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Optimizing the Extraction of Sugars from Sewage Sludge Using Ultrasound Combined with Thermal–Alkali

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Abstract: The extraction and utilization of sugars from readily available and cost-effective sewage sludge increases the economic potential of this residue, contributing to sustainable urban development. The work presented here presents a novel method in which sugars can be directly extracted from sewage sludge following an ultrasound + thermal–alkali pretreatment. The best results indicated that by subjecting the sludge to a 240 W ultrasound for 20 min, followed by alkali digestion using 6 mL of a 2 M NaOH solution at 48 °C for 60 min, it was possible to maximize the yield of crude sugar (34.22 wt.% dry) with the purity of crude sugar at 46.80%, reaching an extraction efficiency of 99.84%. Response surface methodology was used to optimize the crude sugar yields based on experimental data, reaching a value of 34.67 wt.% dry when employing an ultrasound exposure time of 12.5 min and 6 mL of the NaOH solution for a digestion time of 57.5 min; these results were considered consistent with the experimental data.

Keywords: sewage sludge; sugar extraction; optimization; thermal–alkali treatment; ultrasound

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1. Introduction

Sewage sludge (SS) is a ubiquitous side-product of municipal and industrial wastewater treatment plants. Sludge treatment in municipal wastewater plants is a complex operation with possible environmental implications whose cost can exceed half of the total operational cost of the unit [1,2]. SS consists of organic chemicals, inorganic compounds, nutrients, pathogens, and large amounts of water [3,4]. Properly handling such sludge is extremely important for minimizing its impact on the environment and society. Considering that this sludge residue is produced all over the world, the reutilization of this waste material is a relevant subject. With the global shortage of natural resources, the extraction of renewable nutrients from waste biomass is getting increasing attention [5]. The organic matter present in SS may, therefore, be envisaged as a relatively cheap, readily available feedstock, which can make a potential renewable source production pr ofitable in its components.

The extraction and utilization of organic compounds, such as proteins and lipids from disintegration, can be found in the literature [2,6,7]. The extraction of SS mostly focused on waste-activated sludge, with the mechanism of extracellular polymers and intracellular carbon sources released from tough cell walls by disintegration. Other researchers also reported that primary SS contained large amounts of easily degradable organic compounds [8]. It has been reported that 80% of primary SS contains extracellular polymeric materials, including polysaccharides, proteins, and phospholipids [9,10]. Dignac et al. reported that the polysaccharides (alongside proteins) account for approximately 70% to 80% of the extracellular organic carbon, suggesting the possibility of polysaccharide recovery [11]. Recently, the search for various polysaccharides from natural sources such as plants and seaweeds has become a hot topic because of the physiological functions of polysaccharides, such as anti-oxidation and anti-bacterial functions [12]. In addition, the extracted sugars can also be considered as an emerging valuable resource as an energy precursor for biofuel production, such as bioethanol and biohydrogen [13]. For instance, Imman et al. extracted sugars from pineapple leaves for bioethanol production, detecting low amounts of by-products, mainly ethanol (5 wt.%) and solids [14]. Balakrishnan et al. applied the extracted sugars from Napier grass for biohydrogen generation. The results showed that 763.34 mL H_2 could be achieved under suitable conditions [15]. However, the research on sugar extraction from SS is few, and the choice of extraction method affects the yield or recovery efficiency of sugars. Traditional sugar extraction methods from biomass are comprised of hot water extraction followed by alcohol precipitation, enzyme extraction, and water leaching, processes marred by a large solvent consumption, time-consuming digestions, and poor extraction efficiency [16–18]. Hence, it is necessary to explore sustainable extraction techniques, such as thermal–alkali solvation. The principle of a thermal–alkali treatment is the solubilization of insoluble organic matter as well as the destruction of the cell wall and floc structure. In addition, hydrolysis of protein and nucleic acid matter promotes the conversion of organic matter to a liquid phase [19,20]. Su et al. studied the concentrations of carbohydrates and proteins under different alkaline conditions and concluded that the distinct solvation rate can be explained by the competition between solubility and degradation [21]. Dave et al. also found that the maximum reducing sugar yields were obtained under optimal conditions during thermal–acid hydrolysis. It turned out that the thermochemical treatment method can promote sugar extraction by breaking down the macroalgal cell wall of polysaccharides [22]. Jin et al. explored how Na^+ and K^+ in alkali-treated sludge can be exchanged with extracellular polymer ions, loosening the extracellular polymer structure and promoting the dissolution of polysaccharides [23]. As Na^+ and OH^- inhibit the solubility of hydrolysis products, the effect of alkali treatment on sugar extraction requires further study.

