

## Article

# Application of Resident Disease Screening Paradigm on Early Warning of Instability of Anaerobic Digestion of Food Waste

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## Abstract

Early warning has been widely proven to be reliable in lowering the risk of instability for biological processes. However, it is very difficult for the warning systems used currently to achieve satisfactory accuracy, timeliness and universality simultaneously. This study developed a novel early warning system for instability in food waste anaerobic digestion (FWAD) by bioimitating the human disease screening paradigm. It consists of single, comprehensive and microbiological indicators. Findings showed that single-factor early warning systems resembled acute patient diagnosis, having high accuracy but low timeliness and poor universality. Each of the chosen single indicators showed distinct early warning performances and clear preferences for diverse inhibitions concerning the instabilities of high organic load rate, high ammonia, and high fat in FWAD. Then, a new comprehensive indicator was developed using the entropy weights of several indicators. Confirmatory tests revealed that the comprehensive indicator-based early warning system was analogue to the resident sub-health diagnostic regarding superior foresight and good operability but poor targeting. Therefore, an early warning system based on microbial changes was proposed for potential instability in FWAD by bioimitating human periodic physical examination. The sensitive bacteria were identified as *norank\_o\_norank\_c\_Dojkabacteria* and *Rikenellaceae\_RC9\_gut\_group*. Enlarged tests showed that the developed system could be used for emergent, indistinct and potential early warnings simultaneously while avoiding the shortcomings of existing systems. More precious, this study provided a paradigm for developing early warning systems of FWAD, which is also suitable to be applied to the intelligent systems that rely on automated machine learning.

**Keywords:** anaerobic digestion; food waste; early warning; instability; paradigm

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## 1. Introduction

By 2025, the world's yearly food waste (FW) production is expected to exceed 2.5 billion tons considering rapid socioeconomic advancements [1]. Anaerobic digestion (AD) is a very promising technique for treating FW, which can potentially reduce FW while

utilizing resources and being safe [2]. However, the high oil content, strong organics concentration and abundant nitrogen-containing components make the AD process of FW highly unstable, readily inhibiting via intense fat stress, severe organic load shock and high-concentration ammonia [3,4]. Especially with the improvements in the current FW recycling system, FW treatment plants are constantly attempting to operate at ever-higher loads to cope with the burden of growing FW production. Because of these circumstances, early warning of instability in FW AD processes is especially crucial and challenging.

At present, there is already an array of early warning approaches to assess the instability risks of the AD system, and new methods are continuously being developed. However, it is difficult to find a widely applicable early warning method for instability in food waste AD processes. At present, the warning method of tracking changes in single indicators, which can range from simple pH and biogas composition to complex volatile fatty acids (VFAs), alkalinity, etc., is most often used to predict the operational status of bioreactors [5–7]. However, these single indicators frequently differ depending on substrates and operating conditions, and the same indicator would present different early warning thresholds at different bioreactors. Therefore, to measure the stability of the AD system, several multiple-state parameters and models are being developed by establishing non-linear relationships between them. Nevertheless, despite improvements in universality and foresight, the accuracy of those comprehensive indicators has declined [8,9]. Most significantly, the lack of weighting the state parameters that make up the stability assessment tool prevents it from more realistically quantifying stability and providing an earlier warning [10].

Generally, the current early warning methods for AD instability can be broadly categorized into three groups, namely warning systems based on single indicators, comprehensive indicators, and microbial and by-product indicators. Moreover, each of them has advantages and disadvantages, as well as excellent complementary qualities. Firstly, warning systems that rely on single-indicator monitoring often have the benefits of strong targeting and easy operability, but they also have the drawbacks of limited application scope and weak timeliness [5–7]. The AD system would quickly degenerate in many real-world engineering projects within 1–2 days following sharp drops in biogas production and methane concentration. The optimal time to re-stabilize the system is typically missed when biogas is utilized to detect AD system instability [11,12]. Since the released high-concentration ammonia would increase the system's buffering capacity and cause delayed pH changes, pH is also found to be unsuitable as a warning indicator for instability in AD processes of food waste and chicken manure [13,14]. The warning thresholds of VFAs also varied significantly between AD systems, even though much research has demonstrated that they are useful indicators for warning of instability in AD systems [6,15]. As a result, developing comprehensive indicators that are more timely and broadly applicable has emerged as a novel approach to early warning of biological reaction process instability. Nevertheless, the currently developed comprehensive indicators for early warning typically have low maturity, poor accuracy and poor traceability. Cook et al. [10] developed a comprehensive evaluation index that included methane content, pH, alkalinity, free ammonia, VFAs, total ammonia and long-chain fatty acids. Validation results from six AD bioreactors demonstrated that this comprehensive index's accuracy was subpar and that additional improvements were required, particularly regarding the sensible distribution of weights for each index. Finally, early warning systems that rely on microbiological monitoring typically demonstrate good traceability, providing opportunities to identify the root causes of instability and proactively develop preventative actions [16,17]. Their low accuracy and complexity in on-site operation are common drawbacks. Prior research revealed that microorganisms may be categorized into three groups: resistant, resilient and redundant microbes, depending on their response to instability. The first two

categories were ascribed to the low accuracy of microbial warning indicators, while changes in the latter were closely related to the stability of the AD system [18,19]. In addition, developing AD instability early warning systems that integrate multi-level and multi-dimensional monitoring indicators is a key focus for the future development of the industry [20,21]. In particular, machine learning technology, as an intelligent auxiliary means, could simplify the modeling process and empower automatic process control. Even if the system integrates multiple types of parameters and becomes more complex in structure, it can still achieve more comprehensive and accurate identification and early warning of AD system status [22,23].

Based on literature research and classification analysis, we were surprised to find that the three levels of instability warning methods for the AD system described above have startling similarities with the resident disease prevention and control system, which includes disease diagnosis, subhealth diagnosis and periodic physical examination. In a sense, both the human body and AD bioreactors belong to relatively independent ecosystems. Moreover, studies on disease screening and prevention in the former have been conducted for thousands of years, and it has already developed a very reliable early warning system that is valuable to learn from and use in the latter. Thus, this study aimed to develop an early warning system for instability in the food waste AD process by bioimitating the human disease screening paradigm. Firstly, the shortcomings of poor timeliness and narrow applicability of the single warning indicators were reenacted by applying single indicators of pH, VFA, VFA/TA, VFA/BA, VFA, BA/TA and IA/PA to the early warning of food waste AD instability caused by inhibitions from high OLR, high-concentration ammonia and high fat content. Then, a novel comprehensive indicator was established through the weighting method, and its effectiveness in providing early warning for AD instability in food waste was evaluated. During the food waste AD instability process, routine microbiological testing was also conducted to reverse-develop microbial indicators for early warning, particularly the tracking of sensitive microbial communities. Subsequently, a novel system was proposed for the food waste AD process that consists of three parts at different in-depth levels: early warnings for emergent, indistinct, and potential instabilities, respectively. These parts correspond to disease cure, sub-health diagnosis, and periodic physical examination of the human disease prevention and control system, and the effectiveness of this system was verified through pilot-scale experiments. More importantly, this study offered a novel paradigm for developing early warning systems that can be used for other biological reaction processes besides food waste AD.

## 2. Materials and Methods

### 2.1. Substrates and Inoculated Sludge

Food waste (FW) and the inoculum used in this study were obtained from a large-scale FW anaerobic digestion plant in Wuxi City, China. Following sampling, the FW was kept at 4 °C in a refrigerator and the inoculum was kept at 38 °C. Pretreatment procedures for the acquired FW included sorting, pulping, sand removal, and oil extraction. Total solids (TS) of 99.26–105.63 g/L, volatile solids (VS) of 89.37–94.58 g/L, pH of 3.70–3.90, chemical oxygen demand (COD) of 91.42–104.73 g/L, total ammonia nitrogen (TAN) of 68.20–71.7 mg/L and volatile fatty acids (VFAs) of 274.42–328.16 mg/L were the main characteristics of the used FW.

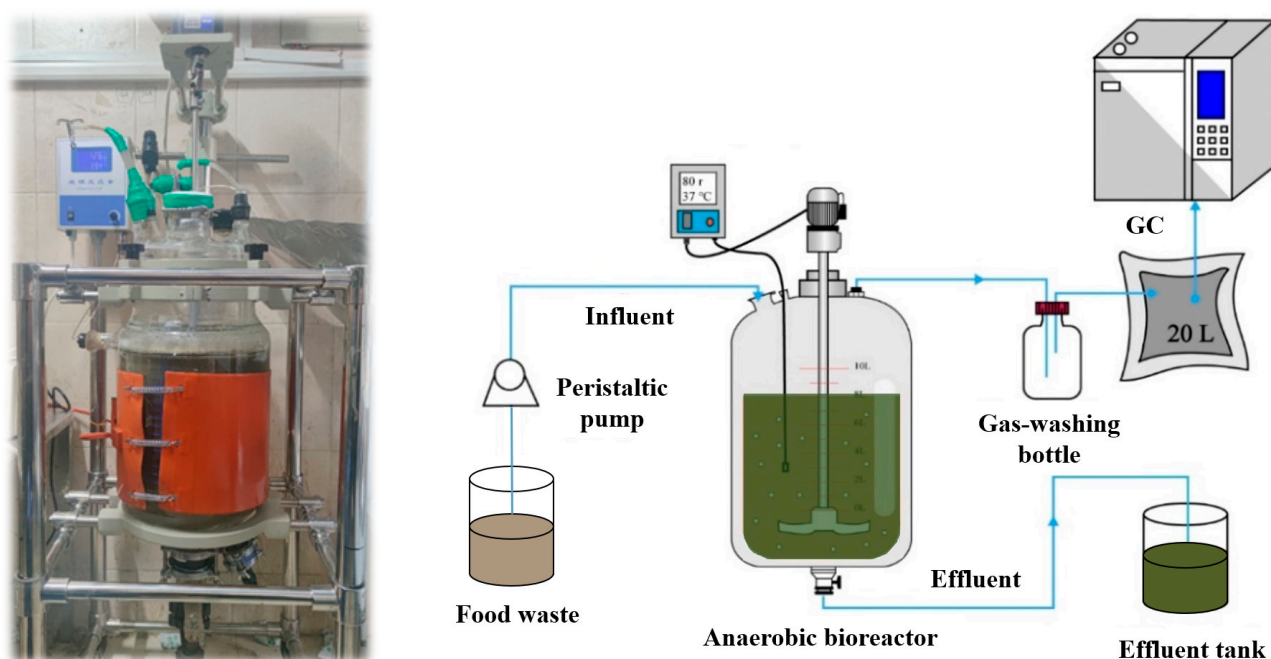
### 2.2. Simulation Experiments on the Instability Processes of Food Waste AD

