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Hydrolase Production via Food Waste Fermentation and Its Application to Enhance Anaerobic Digestion of Sewage Sludge

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Abstract: In the present study, a novel strategy for the effective production of hydrolase via fermentation of food waste was developed to improve methane production from anaerobic digestion of waste activated sludge (WAS). Via the pre-fermentation of food waste, hydrolase could be efficiently enriched and then directly used to enhance sludge hydrolysis with no need for extraction and purification of the enzymes. The results of this study indicate that the activities of the predominant hydrolase, mainly including protease and amylase enriched in the pre-fermented food waste, could reach 4861.10 U/g and 3909.14 mg/(mL·min), respectively. The elevated activities of hydrolases evidently enhanced sludge hydrolysis by more than 50% with the addition of 15 g fermented food waste per 200 g sludge. The released organic matter presented much better biodegradability, of which the BOD_5/COD (B/C) increased from 0.33 of the raw WAS to 0.41 of the pretreated sludge. Moreover, methane production from sludge digestion was substantially improved and increased from 2140 mL to 7187 mL by adding 30 g fermented food waste into 200 g sludge, about 24.3% of which was contributed by the addition of the enriched hydrolase. The preliminary economic assessments of this present study indicate the net profit of sludge digestion of 7.99 USD/m^3 sludge is likely to be harvested via applying this strategy. Furthermore, the results in this present study provide another innovative route to further optimize the conventional co-digestion process of WAS.

Keywords: waste activated sludge; food waste; pretreatment; anaerobic co-digestion; pre-fermentation for hydrolase production

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1. Introduction

In recent years, the production of waste activated sludge (WAS) with a water content of 80% has rapidly increased in wastewater plants (WWTPs) and exceeded 65 million tons in China [1]. WAS disposal has become an impending issue to be tackled. However, the bulky sewage sludge brings not only challenges but also opportunities. WAS is also a promising resource with abundant carbon sources and nutrients that present vast potential to be explored. There are already many technologies that have been developed for sludge treatment in the past decades. Nevertheless, anaerobic digestion has become a predominant technology for sludge treatment due to the feasibility of bioenergy recovery and simultaneous effective reduction and stabilization of organic matter. Therefore, it has attracted widespread attention [2–4].

Carbon sources in sludge are mostly entrapped in the matrix of sludge flocs and are difficult for microorganisms to use directly. Thus, pretreatment has recently become an indispensable step for sludge anaerobic digestion [5–7]. Extensive studies indicate that the performance of sludge digestion could be improved by 50–80% after the application of pretreatment processes [8,9]. The solubilization of organic substances, including protein,

carbohydrates, lipids, and nucleic acids, can be enhanced by sludge pretreatment, which thus greatly improves the efficiency and performance of anaerobic digestion [10].

The recent development of sludge pretreatment methods could be generally divided into physical, chemical, and biological processes, as well as the integration processes with the aforementioned. Compared with physical and chemical pretreatments, biological pretreatment presents some valuable advantages, such as milder operational conditions, fewer by-products, lower energy consumption, etc. [11–14]. The previous study [14] showed that good efficiency in WAS pretreatment could be indeed obtained by the addition of industrial protease and/or chemicals. For example, Wawrzynczyk et al. [15] reported enzymes, sodium tripolyphosphate, and cation exchange resins evidently enhanced the protein solubilization from WAS. However, the costs of industrial enzymes are still high. Thus far, seemingly, the enzymes are difficult to apply widely to sludge pretreatment. Moreover, industrial hydrolase is relatively pure and often limited to one type of specific enzyme, so it is very difficult to achieve a high hydrolysis rate during the pretreatment of sewage sludge with complex components, including protein, polysaccharides, fats, etc.

A cost-effective approach to hydrolase production from waste organics seems a promising alternative [14,16–19]. In addition to sewage sludge, food waste is another typical municipal solid waste with large output and characterized by good biodegradability. Moreover, similar to sewage sludge, there are also abundant organics with a wide variety of food waste, namely protein, carbohydrates, lipids, etc. [15]. Therefore, food waste could be a readily available and cheap substrate for the production of composite hydrolase, which is suitable for sludge pretreatment. Several studies [18–21] reported some microorganisms could produce certain enzymes by using different substrates. For example, *Aspergillus oryzae* could produce amylase, protease, cellulase, pectinase, and lipase by fermentation with the substrates of potato skins, waste bread, apples, grapes, and olive oil, respectively, which often take rather high fractions in food waste simultaneously [18–21]. Finally, food waste is commonly used as a substrate for co-digestion with sludge to improve methane production, which indicates the hydrolase obtained from food waste fermentation would not lead to generating any noticeably inhibitory components [22–25].

To date, while the previous studies [18–22] have touched on this topic, the studies have not dug deeply into the specific approach adopted in our present research. Therefore, in this study, based on the fact that organic matter in food waste has good bioavailability and meets the needs of the hydrolase production for sludge pretreatment, a novel strategy of cheap hydrolase production from food waste was developed to provide an economically practicable and applicable approach to sludge biological pretreatment. In this study, it was hypothesized that a pre-fermentation process for hydrolase production from food waste ahead of the traditional co-digestion of sludge and food waste would substantially enhance the hydrolysis of sewage sludge and improve the performance of sludge digestion overall with barely any increase in cost. Furthermore, the hydrolase could also be easily harvested to provide a cheap bio-composite hydrolase. Thus, a novel attempt in this study was proposed for the further optimization of existing co-digestion technology. Overall, the objective of this study is to develop a cost-effective and efficient approach to generate hydrolase for the enhancement of sludge hydrolysis. In this study, first, a pre-fermentation process of food waste by *Aspergillus oryzae* to produce hydrolase was employed and examined. Subsequently, the optimal conditions and the stability of the hydrolase produced from the fermentation process were investigated in-depth. Then the efficiency and overall performance of the hydrolase in enhancing the sludge pretreatment and methanogenic process were examined. Furthermore, a preliminary economic assessment of the proposed scheme of integrated technology with pre-fermentation and co-digestion to enhance sludge pretreatment and methane production was implemented. By applying this technology to conventional co-digestion processes in this study, the feasibility and contribution of this technology in evidently promoting methane production were confirmed.

2. Materials and Methods

2.1. Substrates

WAS was used as the substrate for anaerobic digestion and was taken from the sludge storage tank of a local WWTP in the city of Wuxi, China. The fresh WAS was pre-concentrated before use. The concentrated WAS had a pH of 6.5–7.5, total solids (TS) of 15.3 ± 0.4%, volatile solids (VS) of 8.2 ± 0.1%, soluble protein of 250.4 ± 10.9 mg/L, and soluble polysaccharides of 296.0 ± 14.8 mg/L.

