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Unraveling Anaerobic Digestion Instability: A Simple Index Based on the Kinetic Balance of Biochemical Reactions

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Abstract: Restoration potential (RP) and deterioration potential (DP) were formulated to shed light on the kinetic balance between anaerobic biochemical reactions. RP is gauged by the ratio of the methanogenesis rate (MR) to the acidogenesis rate (AR), while the DP is the sum of the accumulation rate (AcR) and dilution rate (DR) of total VFAs, normalized using the AR. In an anaerobic digester for a mixture of pulverized food waste and liquified sewage sludge, an RP above 1.0 signifies a restorative state in the kinetic balance of anaerobic biochemical reactions across various operational phases, including startup and steady state, and shifts in organic loading rate. Conversely, a DP value of 0.0 or higher denotes a deterioration in the kinetic balance. The instability index (ISI), calculated as the DP to RP ratio, serves as an indicator of an anaerobic digestion state. When the standard deviation of ISI surpasses 0.2, it signifies instability in biochemical reactions; however, an average ISI below 0.05 indicates a stable digestion process. The study underscores the efficacy of RP, DP, and ISI as robust indicators for assessing the stability of anaerobic digestion based on the kinetics of biochemical reactions.

Keywords: kinetic balance; instability index; perturbation; anaerobic digestion; diagnosis

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1. Introduction

Anaerobic digestion is a sustainable technology capable of decomposing organic matter into methane through biochemical reactions, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis [1,2]. The kinetics of biochemical reactions are highly susceptible to variations in substrate type and operational and environmental conditions. The hydrolysis of hardly degradable organic matter often requires physicochemical or biological pretreatment for anaerobic digestion [3–6]. However, the complexity of anaerobic digestion mainly arises from the distinct physiological characteristics of acidogenic bacteria and methanogenic archaea. Acidogenic bacteria metabolize organic monomers rapidly, whereas methanogenic archaea have a much slower metabolism and are more sensitive to changes in environmental conditions, including temperature and pH, and fluctuations in operational parameters such as hydraulic or organic loading rates [2,4,7]. Therefore, successful anaerobic digestion depends on how well the biochemical reactions are kinetically harmonized.

However, the composition of organic waste and its production for anaerobic digestion may undergo significant fluctuations depending on seasonal or climatic changes, and there is a risk of incorporating toxic substances into the waste stream [8–10]. These fluctuations in organic waste and the incorporation of toxic substances can perturb the biochemical reactions involved in anaerobic digestion [10–12]. In addition, while operating anaerobic digesters, mechanical problems often arise. These include pipeline clogging, power and pump failures, problems with heat exchangers and agitators, and corrosion or leakage in the main body of anaerobic digesters [13–15]. These mechanical problems can also perturb the biochemical reactions of anaerobic digestion [10,13].

The perturbation of the biochemical reactions preferentially inhibits methanogenesis, leading to the accumulation of intermediates, such as volatile fatty acids (VFAs), alcohols, and hydrogen [16,17]. Elevated levels of intermediates can intensify the inhibition of methanogenesis through thermodynamic constraints of syntrophic oxidation of VFAs [18–20]. This intensification can exacerbate the kinetic imbalance of biochemical reactions. Severe kinetic imbalances can precipitate the failure of anaerobic digestion, with recovery to a normal state potentially taking a prolonged period, approximately 2–3 months [11,13,21]. Fortunately, implementing simple preemptive measures, such as adjusting hydraulic or organic loading rates and supplementing alkalinity before reaching a critical state, can effectively control kinetic imbalance [16,21]. Hence, monitoring the kinetic imbalance is vital for maintaining a steady state in anaerobic digestion [4,16]. The states of anaerobic digestion can be categorized into stable, metastable, and unstable, dependent on the kinetic imbalance. A stable state indicates that the biochemical reaction steps are kinetically balanced, while metastable states, although imbalanced, can return to stability under conducive conditions. The unstable states exhibit severe imbalances, often culminating in process failure. The assessment of the kinetic imbalance has been primarily based on indicators such as intermediates or end products generated, environmental conditions, and the activity of anaerobic microorganisms [16,21,22]. These indicators include pH, total VFA ratio to alkalinity, acetate-to-propionate ratio, VFA accumulation rate, methane production rate (MPR), hydrogen and methane contents in biogas, anaerobic microbial community change, and thermodynamic analysis [23,24]. However, some parameters during anaerobic digester operation are highly variable, and some of the above indicators are difficult to monitor in real time [1,13].

Additionally, these indicators must be compared to threshold values to assess the stability of anaerobic digestion [11,25]. However, the thresholds for these indicators were less intuitive for abnormal states and varied with the type of organic waste, anaerobic digestion process, and environmental and operating conditions [16,21]. On the other hand, the interplay between acidogenesis, which produces VFAs, and methanogenesis, which consumes VFAs for methane production, can provide more intuitive insights into the kinetic balances of the biochemical reactions and the accumulation and conversion rates of VFAs.

In light of the limitations associated with traditional indicators for anaerobic digestion, this study introduces a novel and simple instability index grounded in analyzing the kinetic balance of biochemical reactions in various operational modes of an anaerobic digester, providing a pioneering approach to assess the stability of anaerobic digestion processes effectively. The approach was meticulously validated under different operational scenarios, including the startup and steady-state phases, interruption and resumption of substrate supply, and varying impulse organic loading rates (OLRs), establishing its efficacy in monitoring the stability of anaerobic digestion with enhanced precision and reliability.

2. Materials and Methods

2.1. Substrate, Anaerobic Digester, and Operation

A mixture of pulverized food waste (PFW) and hydrothermal liquified sludge (HLS) was used as the substrate for anaerobic digestion. The HLS was prepared by heating a waste-activated sludge from a wastewater treatment plant (Incheon, Republic of Korea) at 190 °C for 30 min. For PFW, food waste was collected from a university cafeteria, ground using a home blender (HC-BL2200M, Busan, Republic of Korea), and stored in a refrigerator at 4 °C until use. Anaerobic digestion sludge was collected from a municipal wastewater treatment plant (Busan, Republic of Korea), screened to remove impurities, and used as the inoculum. Table 1 summarizes the physicochemical properties of the substrate and inoculum, including pH, alkalinity, total volatile fatty acids (VFAs), total solids (TSs), volatile solids (VSs), total chemical oxygen demand (COD), and soluble COD (SCOD).