Due to the quantity of extracellular polymers in sludge, it is one of the main components after microorganisms and water. Extracellular polymers are an important material basis for sludge flocs to maintain a stable structure, which means the dissolution of sugars need accelerate the rate of sludge cracking. Ultrasound-assisted extraction (UAE) has been investigated to promote the dissolution of PSs through biodegradation or the mechanical destruction of cell walls [24]. UAE employs sound waves at high frequencies (>20 kHz), consisting of a series of compression and rarefaction cycles that can propagate through different media and generate cavitation bubbles in areas of negative pressure. Ultrasound resonance occurs when it passes through small floc particles, whose collapse leads to the fragmentation of the macromolecular structure, the collision and bonding of particles, enhancing the solubility of organic matter [25]. This technique has seen growing application in diverse industries, including SS valorization, as reflected in the study of Gong et al., who applied ultrasound and Fenton reagent, and whose results indicated that ultrasound could significantly increase the extracellular polymer concentrations in the supernatant [26], and that of Xu et al., who found that ultrasounds can destroy sludge flocculates and cell structures [24]. In addition, the effect of ultrasonic intensity on sludge water content was slightly greater than that of ultrasonic time. Preliminary studies by Gao et al. provided evidence that the extraction yield of proteins (36.6 wt.% dry) from SS was enhanced largely under the combination of UAE and hot alkali treatment, as well as reporting an improvement of 93.4% [6].The results here presented notwithstanding, the use of UAE for the extraction of sugars from unconventional materials has not attracted much attention from the scientific community at large. Because of this, the objectives of this work were as follows: (1) using raw sludge, to investigate the effect of ultrasound treatment at different powers coupled with the addition of an alkaline solution at different digestion times; (2) to characterize the extracted sugar product; (3) to illustrate the effect of pretreatment(s) and discuss possible mechanisms to facilitate the extraction of sugars. Results from this study help bridge the research gap and provide an alternative approach to biowaste-to-resource valorization.

2. Materials and Methods

2.1. Materials

Primary sludge was received from a wastewater treatment plant in Nanyang City, Henan Province, China. Protein, carbohydrate, and lipid contents in the feedstock were analyzed using the Kjeldahl method, the DuBois method, and Soxhlet extraction, individually [27]. The ash content was determined by combustion at 550 °C for 4 h. The basic properties of the SS are listed in Table 1.

Table 1. Characterization of the sewage sludge.

Moisture	Ash	Organic composition (wt.% daf.) [a]			
(wt.%)	(wt.% dry)	Proteins	Carbohydrates	Lipids	Others
97.98	39.75	44.08	16.04	17.37	22.51
Elemental content (wt.%) [b]					HHVs [c]
C	H	N	O [b]	S	(MJ/kg)
29.54	5.61	5.26	18.84	0.79	14.88

Notes: [a]: daf.: dry, ash-free basis; [b]: calculated by difference; [c]: HHVs (Higher heating values) calculated by the Dulong formula [28].

Sodium hydroxide, calcium hydroxide, concentrated sulfuric acid, phenol, glucose, absolute ethanol, hexane, methanol, and acetone were purchased from the Tianjin Kemiou Chemical Reagent Company.

2.2. Experimental Procedure

2.2.1. Extraction of Sugars

A 50 mL portion of unconditioned sludge was placed in a 100 mL flask. Ultrasound exposure was performed using an ultrasonic bath, in which two power settings were considered (240 W vs. 600 W), and was performed prior to alkali treatment if applicable. The alkali treatment was performed using a 2 M solution (NaOH or $Ca(OH)_2$) added to each flask, and alkali digestion was performed using an oscillating shaker at 48 °C. The choices of NaOH addition, digestion time, ultrasound power, and exposure time were carefully considered based on both theoretical rationale and empirical evidence from previous research [24,29,30]. Determining the optimal range of these parameters helps achieve efficient extraction of crude sugars. NaOH addition influences reaction kinetics and process efficiency. The selected digestion time can ensure ample contact between NaOH and the sludge while mitigating excessive degradation or side reactions. Higher ultrasound power and longer exposure times were explored within reasonable limits to maximize extraction efficiency. We have cited pertinent references to substantiate the rationality behind selecting the process parameters.

The mixture was centrifuged after digestion, and the aqueous supernatant was concentrated to ¼ of the volume. A 30 mL ethanol (95%) portion was added to the supernatant and kept for 12 h. The cooled product was washed three times and centrifuged after adding 2 mL acetone and 3 mL ethanol. The separated solid phase was freeze-dried and regarded as crude sugar.

2.2.2. Purity of the Extracted Sugars

The pure sugar content in the extracted sugars was estimated by the DuBois method, using glucose as the standard [31,32].

