stirred tank reactor (CSTR) conditions. The CSTR conditions with recycling of disintegrated digestate

resulted in an sCOD of untreated manure digestate of 21.2–23.6 gO₂/L, while the sCOD of untreated Dig29 was 12.0 gO₂/L [14]. The CSTR-based acclimation, hence, resulted in a lower relative sCOD solubilization at similar SEs compared to batch-based digestate aging.

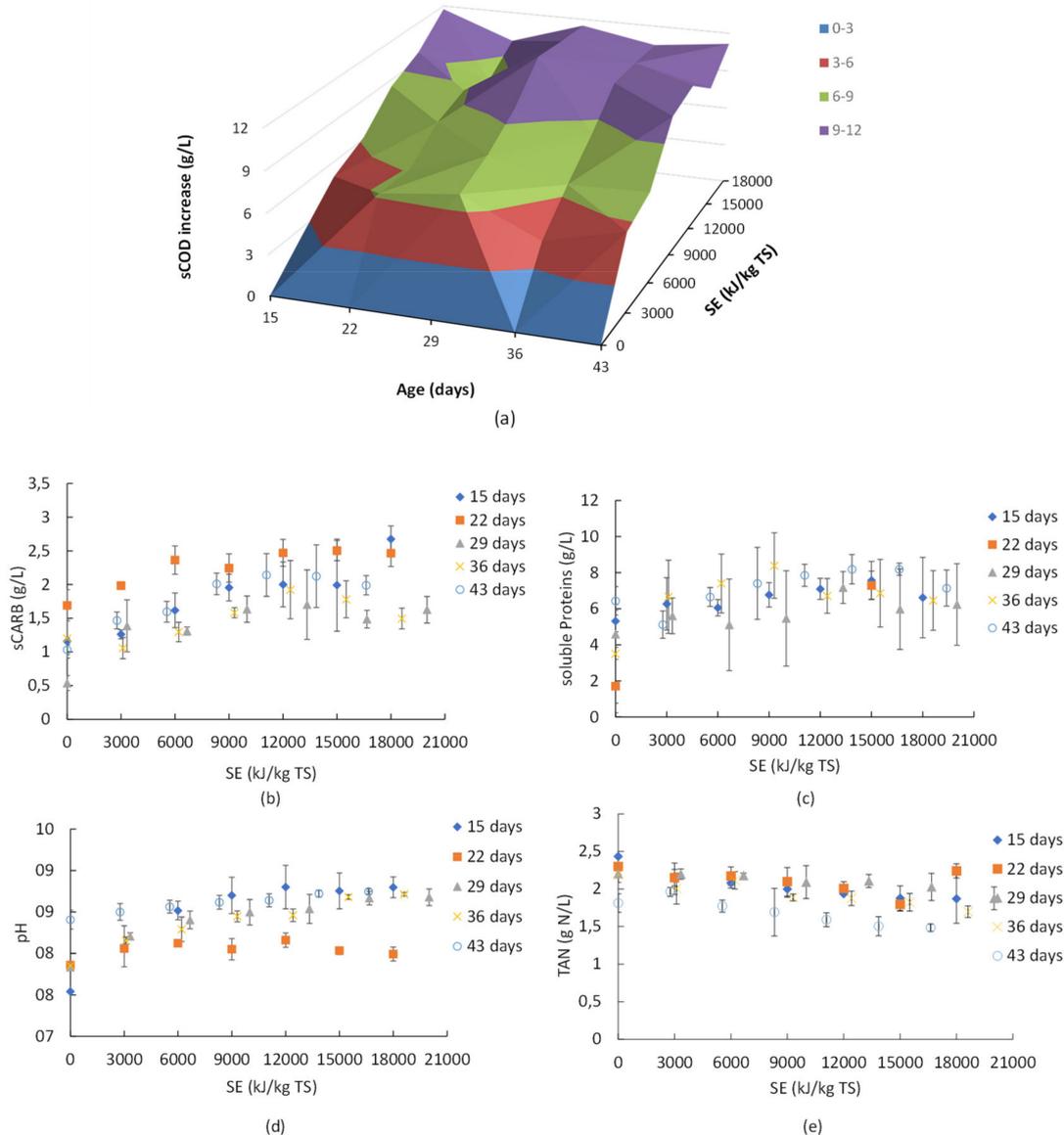


Figure 2. The effect of ultrasound (US) disintegration on biochemical parameters with (a) the absolute soluble chemical oxygen demand (sCOD) increase; (b) the soluble carbohydrate (sCARB), (c) the soluble protein content sPROT, (d) the pH and (e) the total ammonia nitrogen (TAN) concentration profiles in function the applied SE and the digestate age.

The sPROT was not significantly increased by ultrasonication (Table 4), although a 2.5-fold increase was observed for Dig29 at 3333 kJ/kg TS compared to no disintegration (Figure 2). The sCARB increased significantly for all digestate ages. The maximum increase in sCARB was 1.2 ± 0.4 g/L, which was a 3.2-fold increase for Dig29 at an SE of 13,335 kJ/kg TS, after which the sCARB no longer significantly increased with increasing SE. These results are in accordance with the findings of Boni et al. (2016), who found a 2–14-fold increase in sCARB while ultrasonically digesting solid waste digestate at 50–50,000 kJ/kg TS [10].