Table 1. Characteristics of the hydrothermally liquified sludge (HLS), pulverized food waste (PFW), and their mixture used as the substrate and the inoculum.

Parameters	HLS	PFW	Mixed Substrate	Inoculum
pH	7.64 ± 0.02	5.46 ± 0.04	5.73 ± 0.04	7.58 ± 0.03
Alkalinity (g/L $CaCO_3$)	12.8 ± 1.0	2.6 ± 1.0	4.66 ± 0.53	3.0 ± 0.1
VFA (g COD/L)	8.0 ± 0.6	1.4 ± 0.4	3.30 ± 0.19	0.7 ± 0.0
COD (g/L)	64.9 ± 2.0	112.1 ± 8.4	88.07 ± 6.68	12.5 ± 0.0
SCOD (g/L)	58.5 ± 3.4	42.8 ± 7.3	47.79 ± 6.09	53.5 ± 1.6
TS (g/L)	58.4 ± 1.7	103.6 ± 4.4	72.76 ± 1.74	118.7 ± 3.0
VS (g/L)	43.7 ± 1.3	87.02 ± 2.5	56.27 ± 2.29	65.7 ± 2.2

A drum-type horizontal anaerobic digester (50 L working volume, 30 cm diameter, and 120 cm length) was used for the anaerobic digestion experiment. The anaerobic slurry was stirred by rotating a blade mounted on a horizontal shaft at 200 rpm using a DC motor. The slurry temperature was maintained at 35 °C with a heating band around the outer surface of the anaerobic digester. A wet gas meter (W-NK, Inagi-shi, Japan) was connected to the venting valve of the anaerobic digester to monitor biogas production. During operation of the anaerobic digester, the substrate was manually introduced to the front once daily, with the digestate being discharged from the opposite end. The substrate supply was gradually increased and maintained at a hydraulic retention time (HRT) of 20 days or 30 days for stable operation of the anaerobic digesters. In the experiment for evaluating the kinetic imbalance of biochemical reactions, low-impulse OLRs (150% and 200% for a day) were used first after startup. Substrate supply was interrupted for 20 days and resumed, followed by applying higher impulses (300%, 500% for a day) of OLR.

2.2. Analysis

During the operation of the anaerobic digester, a daily digestate slurry sample was taken, and its pH was analyzed using a pH meter (YSI 1200, OH, USA). Alkalinity and VFA were measured using titration methods [1,12]. Other state variables like TSs, VSs, COD, and SCOD followed the standard methods for analysis. The biogas production rate was monitored using a wet gas meter (W-NK, Inagi-shi, Japan), while the biogas composition, specifically methane and carbon dioxide, was analyzed using a gas chromatograph (Series 580, GawMac Instrument Co., Bethlehem, PA, USA) equipped with a thermal conductivity detector and Porapak-Q column (6 ft × 1/8 in, SS).

Statistical relationships among the state variables were assessed using Pearson's correlation matrix. This was achieved using the 'corr' function from the Pandas library in Python. To explore causal relationships between the variables, Granger causality analysis was applied. A *p*-value less than 0.05 in the Granger causality matrix was considered significant for establishing causality [26]. This analysis was conducted using the 'granger causality tests' function from the 'statsmodels' package in Python.

2.3. VFA Balance in Anaerobic Digestion

The mass balance for VFAs in a completely mixed anaerobic digester can be expressed as Equation (1).

$$\mathrm{V(dVFA/dt)} = \mathrm{Q(VFA_i - VFA_e) + V(r_a - r_m)} \tag{1}$$

where V is the volume of the anaerobic digester (L) and Q is the substrate supply rate (L/d). r_a is the acidogenesis rate (AR) (g HAc/L·d) and r_m is the methanogenesis rate (MR) (g HAc/L·d). VFA is the total VFA (g HAc/L) and the subscripts i and e denote influent and effluent. dVFA/dt is the accumulation rate of VFA (AcR) and (Q/V) × VFAe is the dilution rate of VFA (DR). Based on the stoichiometric equation for methane production, one mol of methane is theoretically produced from one mol of acetate or four mol of hydrogen [7].

The amount of methane acetoclastic methanogenesis accounts for about two-thirds of total methane production [27]. The electron transfer efficiency for methane production from organic matter varies significantly depending on the substrate types, electron transfer route, and anaerobic digestion processes but is typically 59.9–90.7% [2,7,11,12]. The conversion factor (f_c) for estimating VFA converted to methane (g HAc/L CH_4) is about 1.97–2.98. Thus, the MR can be obtained from Equation (2).

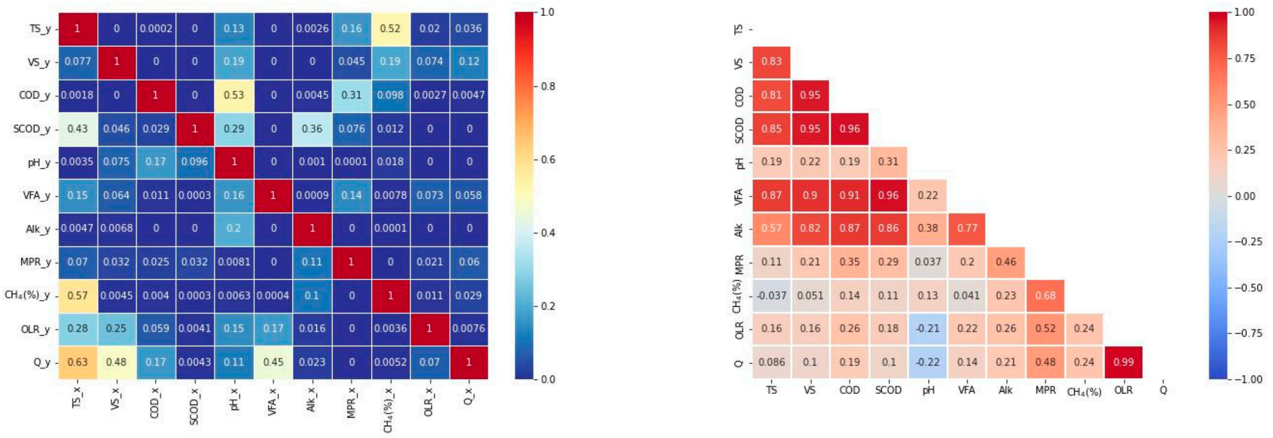
$$r_m = f_c \times MPR \tag{2}$$

where MPR is the methane production rate (L CH_4/L·d). Ignoring electron transfer losses, the AR was estimated from the sum of the AcR, DR, and MR.