2.2.3. Response Surface Methodology

As described, a series of independent variables may affect the result of the pre-treatment process, and multivariate statistical models provide a tool to determine the weight factor of a single variable, as well as the interaction of the different variables, with the ultimate goal to optimize the desired result. Response surface methodology (RSM) can estimate the multivariate polynomial fitted to the different variables using a series of designed factors [14,33]. The result to be optimized is the yield of crude sugars, while the single variables employed were the amount of alkali addition, the alkali digestion time, and the ultrasound time.

2.2.4. Characterization

The surface structure of the disintegrated sludge and the porous structure of sugars were observed by scanning electron microscopy (SEM, JSM-7900, Tokyo, Japan). The functional groups of extracted sugars and sludge residues were characterized via Fourier-transform infrared spectroscopy (FTIR, ALPHA II Bruker, Ettlingen, Germany).

2.3. Data Definition

The crude sugar yield was calculated as the mass of extracted sugars over the mass of organic matter in the original sludge, cited from other resources [12,34], as Equation (1),

$$\text{Crude sugar yield } \% = \frac{M_{extracted\ sugars}}{M_{sludge\ organic\ matter}} \times 100\% \quad (1)$$

The purity of sugars was calculated as the mass of pure sugars divided by the mass of extracted sugars, as Equation (2),

$$\text{Purity of sugars } \% = \frac{M_{pure\ sugars}}{M_{extracted\ sugars}} \times 100\% \quad (2)$$

The extraction efficiency was estimated as the mass of extracted sugars and the mass of total sugars in the original sludge, referred from Jawaher's work [35], as Equation (3),

$$\text{Extraction efficiency } \% = \frac{M_{pure\ sugars}}{M_{total\ sugars}} \times 100\% \quad (3)$$

All the experiments were carried out in duplicate to confirm the reproducibility of the results. Each data point was represented by mean and standard deviation.

3. Results and Discussion

3.1. Effects of Alkali Amount on the Efficiency of Sugar Extraction

Figure 1 shows the effects of adding an increasing amount of alkali solution on the yield of crude sugars. Without adding alkali, the crude sugar yield and extraction efficiency are 1.93% and 4.53%, individually. As the addition of alkali volume is increased from 2 to 6 mL, all parameters increase, to a maximum of 28.17%, 46.75%, and 82.11% (Figure 1a–c) for the yield, purity, and extraction efficiency, respectively. With a further addition of alkali solution, all parameters lower, negligibly in the case of NaOH but markedly in the case of $Ca(OH)_2$. This may be attributed to the different pHs of both alkaline solutions, as a higher pH can enhance the hydrolysis of organic compounds; on the other hand, $Ca(OH)_2$ can promote the sedimentation of extractable materials; the Ca^{2+} ion re-flocculates with organics, thus slowing down the hydrolysis of carbohydrates, leading to a lower sugar extraction efficiency [21]. NaOH can ionize the charged groups and increase its solubility by enhancing repulsion between the polymer matrix components [36]; however, after the estimated maximum of 6 mL, hydrolysis of the desired compounds surpasses the production of these from the hydrolysis of larger molecules, leading to a loss of recoverable material and a decrease in the overall efficiency of the process. The decrease in extraction efficiency can be ascribed to the reflocculation and combination of many decomposed substances [37]. In fact, reducing sugars from the hydrolysis of polysaccharides are important substrates for polyphenol synthesis [38].