Both the sCARB and the sPROT concentrations correlate with the increase in sCOD due to ultrasonication (Figure 3). This result is consistent with other studies that found a high correlation between the soluble organic nitrogen and the soluble COD [8,26].

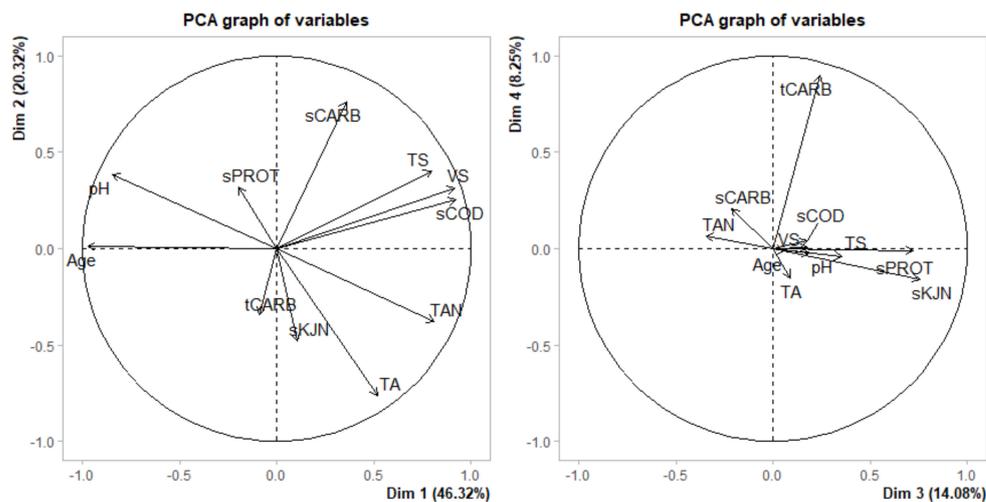


Figure 3. PCA biplot depicting the interdependencies of the biochemical parameters during US disintegration.

With one notable exception, the pH increased due to US in all digestates. Furthermore, the pH converged, regardless of the initial value, to a pH of ~ 8.7 . The exception in Dig22 could not be explained with the available data (Figure 2). The TA of Dig15 at 18,000 kJ/kg TS was 6.8 ± 0.2 g HCO_3^- /L, which was significantly lower (-24%) than the untreated Dig15 at 9.0 ± 0.17 g HCO_3^- /L. The increase in the pH to a value of 8.7, and the decrease in TA, can be the result of CO_2 purging due to ultrasonication. US is known for exhausting dissolved gases from liquids [15]. Hence, it is possible that dissolved CO_2 was being degassed from the liquid, causing an equilibrium shift of dissolved (bi-)carbonates which increased the pH. The underlying mechanisms of degassing can be considered both thermal (i.e., decreases solubility of CO_2 with increased temperatures) and athermal (i.e., micro-convections and microjets causing the migration of a gas-filled cavity towards the surface of the bulk mass) [15,27–29].