3. Results

3.1. Kinetic Response of Anaerobic Digestion to the Fluctuations in OLR

In anaerobic digestion, each microbial metabolism uniquely responds to various environmental and operating conditions, including pH, temperature, and organic loading rate [28,29]. Changes in these conditions can upset the kinetic balance of anaerobic biochemical reactions, which are reflected in the state and performance variables, including pH, alkalinity, VFAs, TSs, VSs, COD, SCOD, MPR, and CH_4 (%) [11,28]. In the Granger causality analysis, the *p* values of OLR were less than 0.05 for nearly all the state and performance variables (Figure 1a). This is the reason why OLR is universally used as a manipulated variable to operate the anaerobic digesters [30,31]. Furthermore, although some state and performance variables appeared to cause changes to each other, their Pearson's correlation coefficients were not significant (Figure 1b). However, it is noteworthy that the *p*-value of VFA in the Granger causality analysis was also 0.05 or less than most other state and performance variables. This confirms that VFA is essential in explaining the kinetic perturbations of anaerobic biochemical reactions [17,21].



(**a**) (**b**)

Figure 1. (**a**) Granger causality matrix for the state and performance variables and operating conditions in anaerobic digestion and (**b**) their Pearson's correlation matrix.

The mass balance (Equation (1)) illustrates that acidogenesis ferments hydrolyzed monomers into VFAs. The VFAs are then broken down into acetate and hydrogen through syntrophic oxidation and converted into methane [11,32]. Nonetheless, when not all VFAs are converted to methane, they build up in the anaerobic digester, increasing VFA levels or discharging through the digestate effluent [32].

The startup, steady-state, and low-impulse OLRs. During the startup of the anaerobic digester, the AR and MR increased in response to the stepwise increase in OLR and their fluctuations were insignificant (Figure 2a). This suggests that the inoculated anaerobic microorganisms were well adapted to the new environment. In the subsequent phase, with the anaerobic digester operating at HRTs of 20 and 30 days for a prolonged period, the MR not only correlated with OLR but also approached the AR. Meanwhile, both the AcR and DR were low and stable. This behavior underscores that anaerobic biochemical reactions, specifically acidogenesis and methanogenesis, achieved a kinetic balance when OLR was sustained over an extended duration [16,33].