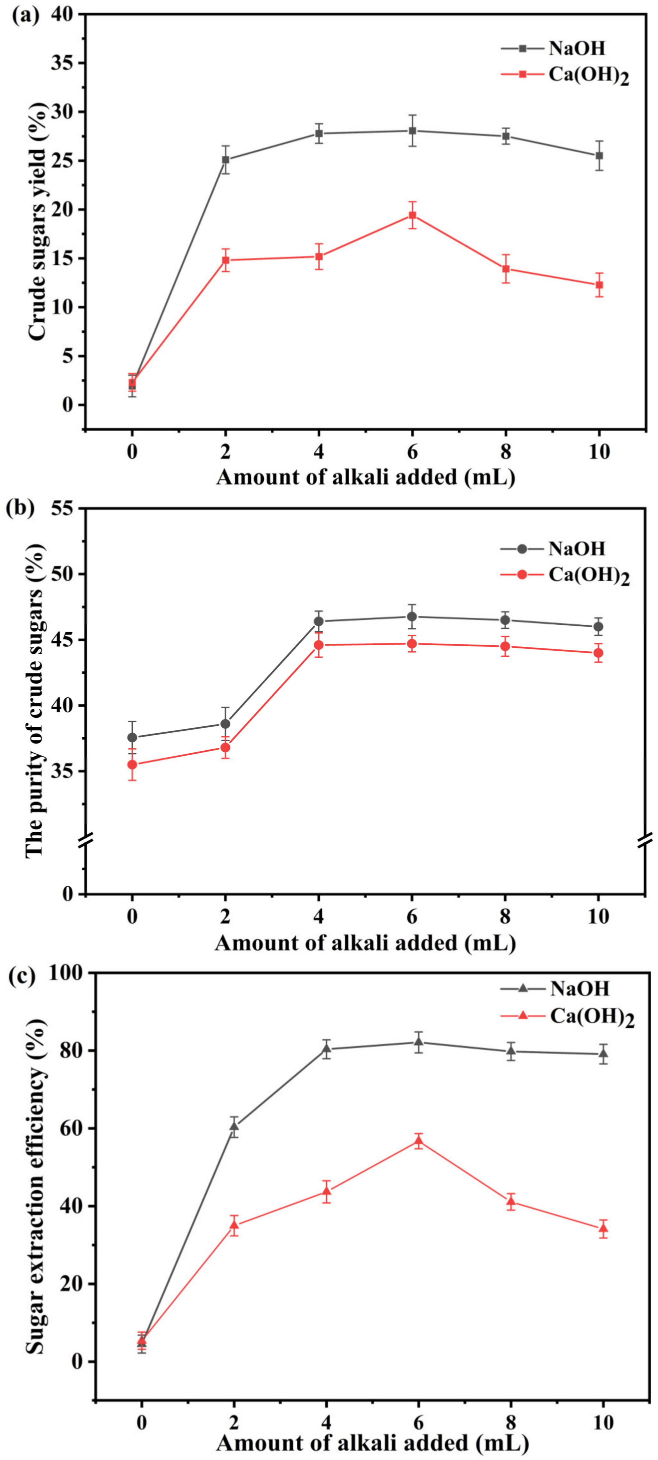

Figure 1. Effect of alkali addition on (**a**) sugar yield; (**b**) purity; (**c**) sugar extraction efficiency. Alkali digestion time: 45 min.

3.2. Effects of Alkali Digestion Time on the Efficiency of Sugar Extraction

In this section, a fixed amount of NaOH solution (6 mL) was employed, and the digestion time was varied (0–75 min). Figure 2 shows that the sugar yield increases from 0.57% to 26.85% up to a digestion time of 30 min, stabilizing afterward until the 60 min mark; this duration can be enough to maximize the degradation of the macromolecules present in the SS, thus releasing soluble carbohydrates, while an overlong reaction may lead to the hydrolysis of the soluble sugars. The trends of purity and extraction efficiency align with the yield of crude sugars, reaching maximum values at 30 min of 47.36% and 83.44%, respectively.

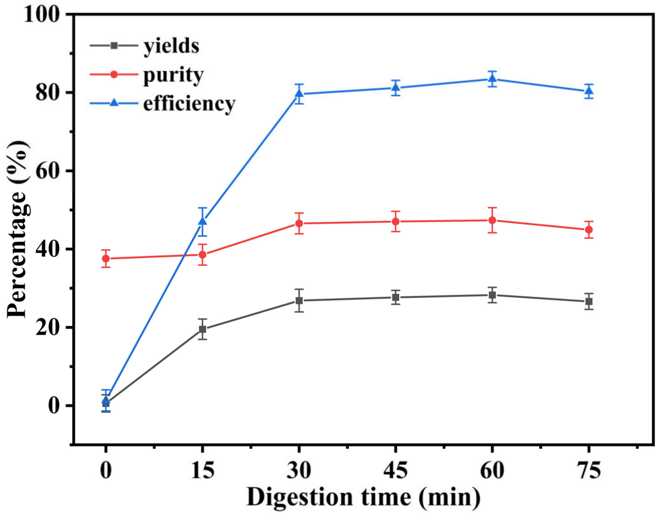

Figure 2. Effects of digestion time on sugar extraction under the 6 mL NaOH addition.

3.3. Effects of Ultrasounds on the Efficiency of Sugar Extraction

In this section, prior to alkali digestion, the sludge samples were subjected to ultrasound treatments at different power levels and during varying exposure times, always followed by alkali treatment using 6 mL of NaOH solution and a digestion time of 60 min.

At a power of 600 W (Figure 3a), the exposure time varied from 0 to 150 s, and ultrasound exposure led to a lowering in the crude sugar yield, when compared to the blank case, to a minimum of 19.66 wt.% at 90 s of exposure. The extraction efficiency peaks at 30 s (60.02%) but decreases sharply afterward, while the maximum purity is found at 60 s.