Food waste was taken from the second canteen of Jiangnan University. After sorting and removing some grease, the food waste was dried under 105 °C for 24 h, crushed and then stored in a refrigerator at 4 °C. When in use, the dry powder of food waste with TS of 99.6%, vs. of 91.0%, total carbohydrate of 50.4%, total protein of 21.6% and lipid of 25.9% in mass was conditioned to a required moisture content (i.e., 50.0% ± 0.5%) by adding distilled water.

2.2. Inoculation

Anaerobic granular sludge from an up-flow anaerobic sludge blanket (UASB) for citric acid wastewater treatment was collected as the seeding sludge for sludge digestion. The fresh seeding sludge was also pre-concentrated before use. The concentrated seeding sludge presented a pH of 6.77 ± 0.05, total solids (TS) of 8.5 ± 0.2%, volatile solids (VS) of 7.1 ± 0.1%, soluble protein of 130.0 ± 8.0 mg/L, and soluble polysaccharides of 154.2 ± 9.4 mg/L.

The *Aspergillus oryzae* (*CGMCC* 3.4437) used in this experiment for hydrolase production from food waste fermentation was purchased from General Microbiology Center of the China Microbial Species Preservation and Management Committee. The approach of cultivation of the *Aspergillus oryzae* was referred to in the study [22]. However, the composition of the *Aspergillus oryzae* seed medium was adapted and is shown in Table S1 in the Supplementary Materials.

The spore suspension of *Aspergillus oryzae* was prepared as follows: first, the conidia were harvested from the cultivation medium plates and then added into 5 mL of inactivated deionized water. Then, the suspension was transferred to a 15 mL sterilized centrifuge tube. The obtained spore suspension was then further diluted 100 times with sterilized deionized water and preserved in a 4 °C refrigerator for further use. The concentration of the spores measured using a hemocytometer was 1.0×10^4 spores mL^{-1}.

2.3. Hydrolase Production from Food Waste Fermentation

Twenty grams of waste food was adjusted into a water content of 50.0 ± 0.5% and then added into a conical flask with a volume of 250 mL, which was the unsterilized food waste medium. Then, 20 g of the waste food was sterilized in the autoclave at 121 °C for 20 min and subsequently adjusted into a water content of 50.0 ± 0.5% by sterile water before adding into the conical flask with a volume of 250 mL, which was the sterilized food waste medium. Afterward, 1.0 mL suspension with a concentration of 1.0×10^4 *Aspergillus oryzae* spore per milliliter was added into the unsterilized and sterilized food waste medium, respectively. Hydrolase production by aerobic fermentation and its stability was investigated under various pHs ranging from 5.0 to 8.5 and temperatures of 25, 30, 35, 40, and 45 °C, respectively, and samples were taken at certain intervals to analyze enzymatic activity. More detailed experimental conditions on the features of the hydrolases are depicted in Tables S2 and S3 in the Supplementary Materials.

2.4. Sludge Pretreatment by Hydrolase in Pre-Fermented Food Waste

The performance of hydrolase contained in pre-fermented food waste in sludge pretreatment was investigated by three groups of experiments, namely the experimental group (I), reference group (II), and control group (III). In group I, seven Erlenmeyer flasks with a volume of 500 mL were filled with 200 g WAS with a water content of 92.5% and then pre-fermented food waste rich in hydrolase with dosages of 0, 5, 10, 15, 20, 25, and 30 g

were added into the flasks, respectively. The volume of the substrate in all shake flasks was finally adjusted to 230 mL with distilled water. In groups II and III, all of the operational conditions and steps were kept the same as those in group I, except that the pre-fermented food waste was substituted by food waste in group II and WAS in group III, respectively. When the stirring speed was 120 rpm, and the temperature was 40 °C, samples taken from beaker flasks at the interval of about 30 min were analyzed. The results indicated that the increase of soluble COD (sCOD) concentration was not substantial 24 h after the experiment started. The data presented in this work were obtained on the 24th hour.

2.5. Anaerobic Digestion for Methane Production

Batch experiments were adopted to investigate the acceleration of hydrolase in the pre-fermentation of food waste to sludge digestion. Nine Erlenmeyer flasks with a volume of 1.0 L were filled with 200 g WAS with a water content of 92.5%. Then, 30 g food waste (I), 30 g inactivated fermented food waste (II), 30 g fermented food waste (III), 25 g fermented food waste (IV), 20 g fermented food waste (V), 15 g fermented food waste (VI), 10 g fermented food waste (VII), 5 g fermented food waste (VIII), and 0 g fermented food waste (IX) was added, respectively. All samples were adjusted to 230 mL with distilled water and then placed in an incubator shaker for 24 h at 40 °C and at 120 rpm. Then, the pH was adjusted to around 7.5 by diluting HCl and NaOH. Inocula were added at the amount of 25% of the total sample, which was calculated based on volatile solid (VS). The dissolved oxygen in samples and the headspace of the flasks were purged by sparging gaseous nitrogen for 30 min to maintain strict anaerobic conditions. In the whole process of digestion, Erlenmeyer flasks were placed in an orbital shaker with a rotation speed of 120 rpm and a temperature of 35.0 ± 0.1 °C. The volume and composition of the biogas produced in Erlenmeyer flasks were analyzed at certain intervals. All the experiments were carried out independently in triplicates. Moreover, the inactivated fermented food waste was obtained by placing the fermented food waste in the *Aspergillus oryzae* at 50 °C for 24 h.

Semi-continuous tests were implemented to investigate the effects of the application of hydrolase produced from food waste pre-fermentation on conventional co-digestion. An Erlenmeyer flask of 1.0 L was used as the reactor for sludge digestion. Firstly, in the first 25 days, this reactor with a substrate of 230 g sludge was operated in batches as a start-up. Then, in the 25–75 days (stage I), the semi-continuous operation was adopted. That is, every day, 25 g of digested sludge was discharged, and 25 g of fresh sludge was fed. The organic loading rate (OLR) of the reactor was controlled at about 7.3 kgCOD/m^3·d. During the 75–100 days (stage II), the reactor was also semi-continuously operated at an OLR of 7.3 kgCOD/m^3·d, but the substrate of 25 g sludge was substituted by the mixture of 3.26 g pre-fermented food waste and 21.74 g sludge. During the 100–130 days (stage III) and 130–160 days (stage IV), when other operational conditions remained the same, the OLRs were elevated to 14.6 kgCOD/m^3·d in stage III and then 43.8 kgCOD/m^3·d in stage IV, respectively.