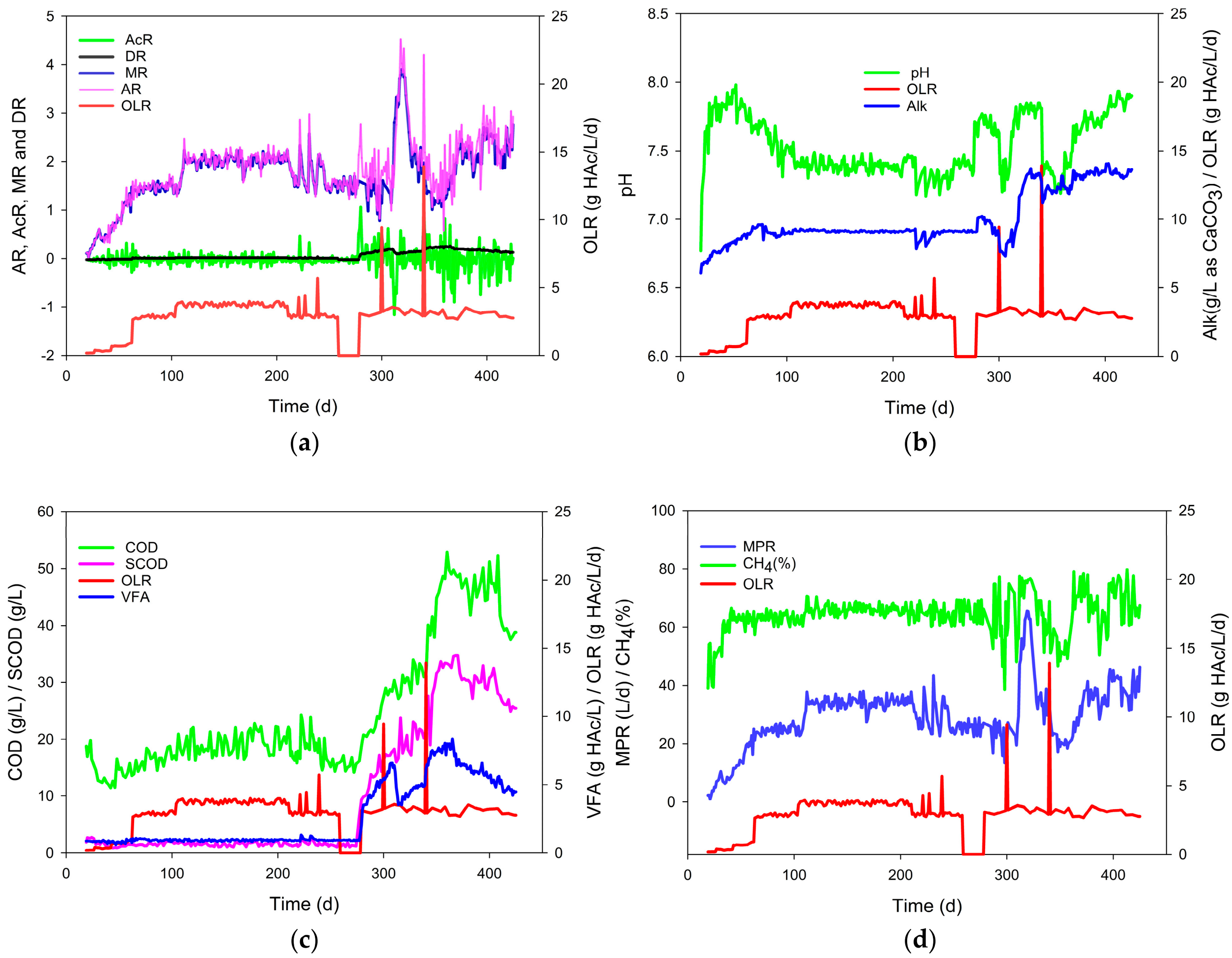


Figure 2. (**a**) Behaviors of the acidogenesis rate (AR), methanogenesis rate (MR), accumulation rate of VFAs (AcR), and dilution rate of VFAs (DR); (**b**) pH and alkalinity (Alk); (**c**) total chemical oxygen demand (COD), soluble COD (SCOD), and total volatile fatty acids (VFAs); and (**d**) methane production rate (MPR) and methane content (CH_4 (%)) in biogas depending on the organic loading rate (OLR) changes.

A low-impulse OLR of 150% applied on the 221st day caused transient fluctuations in some state variables, including pH, alkalinity, SCOD, and VFAs. However, the MR was still close to the AR and they correlated with each other. Although a small peak in AcR was observed in response to the low-impulse OLR, it quickly disappeared. It appears that a low-impulse OLR had a minor impact on the kinetic balance, which was promptly buffered by the activity of methanogenic archaea. Interestingly, the repeated 150% impulse OLR made the AcR peak smaller than the first peak, after which no clear AcR peak appeared even at 200% impulse OLR. This suggests that the methanogenic species became more diverse when small impulse OLRs repeated, increasing their resilience to the external perturbations in OLR [33].

Interruption and resumption of substrate supply. The interruption of substrate supply from day 259 did not change the state and performance variables noticeably for 20 days (Figure 2). However, there was a significant increase in pH, alkalinity, COD, SCOD, and VFAs as the substrate supply resumed (Figure 2c). Furthermore, the MPR and the methane content in biogas fluctuated significantly (Figure 2d). These results indicate that there was a kinetic imbalance of anaerobic biochemical reactions, which was reflected in the upward peaks of the AR and AcR, the downward peaks of the MR, and the gradual increase in the DR (Figure 2a). Under limited available substrates, acidogenic bacteria can use various survival strategies, including competition for substrates, altered metabolic pathways and gene expression, and use of different substrates [34]. However, methanogenic archaea have a higher substrate specificity, resulting in smaller community size and diversity than acidogenic bacteria [2,7,34]. This indicates that methanogenic archaea with slow metabolic rates do not readily recover their community size and diversity in response to rapid fluctuations in acidogenesis [16,35].

High-impulse OLRs. On the 300th day, the high-impulse OLR of 300% led to a short-term increase in SCOD and VFAs while causing a decrease in both pH and alkalinity (Figure 2b,c). Notably, this high-impulse OLR intensified the fluctuation of methanogenic attributes, including the MPR and the methane content of the biogas (Figure 2d). This implies that such a high-impulse OLR overly accelerated the processes of hydrolysis and acidogenesis, whereas methanogenesis did not respond as swiftly. However, in the long-term, from the 310th day, there was a rapid decline in VFAs, paralleled by a marked increase in MPR. As a result, notable downward peaks were observed in the AcR and upward peaks in the MR (Figure 2a). The dominant species in the methanogenic community seems to have transitioned into the species with higher metabolic rates due to prolonged exposure to high VFA concentrations. It is known that the metabolic rate of *Methanosarcina* spp. is higher than that of *Methanosaeta* spp., but the substrate affinity is higher in *Methanosaeta* spp. [36,37]. Here, the AR fluctuated in conjunction with MR. This suggests that a high level of VFAs may simultaneously inhibit hydrolysis and acidogenesis processes [38]. Based on the observed behaviors of AR, AcR, and MR from the 310th day, the rapid methane conversion of VFAs appears to restore the kinetic balance between acidogenesis and methanogenesis.

On the 340th day, the application of a high-impulse OLR of 500% led to significant short-term changes over approximately three weeks, as evidenced by the increase in state variables like COD, SCOD, and VFAs (Figure 2c). Conversely, there was a notable decrease in the MPR and the methane content of the biogas (Figure 2d). Thus, the peak of the AR was significantly higher than MR. This suggests that the high-impulse OLR of 500% considerably perturbed the kinetic balances of the anaerobic biochemical reactions. However, alkalinity was maintained at about 12 g/L as $CaCO_3$, even after a slight drop immediately after the high-impulse OLRs, keeping the pH level above 7.2 (Figure 2b). This is why significant kinetic imbalance did not lead to the worst scenario of failure of anaerobic digestion [30,39]. It suggests that above pH 7, methanogenesis is not significantly inhibited even at a high level of VFAs. However, in the long term, after applying a 500% impulse OLR, the downward peak of AcR began to rise from about week 3, and the MR gradually approached the AR. This suggests that the kinetic balance of anaerobic biochemical reactions was gradually improving. However, even after approximately 3 months, VFA was still high at 4.5 g/L and did not return to the low and stable values observed before applying the high-impulse OLR.

3.2. Restoration and Deterioration of the Kinetic Balance in the Biochemical Reactions

In anaerobic digestion, kinetic balance of anaerobic biochemical reactions, including acidogenesis and methanogenesis, is essential to maintaining process stability [11,13]. When the MR closely approximates the AR, it indicates that the state of anaerobic digestion is kinetically stable. However, a kinetic imbalance arises as the MR is smaller than the AR, increasing the AcR and DR. When the imbalance intensifies, it leads to severe instability of anaerobic digestion, with a risk of eventual failure. Conversely, the MR surpasses the AR as the kinetic balance improves, and AcR and DR decrease. These highlight the vital role of the MR and AR in restoring the kinetic balance of anaerobic biochemical reactions [21,40].