Food waste was anaerobically digested using ten groups of anaerobic bioreactors (designated A, B, C, D, E, F, H, I, J and K) with a total volume of 700 mL and a working volume of 500 mL. Each bioreactor was equipped with motorized automated mixers that

rotated at 100 rpm. All bioreactors were operated at  $37 \pm 1$  °C in semi-continuous mode with daily feeding at the top and discharging at the bottom. The air pockets collected the biogas produced from the air outlets at the top of the bioreactors. The bioreactors were started at an organic loading rate (OLR) of 3.0 g VS/L/d, a TAN level of below 1500 mg/L and a fat content of roughly 2% (20.0 g/kg TS). Firstly, four groups of bioreactors were selected to simulate the instability processes of food waste AD caused by high OLRs. Each of the four bioreactors (A, B, C and D) were operated at OLRs of 4.5, 5.0, 5.5, and 6.0 g VS/L/d, respectively. In a similar manner, three more bioreactor groups were chosen to simulate the instability processes caused by high-concentration ammonia inhibitions. In bioreactors E, F, and G, ammonium chloride was continually supplied, and the TAN in each was progressively increased to 4000, 5000, and 6000 mg/L, respectively. Finally, the remaining three bioreactor groups were selected to simulate the instability processes caused by high-concentration fat inhibitions. The three bioreactors (H, I, and J) were configured to have fat contents of 15% (150 g/kg TS), 20% (200 g/kg TS) and 25% (250 g/kg TS), respectively. During the experiments, the variations in pH, volatile fatty acids (VFAs), total alkalinity (TA), bicarbonate alkalinity (BA), intermediate alkalinity (IA), partial alkalinity (PA), TS, VS, and COD within the bioreactors were measured, as were the changes in the amounts and compositions of the biogas produced daily. Every bioreactor's stability was assessed. Three parallels were established for every group to ensure accuracy. Additionally, based on the obtained data in bioreactors A–J, the comprehensive anaerobic digestion stability index was established by the weighting method [10].

### *2.3. Verification of the Comprehensive Indicator's Performance in Early Warning*

Two bioreactors, designated R and S, with working volumes of 1.2 L and 10 L, respectively, were operated for food waste AD in order to verify the performance of the developed comprehensive indicator in early warning. The structure of bioreactor R was identical to that of the ten anaerobic bioreactor groups that were previously discussed. The physical image and schematic diagram of bioreactor S are shown in Figure 1. Once a day, food waste was semi-continuously pumped into bioreactor S at the top after the equivalent amount of digested FW was discharged at the bottom. The temperature was maintained by a heating jacket. Other operating parameters, such as TAN (less than 1500 mg/L), temperature ( $37 \pm 1$  °C), rotating speed (100 rpm) and fat content (roughly 2%), were kept constant in addition to the OLRs. Additionally, a mode of gradually increasing OLR by 0.5 g VS/L/d all at once was implemented in bioreactors R and S until instability developed in order to realistically meet the operating conditions in real-world applications. The stability of each bioreactor was assessed during the trials by measuring the variations in pH, VFAs, TA, BA, IA, PA, TS, VS and COD, as well as the changes in biogas production.



**Figure 1.** Physical picture (left) and schematic diagram (right) of bioreactor S.

#### 2.4. Physical and Chemical Analysis

The conventional parameters pH, TS, VS, COD and  $\text{NH}_4^+\text{-N}$  were determined following the standards provided by the State Environmental Protection Administration of China [24]. pH was measured using a Mettler Toledo pH meter. TS and VS were measured using the drying and weighing method. COD was measured using HACH analysis and ammonia nitrogen was measured using the phenol-sodium hypochlorite colorimetric method. Gas production was calculated according to stomatal volume, and methane content was determined by gas chromatography (GC9790II, Fuli, Wenling, Taizhou, China). Volatile fatty acids (VFAs) were determined using the gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan), and total VFA concentration was obtained by adding the concentrations of different acids. Total alkalinity (TA), bicarbonate alkalinity (BA), intermediate alkalinity (IA) and partial alkalinity (PA) were analyzed by potentiometric titration [25]. The phenol-sulfuric acid method measured polysaccharides, and protein was tested using the BCA assay protein kit. Extracellular polymeric substances (EPSs) were extracted by a modified thermal separation method. The value of free ammonia nitrogen (FAN) was calculated according to Formula 1.

$$FAN = TAN \times \left( 1 + \frac{10^{-pH}}{10^{-(0.09018 + \frac{2729.92}{T})}} \right) \quad (1)$$

where TAN is the total ammonia nitrogen concentration (mg/L), and T is the thermodynamic temperature with a value of 310.15 (K) at 37 °C.

#### 2.5. Microbial Community Analysis

For microbiological analysis, samples were taken from the digestion solutions of the bioreactor S on days 0, 30, 50, 60, 70, 80, 90 and 100 of the experiment based on the time nodes of OLR increase and methane production conditions. The bioreactor number and sampling date were included in the sample names. The microbial community structures in anaerobic digestion were analyzed by 16S rRNA sequencing. DNA extraction, amplification and purification were the primary sequencing processes. The primers used for PCR amplification were 338F, 806R, 524F10extF and Arch958RmodR. 16S rRNA genes of

bacteria and archaea were sequenced in the 338F\_806R/524F10extF\_Arch958RmodR region. The Illumina MiSeq platform (Majorbio Bio-Pharm65 Technology Co. Ltd., Shanghai, China) was used to analyze the 16S rRNA genes of bacteria and archaea.

### 3. Results

#### 3.1. Early Warning for Emergent Instability in Food Waste AD by Bioimitating Human Disease Diagnosis

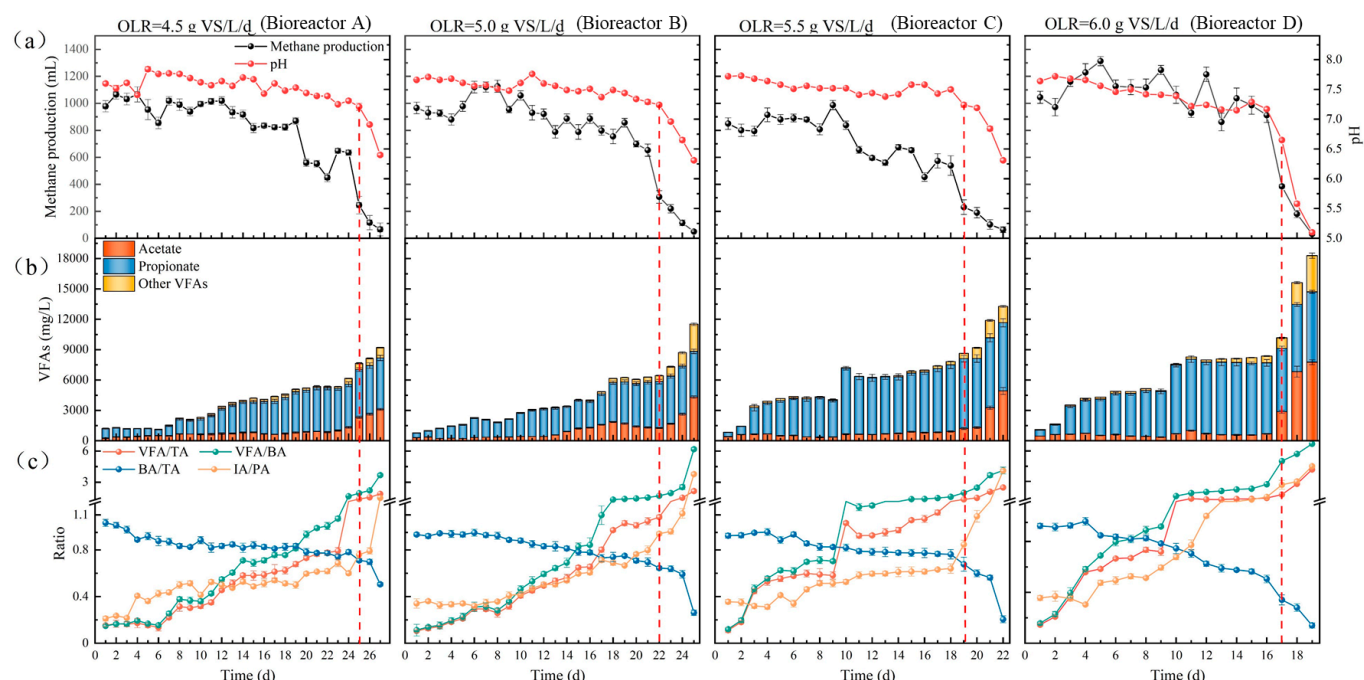
The AD system's emergent instability process is comparable to a person becoming ill, both of which require prompt and targeted treatment. Furthermore, physical signs like body temperature, blood pressure, and cough frequency are often employed for diagnosing the latter. Still, some straightforward and easy-to-use markers are also necessary for the prompt assessment of the former. The basis for early warning of emergent destabilization in the AD system is a single early warning indicator, characterized by easy detection and good response but weak universality and timeliness. In previous studies, the AD system was frequently monitored using specific indicators, such as the free ammonia content, alkalinity, and accumulated VFAs. These single indications and simple combinations were chosen for use in this study's early warning system for emergent destabilization of the food waste AD system because of the respective inhibitions from OLR, ammonia, and fat.

##### 3.1.1. Early Warning of High OLR Inhibition Based on Single Indicators

VFAs, VFAs/TA, and VFAs/BA could serve as reference values for the early warning system if rising OLR inhibition causes emergent instability in food waste AD. At a relatively high OLR inhibition of over 5.5 kg VS/m<sup>3</sup>·d, the early warning for VFA, VFA/TA and VFA/BA was 0–7, 1–9 and 1–9 days in advance, respectively. Furthermore, the higher the OLR, the better the early warning performance. However, the early warning for both VFA/TA and VFA/BA was only 1.0 day in advance when OLR was less than 5.0 kg VS/m<sup>3</sup>·d. Firstly, as illustrated in Figure 2, four bioreactors for food waste AD (designated A, B, C and D) were operated at a high OLR (4.5, 5.0, 5.5 and 6.0 kg VS/m<sup>3</sup>·d, respectively). According to the results of Figure 2a, the bioreactors were in stable operation for a shorter period and reached instability at a faster rate when the OLR increased. When the instability threshold of the food waste AD system was established as a 50% drop in methane production to normal levels [6], bioreactors A, B, C, and D reached instability on days 25, 22, 19, and 17, respectively. The pH value, one of the most often utilized warning indicators recently, was also nearly comparable regarding the shifting trends of methane production. This might be due to the excessive release of ammonia from food waste AD, which acted as a buffer and kept the pH level comparatively constant [14].