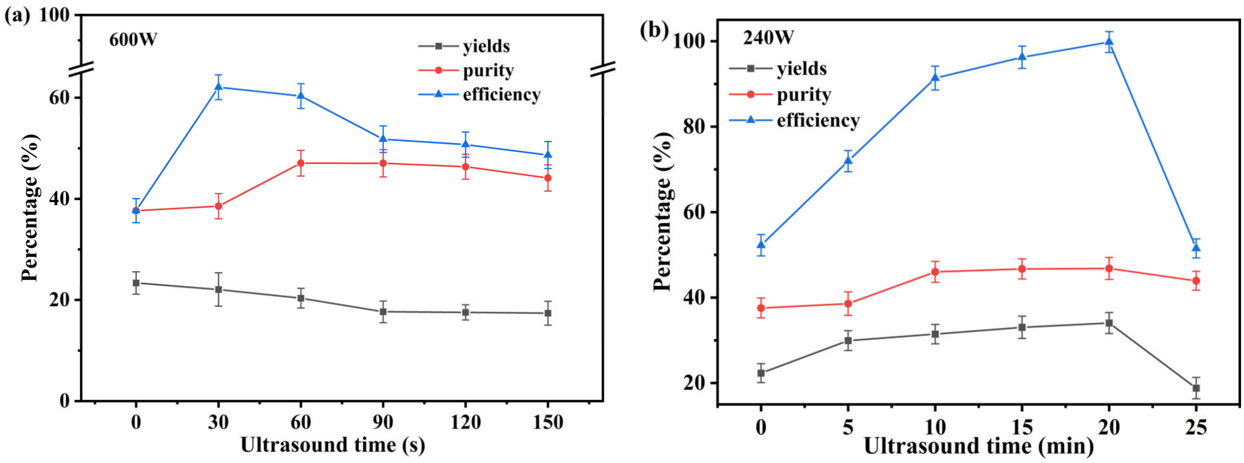

Figure 3. Effect of ultrasound power and time on the efficiency of sugar extraction: (**a**) 600 W; (**b**) 240 W. All experiments were performed after the addition of 6 mL of NaOH solution. Extraction time: 60 min.

The results when considering an ultrasound power of 240 W (Figure 3b) are vastly different from those found at 600 W. Due to lower power, longer exposure times were considered (0–25 min). Before 20 min, the sugar yield increases with the increasing exposure time, mainly due to the dissolution caused by ultrasonic thermal effect [37,39]. All parameters were maximized at an ultrasound treatment time of 20 min, with maximum values of sugar yield, purity, and efficiency of 34.22%, 46.80%, and 99.84%, respectively. Notably, the sugar yield at 240 W was approximately double the one obtained at 600 W.

Faced with these results, we conclude that an excessively high ultrasound power can degrade soluble sugars, while a lower power may assist in disrupting the macromolecular structure, aiding in the solution of sugars without their degradation. After 20 min of exposure, all parameters fall, which may be attributed to a spontaneous increase in temperature leading to the degradation of extracted sugars even at a lower power.

3.4. Process Optimization Using RSM

The RSM utilized a central composite design, incorporating three distinct factors (alkali digestion time, amount of alkali solution added, and ultrasound time), each with three levels: high (+1), central (0), and low (−1). To ensure a good estimation of the experimental design, 17 runs were conducted. To assess the performance of extracting crude sugar from activated sludge, the crude sugar yield (%) was selected as the response variable (y) for optimizing the operating variables to predict the optimal response value. The range of factors considered are alkaline digestion time (0–75 min), amount of alkaline solution added (0–10 mL), and ultrasound time (0–25 min).

The parameters that have the greatest influence on sugar extraction are NaOH addition, alkali digestion time, and ultrasound exposure time as discussed in the above sections. Regarding the single factor test, the best results indicated that subjecting the sludge to a 240 W ultrasound for 20 min, followed by alkali digestion using 6 mL of a 2 M NaOH solution for 60 min. It is important to find the optimum, i.e., the maximum crude sugar yield achieved from the combined methods. The parameters were optimized by RSM based on the Box–Behnken design. The calculated list of experiments is shown in Table S1.

According to Figure 4a, the numerical correlation between the predicted and experimental values was 96.44%, and the adjusted R2 was 91.86%, suggesting that the model can accurately predict the crude sugar yield. Figure 4b shows the residuals versus the predicted response, which fit well within the predicted range values. According to Table S2, if the *p*-value of the significant parameter is < 0.05 and if the model simulation is valid, the secondary terms (NaOH addition, digestion time, and ultrasound exposure time) had a significant influence on the crude sugar yields.