2.6. Analytical Methods

Samples were pretreated with GF/C glass microfiber of 0.45 μm. Conventional indexes, including COD, BOD_5, VSS, TSS and sludge moisture content, were analyzed according to the standard methods issued by the State Environmental Protection Administration of China [26]. The particle size of the sludge was measured by a BT-2003 laser particle size analyzer, which works on the principle of laser detraction. The functional groups of organics in sludge were determined by Fourier transform infrared spectroscopy (FTIR). The gas component was analyzed by gas chromatography (GC9790II, FULI, Wenling City, Zhejiang, China) with a thermal conductivity detector (TCD) and a column packed with stainless steel (AE. TDX-01, 2 m × 3 mm). The volume of the produced biogas was measured by displacement of saturated aqueous NaCl in a graduated measuring cylinder [27]. The gas volume was calibrated to standard conditions (273 K, 1 atm) after measurement.

The activity of protease was measured by the Folin method [28]. Protease in the pre-fermented food waste (1.0 g) was firstly mingled into the solution by adding 100 mL phosphate buffer solution with a pH of 7.5. Then, a crude protease solution could be obtained by filtering with the gauze of eight layers. The activity of amylase was measured by DNS colorimetric method [29]. Amylase in the pre-fermented (1.0 g) was first mixed into the solution by adding 100 mL phosphate buffer solution with a pH of 7.0. Then, crude amylase solution could be obtained by filtering with the gauze of eight layers. In this study, amylase activity was expressed as mg maltose/(mL·min), which is also equivalent to 2.92 μmol/(mL·min).

The concentration of free DNA in the supernatant of sludge was measured to analyze the degree of sludge cell rupture [13]. Firstly, the supernatant of sludge was obtained by centrifuging at 8000 r/min for 10 min under a temperature of 4 °C. Secondly, the solution of phenol/chloroform/isoamyl alcohol (25:24:1) was added into the supernatant at the volume rate of 1:1. After becoming completely mixed, the supernatant was centrifuged at 12,000 r/min for 10 min under a temperature of 4 °C, and the obtained supernatant was labeled as supernatant 1#. Thirdly, the solution of chloroform/isoamyl alcohol (24:1) was added into supernatant 1# at the volume rate of 1:1. After completely mixed, the supernatant 1# was centrifuged at 12,000 r/min for 10 min under a temperature of 4 °C, and the obtained supernatant was marked as supernatant 2#. Fourthly, isopropyl alcohol was added into supernatant 2# at the volume rate of 0.6:1. After being completely mixed, the supernatant 2# was settled for 30 min under a temperature of 4 °C and then centrifuged at 13,000× *g* for 20 min under an ambient temperature. The precipitation, labeled as precipitation 1#, was washed with 1 mL of 70% ethanol and then centrifuged at 13,000× *g* for 10 min under the ambient temperature. After being dried, Tris EDTA (TE) buffer solution of about 50 μL was added into the precipitation, labeled as precipitation 2#. After being completely mixed the solution was used to analyze the concentration of DNA by NanoDrop 2000 (Thermo, Wilmington, NC, USA).

VFAs with the pretreatment of filtrating samples through 0.45 μm filter membranes were measured by a gas chromatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with an auto injector (AOC-20i, Shimadzu) [12]. The detector was a flame ionization and the column was a fused silica capillary (PEG-20M, 30 m × 0.32 mm × 0.5 μm, China); 4-methyl-valeric acid was added as an internal standard and the samples were acidized by 3 M phosphoric acid. The initial temperature of the GC column was 80 °C and was held for 3 min, afterwards increased by 15 °C/min to a final temperature of 210 °C, and then held for 2 min. Both temperatures of the injection port and the detector were set at 250 °C. The total VFA concentrations were calculated by summing up each individual VFA. Each sample was analyzed in triplicate, and the standard deviations of all analyses were always less than 5%.

3. Results

3.1. Hydrolase Production from Food Waste Fermentation

Hydrolase could be efficiently produced by *Aspergillus oryzae* using food waste as the substrate. First, the activities of protease and amylase were tracked over time to investigate the efficiency of hydrolase production under pH of 7.0 and 40 °C. As shown in Figure 1, effective production of protease took approximately 85 h, and its activity in the fermented food waste reached the maximum of approximately 6646.75 U/g. Amylase production needed about 108 h, and its activity in the fermented food waste reached the maximum of approximately 5568.40 mg/(mL·min). Moreover, the substrate seemed to have a significant effect on hydrolase production. The maximum of both protease activity of 6646.75 U/g and amylase activity of 5568.40 mg/(mL·min) from the fermentation of sterilized food waste was all evidently higher than those of the unsterilized food waste, i.e., protease activity of 4861.10 U/g and amylase activity of 3909.14 mg/(mL·min), although their rates in hydrolase production were almost the same. However, the activities of protease and amylase produced by unsterilized food waste also reached the maximum values of

4861.10 U/g and 3909.14 mg/(mL·min), respectively. The two previous studies indicated protease activity was below 5.0 U/g during sludge fermentation for VFAs production, and the hydrolysis of residual organics could be reactivated by adding external protease at the end of the sludge fermentation process when the activity of protease was only at the level of about 17.5 U/g [11,30]. Therefore, the results indicated the hydrolase in the fermented food waste could be directly used to accelerate sludge digestion, avoiding tedious extraction processes. Fermented food waste with hydrolase would be used as a cost-effective biological alternative for industrial hydrolase.