The TAN decreased significantly in Dig15, Dig36 and Dig43 by 23%, 22% and 18%, respectively, at the maximum applied SEs (Table 1). The TAN removal is attributed to degassing via thermal and non-thermal effects, similar to CO_2 removal. This occurrence was also reported by Cho et al. (2014), who found a maximum 55% TAN removal after ultrasonication with a frequency of 28 kHz at pH 11 [27]. The decrease in TAN is, however, in contrast to previous studies [9,14], where no TAN removal was found during US disintegration. Azman et al. (2020) applied the same US reactor set-up as the one used in this study with lower SEs, i.e., 1500 and 3000 kJ/kg TS compared to 3000–20,000 kJ/kg TS [14]. Somers et al. (2018) used a different US reactor set-up which operated at lower temperatures compared to this study [9].

4. Discussion

4.1. Effect of Digestate Age on US Disintegration Efficiency

In this study, “higher US disintegration efficiency” is defined as “producing a comparatively greater effect per applied SE”. The pH data of Dig22 were removed for this analysis because the unexplained discrepancies in the pH data of Dig22 compared to Dig15 and Dig43 resulted in unfounded significant differences between the digestate ages. From Table 5, it can be derived that the digestate age did significantly influence the US efficiency for all investigated parameters at an SE of ± 3000 kJ/kg TS. However, the significance of this influence rapidly diminishes when the SE is increased (Table 5), at least in terms of

sCOD solubilization. Tukey's test revealed that all digestates except Dig36 significantly solubilized more COD per kJ/kg TS compared to Dig15. The deviating results of Dig36 cannot be explained on the basis of the available data. Since the US disintegration efficiency is higher in the older digestates (i.e., Dig22, Dig29 and Dig43 compared to Dig15), it must be concluded that the differences in initial biochemical parameters of the digestates as a result of the digestate age had a significant influence on the sCOD solubilization at digestate ages below 22 but no more above. Thus, in general, the effect of digestate age on US disintegration efficiency diminishes with increasing digestate ages.

Table 5. ANOVA results for the efficiency of US disintegration per digestate age. Bold *p*-values are significant.

SE (kJ/kg TS)	sCOD	TAN	sKJN	sPROT	sCARB	pH
~3000	* 0.035	0.011	0.218	* 0.033	0.001	0.014
~6000	* 0.169	0.096	0.493	0.140	0.173	0.006
~9000	0.421	0.878	0.676	0.736	0.022	0.259
~12,000	0.666	0.902	0.749	0.218	0.173	0.978
~15,000	* 0.717	0.593	0.493	0.667	0.944	0.019
~18,000	0.384	0.019	0.985	0.750	0.000	0.732

* *p*-value obtained with the Kruskal–Wallis test.

The only difference in sPROT solubilization efficiency was found between Dig43 and Dig36 at an SE of ± 3000 kJ/kg TS. However, the sPROT and sKJN analyses were categorized by high standard deviations, as shown in Figure 2, which negatively influenced the ANOVA results. The sCARB solubilization efficiency was only influenced by the digestate age between Dig29 and Dig15 and at the lowest applied SEs. At the highest SE, there was a significant influence of the digestate age on the sCARB solubilization efficiency, where the highest sCARB solubilization per SE occurred in Dig15. This might have been explained by a higher tCARB content in Dig15 of 7.7 ± 1.2 g/L than digestates of higher ages, as the tCARB is converted to methane during digestion [30]. However, the decrease in tCARB as a function of digestate age was not statistically significant during this study. The digestate age also seemed to have an effect on the TAN removal and pH increase. However, the results of Tukey's test indicate that this effect is sporadically divided across the SEs and digestate ages and may, thus, be more an influence of the initial pH and the temperature increase during US. As previously mentioned, the pH converges to ~ 8.7 , indicating that only the starting value had an influence on the slope of the pH increase in function of the SE. The results of Tukey's test can be found in the Supplementary Materials Table S1.