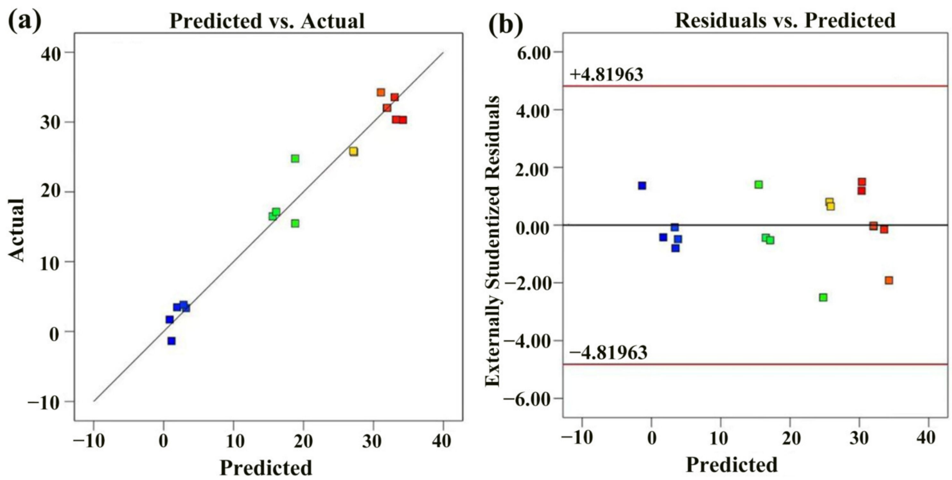

Figure 4. Model prediction: (**a**) correlation and (**b**) residual distribution.

The 3D surface plots comparing the effect of the various parameters on the crude sugar yields can be seen in Figure 5. The surface plots permit the prediction of the maximum response point of each factor. The yield of crude sugars seems to increase with higher additions of NaOH solution up to a value of 6.4 mL (Figure 5a,b); it seems to increase with higher digestion times up to 60 min (Figure 5a,c); and it seems to maximize at an ultrasound exposure time of 16 min (Figure 5b,c).

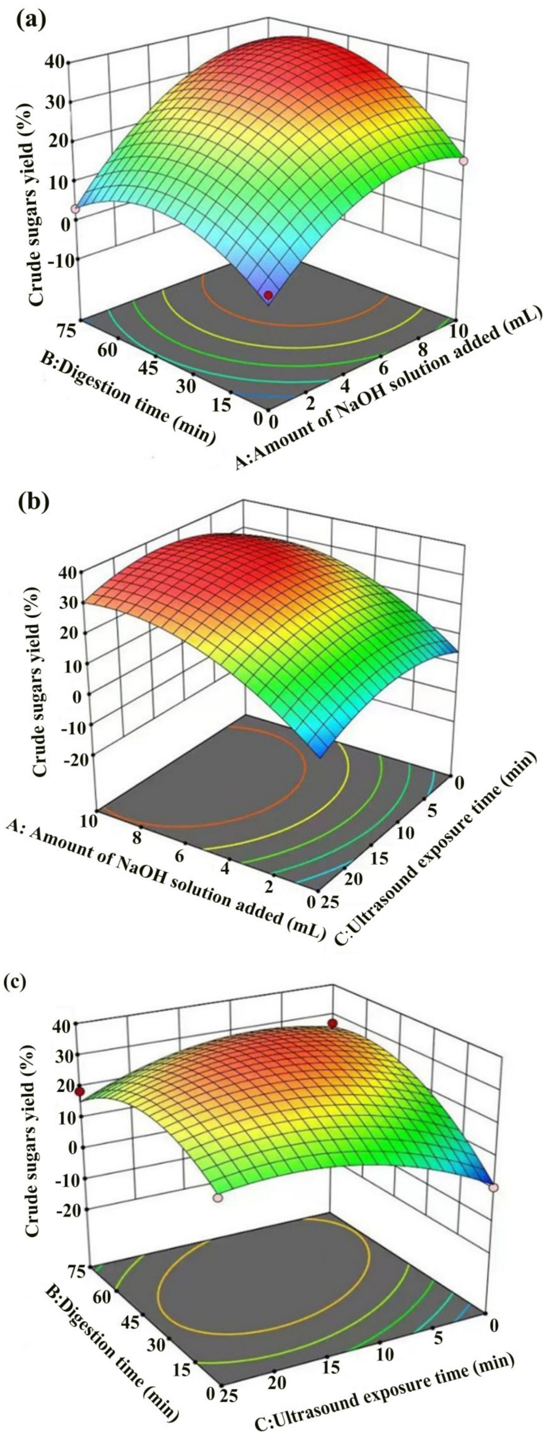

Figure 5. Response surface plots of crude sugar yield: (**a**) NaOH solution added and digestion time; (**b**) NaOH solution added and ultrasound exposure time; (**c**) ultrasound exposure time and digestion time.

