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Lab-scale biogas production from co-digestion of super-intensive shrimp sludge and potential biomass feedstocks

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This study evaluated biogas production potentials from local biomass feedstocks comprising of Abstract: rice straw (RS), steamed lemongrass (SL), bagasse (BA) and desiccated coconut (DC) on superintensive shrimp sludge (SS) anaerobic digestion. A series of batch anaerobic digestion experiments was conducted at an organic loading rate of 50 g-VS L⁻¹ and a C/N ratio of 25 under mesophilic conditions. The results indicate that co-digested biomass substrates are more suitable than single sludge except for DC supplementation, which exhibited a severe pH inhibition for methanogenesis activities. A reactor supplemented with BA achieved the highest overall biogas production (126.78 L kg-VS_{added}⁻¹), which increased biogas yields 53.70% compared to a monosludge reactor. Furthermore, reactors with RS and SL increased biogas yields by 26.40% and 29.21%, respectively. Irrespective of initial materials, the H_2S concentration in biogas compositions was measured at very high levels (23,710 - 65,040 ppm) after 10-15 days of digestion, while a decreasing trend was recorded for the remaining digestion period (16 - 60 days), yet still maintained relatively high levels (5,873 - 9,155 ppm). The study suggests that future works should focus on pH neutralization within the reactor with DC substrates and H₂S removal in biogas composition.

Keywords: Batch anaerobic digestion, Biogas production, Biomass feeding, Co-digestion, Shrimp sludge, Super-intensive shrimp cultivation

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1. INTRODUCTION

Super-intensive shrimp aquaculture is increasingly practiced in the coastal provinces of the Vietnamese Mekong Delta region. The production model produces a large amount of shrimp sludge due to highdensity stocking and high daily water exchange. The effectively untreated sludge is discharged directly into waterbodies resulted in environmental contamination. Developing alternative methods for processing this sludge is urgently required, with respect to limiting both cultivation-related risks and environmental degradation. Although anaerobic digestion is recognized as a well-known technology to treat biodegradable wastes [1], it is still a novel technical measure for effective treatment of sludge produced from super-intensive shrimp farms with many challenges remaining to recuperate renewable energy effectively [2,3]. Implementation of the technology in processing super-intensive shrimp sludge has received much attention in recent years due to its applicable characteristics, which offer important benefits for supplying decentralized renewable energy. However, biogas production from the sludge is generally as less efficient [4]. This could be attributed to its low carbon source and high nitrogen content [5], which cause a C/N ratio imbalance; simultaneously, the high salinity is also considered as a major concern in anaerobic digestion technologies [6,7]. Salinity is however difficult to tackle due to the hightech and high costs involved, while carbon deficiency is normally supplemented by additional biomass inputs [8,9]. Thus, a number of studies have focused on the selection of supplementary materials to combine with shrimp sludge to reach an improved C/N ratio of 25 - 30, required for optimizing anaerobic digestion [8,10,11].

In the Vietnamese Mekong Delta, agricultural production plays a leading role in local livelihood activities, and contributes up to 50% of national gross domestic product [12]. As such, agricultural residues are abundant in almost all localities; especially, rice straw which is annually produced at approximately 26 Mt year⁻¹ [13]. In parallel, large quantities of residual biomass such as bagasse from sugar production, steamed lemongrass leaves from essential oil production, and desiccated coconut from candy production are considered unutilized potential sources [14]. Combining locally available biomass and shrimp sludge with a more favorable C/N ratio potentially promotes biogas production. Several previous studies have undertaken co-digestion between potential biomass inputs and shrimp sludge for biogas production [12,14]. However, these experiments have mostly concentrated on accelerating biogas production conditions by supplementing methanogenesis bacteria and stainless-steel fermenters. However, the optimization of the C/N ratio by co-digestion between shrimp sludge and additional biomass feedstocks abundant in the Vietnamese Mekong Delta has not been thoroughly addressed in previous studies. Thus, this study investigates the use of different biomass feedstocks and superintensive shrimp sludge at optimal C/N ratio for biogas production under batch mesophilic anaerobic digestion. The study provides significant recommendations for selecting potential biomass to combine with shrimp sludge for biogas production in coastal areas.

2. MATERIAL AND METHOD

2.1. Materials

Shrimp sludge (SS) was mixed with the four different locally sourced biomass inputs. These were rice straw (RS), steamed lemongrass (SL), bagasse (BA) and desiccated coconut (DC). For the inoculum, the shrimp sludge (SS) was collected from siphoning ponds of a super-intensive white-leg shrimp farm at Tien Giang province (10°14'05.1"N, 106°43'18.6"E). The sludge was taken following 70 days of shrimp culturing and mainly consisted of daily shrimp excreta and leftover-settled feed. For the additional biomass, (1) RS was obtained from a rice paddy field after harvesting (variety IR50404); (2) SL was collected from a local private company that extracted essential oil from the leaves by steaming

leaves at the temperature of 121°C and pressure of 1.2 Kpa within 3 hours; (3) BA was gathered from drinking retail shops after juicing, whereas (4) DC was collected from a local coconut factory after squeezing coconut milk. Once collected each biomass material was chopped into small pieces of less than 2 cm. The inoculum and biomass feedstocks were independently mixed and consequently stored at 4°C before starting the experiment. The C/N ratio of SS, RS, SL, BA, and DC was 16.80, 75.14, 60.70, 103.00 and 53.59, respectively. The characteristic of the inoculum and substrates are shown in Table 1.