Secondly, as illustrated in Figure 2b, VFAs concentration demonstrated a specific performance in early warning of emergent instability in food waste AD. VFAs first accumulated slowly in the artificially generated environment with high organic loading. As OLR increased, there was a noticeable surge in VFAs concentration, which served as an early warning signal of system instability. The VFA concentrations jumped on days 25, 18, 10, and 10 at bioreactors with OLRs of 4.5, 5.0, 5.5, and 6.0 kg VS/m<sup>3</sup>·d, indicating early warning 0, 4, 9, and 7 days in advance, respectively. As illustrated in Figure 2c, the ratios between alkalinity and VFAs may also be necessary in early warning of emergent instability in food waste AD. However, their performance varied greatly and followed the pattern of having a good warning effect under high load and a poor effect under low load. The changing trends of VFAs/TA and VFAs/BA values were basically synchronized. For bioreactors with OLRs of 4.5, 5.0, 5.5 and 6.0 kg VS/m<sup>3</sup>·d, the jumping increases occurred on days 24, 17, 10 and 10, indicating early warning 1, 5, 9 and 7 days in advance, respectively.

It was nearly impossible to identify points of sudden increase in the BA/TA and IA/PA curves, indicating that they were unsuitable as markers of emergent instability in food waste AD. The AD system's buffer equilibrium and resistance to external disturbances were reflected in the ratio between alkalinity and VFAs, whose fluctuation was suggestive of the system's slow instability and eventual collapse.

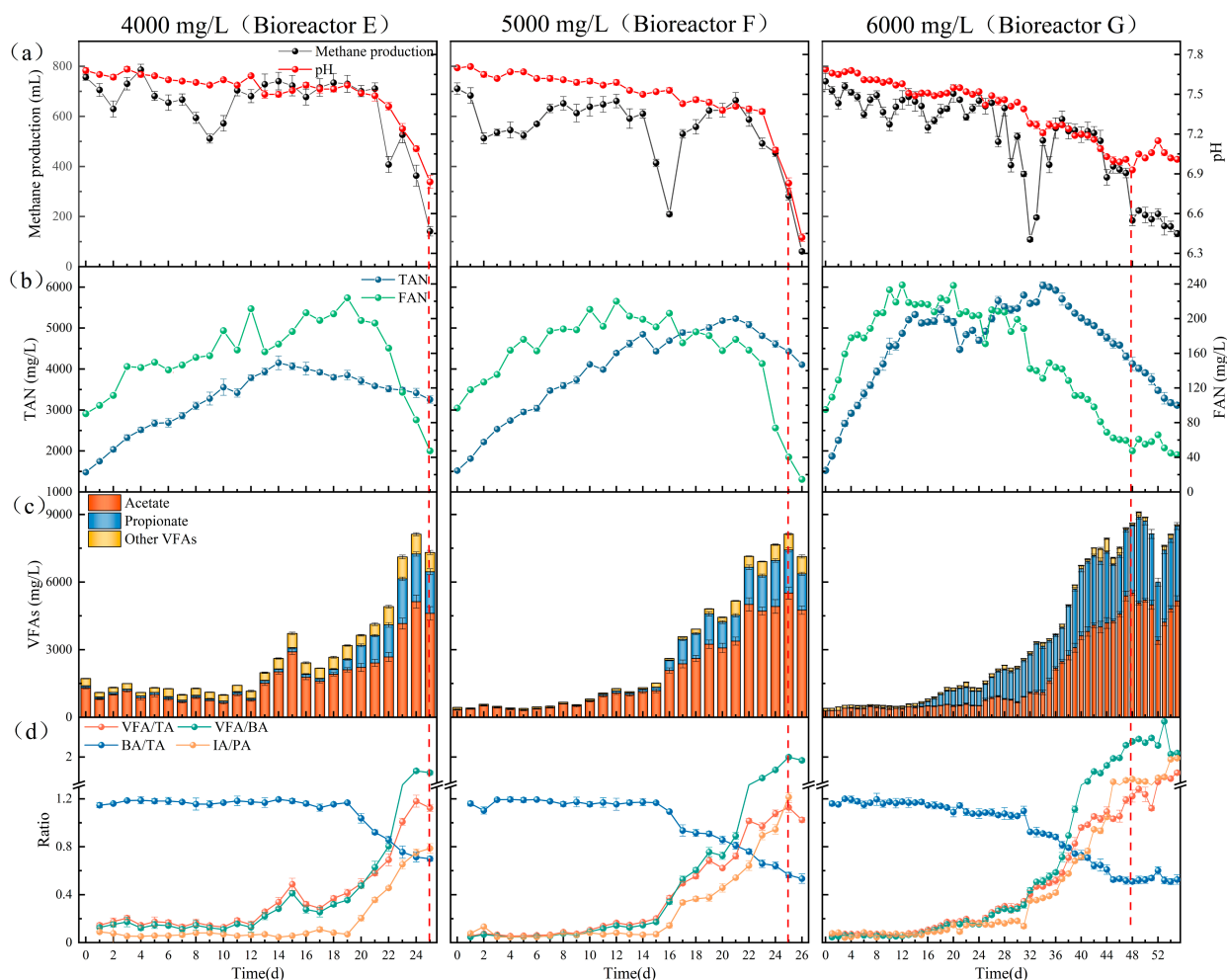


**Figure 2.** Operation parameter responses of bioreactors under high OLR inhibition. (a) Evolutions of methane yield and pH; (b) acetate, propionate, and other VFAs; and (c) VFAs/TA, VFAs/BA, BA/TA, IA/PA. The date of instability is shown by the red dotted line.

### 3.1.2. Early Warning for High Ammonia Inhibition Based on Single Indicators

VFAs/TA, VFAs/BA, VFAs, BA/TA and IA/PA could serve as reference values for early warning of emergent instability in food waste AD due to high ammonia inhibition. Their performances ranged from high to low, and the early warning was 9–16, 9–16, 9–12, 6–15 and 6–15 days in advance, respectively. As illustrated in Figure 3a,b, bioreactors E, F, and G lost stability and collapsed on days 25, 25, and 48, respectively, under the continuously growing inhibition of high-concentration ammonia. The pH, FAN, and TAN values were not appropriate for an early warning system for high ammonia inhibition in the food waste AD system since the fluctuations in these parameters and methane production were highly consistent. As seen in Figure 3c,d, VFA concentration presented a certain effect, serving as an early warning of emergent instability in food waste AD due to high ammonia inhibition. The VFA concentrations jumped on days 13, 16, and 38 in response to the rising ammonia concentrations in bioreactors E, F, and G, indicating early warning 12, 9, and 10 days in advance, respectively. The changing trends of the VFA/TA and VFA/BA values were also basically synchronized, with spikes occurring on days 15, 16, and 32 in bioreactors E, F, and G, respectively, indicating early warning 10, 9, and 16 days in advance. The changing trends of the BA/TA and IA/PA values were likewise nearly synchronized, with early warning 6, 8, and 15 days ahead, respectively. Therefore, the results showed that the instability in food waste AD produced by high ammonia inhibition could be effectively warned about with more warning indicators and earlier warning days compared to high OLR inhibition. Additionally, it is worth noting that in this study,

the addition of ammonium chloride was used to adjust the concentration of TAN, which also had a certain inhibitory effect from chloride ions.



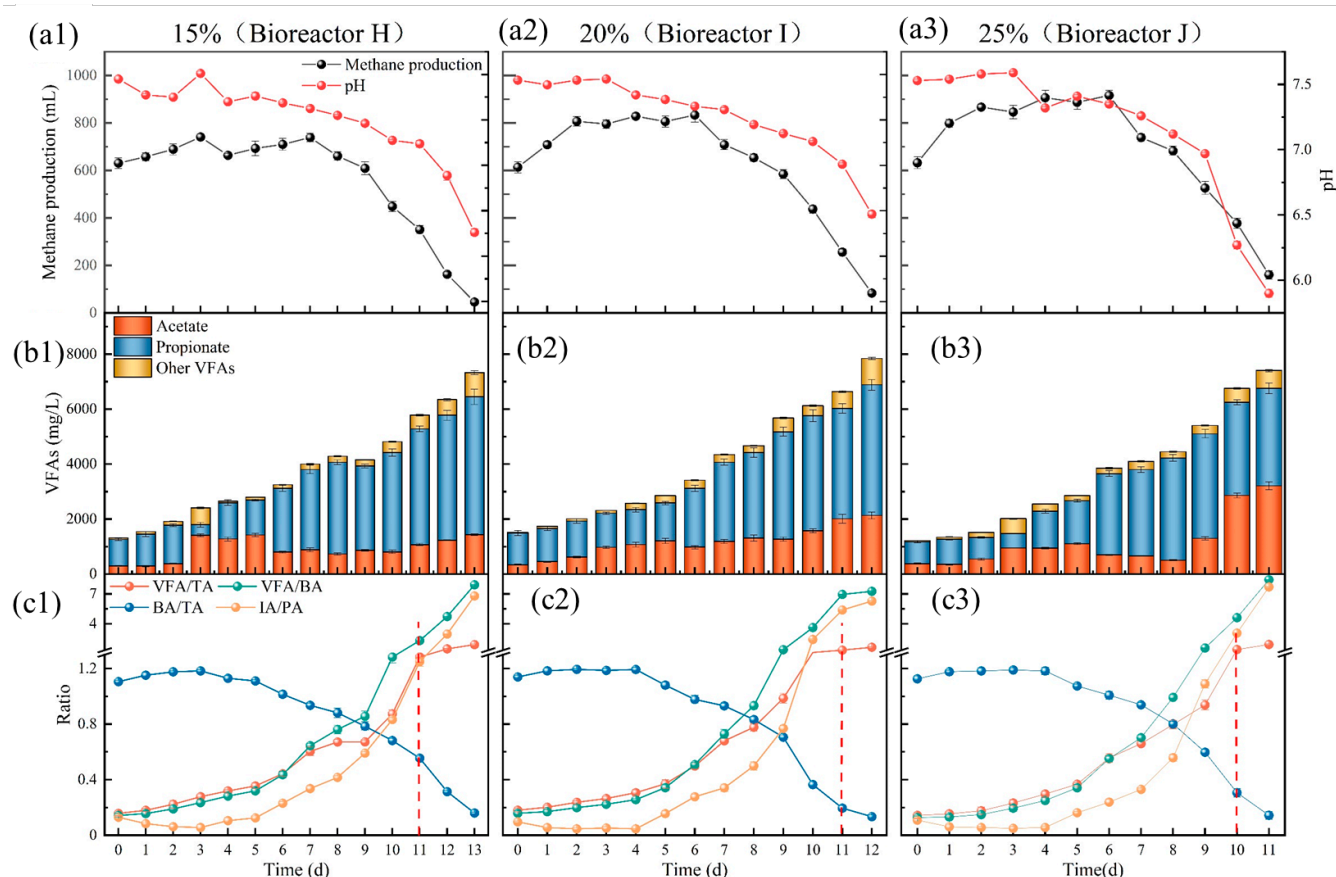
**Figure 3.** Operation parameter responses of bioreactors under high-concentration ammonia inhibition. (a) Evolutions of methane yield and pH; (b) TAN and FAN; (c) acetate, propionate, and other VFAs; and (d) VFA/TA, VFA/BA, BA/TA and IA/PA. The date of instability is shown by the red dotted line.