Hence, the potential for restoring the kinetic balance, referred to as restoration potential (RP), was defined as the ratio of the MR to the AR (Equation (3)).

$$\mathrm{RP} = \mathrm{MR}/\mathrm{AR} \tag{3}$$

While operating the anaerobic digester, the kinetic balance depends on the changes in the OLR change, which are reflected in the RP fluctuation (Figure 3a). The RP values with the OLR changes were statistically analyzed and summarized in Table 2. The RP fluctuated from 0.70 to 1.52 during the startup period, reflecting the perturbations in the AR and MR due to the stepwise increase in OLR. However, the average of the RP values was close to the baseline at 1.0, and the standard deviation was only 0.15, indicating that the kinetic balance was slightly disturbed, but the anaerobic digestion process was stable (Figure 3a). Then, when OLRs were fixed for prolonged times, the fluctuation of the RP ranged from 0.78 to 1.14. In addition, the average of the RP was close to the baseline at 0.98, and the standard deviation was 0.05, indicating that the anaerobic biochemical reactions were kinetically balanced. When impulse OLRs of 200% or lower were applied, the average RP remained at 0.99 with a standard deviation of 0.05, indicating minimal fluctuations in RP.

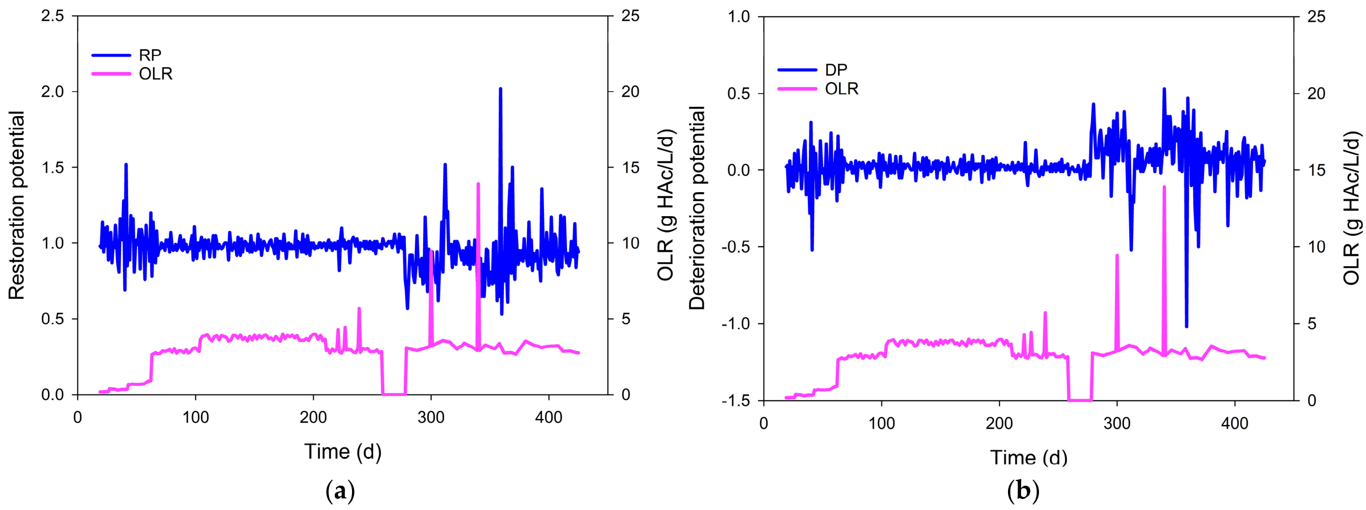


Figure 3. Changes in (**a**) restoration potential (RP) and (**b**) deterioration potential (DP), depending on the OLRs.

On the other hand, the interruption of the substrate supply for approximately 20 days (0.67 HRT) did not significantly affect the RP value. The depletion of the available substrate after substrate supply interruption appeared to limit all microbial metabolisms of acidogenic bacteria and methanogenic archaea, reducing both the AR and MR [33,41]. Then, the abrupt resumption of substrate supply further fluctuated the RP from 0.58 to 1.18. The average of the RP considerably decreased to 0.84, and the standard deviation was 0.14. This suggests that the abrupt resumption of substrate supply after interruption significantly disrupts the kinetic balance. It is known that the response of anaerobic microorganisms to the limited substrate available relies on the physiological properties of the anaerobic microorganisms [41]. Generally, acidogenic bacteria with high metabolic rates increase AR, while methanogenic archaea with slow metabolic rates struggle to match the AR [2,4].

An impulse OLR of 300% reduced the RP peak to 0.62 in the short term, indicating a severe kinetic imbalance in anaerobic biochemical reactions, as confirmed by the increase in AR and decrease in MR. However, on the 12th day, after applying the impulse OLR at 300%, the RP peaked at 1.52 in the long term. This high RP peak signals the onset of the restoration to a more balanced kinetic state. However, when a higher-impulse OLR of 500% was applied, the RP peak decreased significantly to 0.53 in the short term. In the long term, although the RP rebounded to around 2.02 after approximately 20 days, it fluctuated for three months without reaching a stable value near 1.0. The high-impulse OLR of 500% severely perturbed the kinetic balance of anaerobic biochemical reactions, increasing the instability of anaerobic digestion. These findings suggest that RP can serve as an indicator to access the restoration potential of perturbed biochemical reactions.

Table 2. The behavior of the AR, MR, AcR, and DR; the range, mean, and standard deviation of the RP and instability index (ISI); and the state of the anaerobic digestion process.