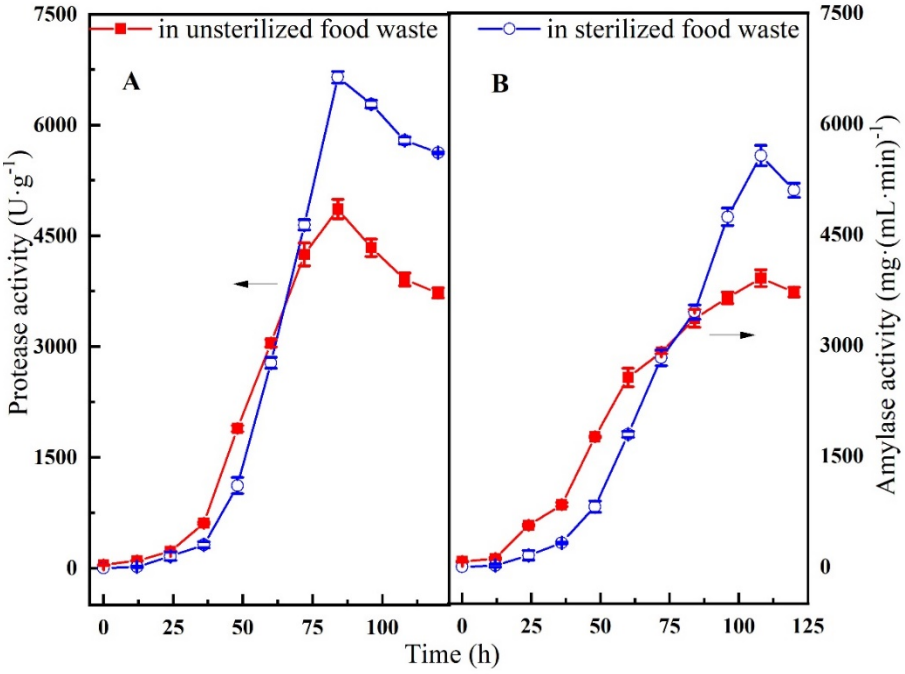

Figure 1. Fermentation of food waste for protease (**A**) and amylase (**B**) production by *Aspergillus oryzae* under pH of 7.0 and 40 °C.

3.2. Optimal Conditions of the Hydrolase

During sludge digestion, the key parameters of the optimal conditions of the hydrolase mainly included temperature and pH. As shown in Figure 2A,B, the activities of both protease and amylase extracted from fermented food waste were indeed evidently influenced by temperature and pH. In the range of 25–45 °C, the temperature slightly affected the activity of protease and the optimum temperature was around 40 °C. In a wide range of 30–80 °C, the influence of temperature on the activity of amylase was not significant, though the optimum temperature was around 50 °C. Therefore, the results indicated the hydrolase produced by *Aspergillus oryzae* from food waste seemingly had a rather broad spectrum of working temperature, and the optimum temperature range was 40–50 °C.

Moreover, seemingly the activity of hydrolase was not severely interfered by the pH of the niche. However, alkaline conditions seemed to be more conducive to hydrolase activity. In the range of 6.0–8.5, the effect of pH on protease activity was not substantial, and the optimum pH was around 7.0. However, its activity dropped by over 70% rapidly when pH was adjusted from 6.0 to 5.5 (Figure 2C,D). Compared with protease, the influence of pH on amylase activity seemed to be more negligible. Therefore, generally, in this study, the optimal conditions of the hydrolase were seemingly relatively broad, and the ideal environment was in the range of 40 and 50 °C and under neutral or slightly alkaline conditions.

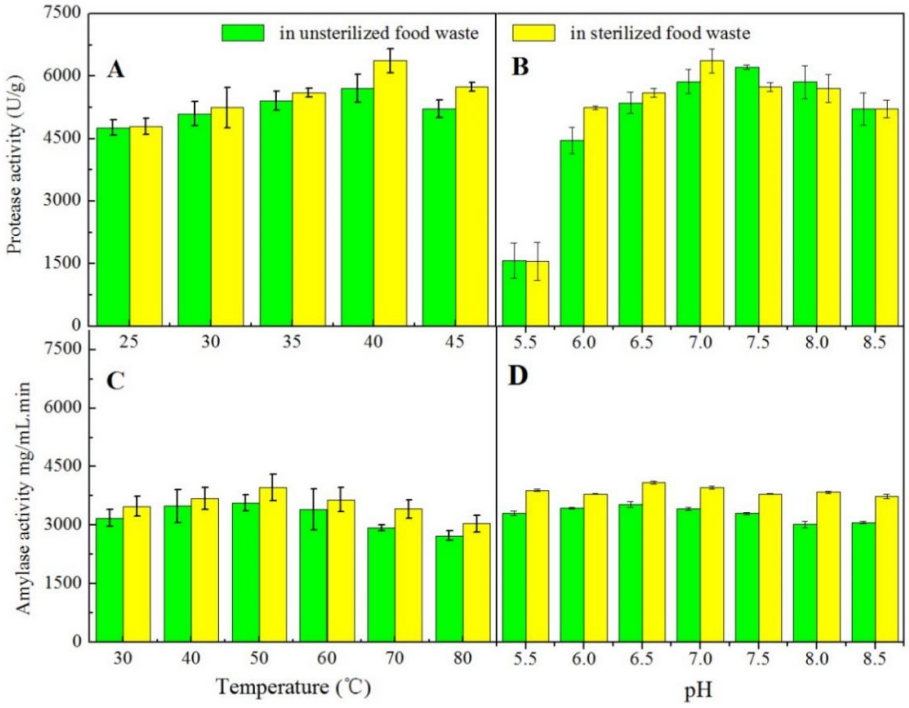

Figure 2. Activities of protease (**A**,**B**) and amylase (**C**,**D**) under different temperatures (**A**,**C**) and pHs (**B**,**D**).

3.3. Stability of Hydrolase

The stability of the hydrolase crudely extracted from fermented food waste could fully fulfill its functions in its application on sludge pretreatment. As shown in Figure 3, the stability of protease was evidently better than that of amylase. The observed half-life of protease was 2.5–7.5 h, while that of amylase was 0.8–2.0 h. Sludge pretreatment via external hydrolase generally needed less than 2 h to have effective hydrolysis [11]. Under the operational conditions during the application of the hydrolase crudely extracted from fermented food waste in this study, the activity of the hydrolase could be well maintained, larger than 50% of the initial activity within the required 2-h period for sludge pretreatment.

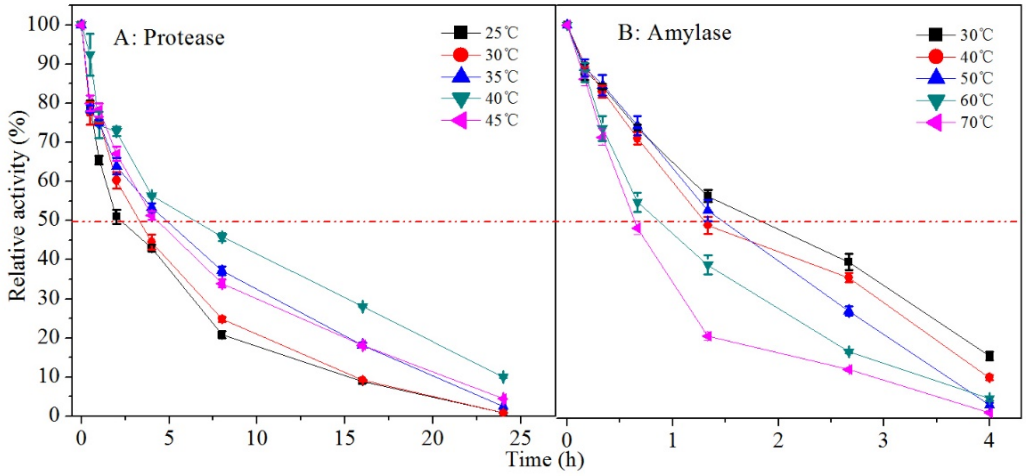

Figure 3. Stabilities of protease (**A**) and amylase (**B**) under different temperatures.