### 3.1.3. Early Warning for High Fat Inhibition Based on Single Indicators

The values of VFAs, VFAs/TA, VFAs/BA, BA/TA and IA/PA could also be applied for early warning of emergent instability in food waste AD due to high fat inhibition. As shown in Figure 4a, the early warning was 3–4, 0–2, 3–5, 5–6 and 5–6 days in advance, respectively. Although the fat contents in bioreactors H, I and J increased from 15%, 20% to 25% of the total organic contents, around the same time, on days 10–11, they lost stability, and the trend of their methane production was basically consistent. The pH value was also unsuitable for use as an early warning for high fat inhibition, and the changes in pH and methane production were very consistent. This could be because of the high ammonia concentration in the food waste AD system, which results in high alkalinity and a strong pH buffering ability. Furthermore, as seen in Figure 4b,c, it was challenging to determine the jump points of the VFA, VFA/TA, VFA/BA, BA/TA, and IA/PA shifting curves because they were all relatively smooth. All the leaping increases in VFA concentrations under the increasing fat contents in bioreactors H, I, and J occurred on day 7, recognizing early warnings 4, 3, and 4 days in advance, respectively. Using the same approach, the early warning

for VFAs/TA was 1, 2, and 0 days in advance; for VFAs/BA it was 3, 5, and 4 days in advance; and for IA/PA and BA/TA it was the same, at 5, 6, and 6 days in advance.

The results above demonstrated that those single indicators, and simple combinations, could achieve good early warning performance when dealing with severe emergent instability in AD systems. They also presented some superiorities, such as ease of operation, high accuracy, and strong targeting, which gave operators a foundation to quickly develop urgent error correction response strategies, thereby lowering the risks of instability and even eventual collapse in AD systems. However, there were also several noteworthy shortcomings. The early warning time for these indicators was relatively short, and some were only 1–2 days in advance, which could result in insufficient time to adjust operation parameters to prevent system instability. This is comparable to diagnosing human diseases, where medical treatment only starts when symptoms like fever and cough become evident, which leads to poor timeliness. These single indicators, as well as combinations, lacked universality. For example, BA/TA and IA/PA were unsuitable for early warnings of ammonia and OLR inhibitions, and the remaining indicators likewise showed different sensitivities to different reasons for instability. Each indicator would have experienced slight and imperceptible variations due to the multiple interfering factors that frequently played roles concurrently and whose weights fluctuated in AD systems of complex substrates like food waste. Therefore, all indicators' cumulative effects must be considered by developing comprehensive warning indicators.



**Figure 4.** Operation parameter responses of bioreactors under high fat inhibitions. Evolutions of (a1–a3) methane yield and pH; (b1–b3) acetate, propionate and other VFAs; and (c1–c3) VFAs/TA, VFAs/BA, BA/TA, and IA/PA. The red dotted line shows the date of instability.

### 3.2. Comprehensive Early Warning for Indistinct Instability in Food Waste AD by Bioimitating Human Subhealth Diagnosis

The unclear process of instability in the food waste AD system is comparable to human discomfort, which is difficult to describe; both are marked by high uncertainty and unpredictability. Human sub-health diagnosis often requires a comprehensive analysis based on the findings of various physical indicators. Therefore, it is also necessary to establish a comprehensive index suitable for sub-stability evaluation of the food waste AD system to enhance the early warning system's predictability and universality. A novel evaluation system for the stability of the AD process was developed in this study using the weighting method, which was based on the single warning indicators and the straightforward combinations mentioned above. This system quantitatively evaluated the stability of the food waste AD system and showed information about each step of the AD process.

### 3.2.1. Building the Comprehensive Evaluation System

Six indicators were selected to build the comprehensive evaluation system based on the data obtained from bioreactors A–J, namely VFAs, VFAs/TA, VFAs/BA, BA/TA, IA/PA, and FAN (Figure 5a). The thresholds for their effects on food waste AD were established as follows: 3000, 0.35, 0.8, 0.8, 0.6, and 200, respectively (Figure 5b). As shown in Figure 5c,d, individual indicator ratings were calculated using the step functions method. For instance, the stability score of VFAs ( $SI_{VFA}$ ) appeared to be 100 when the concentration of VFAs in the AD bioreactor was below 2000 mg/L, which was less than 80% of its threshold of 3000 mg/L. The overall stability status of the AD bioreactor in this system may be reflected by specific scores (SI) (Figure 5 e,f), which were determined using the data from each stability indicator, e.g.,  $SI_{VFA}$ ,  $SI_{VFA/TA}$ ,  $SI_{VFA/BA}$ ,  $SI_{BA/TA}$ ,  $SI_{IA/PA}$  and  $SI_{FAN}$ . The values of individual indicator ratings were weighted to approximate the bioreactors' actual stability status. The assignment method used the entropy weight method and the following steps.

#### (1) Standardization of the data for each indicator

Using the selected indications of VFAs, VFAs/TA, VFAs/BA, BA/TA, IA/PA, and FAN, the instability time of S1, S2, S3, S4, S5, and S6 could be predicted in advance. The unified values of X1, X2, X3, X4, X5, and X6 were obtained by horizontally unifying the data of those instability warning times by dividing S1–S6 in each bioreactor by the indicator with the best early warning performance ( $S_{max}$ ), as each bioreactor had different instability warning times. The data of various indicators in each bioreactor was standardized into the unified data, named Y1, Y2, Y3, Y4, Y5 and Y6, which could be calculated by using the model below.

$$Y_{ij} = \frac{X_{ij} - \min(X_i)}{\max(X_i) - \min(X_i)} \quad (2)$$

where j represents the number of bioreactors.

#### (2) Calculations of the information entropy of each indicator

The following formulae can be used to determine the information entropy ( $E_j$ ) of a set of data by its definition.

$$E_j = -\ln(n)^{-1} \sum_{i=1}^n p_{ij} \ln p_{ij} \quad (3)$$

$$p_{ij} = \frac{Y_{ij}}{\sum_{i=1}^n Y_{ij}} \quad (4)$$

where  $\lim_{p_{ij} \rightarrow 0} p_{ij} \ln p_{ij} = 0$  is defined if  $p_{ij} = 0$ .

(3) Determining the weight of each indicator

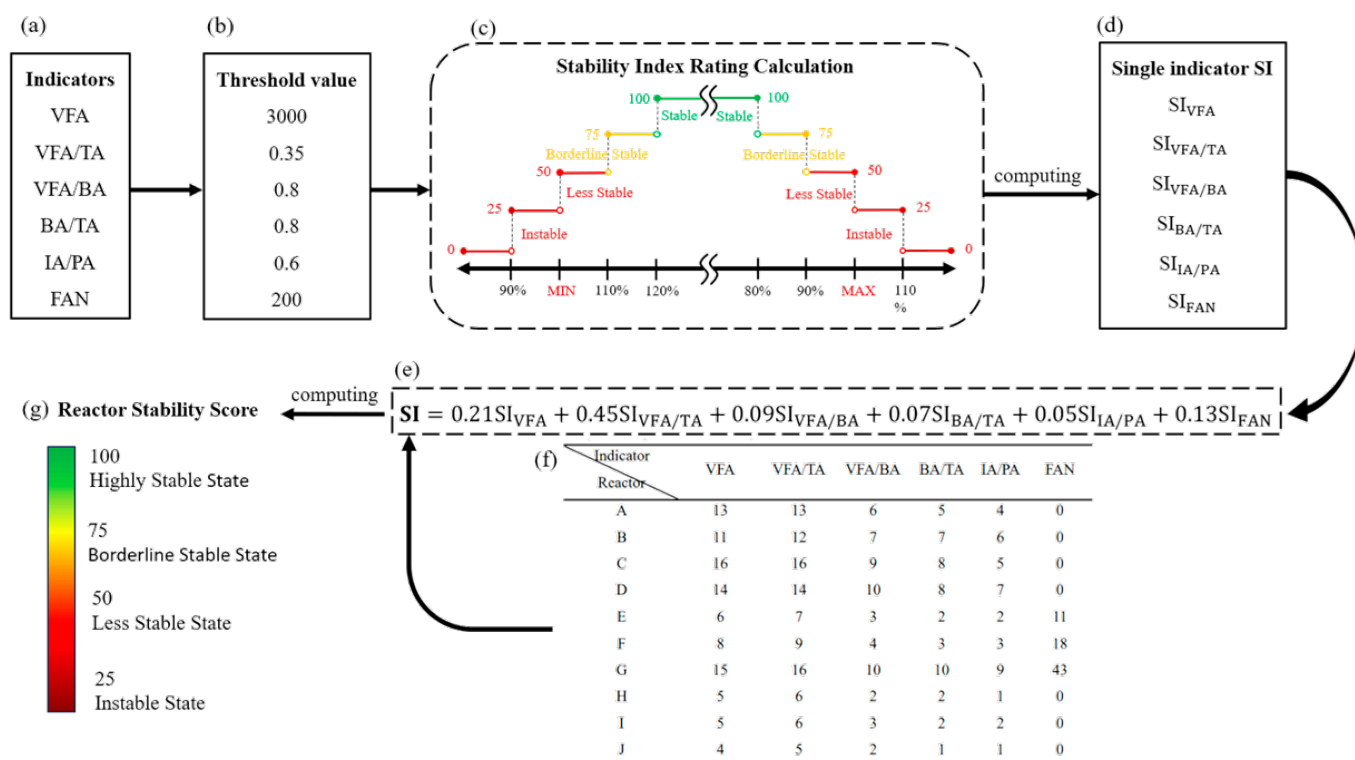
The information entropies of the indicators VFAs, VFAs/TA, VFAs/BA, BA/TA, IA/PA, and FAN were determined and designated as E1, E2, E3, E4, E5, and E6, respectively, based on the formulae for computing information entropy. The weight of each indicator ( $W_i$ ) was obtained by using the following formula.