Condition	Variable Behavior	RP			ISI			AD State
		Range	Mean	Std	Range	Mean	Std	
Startup	AR and MR increased.	0.70~1.52	1.01	0.15	−0.34~0.44	0.01	0.15	Stable
Steady state	MR approached AR; AcR and DR maintained low and stable.	0.78~1.14	0.98	0.05	−0.13~0.28	0.02	0.05	Stable
After low-impulse OLRs (<200%)	MR was close to AR, and a small peak in AcR.	0.82~1.10	0.99	0.05	−0.09~0.22	0.02	0.05	Stable
Interruption of substrate supply	No change.	0.94~1.06	1.00	0.03	−0.06~0.06	0.00	0.03	Stable
Aftermath following resumption of substrate supply	Upward AR, AcR, DR, and downward MR.	0.58~1.18	0.84	0.14	−0.15~0.74	0.23	0.21	Unstable
Aftermath following the impulse OLR (300%)	(i) Short term: SCOD and VFA increased; pH and Alk decreased.	0.62~1.52	0.93	0.23	−0.34~0.61	0.13	0.24	Metastable
	(ii) Long term: downward AcR; upward MR, AR.	0.83~1.21	0.95	0.09	−0.17~0.20	0.06	0.10	Metastable
Aftermath following the impulse OLR of 500%	(i) Short term: AR higher than MR.	0.53~2.02	0.87	0.29	−0.51~0.89	0.24	0.30	Unstable
	(ii) Long term: the downward of AcR began to increase.	0.75~1.50	0.96	0.15	−0.33~0.33	0.06	0.14	Metastable

In anaerobic digestion, a high concentration of VFAs can inhibit methanogenesis, while high substrate supply rates can lead to the washout of methanogenic archaea from anaerobic digesters [42,43]. This indicates that the increased AcR and DR can further worsen the kinetic balance of the anaerobic biochemical reactions [42,43]. Therefore, the deterioration potential (DP) of kinetic balance can also be defined as the ratio of the sum of the AcR and DR to the AR (Equation (4)).

$$DP = (AcR + DR)/AR \tag{4}$$

During the operation of the anaerobic digester, the DP value exhibited positive or negative peaks around the baseline of zero, indicating the deterioration or restoration of kinetic balance, respectively (Figure 3b). It is important to note that the sum of the MR, AcR, and DR is equal to the AR (Equation (5)). Thus, similar to the RP, the DP values above its baseline indicate a state of kinetic balance associated with deterioration. This suggests that the DP can also serve as an indicator to assess whether the kinetic balance of biochemical reactions is being perturbed.

$$AR = MR + AcR + DR \tag{5}$$

3.3. Instability Index (ISI) of Anaerobic Digestion

In anaerobic digestion, RP or DP provides valuable information about the kinetic balance of the biochemical reactions. However, the values of RP or DP were not linearly correlated with the perturbations of the monitoring variables (Figure 2). For example, the fluctuation ranges in the RP and DP decreased considerably after the high RP and DP peaks, but the kinetic imbalance of biochemical reactions persisted. Hence, it was challenging to quantitatively assess the stability of the anaerobic digestion process solely based on the RP or the DP. It is worth noting that the RP and DP are complementary parameters. So, as one increases, the other parameter decreases, indicating that combining the DP and RP better quantifies the instability of the anaerobic digestion process based on the kinetic imbalance. As shown in Equation (6), the DP ratio to the RP can serve as an instability index (ISI) to assess the state of the anaerobic digestion process.

$$\mathrm{ISI} = \mathrm{DP}/\mathrm{RP} = (1 - \mathrm{RP})/\mathrm{RP} = (\mathrm{AR} - \mathrm{MR})/\mathrm{MR} \quad (6)$$

The ISI gradually increases as the RP value decreases to 0.5 and increases significantly for the RP values below 0.25. More specifically, the ISI is the ratio of the difference between AR and MR divided by MR. This suggests that ISI can be reliably employed to assess the instability of the anaerobic digestion process over a wide range.

The ISI provided a good indication of the overall anaerobic digestion state regarding kinetic imbalance during anaerobic digester operation (Figure 4). In general, the anaerobic digester is initiated by increasing the OLR stepwise after inoculating it with seed sludge [25,43]. However, as mentioned above, the inoculated anaerobic microorganisms must acclimate to the new environment and the stepwise increase in OLR [12,44]. The anaerobic biochemical reactions can thus be perturbing, leading to kinetic imbalances during the startup period. The ISI fluctuated between −0.34 and 0.44 for the startup period, and the standard deviation was 0.15 (Table 2). This indicates that the fluctuation in ISI explains the variation in kinetic imbalance of the anaerobic biochemical reaction during startup. However, despite these fluctuations in ISI, the average value of the ISI peaks was close to the baseline, and there were no state and performance variables of anaerobic digestion with apparent abnormalities. This suggests that during the startup period, the fluctuation in ISI peak reflects the perturbed kinetics of the anaerobic biochemical reactions, and the average value of the ISI describes the stability of the anaerobic digestion process.

When the HRT was maintained at 20 or 30 days for a prolonged time, the OLR was 2.68 ± 0.11 g COD/L·d or 3.75 ± 0.12 g COD/L·d, respectively. During this period, the fluctuation of the ISI was slight, ranging from −0.13 to 0.28. Their average and standard deviation were only 0.02 and 0.05. These observations suggest that the anaerobic biochemical reactions maintained kinetic balance, reflecting a stable state in anaerobic digestion [16,28,45]. The response of each anaerobic biochemical reaction to an impulse OLR is different. Therefore, an excessive impulse OLR can directly cause kinetic imbalance. However, low-impulse OLRs of 150% and 200% applied to a steady state resulted in a small transient ISI peak, indicating the kinetic imbalances in the anaerobic biochemical reactions were only slight.

The steady provision of the substrate supply was suddenly interrupted for about 20 days (0.67 HRT). However, almost no abnormal behavior in monitoring variables was observed, resulting in minimal fluctuations in the ISI. On the other hand, the resumption of interrupted substrate supply suddenly increased alkalinity, pH, COD, SCOD, and VFA levels (Figure 2b,c). Moreover, it resulted in a decrease in the MPR and an increase in the variability of biogas methane content. The high ISI peak of 0.74 effectively reflected the fluctuations in these state and performance variables, corresponding to the RP of 0.59. The ISI peaks repeated in the range of −0.15 to 0.74, and the average and standard deviation rose to 0.23 and 0.21, respectively. These indicate that the imbalance of biochemical reactions increased after the abrupt resumption of substrate supply. As a result, the anaerobic digestion process did not restore stability for a while.