Moreover, the ambient temperature presented significant influences on the stability of the hydrolase produced by food waste fermentation, and the effect also seemed to be divergent on protease and amylase. The temperature conducive to the stability of protease was around 40 °C; in the range of 25–45 °C, the lower or upper limits of the temperatures

substantially affected its stability. While the temperature conducive to the stability of amylase was at about 30 °C and in the range of 30–70 °C, as the temperature increased, its stability decreased. Therefore, the optimal temperature of this hydrolase crudely extracted from fermented food waste would be good to be controlled at 30–40 °C as much as possible.

3.4. Performance of Hydrolase in Sludge Pretreatment

3.4.1. Solubilization of Organic Matter

The release of organic matter from sewage sludge was greatly accelerated by adding the pre-fermented food waste rich in hydrolase. The concentrations of soluble COD (sCOD) in the supernatant of the pretreated sludge were improved with the increase of the added dosage of the pre-fermented food waste (Figure 4A). Comparing the mixed sample of food waste and sludge (II) with the sample of sole sludge (III), the supernatant of the former presented with a higher SCOD concentration due to the contribution of the SCOD present in the food waste. Further comparing the mixed sample of food waste and sludge (II) with the mixed sample of pre-fermented food waste and sludge (I), the SCOD concentration in the latter was significantly higher than the former, implying that the hydrolase contained in pre-fermented food waste played an important role in the release of organics.

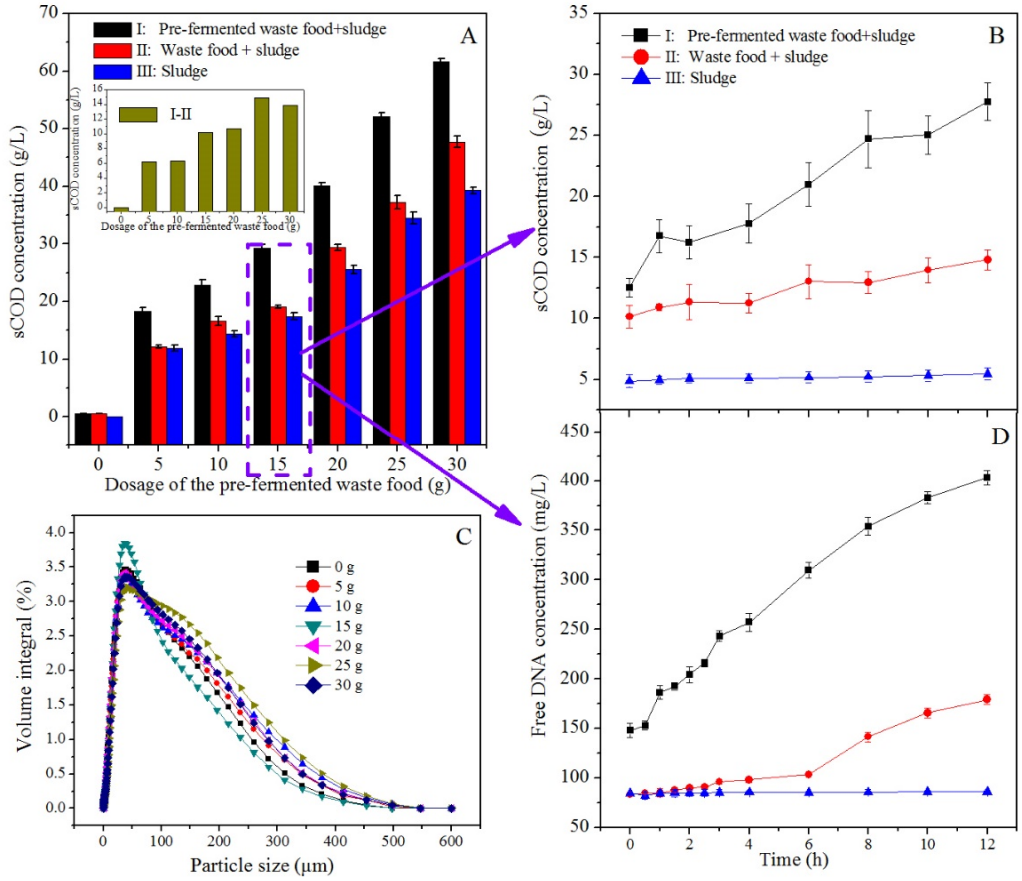

Figure 4. Performance of hydrolase in sludge pretreatment, including carbon release (**A**) and particle size (**C**) with different hydrolase additions, as well as the changes of sCOD (**B**) and DNA (**D**) in supernatant during pretreatment.

Meanwhile, the performance of hydrolase in sludge pretreatment could also be confirmed via the changes in the particle size of the sludge. As shown in Figure 4C, sludge particles strikingly decreased by hydrolase. The particle size of the pretreated sludge was presented to be the minimum when the addition dosage was 15 g fermented food waste per 200 g sludge, which indicated at this condition, the sludge had been sufficiently hydrolyzed. As further adding fermented food waste, the performance of sludge hydrolysis was only

slightly further improved, which might be attributed to the fact that the large particles present in the pre-fermented food waste probably also interfered with the detection of sludge particle size as well as the hydrolysis process.

Furthermore, as shown in Figure 4B,D, the concentrations of sCOD and free DNA in the supernatant of the pretreated sludge (I) also kept increasing sharply within 12 h of the experiment. However, the concentrations of sCOD and free DNA basically remained stable in the sample of sole sludge (III) and only slightly increased in the mixed sample of food waste and sludge (II). Therefore, the results showed that the hydrolase from food waste fermentation not only played an important role in sludge hydrolysis, rupturing sludge cells and dissolving organic matter, but also its activity could be maintained at a high level for a long period in semi-continuous operation, obtaining a much longer observed half-life than that mentioned above. Presumably, if applied together with fermented food waste rather than after extraction, the activity of protease could be more stable.