The effect of digestate age on the US digestate disintegration can be explained by the variations in initial biochemical parameters before ultrasonication. As discussed, the TS value decreased with digestate age. Dig 15 had the highest TS with 7.5% and it decreased to 6.4% for Dig 29, where the US efficiency, in terms of sCOD solubilization, was the highest. This indicated that the high TS content of Dig15 resulted in lower US efficiency. In previous studies, a TS value of 2.3% to 3% of waste-activated sludge (WAS) was found to be the most optimal range for efficient US disintegration, while an increase to a TS of 7% resulted in a threefold increase in energy consumption for similar disintegration [15]. Similarly, Akin et al. (2006) discovered that the particle size reduction efficiency, as a result of US disintegration, was higher at lower TS values [31]. Another explanation may be related to the structural organization of the COD in the organic matter. However, as the COD is a lumped parameter, a more comprehensive fractionation of the COD has to be conducted to obtain more insight into the effect of digestate age on the US efficiency.

It can be concluded that there is a significant influence of digestate age on the US disintegration efficiency. However, this effect is relatively small compared to the influence of the SE and will thus be nullified when high SEs are applied to the digestate.

US disintegration is often compared to hydrodynamic disintegration (HD), which is reported to have a lower energy consumption compared to US disintegration [32,33]. Żubrowska-Sudoł et al. (2020) reported an sCOD increase of 6.5–185% at a HD energy dose of 210 kJ/L for maize silage [33], while in this study, the manure digestate of 22 days ultrasonicated with 3000 kJ/kg TS or 213 kJ/L resulted in an increase in the sCOD of 41%. The obtained sCOD increases in this study are in the range of HD pre-treatment results; however, to the best of the authors' knowledge, no study has yet investigated the effect of HD disintegration on the digestate of an anaerobic digester.

4.2. Numerical Modeling of US Disintegration

A numerical model describing the relationship between the absolute increase in sCOD after ultrasonication (called sCOD_{us}) and the input parameters (i.e., SE, sCOD₀, TS, VS, TS, VS, TAN, sKJN, sPROT, tCARB, sCARB, TA and pH) of Dig15 to Dig 43 was constructed using the PLS methodology. The data can be found in Table S2. Furthermore, a numerical model predicting the absolute increase in sPROT and sCARB after ultrasonication was also constructed. However, the resulting model for sPROT and sCARB could only explain a maximum of 32% and 23% of the variance in the X-matrix, respectively. The quality of this model was too low, and it was, hence, discarded. During the deconstruction of the X-matrix, an appropriate number of LVs was selected on the basis of the RMSEC and Q²cum (Figure 4). Two LVs were selected for the sCOD model. Selecting a higher amount of LVs did not decrease the RMSEC sufficiently and would have resulted in overfitting of the data and, thus, decreased the predictive power of the model. The Q²cum reached a maximum of 0.692 at two LVs, which can be considered to have good predictivity [22]. The resulting coefficients and the variable ranges in which these coefficients are appropriate are depicted in Table 6. The standardized coefficients, in Figure 5, were used to investigate the influence of the variables on the sCOD_{us}.