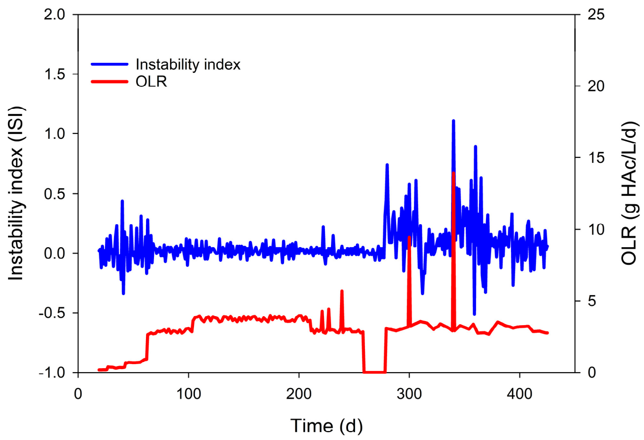


Figure 4. Instability index (ISI) of anaerobic digestion depending on the fluctuation in OLRs.

In the short term, after applying the high-impulse OLR of 300%, the state and performance variables, including COD, SCOD, and VFAs, increased continuously for the initial six days (Figure 2c). The pH and alkalinity levels decreased temporarily and then increased (Figure 2b). The perturbations of these variables were well reflected in the ISI fluctuation, which increased from −0.34 to 0.61 and averaged 0.13. This indicates that the impulse OLR of 300% caused substantial destabilization of the anaerobic digestion process by perturbating the kinetic balance. However, the ISI peak decreased between −0.17 and 0.20 over the long term, averaging 0.06, with slight fluctuations over the subsequent 25 days. A negative peak in the ISI value represents an increase in RP due to the MR that raised above the AR.

Unlike the impulse OLR of 300%, a significant ISI peak of 0.61 appeared immediately after the impulse OLR of 500%. In the short term, the range of ISI fluctuation for about three weeks was significant, ranging from −0.51 to 0.89, and the average value was 0.24. This means that the high-impulse OLR aggravated the kinetic imbalance between the anaerobic biochemical reactions. Thus, the instability of the anaerobic digestion process increased accordingly. However, the ISI fluctuated from −0.33 to 0.33 in the long term, and the average decreased to 0.06 after three weeks. This indicates an improvement in state stability despite the considerable kinetic imbalance.

4. Discussion

In anaerobic digestion, fluctuations in environmental and operating conditions and malfunctions of auxiliary equipment can disrupt the kinetic balance between anaerobic biochemical reactions [11,12,33]. The kinetic imbalance can destabilize the anaerobic digestion, potentially leading to operational failure. Thus, it is crucial to implement appropriate measures to restore this balance [45,46]. As a result, assessing whether the biochemical reactions are kinetically balanced is essential for operating an anaerobic digester in a stable state. So far, numerous approaches have been explored to assess the stability of anaerobic digestion [23,42,45]. These approaches primarily rely on indicators that utilize specific anaerobic intermediates and products. These include pH, alkalinity (partial, intermediate, and total), VFAs (acetate, propionate, and total), and partial pressures of hydrogen and methane in biogas [23,25]. However, the effectiveness of these indicators is influenced by various factors, such as substrate types, anaerobic digestion processes, and environmental and operational conditions [11,16]. The kinetic balance between the anaerobic biochemical reactions, including acidogenesis and methanogenesis, provides insights into the stability of the anaerobic digestion process [33]. Recently, an indicator was introduced that utilizes the ratio of AcR to MPR, drawing on the kinetic balance, to evaluate the stability of anaerobic digestion. This indicator employs MPR fluctuations as an auxiliary measure, offering directional insights into stability [24]. While this indicator presents several benefits, its ability to provide comprehensive information is limited, and its implications can sometimes be ambiguous.

Under various operational modes, such as startup, steady-state operation, low-impulse OLR, and interruption of substrate supply, the average RP values were close to the baseline of 1.0, and the fluctuation range of RP was narrow. These indicate that the states of anaerobic digestion were good, although the biochemical reactions were kinetically slightly perturbed (Figure 5a). Across the other modes, including the resumption of substrate supply and high-impulse OLRs of 300% and 500%, the average RP value below the baseline suggests deterioration in the biochemical kinetic balance. This indicates that when the stresses suppress the MR, the RP decreases; however, the RP increases as the kinetic imbalance is restored. The RP or DP can be a good indicator for assessing the kinetic imbalance of anaerobic digestion. However, no linear correlation of the RP or DP was observed with the variability in state and performance variables and, thus, the stability of anaerobic digestion. In addition, the values of the RP and DP are relatively small near the baselines, making it inconvenient to quantitatively determine the stability of anaerobic digestion.

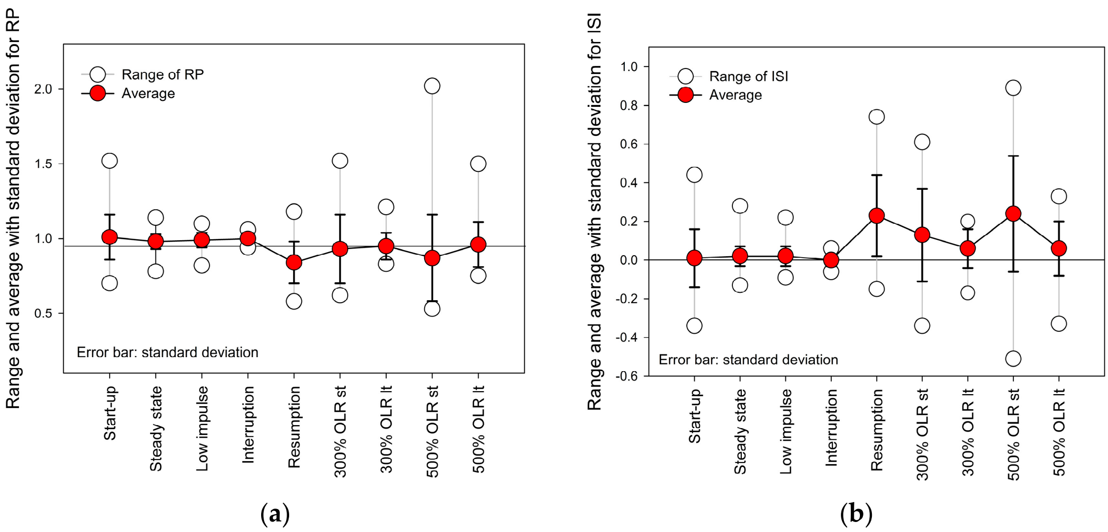


Figure 5. Changes in range, average, and standard deviation(std) of (**a**) RP and (**b**) ISI depending on various operational modes.