$$W_i = \frac{1 - E_i}{k - \sum E_i} (i = 1, 2, \dots, k) \quad (5)$$

The calculated weights of VFAs, VFAs/TA, VFAs/BA, BA/TA, IA/PA and FAN were 0.21, 0.45, 0.09, 0.07, 0.05 and 0.13, respectively, generating a novel formula for reflecting the bioreactor stability score (SI).

$$SI = 0.21SI_{VFA} + 0.45SI_{VFA/TA} + 0.09SI_{VFA/BA} + 0.07SI_{BA/TA} + 0.05SI_{IA/PA} + 0.13SI_{FAN} \quad (6)$$

According to Figure 5g, the AD bioreactor was deemed highly stable when the total score (SI) was 76–100. The bioreactor was considered in a borderline stable state, and the operator received a warning when SI was between 51 and 75. Additionally, it indicated that the AD bioreactor had a poor ability to cope with external impacts and that corresponding adjustments in operating conditions were necessary. A more urgent alarm was sounded when the SI value dropped to 26–50, indicating that the AD bioreactor had further lost stability and had entered a less stable condition. The AD bioreactor was deemed entirely collapsed when the score hit 0–25; at this point, it issued a warning and recommended stopping operations.



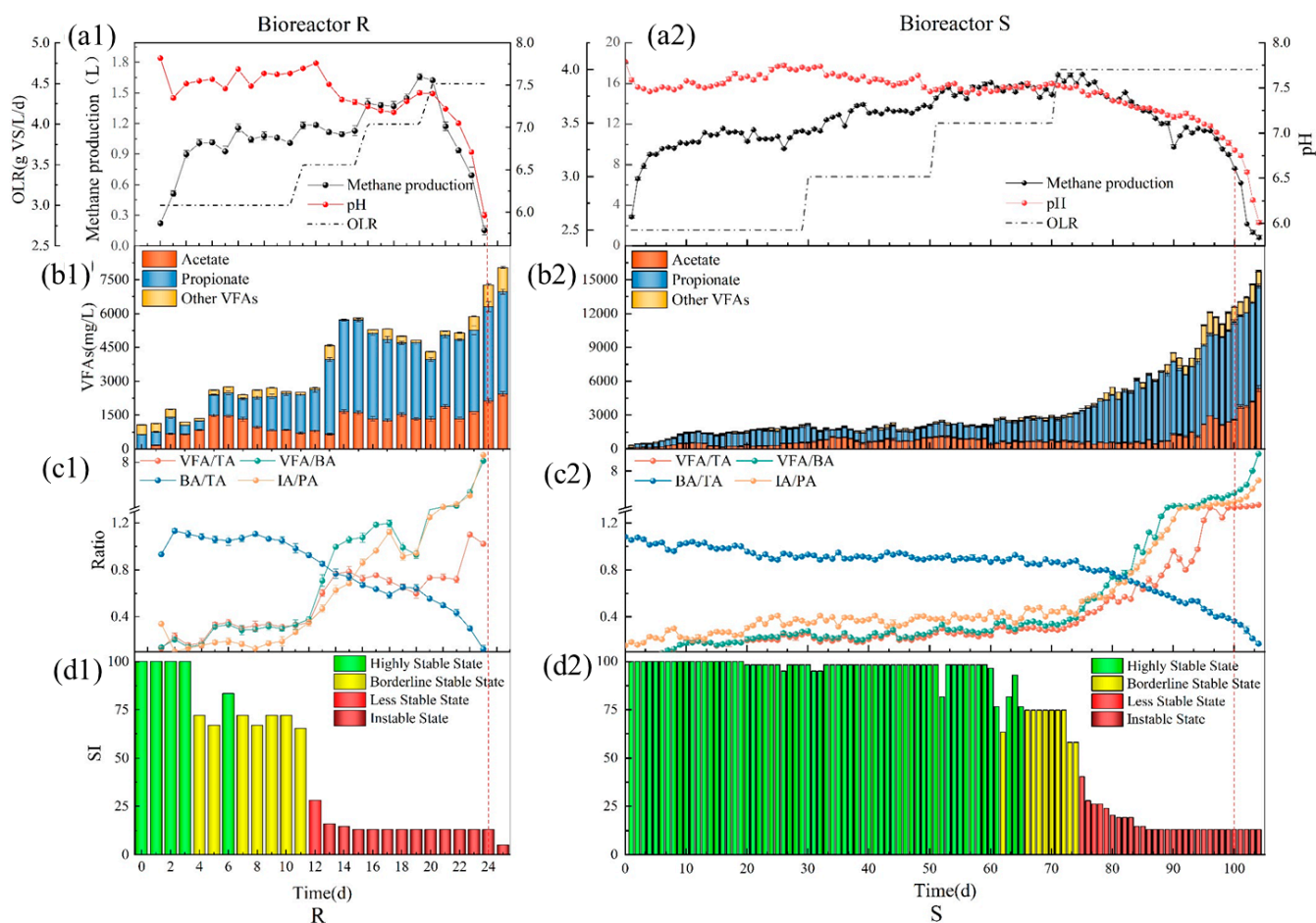
**Figure 5.** Process of building the comprehensive evaluation system for the stability of food waste AD, including (a) stability indicators, (b) warning threshold value, (c) stability index rating calculation, (d) single-indicator stability score, (e) total stability index score, (f) early warning effect of stability index in different bioreactors and (g) bioreactor stability status.

### 3.2.2. Performance of the Comprehensive Evaluation System in Providing Early Warning for Indistinct Instability in Food Waste AD

The developed comprehensive index performed significantly better in early warning, particularly in terms of foresight, quantifiability, and discriminability, compared to the single indicators. Bioreactors R and S, two bioreactors with effective volumes of 1.2 L and 10 L, respectively, were operated for food waste AD to verify the performance of the developed comprehensive evaluation system in early warning. A mode of gradually increasing the organic load in bioreactors R and S until instability developed was chosen to realistically fit the operating conditions in real-world applications. Bioreactor R operated stably when the organic load increased from 3.0 to 3.5 and 4.0 g VS/L·d (Figure 6(a1)). Further raising the organic load to 4.5 g VS/L·d caused bioreactor R to collapse right away, and on day 22, methane production dropped to less than 50% of the expected value. The early warning for pH, VFAs, VFAs/TA, VFAs/BA, BA/TA and IA/PA was 9, 9, 9, 9, 13 and 13 days in advance, respectively (Figure 6(a1,b1,c1)). However, as Figure 6(d1) demonstrates, the comprehensive indicator may offer even more detailed information regarding bioreactor R's instability. On the fourth day, bioreactor R's stability score (SI) fell below 75, indicating the bioreactor had entered a borderline stable state. As a result, the early warning period was extended to 18 days in advance. Simultaneously, the data demonstrated that the bioreactor's instability at this point was a sub-stability state, which could be completely restored. The SI of the bioreactor did return to normal on the sixth day. However, the bioreactor's instability would have been inevitable if the high OLR operating state had remained unchanged. Following a brief period of stability on day 7, the bioreactor once more reached a borderline stable state. Furthermore, bioreactor R kept the borderline steady for 5 days while operating at high OLR. On day 12, it ultimately entered a less stable condition, with an SI of less than 50, and on day 13, it quickly entered an unstable state, with an SI of below 25. Therefore, results showed that the early warning system based on this developed comprehensive index increased the number of advance warning days and quantitatively monitored the entire process of the AD bioreactor losing stability.

In addition, it was unlikely to quickly increase the OLR of the AD bioreactor during a short period in actual projects, where the OLR is often gradually increased over a comparatively long time interval to lower the risk of bioreactor instability. Therefore, over 110 days, the OLR was progressively raised from 2.5 to 3.0, 3.5, and 4.0 g VS/L·d in AD bioreactor S (effective volume 10.0 L), in order to represent the real-world application in food waste AD more accurately. Figure 6(a2) illustrates that bioreactor S operated stably when the OLR was below 4.0 g VS/L·d. However, after the OLR reached 4.0 g VS/L·d., the methane yield gradually decreased and reached 50% of the normal level on the 100th day, indicating that bioreactor S had lost stability. Interestingly, it was challenging to identify significant transition points because all the variation curves for pH, VFAs, VFAs/TA, VFAs/BA, BA/TA, and IA/PA were relatively smooth, as seen in Figure 6(a2,b2,c2). Therefore, this suggested that warning systems based on a single indicator would no longer be effective when the operation conditions, such as OLR increase, change relatively slightly. However, as shown in Figure 6(d2), the developed comprehensive evaluation system presented good performance in early warning of indistinct instability in food waste AD. Bioreactor S reached a borderline stable state, with a stability score (SI) of below 75, on the 62nd day and then returned to the stable state in the following 3 days. Bioreactor S continued to operate at a high OLR of 3.5 g VS/L·d, exhibiting low resistance to OLR shocks. On the 66th day, it once more entered a borderline stable state. Bioreactor S eventually entered a less stable state, with an SI of below 50, on day 75 and quickly transitioned into an unstable state, with a SI of below 25, on day 80 as the OLR further increased to 4.0 g VS/L·d. Therefore, the results indicated that the system developed based on the comprehensive indicator not only achieved a 38-day advance warning, but also provided detail

feedback regarding the entire process of a bioreactor losing stability. Especially for situations where instability was indistinct, its superiority was even more prominent.



**Figure 6.** Operation parameter responses of bioreactors R (left) and S (right) under OLR increases, including evolutions of methane yield and pH (a1,a2); VFAs (b1,b2), VFAs/TA, VFAs/BA, BA/TA and IA/PA (c1,c2); and SI (d1,d2). The red dotted line shows the date of instability.