The multivariate polynomial that results from RSM is found in Equation (4), where A, B, and C stand for the amount of added NaOH solution, digestion time, and ultrasound exposure time, respectively. The ANOVA analysis of RSM can be found in Table S2.

$$\begin{aligned} llYield\ [wt.\%\ dry] &= 5.17790 \times \mathrm{A} + 0.758560 \times \mathrm{B} + 1.67856 \times \mathrm{C} \\ &+0.017422 \times \mathrm{AB} + 0.017287 \times \mathrm{AC} - 0.013768 \times \mathrm{BC} \\ &-0.361012 \times \mathrm{A}^2 - 0.006983 \times \mathrm{B}^2 - 0.045916\ \mathrm{C}^2 \end{aligned} \quad (4)$$

This study leads to an optimum value of 34.67% for the yield of crude sugars, corresponding to an addition of 6 mL of the NaOH solution, a digestion lasting 57.5 min, and a 12.5 min ultrasound exposure time at 240 W. Based on these results, a further experiment was conducted leading to a yield of crude oil of 34.22 wt.%, indicating the viability of the RSM results.

3.5. Surface Structure Characterization

When investigating the extraction of sugars from SS, not only the influence of controllable variables on the yield of desired product is relevant but also the evolution of the feedstock structure during the chemical treatment.

It is not only the controllable variables that affect the yield of the target product but also the evolution of the raw material structure during the chemical treatment process.

Raw sludge residue without any treatment shows a regular shape, presenting a relatively smooth surface structure, without any folds and cracks, as shown in Figure 6a. After 60 min of thermal–alkali decomposition, the surface structure of the residue is destroyed in the irregular shape in Figure 6b. However, when ultrasounds were combined with the alkali treatment (Figure 6c), the surface structure appeared different from the other cases, as if it had been fractured irregularly. This indicates that this combination of methods inflicts the maximum damage to the solid structure, enhancing the dissolution of sugars and leading to the highest extraction efficiency.

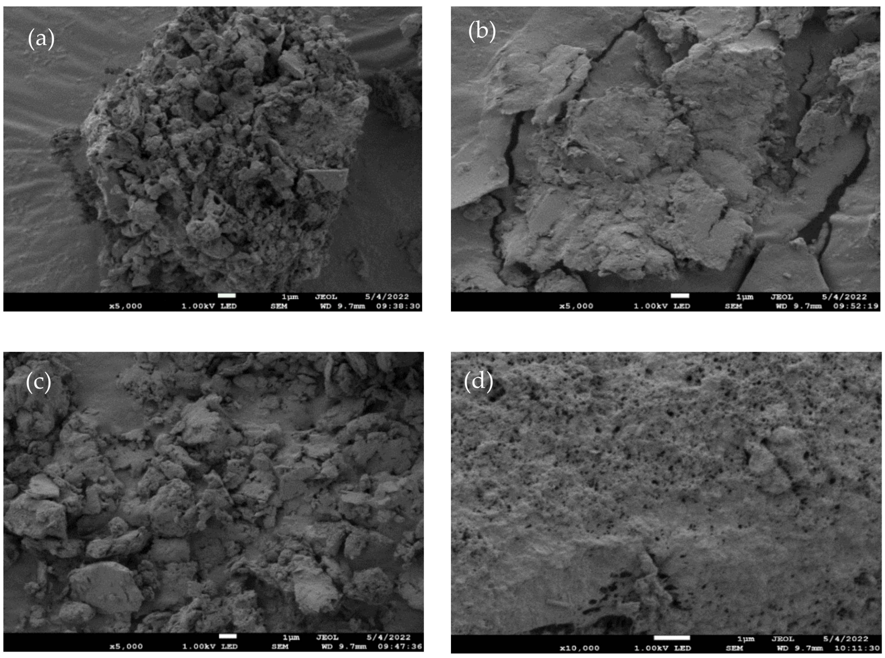

Figure 6. The SEM images:(**a**) raw SS; (**b**) thermal–alkali, for 60 min; (**c**) thermal–alkali and ultrasound, 20 min; (**d**) extracted sugars under the condition of the 6 mL NaOH addition, digestion time 60 min, and 20 min ultrasound time.

SEM was also used to characterize the surface structure of the extracted sugars, and Figure 6d displays the SEM images of the extracted sugars from the alkali–ultrasound combined treatment. The porous structure of the sugar can be observed. The structure of the crude sugar is powdery; the surface is distributed with many irregular tiny voids; and the external shape is smooth.