However, it is worth noting that RP and DP have a complementary relationship. So, ISI (instability index), the DP ratio to the RP, can assess the instability of anaerobic digestion even if the kinetic imbalance is slight (Table 2). For the startup period, the ISI fluctuated from −0.34 to 0.44, but the standard deviation was only 0.15, and the average was close to the baseline value at 0.01 (Figure 5b). During steady-state operation at 20 and 30 days of HRT, the ISI range decreased to between −0.13 and 0.28, and the standard deviation and average were only 0.05 and 0.02, respectively. The variances of the ISI in range and standard deviation during these operational modes indicate that the kinetics of the bioelectrochemical reactions were slightly unstable. On the other hand, the average values close to zero suggest that the anaerobic digestion state was comparatively stable. In the periods following the resumption after substrate supply and high-impulse OLRs of 300% and 500%, the state and performance variables fluctuated significantly. These fluctuations were evident in the range of 0.89–1.40 and a standard deviation of 0.21–0.30. Additionally, the average values of the ISI increased to 0.13–0.24. This indicates that stresses due to excessive OLR changes cause a severe kinetic imbalance between the anaerobic biochemical reactions, exacerbating the instability of the anaerobic digestion process.

The descriptions of the RP, DP, and ISI related to the kinetic balance and state instability of anaerobic digestion can be summarized as follows (Figure 6): (i) For anaerobic digestion, an RP value above 1.0 suggests a restoring state in kinetic balance while below 1.0 implies a deteriorating state. The DP and RP are complementary values. (ii) The range and standard deviation for the ISI assess the kinetic imbalance between anaerobic biochemical reactions.

A standard deviation of 0.2 or above indicates a significant kinetic imbalance. (iii) The average ISI gauges the instability of anaerobic digestion. A value below 0.05 suggests a highly stable state. (iv) An average ISI between 0.05 and 0.2 indicates a restorable metastable state. (v) An ISI of 0.2 or higher represents an unstable state of anaerobic digestion. The RP and ISI are believed to be valuable indices to assess the instability of the anaerobic digestion process based on the kinetic imbalance. However, further exploration and verification are required to assess the state of anaerobic digestion using RP, DP, and ISI across various substrates, temperatures, and other conditions.

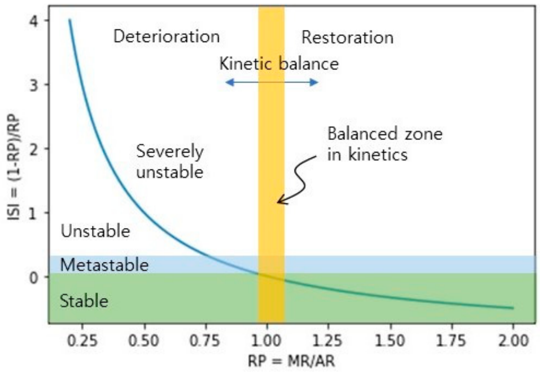


Figure 6. Summary of changes in the RP and ISI for the restoration and deterioration potentials for kinetic balance and instability of anaerobic digestion.

The ISI can be obtained from substrate supply rate, VFAs, and MPR when operating anaerobic digesters. An electromagnetic flowmeter can measure the substrate flowrate online [47,48]. The VFAs can be effectively monitored using an automated titrator, streamlining the process and ensuring precise measurements. However, automatic VFA titrators are expensive and prone to mechanical issues, such as clogging in the pipeline [1]. MPR can estimate the biogas production rate and the methane content in the biogas. However, biogas with high moisture and hydrogen sulfide levels can cause corrosion to the methane sensor [48]. Fortunately, the online data for pH, oxidation and reduction potential (ORP), and electric conductivity (EC) in the liquid phase can be obtained without severe issues using electrochemical sensors [49]. With a hybrid deep learning model like a convolutional neural network–long short-term memory, the status variables, such as VFAs and MPR, can be predicted in real-time from the online sensor data of pH, ORP, and EC, with a high determinant correlation coefficient of 0.98 [1]. Thus, a deep learning model can estimate the ISI in real time by predicting the state variables such as VFAs and MPR from online sensor data. Anaerobic digesters could be operated stably by continuously monitoring the ISI, early detection of abnormal symptoms, and taking prompt corrective actions.

5. Conclusions

The kinetic balance of biochemical reactions in anaerobic digestion ensures stable operation. The restoration potential (RP), representing the ratio of methanogenesis rate (MR) to acidogenesis rate (AR), along with the complementary parameter, deterioration potential (DP), plays a vital role in assessing the kinetic balance. RP values above 1.0 indicate a state of kinetic balance favoring restoration, while DP values above 0.0 indicate a state of kinetic balance associated with deterioration. The instability index (ISI), defined as the DP ratio to the RP, is a valuable tool for assessing the instability of anaerobic digestion. A wide fluctuation range with a standard deviation of ISI above 0.2 indicates an unstable kinetic balance of biochemical reactions, whereas an average ISI value below 0.05 suggests a more stable anaerobic digestion process. Monitoring the ISI allows for early detection of any deterioration in anaerobic digestion status, and it significantly contributes to maintaining the stable operation of anaerobic digesters by enabling prompt measures to address potential issues.

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