3.4.2. Improved Biodegradability of the Pretreated Sludge

The biodegradability of sewage sludge could be greatly improved by the hydrolase in the fermented food waste, which was also confirmed in the study on the residual sludge by the addition of protease [30]. The BOD_5/COD rates (B/C) of sewage sludge and fermented food waste were 0.29 and 0.38, respectively. As shown in Figure 5A, when 15 g of fermented food waste was added to 200 g of sewage sludge, the B/C of the mixed sample (I) was 0.33. However, with the addition of hydrolase, the B/C rate of the mixed sample (I) increased during the operation of 12 h and eventually even surpassed that of the fermented food waste (IV), reaching 0.42. The improvement could be attributed to the substantial enhancement in the degradation of macromolecules, such as proteins with low biodegradability, reported in the study [30]. Although the studies [11,30] indicated that the thermal or thermal-alkaline and biological pretreatments (e.g., the addition of commercial enzymes) of sewage sludge could obtain comparable performance in contrast with the approach adopted in this study and all substantially enhance the biodegradability of sewage sludge, the costs are often rather high due to the relatively high consumption in energy or chemicals and high expenditure in commercial enzymes in the pretreatments, compared with the proposed approach in our present study.

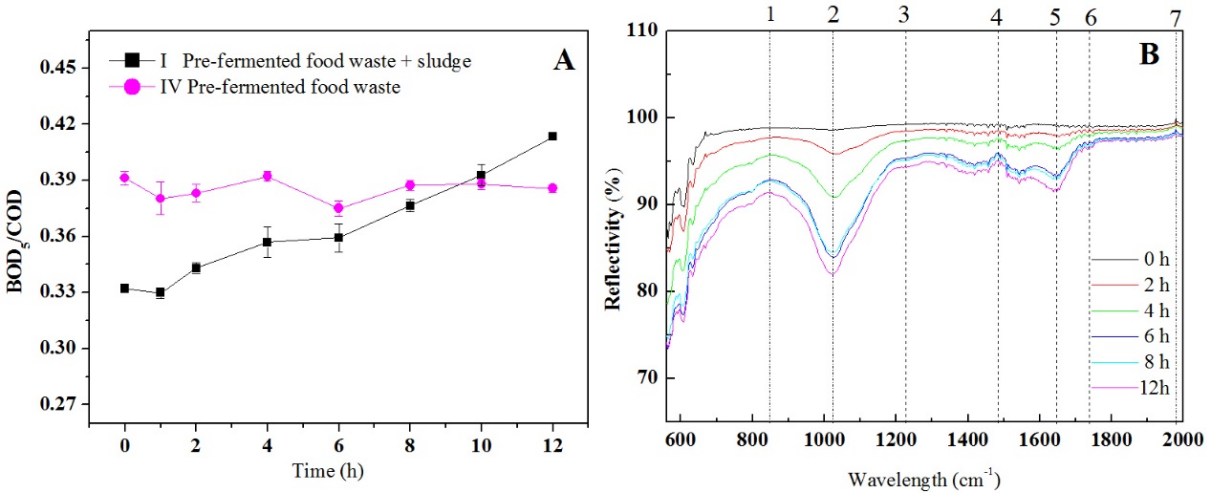

Figure 5. Changes of BOD5/COD (**A**) and features of Fourier infrared spectrum (**B**) of the sludge treated by the hydrolase in fermented food waste.

Moreover, in order to clarify the mechanism of hydrolase in promoting sludge biodegradability, the characteristics of the Fourier infrared spectrum of the organics in the supernatant of the pretreated sludge were detected at different intervals. With the extension of the pretreatment time, the reflectivity of the Fourier infrared spectrum appeared two obvious peaks at

1000–1050 cm^{-1} (peak 2) and 1600–1700 cm^{-1} (peak 5) (Figure 5B). The former represented the bending vibration of the polysaccharide structure, including single bonds of C-C, C-N and C-O, while the latter indicated the existence of amides composed of C=O and C=C, most likely being the amino acids in proteins. Of course, there were also other peaks, such as 1200–1250 cm^{-1} (peak 3), 1450–1500 cm^{-1} (peak 4) and 1700–1750 cm^{-1} (peak 6), which indicated the existence of C-O-C bonds in phenol ether, C=C bonds in the aromatic ring and C=O bonds in aldehydes and esters, respectively. Therefore, the results indicated hydrolase in the fermented food waste not only promoted the dissolution of organic matter, such as proteins and polysaccharides, in the sludge but also facilitated the decomposition of other macromolecules, thereby improving the overall biodegradability of sludge.

3.4.3. Enhanced methane production from sludge digestion by hydrolase pretreatment

Methane production from sludge digestion could be greatly enhanced by the pretreatment of hydrolase in fermented food waste. The final cumulative methane production was improved from 2140 mL in sample IX with no addition of fermented food waste to 7187 mL in sample III with the addition of 30 g of fermented food waste (Figure 6A). Moreover, by gradually elevating the fraction of fermented food waste from sample IX to III, methane production also increased accordingly. However, the enhanced methane production could be attributed to two aspects, namely, the added hydrolase and the introduced organics of fermented food waste.