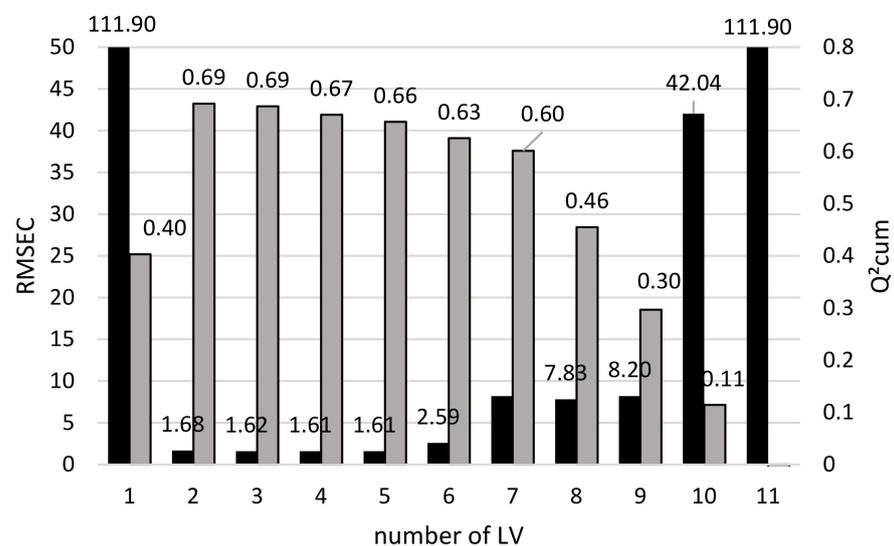
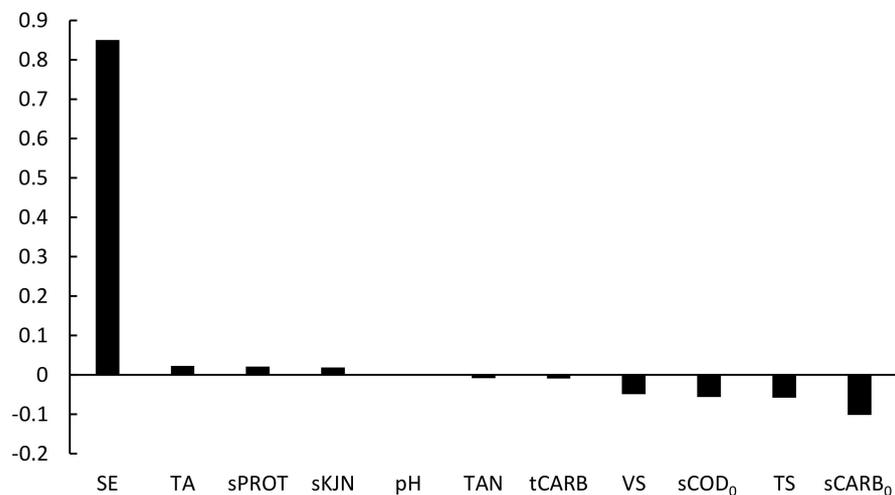


Figure 4. The root mean squared error of cross-validation (RMSEC, black bar) and the Q²cum (grey bar) in function of increasing number of latent variables (LVs).

Table 6. Resulting variable coefficients and corresponding variable ranges and units.

Parameter	Coefficients	Range	Unit
Intercept	7.909	n.a.	g/L
SE	0.001	0–20,000	kJ/kg TS
sCARB ₀	−1.031	0.54–1.69	g/L
TS	−0.502	6.38–7.49	%
sCOD ₀	−0.107	11.95–16.96	g/L
VS	−0.384	4.48–5.64	%
TA	0.253	7.93–8.84	g HCO ₃ /L
sPROT	0.046	1.71–6.84	g-N/L
sKJN	0.299	2.57–3.28	g-N/L
tCARB	−0.036	5.08–7.73	g/L
TAN	−0.157	1.81–2.43	g-N/L
pH	−0.010	7.54–8.41	-

**Figure 5.** Standardized regression coefficients, in decreasing order, for the selected model predicting the sCOD after ultrasonication of digestates, constructed using partial least squares (PLS) methodology using two latent variables.