### 3.3. Regular Microbial Testing of Early Warning of Potential Instability in Food Waste AD by Bioimitating Human Periodic Physical Examination

Humans usually have routine physical examinations every six months or a year, even in the absence of symptoms of illness, to detect and control diseases early. Similarly, it is necessary to conduct regular detection of AD for possible instability risk to prevent the occurrence of instability accidents in the AD system from the source. Microorganisms play a key role in the biotransformation of food waste, and changes in their communities are the main cause of instability in the AD system. Therefore, early warning of potential instability in the food waste AD system might be achieved through routine microbial testing and analysis, especially tracking sensitive microbial communities. The benefits of early warning based on routine microbial testing include strong essence and good foresight. In order to reverse-develop the early warning system for possible instability in food waste AD, a deterministic analysis of sensitive microorganisms was conducted in this study based on the changes in the microbial community during the process of instability in bioreactor S as stated above.

#### 3.3.1. Microbiome Overview During the Process of Instability in Food Waste AD

Eight sludge samples (designated S0, S30, S50, S60, S70, S80, S90 and S100, respectively) were taken on days 0, 30, 50, 60, 70, 80, 90 and 100 based on the time nodes of OLR increase and methane production conditions in bioreactor S (Figure 6(a2)). These samples were subsequently subjected to high-throughput sequencing analysis. A total of 1170,901 uniform 16S rRNA sequence reads were obtained, which were subsequently clustered into OTUs with 97% similarity and assigned to different taxonomic units. These reads were classified into 38 phylums, 85 orders, 329 families, 528 genera and 1143 OTUs. The coverage of all samples was higher than 99%, indicating that most of the microorganisms in the samples were detected. The operation process of bioreactor S was divided into three stages, namely stable (0–49 days), sub-stable (50–77 days) and unstable (78–104) stages, to investigate the evolution mechanism of microbial communities during the process of the AD system losing stability. Bioreactor S was operated at a relatively low OLR of 2.5–3.0 gVS/L·d during the stable stage, exhibiting normal methane production and stable pH values (Figure 6(a2)). The samples of S0 and S30 represented the microbial community at this stage. Bioreactor S was operated at an OLR of 3.5 gVS/L·d during the sub-stable stage (methane production was expected, but pH values began to reduce in a fluctuating manner). The microbial communities at this stage were examined using S50, S60, and S70 samples. Bioreactor S was operated at a high OLR of 4.0 gVS/L·d during the unstable stage (methane production and pH values steadily decreased). The samples of S80, S90, and S100 were used to represent the microbial community. As shown in Figure 7a, microbial hierarchical clustering analysis revealed that the OUTs of S0 and S30; OUTs of S50, S60 and S70; and OUTs of S80, S90 and S100 showed high similarity. The results indicated it was feasible to warn of potential instability in food waste AD early by regular microbial testing.

### 3.3.2. Exploring Shifts in Microbial Communities to Warn Early of Potential Instability in Food Waste AD

During the process of instability in the food waste AD system, the microbial community structure underwent significant changes, which provided an opportunity for early warning, especially for potential instability warning, via exploring the shifts in microbial communities. As shown in Figure 7b,c, the bacterial and archaeal community abundances at the genus level were analyzed for each of the eight samples (S0, S30, S50, S60, S70 and S80, S90 and S100). Representative samples of S0, S30, S60 and S100 were selected at each stage to provide details of the abundance of dominant bacterial phyla and genera (Table 1).

The results showed that the microbial community was stimulated to adapt when unfavorable living conditions occurred (causes of instability). Firstly, the proportion of tolerant microorganisms significantly increased. Most of the bacterial microorganisms in bioreactor S were *Firmicutes* and *Bacteroidota*, which are persistent microorganisms that may produce a variety of metabolic enzymes involved in acidification and hydrolysis. *Firmicutes* abundance even sharply rose to 51.9% during the unstable stage because of its strong tolerance to the high OLR stress. Secondly, there was an increase in the number of microbes that favored stressful environments. As shown in Figure 7c, the abundance of acid-producing bacteria, *norank\_o\_Oscillospirales* [26], increased gradually during the process of instability in bioreactor S. In stable, sub-stable, and unstable stages, its abundance was 1.89%, 2.92%, and 13.0%, respectively. Thirdly, the abundance of some bacteria unsuitable for stressful environments naturally decreased. As seen in Figure 7c, the abundance of *norank\_f\_Bacteroidetes\_vadinHA17*, another type of acid-producing bacteria [27], steadily declined during bioreactor S's process of instability. In stable, sub-stable, and unstable stages, the abundance was 12.4%, 7.12%, and 4.66%, respectively. Moreover, the abundances of *norank\_o\_norank\_c\_Dojkabacteria* and *DMER64*, two types of hydrogen-

producing bacteria, also gradually decreased during the process of instability, suggesting a steady decline in bioreactor S's potential to produce hydrogen. Finally, the abundance of bacteria that produce propionic acid, *Rikenellaceae\_RC9\_gut\_group* [28], was found to exhibit dramatic fluctuations during the process of instability. In the stable, sub-stable, and unstable stages, the abundance was 3.97%, 23.4%, and 5.94%, respectively. This could be because the high OLR pressure during the sub-stable stage encouraged the growth of this type of bacteria, leading to rapid production and accumulation of propionic acid. It consequently inhibited growth and caused a sudden drop in abundance during the unstable stage.

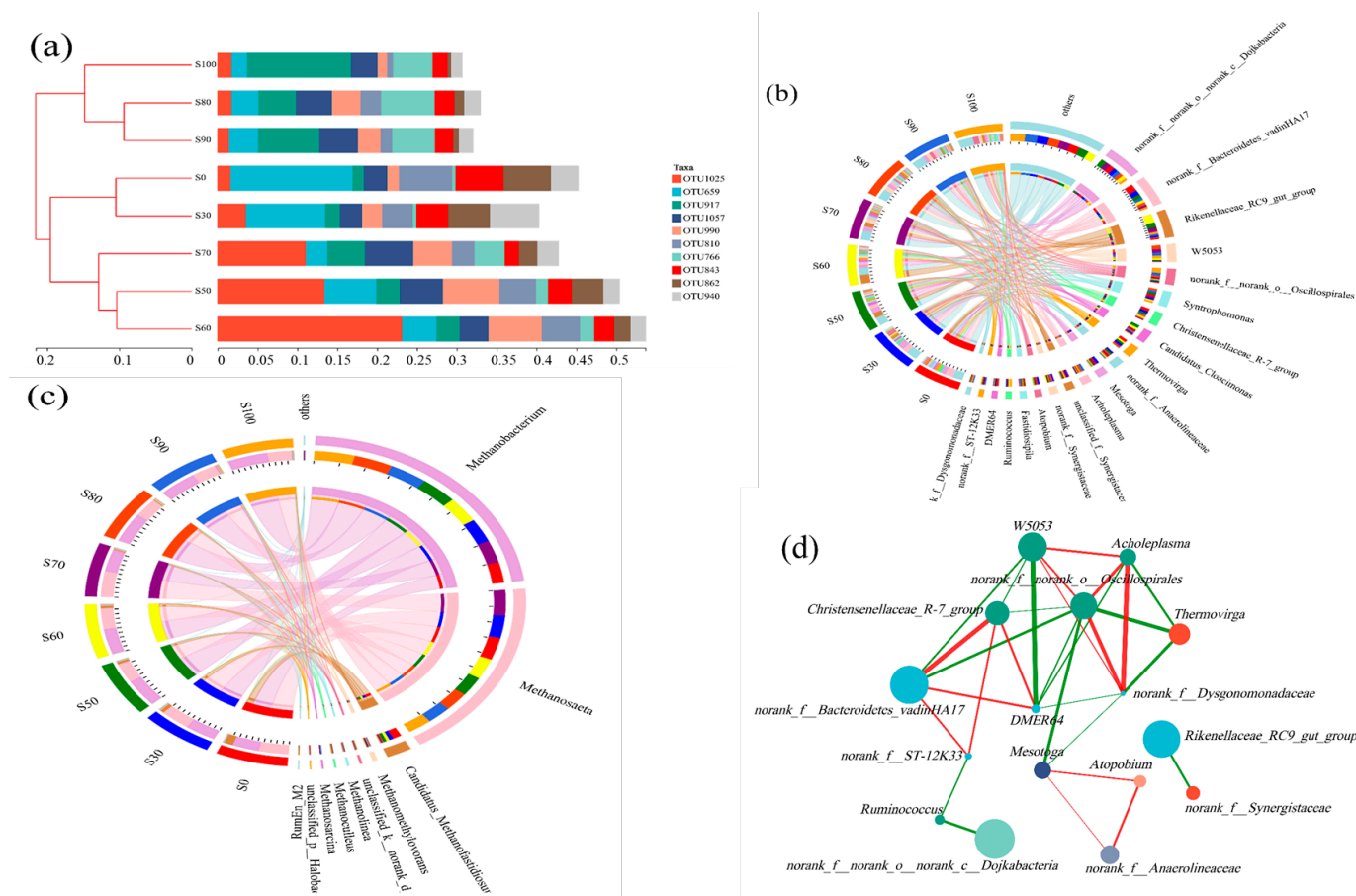
In addition, the main pathway for methane production also changed during the process of instability. As shown in Figure 7c, the methanogenic archaea were dominated by the hydrogenotrophic *Methanobacterium* and the acetateotrophic *Methanosaeta*. During the stable period, the abundances of these two methanogenic archaea remained essentially stable. However, the ratio of the two types of methanogenic archaea gradually changed as the stability of bioreactor S declined. The abundance of the hydrogenotrophic *Methanobacterium* gradually increased and progressively dominated the methanogenesis. These findings were consistent with the earlier research by Goux et al. [29]. This could result from hydrogen-nutrient methanogenic bacteria being more resilient to the stressful environment than acetate-nutrient methanogenic bacteria. One of the leading causes of the AD system's collapse may have been the increasing imbalance in hydrogen partial pressure brought on by the reduced abundance of hydrogen-producing bacteria, resulting in the equilibrium between hydrogen production and utilization being disrupted, and the hydrogen partial pressure was gradually imbalanced. Consequently, the findings suggested that the microbial population experienced frequent alterations throughout the AD system's instability phase, which offered hints for early warning.