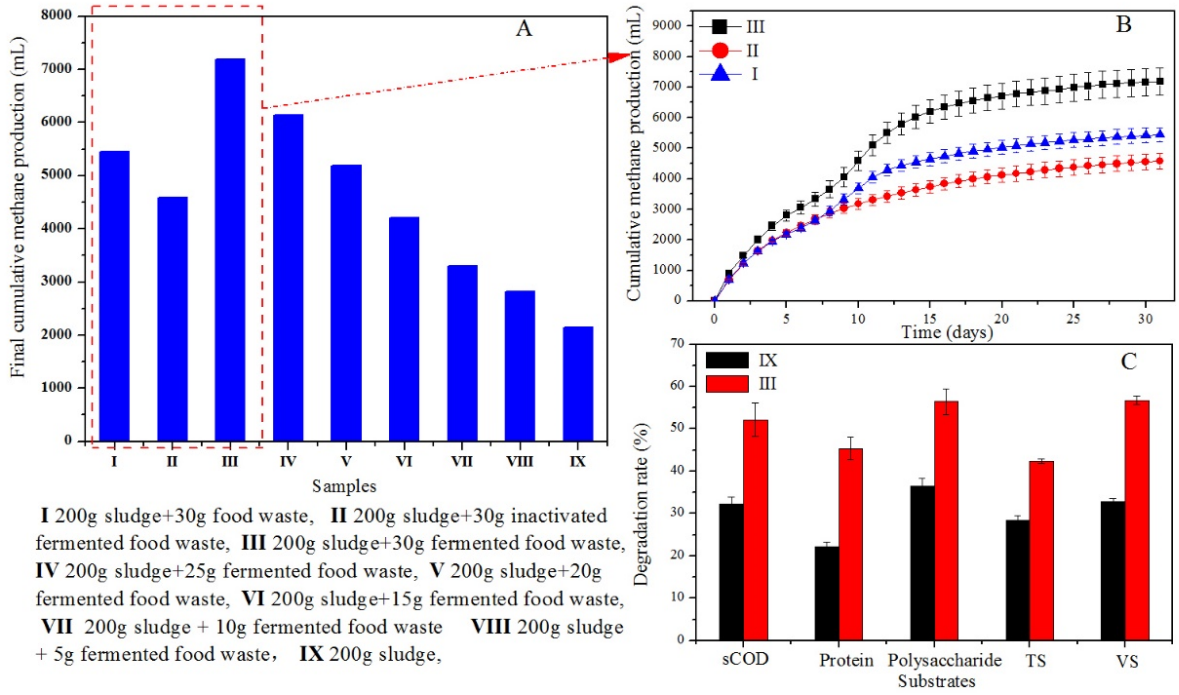

Figure 6. Final methane production by co-digestion of sludge with different amounts of food waste (**A**), cumulative methane production during co-digestion of sludge with elevated amounts of food waste (**B**) and the degradation rate of the substrates (**C**) (Note: gas volume unit (STP)).

Thus, the performance of hydrolase was further confirmed by sludge co-digestion with food waste (sample I), fermented food waste (sample III) and inactivated fermented food waste (sample II), respectively. Figure 6A,B depict that when the total amount of substrate remained the same, methane production of the hydrolase-rich sample III was at the maximum. The methane production of sample II was smaller than that of sample I, which was possibly caused by the volatilization of small molecules during the inactivation process. Therefore, the results evidently showed that the hydrolase in the fermented food waste indeed played an important role in promoting sludge anaerobic digestion.

The presence of hydrolase introduced via the addition of fermented food waste could greatly promote the degradation of organic matter in the sludge. Figure 6C shows the degradation rates of sCOD, protein and polysaccharide were only about 32%, 22%, and 26%, respectively, in the group with sole sludge (sample IX), resulting in low vs. and TS reductions rates that were less than 30% and 33%, respectively. However, by the addition of 30 g fermented food waste (sample III), the degradation rates of sCOD, protein, and polysaccharide reached 52%, 45%, and 56%, respectively, resulting in elevated vs. and TS reduction rates that were more than 42% and 56%, respectively. Therefore, the results indicated this hydrolase cost-effectively harvested from the pre-fermented food waste presented remarkable performance and seemingly is a feasible approach to be applied on sludge pretreatment.

4. Discussion

4.1. Contributors to the Enhanced Methane Production

As mentioned above, methane production from sludge digestion was greatly improved by the addition of fermented food waste. By comparing the results of samples IX, I and III in Figure 6, the enhancement of methane production of sample III could be explained from the following three aspects: namely, common sludge digestion, co-digestion of residues of the food waste, and enhancement resulting from the hydrolase. Firstly, 29.8% of the methane amount (Figure 7) was contributed by sludge digestion which proceeded under the condition without any enhancement measures, just like traditional anaerobic digestion. Then, due to the co-digestion of organics in the fermented food waste, 46.0% of the methane production was enhanced via substrate increase and co-metabolism synergy. Finally, about 24.3% of the methane amount evidently benefited from the hydrolase addition. The results indicated that though the ratio of the fermented food waste to sludge was only about 30 g/200 g, the contribution of hydrolase to methane production reached approximately 25%, whereas, in the conventional co-digestion process, the ratio of food waste to WAS is often as high as one. Thus, in a modified co-digestion process of the two wastes with pre-fermentation to generate hydrolases, the potential of hydrolase production from food waste is supposed to be much larger than 25%, which would lead to a substantial enhancement of WAS hydrolysis and methane production.

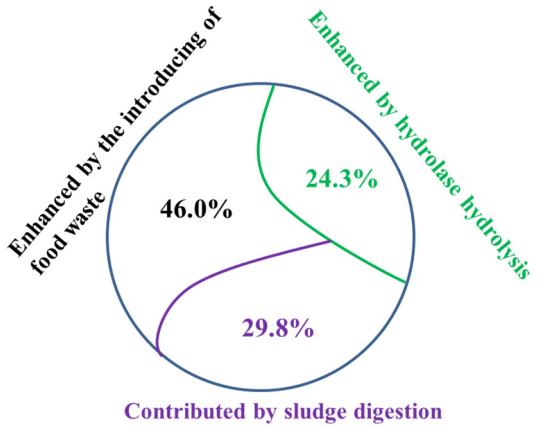

Figure 7. Contributors to the enhanced methane production during sludge digestion with the addition of pre-fermented food waste.

4.2. Economic Evaluation

In order to investigate the economic potential of hydrolase production from the pre-fermentation of food waste and its application on conventional co-digestion modification, the costs and profits of the process were calculated and compared with common sludge digestion (CSD) and conventional co-digestion of sludge and food waste (CDSF), respectively. In this study, only the costs of biogas purification and digester operation were considered, while the expenditure of residual sludge dewatering and disposal was not

considered because of the comparable scenarios in digestate treatment and disposal among the three technologies.

Considering the effects of scaling up, the capacity of sludge digestion was enlarged to 200–230 tons/day with a water content of 90%, and the parameters and inputs were based on the experimental results. The reaction time of sludge digestion was set to 25 days, while that of hydrolase production from the fermentation of food waste was set to 75 h. The cost of digester operation was mainly considered to be from electricity consumption which is based on the amount of disposal of sludge and retention time. Therefore, the three technologies presented similar operational costs due to the same scale, but there is an additional operational cost for hydrolase production from food waste pre-fermentation in the technology of the modified co-digestion of sludge and food waste (MCSF). Moreover, the cost of biogas purification is relative to the amount of biogas. Thus, MCSF would present the highest costs of biogas purification due to the largest biogas production.