3.6. Functional Groups Characterization

FTIR analysis was employed to characterize the functional groups of feedstock and sugar samples. As shown in Figure 7a, the peak at 3438 cm^{-1} is assigned to stretching O-H in carboxyl and hydroxyl groups [12]. The corresponding peaks in the treated sludge residue were weaker than those in the raw sludge, confirming the dehydration that happens via hydrolysis. The observable peaks at 1630 cm^{-1} and 1035 cm^{-1}, related to C=O and C-O bonds, have been weakened after extraction, suggesting the sugar content in the sludge is largely decreased, i.e., most of the sugars have been extracted. The peak at 1035 cm^{-1} after treatment with ultrasound changed most significantly, which was because of the C-O stretching in alcohols [40]. The weakness of this peak found in the treated sludge suggested that the alcohol functional groups were in some way removed. A peak at 3438 cm^{-1}, associated with hydroxyl groups (-OH), is associated with sugar content, as it is also present in Figure 7b. The peak observed at 1035 cm^{-1}, which was assigned with an N-C-O bond, and the peak around 769 cm^{-1}, corresponding to a C-H bond, vary visibly between different pretreatment methods. When reading from bottom to top, these peaks become increasingly less intense, which may indicate that the combined treatment is more effective at disrupting the structure than the alkali treatment alone, corroborating results discussed in the previous sections.

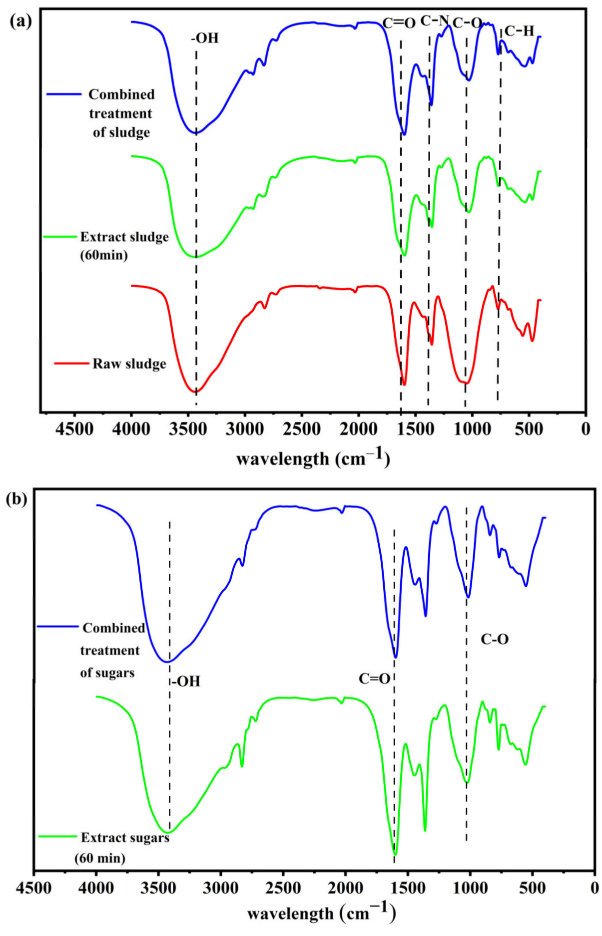

Figure 7. The FTIR spectra of (**a**) raw sludge and treated sludge residue; (**b**) extracted sugars.

4. Conclusions

The effects of the amount of alkali solution added, the digestion time, and ultrasound power on the yield and purity of sugar as well as the extraction efficiency from primary sewage sludge using a combined thermal–alkali and ultrasound treatment have been deeply investigated. The maximum yield of sugar (34.22 wt.% dry) was obtained under the following conditions: 6 mL of NaOH solution, 60 min reaction, and 20 min ultrasound exposure with a power of 240 W. The purity of the extracted crude sugars is around 46.80%, indicating that the method here presented can serve as an alternative source of soluble sugars. Tests using the aforementioned parameters beyond the optimum conditions led to a disruptive effect, explained by the hydrolysis of soluble sugars, resulting in a decrease in sugar yields.

The combination of alkali and ultrasound largely disrupted the solid and macromolecular structure of the sludge, enhancing the dissolution of sugars and further increasing the extraction efficiency. Because sugar content only accounted for around 16 wt.% of the total organic components in the raw SS, around 84 wt.% of the original organics is remaining after extracting the sugars. Consequently, future work should focus on the further valorization of the SS residue, to minimize the production of waste, increase process sustainability, and generate high-value side streams.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16091289/s1. Table S1: Crude sugar yield response surface design and results 6; Table S2: Results of ANOVA analysis of the regression model.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflicts of interest.

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