### 3.3.3. Tracking Sensitive Bacteria to Warn Early of Potential Instability in Food Waste AD

Monitoring changes in microorganisms susceptible to environmental stress could provide an early warning of potential instability in AD systems. There are various microorganisms in AD systems, and they all serve the same function. In other words, the abundance of a certain type of microorganism drastically decreases under environmental stress, which would not necessarily indicate a loss of stability in an AD system. This is because the AD system's stability would be maintained sequentially, as additional microorganisms with the same function would grow and replace the roles of these suppressed microorganisms. Therefore, those sensitive bacteria selected to warn early of potential instability in the AD system should possess unique and irreplaceable functions. In this study, one-way network analysis was adopted to explore the correlation and interactions between sensitive bacteria in the microbial community evolution process caused by instability in the AD system.

The findings suggested that two bacterial groups, *Rikenellaceae\_RC9\_gut\_group* and *norank\_o\_norank\_c\_Dojkabacteria*, could be used as sensitive bacteria for early warning of potential instability in food waste AD. These bacteria, as Figure 7d illustrates, including the acid-producing *norank\_f\_Bacteroidetes\_vadinHA17* and *norank\_o\_Oscillospirales*, as well as hydrogen-producing *DMER64*, were sensitive to environmental stress and changed significantly in abundance during the process of instability. However, they were all strongly correlated with a wide range of microorganisms, suggesting that these bacteria had close associations with other microorganisms and that changes in their abundance would induce corresponding changes in other microorganisms to maintain the stability of the AD system. As a result, these bacteria could not be employed to detect AD system instability early. In contrast, the two bacterial groups, *Rikenellaceae\_RC9\_gut\_group* and

*norank\_o\_norank\_c\_Dojkabacteria*, were each negatively correlated with only one other type of microorganism in the microbial community (Figure 7d). The disparity in their abundances indicated that as the two groups of microorganisms declined, there were insufficient microorganisms with the same function that appeared to displace them to maintain the AD system's steady progression. Therefore, *Rikenellaceae\_RC9\_gut\_group* and *norank\_o\_norank\_c\_Dojkabacteria* were the suitable bacteria for early warning of potential instability in food waste AD.



**Figure 7.** Microbiological analysis chart. (a) Hierarchical cluster analysis of bacterial samples on the OTU level, (b) a Circos plot of bacterial communities on the genus level, (c) a Circos plot of archaeal communities on genus level, and (d) one-way network analysis of microorganisms at the genus level, where the size of the nodes in the graph indicated the size of species abundance. Different colors indicate different species. The color of the connecting lines indicates positive and negative correlations; red denotes a positive correlation and green a negative correlation. The line thickness expresses the correlation coefficient's size; the thicker the line, the stronger the link between the species, and the more lines there are, the closer the link between the species and other species.

**Table 1.** Taxonomic compositions of bacterial communities at the phylum and genus levels (relative abundance, %).

| Phylum/Genus                            | S0   | S30  | S60  | S100 |
|-----------------------------------------|------|------|------|------|
| Bacteroidota                            | 32.1 | 25.4 | 36.6 | 23.2 |
| <i>norank_f_Bacteroidetes_vadinHA17</i> | 18.3 | 12.4 | 7.12 | 4.66 |
| <i>DMER64</i>                           | 5.47 | 2.41 | 0.83 | 0.81 |
| <i>Rikenellaceae_RC9_gut_group</i>      | 2.03 | 3.97 | 23.4 | 5.94 |
| Firmicutes                              | 23.7 | 22.4 | 28.2 | 51.9 |

|                                          |      |      |      |      |
|------------------------------------------|------|------|------|------|
| <i>Christensenellaceae_R-7_group</i>     | 7.76 | 5.21 | 3.44 | 2.91 |
| <i>norank_o__Oscillospirales</i>         | 1.40 | 1.89 | 2.92 | 13.0 |
| W5053                                    | 0.64 | 0.94 | 4.43 | 7.42 |
| Patescibacteria                          | 13.5 | 11.6 | 8.47 | 5.78 |
| <i>norank_o__norank_c__Dojkabacteria</i> | 11.0 | 9.63 | 8.02 | 5.58 |
| Synergistota                             | 12.3 | 11.2 | 8.47 | 5.78 |
| <i>Thermovirga</i>                       | 6.78 | 3.91 | 4.85 | 0.77 |
| Thermotogota                             | 3.44 | 6.33 | 2.16 | 1.79 |
| <i>Mesotoga</i>                          | 3.38 | 6.16 | 1.87 | 1.37 |
| Actinobacteriota                         | 4.63 | 7.69 | 2.45 | 3.39 |
| <i>Atopobium</i>                         | 2.44 | 5.76 | 1.34 | 1.91 |
| Chloroflexi                              | 5.06 | 8.00 | 2.89 | 2.53 |
| <i>norank_f__Anaerolineaceae</i>         | 3.26 | 5.81 | 2.41 | 3.39 |
| Cloacimonadota                           | 1.45 | 2.51 | 6.73 | 1.37 |
| <i>Candidatus_Cloacimonas</i>            | 1.44 | 2.49 | 6.68 | 1.20 |

### 3.4. A Novel Early Warning System for Instability in Food Waste AD via a Biomimetically Simulating Resident Disease Screening Paradigm

#### 3.4.1. Proposal of the Novel System

In a sense, both anaerobic digestion bioreactors and the human body are part of relatively independent ecosystems. Research on human disease prevention and control has been ongoing for thousands of years, and a pervasive health maintenance system has already been established. All of these provide inspiration to develop a feasible early warning system for instability in the anaerobic digestion process by bionically simulating a resident disease screening paradigm. A novel system was proposed for the food waste AD process, corresponding to the system of disease cure, sub-health diagnosis, and periodic physical examination of human disease prevention and control. Figure 8 illustrates the proposed novel system, which consists of three parts at different in-depth levels: early warnings for emergent, indistinct and potential instabilities. The aforementioned drawbacks of the three kinds of early warning systems could be avoided by organically combining the emergency warning based on a single indicator, the indistinct warning based on comprehensive indicators, and the potential risk warning based on microbial indicators. This would also allow for simultaneously achieving emergency strategy formulation, potential risk assessment and revealing the cause of the instability in the AD system. Detailed descriptions of the system are listed as follows.

##### Level 1: Early warning for emergent instability

The early warning at this level bio-imitated the diagnostic process after residents developed lesions. It was most suited for diagnosing AD system faults when clearly anomalous conditions emerged. Furthermore, this early warning level primarily relied on single indicators that were very targeted and simple to assess. Its primary function was to provide assistances in formulating emergency error correction strategies for the operation of the AD bioreactor, avoiding the eventual collapse of the AD system. Therefore, this type of early warning exhibited high accuracy and strong targeting but poor foresight.

##### Level 2: Early warning of sub-stability

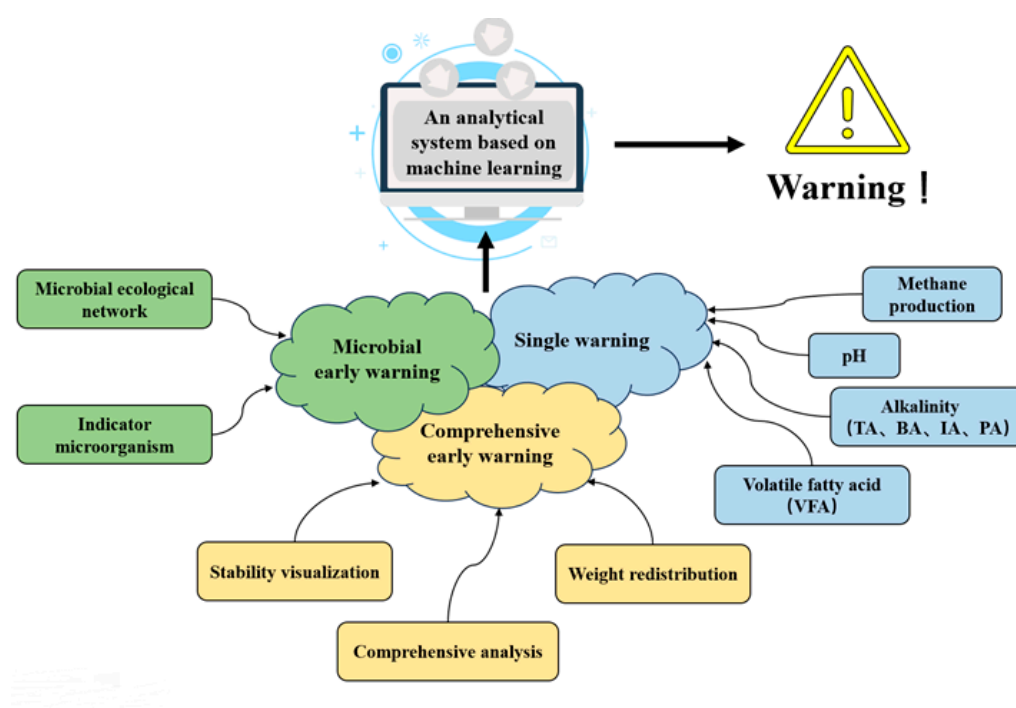
The early warning at this level bio-imitated the sub-health check-up procedures for residents who experienced discomfort but lacked overt symptoms. It was appropriate when the AD system was essentially functioning normally, and it was challenging to identify instability because so few routine indicators changed, and at extremely small magnitudes. In order to make an early forecast of upcoming instability in the food waste AD system, it was necessary to build a comprehensive indicator consisting of multiple single

warning indicators with different weight assignments and analyze the trend of changes within a certain period. The warning at this level mainly relied on relevant models and operational experience to discover hidden abnormal fluctuations and achieve the early warning objective. As a result, this level of early warning displayed traits including high accuracy, high accuracy, and comparatively strong foresight.

#### Level 3: Early warning of potential instability

The early warning at this level bio-mimicked the annual physical examination of residents. It was mainly suitable for periodic inspections conducted at long intervals of several months or even one year while the anaerobic digestion system operated stably. Moreover, the critical state of anaerobic digestion operation must be reflected in the analysis indicators at this level. Consequently, the primary monitoring objects in this study were the microbial composition and dominant microbial communities. The anaerobic digestion system's potential stability and potential risk of losing stability were assessed by comparing it with the earlier detection results in databases. As a result, this early warning level showed excellent foresight but poor accuracy and targeting.