As listed in Table 1, although the MCSF shows the highest total cost of 20.28 USD/m^3 sludge due to its enhanced biogas production, in comparison with the CDSF of 16.42 USD/m^3 and CSD of 13.18 USD/m^3, the MCSF still presents evident economic potential, presenting the largest net profit of 2.64 USD/m^3 sludge under the defined common conditions and scenarios of the technologies. Moreover, it is worth mentioning that the increase in biogas production also means decreases in the amount and disposal cost of residual sludge. Therefore, in view of the current situation that it is difficult for traditional sludge digestion to make profits, this technology of co-digestion of sludge with rich-hydrolase food waste possibly provides a promising direction to elevate the economic benefits of sludge digestion for biogas production.

Table 1. Economic comparison of VFAs and biogas production processes from sludge by anaerobic digestion.

Items	Conventional Sludge Digestion (CSD)	Conventional Co-Digestion of Sludge and Food Waste (CDSF)	Modified Co-Digestion of Sludge and Food Waste (MCSF)
Volume of digester (m^3)	5000	5750	Co-digestion: 5750 Hydrolase production: 94
Mass of treated sludge or waste food (ton/d)	200 (sludge)	200 (sludge) +30 (food waste)	200 (sludge) +30 (fermented food waste)
Cost of biogas purification (USD/m^3 sludge) [a]	2.68	5.92	7.81
Cost for digester operation (USD/m^3 sludge) [b]	10.5	10.5	10.5 + 1.97
Total cost (USD/m^3 sludge)	13.18	16.42	20.28
Amount of biogas (m^3/m^3 sludge)	17.84	39.45	52.08
Price of products (USD/m^3 biogas)	0.44	0.44	0.44
Gross Profit (USD/m^3 sludge)	7.85	17.36	22.92
Net profit (USD/m^3 sludge)	−5.35	0.94	2.64

[a]: The cost of the purification of biogas is 0.15 USD/m^3 biogas. [b]: The price of electricity is 0.14 USD/kWh, and the stirring time is set as 6 h/d.

4.3. Application of Conventional Co-Digestion Optimization

Recent reports and large-scale applications indicate that co-digestion has already proved to be one of the most feasible measures to improve sludge digestion. Moreover, as aforementioned, in case a pre-fermentation of the substrates with relatively high biodegradability, such as food waste, was adopted for hydrolase production prior to a traditional anaerobic co-digestion process, the performance of methane production would be further

accelerated. By adding pre-fermented food waste into sludge digestion processes, methane production was improved by approximately 180% from about 160 mL/day in stage I to about 446 mL/day in stage II (Figure 8). Furthermore, the efficiency of sludge digestion was also enhanced. When the organic loading rates (OLR) were substantially elevated from 7.3 kg/m^3·d in stage II to 14.6 kg/m^3·d in stage III and even further to 43.8 7.3 kg/m^3·d in stage IV, the methane production rate also proportionally increased. Therefore, this proposed technology of partly pre-fermenting substrates for hydrolase production seems to be feasible to be applied to the optimization of conventional co-digestion processes.

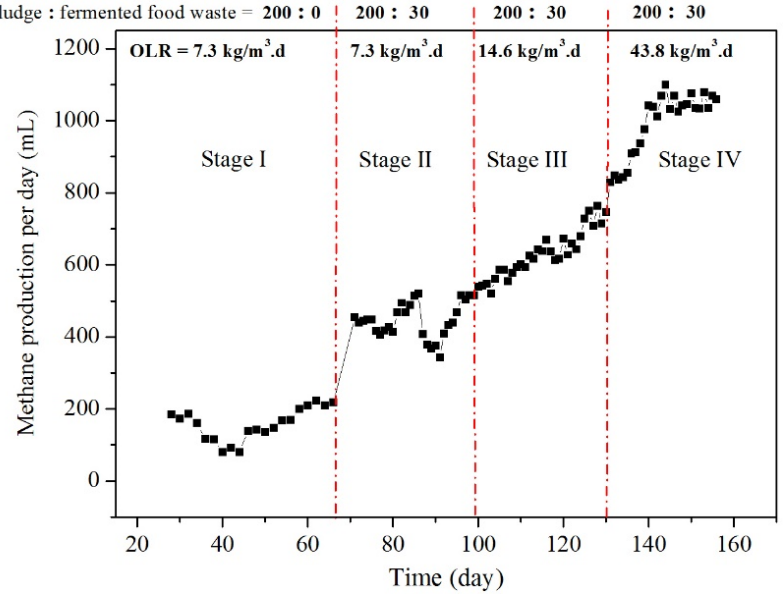

Figure 8. Application of hydrolase production from the fermentation of food waste to enhance conventional anaerobic digestion of sewage sludge.

5. Conclusions

This study presents a novel and cost-effective strategy for hydrolase production from organic waste to enhance biological sludge hydrolysis and provides a proof-of-concept for the optimization of conventional co-digestion process. Hydrolase produced by *Aspergillus oryzae* using food waste as substrate presented rather good stability and performance in sludge pretreatment. The following remarks can be concluded:

(1) Hydrolase was efficiently produced by *Aspergillus oryzae* using food waste as substrate. The relatively high and stable activities of protease and amylase in the fermented food waste indicated the fermented food waste enriched with hydrolase could be directly used to accelerate sludge co-digestion and the processes of extraction and concentration of hydrolases were no need.

(2) The optimal conditions of the hydrolase produced from the fermentation of food waste were 40–50 °C and neutral or slightly alkaline condition, well matching the usual conditions of anaerobic sludge digestion.

(3) Both of the quantity and quality of organic matter released from sewage sludge were greatly improved by the addition of the pre-fermented food waste rich in hydrolase, finally generating significant enhancement of methane production, and 46.0% of the enhancement was contributed by the addition of food waste and 24.3% was contributed by the hydrolase.

(4) The preliminary economic assessment indicates that the proposed MCSF process presented relatively high economic potential under the other defined scenarios in comparison with the other processes. The feasibility of implementing this technology of food waste pre-fermentation for hydrolase production on the optimization for conventional co-fermentation of sludge and food waste was proven, and the economic benefits seemed to be also very attractive.

(5) With regard to the limitations of the work and further research, to advance the understanding of the enhancement mechanism of the proposed process, it would be of great necessity to further explore how the pre-fermentation (i.e., addition of hydrolase-rich pre-fermented food waste to WAS) enhanced the methanogenic process, particularly at the enzymatic and microbial levels of the anaerobic co-digestion process.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9060526/s1, Table S1: The composition of *Aspergillus oryzae* seed medium; Table S2: Assays on enzymatic properties of protease under various conditions; Table S3: Assays on enzymatic properties of amylase under various conditions.

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

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