It is clear that the applied SE had the most influence on the sCOD_{us}. This result was expected as the SE is reference parameter and the most influential parameter with respect to the disintegration of the organic matter [15]. The four next influential parameters were all negatively correlated with the solubilized sCOD after ultrasonication. These were, in order of importance, the sCARB₀, TS, sCOD₀ and VS. As expected, TS was negatively correlated with sCOD_{us} because the TS contents of this study were outside the optimum range observed for WAS disintegration (i.e., 2.3–3.2% TS). Higher TS contents decrease the US efficiency because the particles can absorb acoustic energy without disintegrating [15]. The VS content was strongly correlated with the TS content, but its impact on the model was relatively small. This effect can be a result of the sCOD_{us} originating from the VS. A relatively higher VS/TS results in relatively higher COD solubilizations [34]. It is hypothesized that the sCOD₀ and sCARB₀ were negatively correlated with the sCOD_{us} because they also contributed to the absorption of acoustic energy, although more research is needed to verify this hypothesis. The other parameters all have similarly low contributions to the numerical model. The obtained model had an R² value of 0.8 and nearly all of the values lie within the 95% confidence interval (Figure 6). Thus, the numerical model can be considered to be of good quality [21].

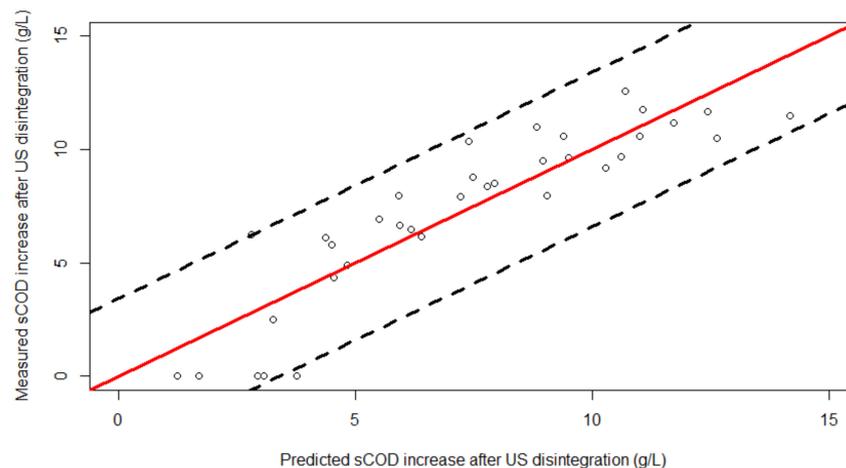


Figure 6. The predicted and measured sCOD increase after US disintegration. The red line has an angle of 45° ; the dotted lines are the 95% confidence interval.

5. Conclusions

In this study, dairy manure digestates of five different digestate ages, i.e., 15, 22, 29, 36 and 43 days, were subjected to US disintegration. The digestate age was significantly negatively correlated with the sCOD, TS and VS and positively correlated with the TAN, TA and the pH of the digestates before ultrasonication. US effectively disintegrated all the digestates, which resulted in an increased sCOD by a maximum of 98% g/L and a 3.1-fold increase in sCARB in Dig29. The pH generally increased due to US in all digestates. The digestate age slightly influenced the efficiency of US disintegration with the applied SE of ~ 3000 kJ/kg TS. Above this SE, the effect of the digestate age on US efficiency quickly diminishes. Hence, the impact of the digestate age on the US efficiency was comparatively lower compared to the impact of high SEs. When using US as a means for manure digestate disintegration in a post-digester set-up, a digestate age of over 15 days and an SE of 3000 kJ/kg TS are recommended. SEs over 3000 kJ/kg TS did not result in higher disintegration efficiencies. Future research should evaluate the efficiency of US digestate disintegration on the biochemical methane potential, which is a crucial parameter to optimize before the implementation of US digestate disintegration in the anaerobic digestion sector. A numerical PLS model for sCOD solubilization was constructed. This model determined the most influential parameters as the SE, sCARB₀, TS, sCOD₀ and the VS, in order of absolute impact.

Supplementary Materials: The following are available online at <https://www.mdpi.com/1996-1073/14/6/1640/s1>. Table S1: The results of Tukey's test comparing the different digestate ages on the US disintegration efficiency per applied specific energy. Significant *p*-values are depicted in bold; Table S2: The data used for the prediction of the sCOD after ultrasonication (i.e., sCOD_{us}) of dairy manure digestate.

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