Most significantly, the three early warning systems mentioned above should be implemented concurrently, and their outcomes can be compared to one another, much like the resident disease prevention and control system. As shown in Figure 8, the feedback information or values from the three systems would be consolidated and sent to the data processing center. Following analysis, warning signals would then be sent to the digital display equipment.



**Figure 8.** Biomimetic simulating resident disease screening paradigm as an early warning system for food waste AD instability.

#### 3.4.2. Verification of the New System in Early Warning Instability

A pilot-scale continuous stirred tank bioreactor (CSTR) with a volume of 30.0 L and operating for food waste AD was chosen to verify the superiority of the developed warning system (Figure 9d). In the CSTR, the approach of adding biochar was adopted to improve the stability of methane production from food waste during anaerobic digestion. This study focused on the entire operation process of the CSTR bioreactor from stable operation to stability loss due to high OLR, and finally stability restoration with the

addition of biochar. As illustrated in Figure 9a, methane production significantly increased over the next four days after the OLR of the steadily operating CSTR bioreactor was raised from 3.0 to 4.0 kg VS/m<sup>3</sup>·d on the seventh day. The CSTR lost instability on the 17th day under high OLR, while the food waste AD system's instability criterion was specified as a 50% drop in methane production to normal levels [6]. When biochar was added to the CSTR on the 17th day, the pH value rose immediately. On the 28th day, methane production from the CSTR bioreactor reached a high level after 9 days of stable operation at a low level. On the 36th day, methane production returned to normal under high OLR operation.

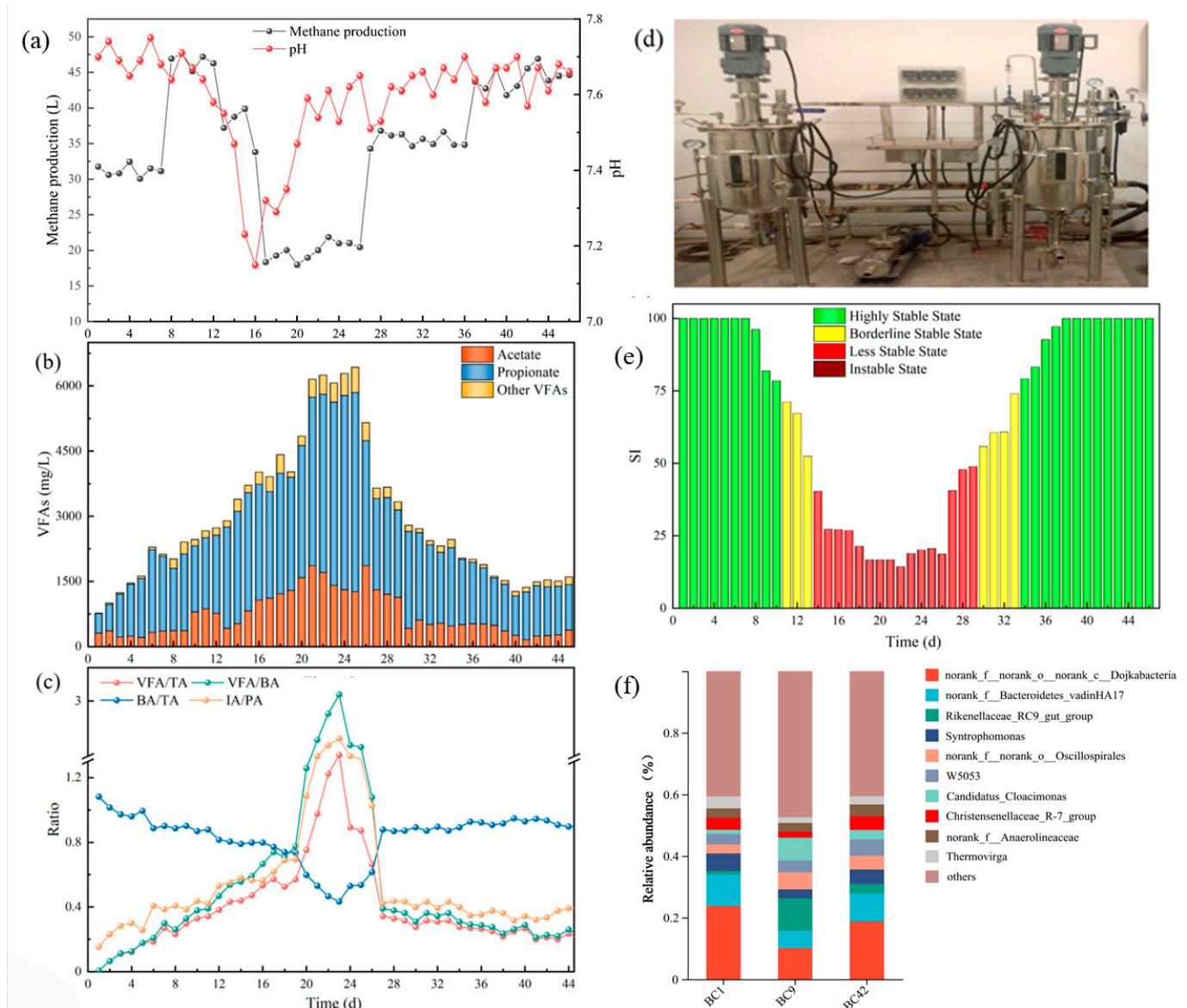
These single indicators' performances and simple combinations appeared unsatisfactory as a conventional early warning method for AD instability. As shown in Figure 9a–c, although all values of pH, VFAs, VFAs/TA, VFAs/BA, IA/PA and BA/TA correspondingly increased and decreased during the loss and recovery processes of CSTR stability, respectively, before the stability loss of the CSTR on the 17th day, only insignificant jumping points of pH on day 12, VFAs on day 6, IA/PA on day 6, BA/TA on day 5, VFAs/TA on day 7 and VFAs/BA on day 7 appeared, which might be used in a constrained manner as signals for early warning of instability. On days 20 and 21, when the CSTR had lost stability, there were instead noticeable jumping points in the curves of those values. Thus, the results indicated that the operability of single indicators and simple combinations was not strong enough to warn of AD instability early in this test.

The comprehensive warning indicator outperformed single warning indicators regarding visualization and foresight when utilized as an early warning system for AD instability. As shown in Figure 9e, the degree of instability and the state of the CSTR's recovery are indicated by different colors. Moreover, the comprehensive indicator also had good foresight in early warning of AD instability. The SI value of the CSTR reached below 75 on day 11, triggering a yellow warning that signaled the implementation of a 6-day-early warning. However, the comprehensive indicator's targeting as an early warning of sub-stability was comparatively subpar. It was challenging to identify the source of the CSTR instability based on changes in the comprehensive indicator. The early warning system of single indicators' pH and VFA changes made it easy to determine the cause, which was an increase in OLR.

Finally, the early warning system for potential instability based on microbial community changes also performed well. Three sludge samples were taken from the CSTR on the 4th, 9th and 42nd days, corresponding to the periods of stable operation, stability loss and stability restoration, respectively (Figure 9f). It was apparent by comparing the microbial community distributions in the three sludge samples that the microbial community in CSRT was highly stable. The primary microbial communities and their abundance on days 4 and 42 presented high similarity despite the CSTR having undergone stability loss and recovery procedures and biochar being added. Moreover, what was even more gratifying was that the screened sensitive bacteria's abundances responded favorably to the CSTR operation's stability. The abundance of *norank\_o\_norank\_c\_Dojkabacteria* decreased, and the abundance of *Rikenellaceae\_RC9\_gut\_group* significantly increased with the CSTR's stability loss on the ninth day. Furthermore, the abundances returned to their pre-instability levels as soon as the CSTR's stability was restored on the 42nd day. Additionally, as Figure 9a illustrates, the CSTR continued operating stably for a long time even after the OLR increased on the seventh day, and methane production increased as well. Nevertheless, it was discovered from the microbial detection data that certain instability changes were taking place at this time. According to the data, monitoring the changes in microorganisms provided super foresight in early warning systems for instability.

Therefore, this verification experiment revealed that the three levels of early warning approaches were organically combined to develop a new early warning system that was

comparable to the paradigm of human disease prevention and control and had notable advantages. (1) The triple guarantee enhanced this warning system's safety and reliability, which virtually eliminated the possibility of false alarms. (2) It may be possible to attain perfect synchronization between insight into apparent phenomena and identification of underlying causes. (3) This system delivers competitive performance in prediction precision, early perception of digester instability, forecasting robustness and application versatility. However, due to the limited volumes of the bioreactors in this study and the differences in FW composition in different sources, these factors may lead to different microbial community structures and reaction processes, ultimately resulting in significant differences in the efficiency of the warning system. Therefore, we need to improve and upgrade the early warning system according to actual conditions.



**Figure 9.** Results of the validation tests for the new early warning system. (a) Changes in pH and methane production; (b) VFAs; (c) VFAs/TA, VFAs/BA, BA/TA, and IA/PA; (d) physical photo of the bioreactor in the laboratory; (e) SI values; and (f) the microbial community shifts throughout the CSTR bioreactor's whole operation process.

## 4. Conclusions

This study proposed a new early warning system by bionically simulating the resident disease screening paradigm for the instability of the anaerobic digestion of food waste. The results revealed that the traditional early warning methods mainly based on single indicators, similar to human disease diagnosis, were appropriate for the cases of emergent food waste AD instability and presented high accuracy, strong targeting, and good convenience but poor foresight. Therefore, this study developed a novel method for obtaining comprehensive indicators via the entropy weight of multiple indicators. The early warning system based on comprehensive indicators presented the advantages of reasonable foresight, high accuracy and reliable operability. However, like the resident sub-health check-up processes, this developed method lost targeting, that is, the comprehensive indicator could not reflect the causes of bioreactor instability. Thus, an additional auxiliary early warning approach for potential instability in food waste AD based on periodic detection of microbial communities was further developed by simulating resident annual physical examinations. This method possessed the characteristics of good foresight, but weak accuracy and poor targeting. Verification experiments showed that the new early warning system demonstrated superiority in terms of high reliability, strong foresight and easy traceability, among other aspects, by incorporating the advantages of the three aforementioned early warning methods while avoiding their shortcomings. More precisely, this study provided a novel paradigm for developing early warning systems for food waste AD. In addition, the multi-layer warning system constructed in this study could be tightly coupled with automated machine learning pipelines to realize closed-loop intelligent control of anaerobic digesters, consistent with the intelligent development trend.

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