	Characteristic	Unit	SS	RS	SL	BA	DC	
	Moisture	%	93.2	27.4	31.2	16.9	42.8	
	Total solid	%	6.80	72.6	68.8	83.1	57.2	
	Volitive solid	%TS	70.7	92.1	90.3	93.4	97.7	
	pН	-	6.62	ND	ND	ND	ND	
	Salinity	mg L ⁻¹	10.2	ND	ND	ND	ND	
	$\mathrm{NH_{4}^{+}}$	mg L ⁻¹	220	ND	ND	ND	ND	
	SO_4^{2-}	mg L ⁻¹	983	ND	ND	ND	ND	
	TC	%TS	63.0	58.6	52.2	53.9	56.2	
	TN	%	3.75	0.78	0.86	0.52	1.05	
	C/N	-	16.8	75.14	60.70	103.00	53.59	

Table 1. Main characteristics of inoculum and substrates

Note: ND (not detected)

2.2. Experimental Design

The reactor used in this study was a 5-L glass bottle. However, the real working volume was set up to 4-L. The digester included a gas collection and outlet sampling systems certainly set onto the plastic lid. The system was fabricated by an inner 6-mm diameter plastic pipe connected directly from the air headspace's bottle to a 5-L aluminum foil bag, while the outlet sampling system was directly inserted into the inoculum, which was $\frac{1}{2}$ the bottle height. The bottle was then tightly closed using a seal tape and a rubber ring placed under the lid. This model was scaled down similarly to the reactor reported by Nam et al. [15]. Before processing, bottles were tested for airtight conditions. Air was pumped into each bottle to a 15-cmH₂O pressure and kept continuously for 2 hours. These airtight-ensured bottles were then used for the experiment. Nitrogen gas was purged in each reactor's headspace for 5 minutes before commencing the experiment to secure the homogeneously initial anaerobic condition. Thereafter, the digester was randomly arranged on a shelf inside a screen-house (Can Tho University, 10°1'39.02"N, 105°45'53.64"E) under mesophile conditions. The ambient temperature recorded during the experiment varied from 24.7 - 31.2 °C. A control reactor A (SS) and four co-digestion treatments were investigated in this experiment, including B (SS+RS), C (SS+SL), D (SS+BA), and E (SS+DC). Each treatment was performed in quintuplicate at the same time. The initial organic loading rate for each reactor was set based on the volatile solid (VS) weight at 50 g-VS L⁻¹, and a C/N ratio by 25 was calculated by adjusting the SS and biomass substrates based on Equation 1. The reactors were adjusted with de-chlorinated tap water to reach the same test volume (4 L). Each digester was manually mixed once to ensure materials sank in the inoculum. The fermentation stage was set to 45 days. A mixture including inoculum, feedstocks, and tap water was supplemented based on the designed experimental conditions (Table 2).

Deremators	Unit -	Reactors					
Farameters		А	В	С	D	E	
SS	g	190.4	112.0	99.9	114.2	98.9	
RS	g	NA	88.0	NA	NA	NA	
SL	g	NA	NA	100.1	NA	NA	
BA	g	NA	NA	NA	85.8	NA	
DC	g	NA	NA	NA	NA	101.1	
C/N co-substrate	-	16.8^{\dagger}	25	25	25	25	
Total VS added	g	190.4	200	200	200	200	
Total volume	mL	4000	4000	4000	4000	4000	

Table 2. Lab-scale experimental design.

Note: NA (not applicable); "[†]" solely shrimp sludge

2.3 Analytical Methods

The total solids (TS), volatile solids (VS), total carbon (TC), total nitrogen (TN), NH_4^+ , and SO_4^{2-} were all determined according to the Standard Methods [16]. The pH and the redox potential of digester liquids were measured directly in the reactors through the sampling outlet using a pH meter (TOA DKK, Japan) and a redox meter (TOA DKK, Japan). The daily biogas was collected in aluminum foil bags and was measured by a gas volume meter (TG 02, Ritter, Germany). The CO_2 and H_2S were measured daily using a Biogas 5000 gas analyzer (Geotechnology, UK). Methane concentration (v/v) was determined by a Shimadzu GC 2014AT (Shimadzu, Japan) gas chromatograph with a thermal conductivity detector (TCD) and a 60/80 Carboxen-1000 column (L x O.D x I.D: 4.57 m x 3.1 mm x 2.1 mm). The operational temperatures of the injection port, column oven, and detector were 240°C, 180°C, and 240°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 10 mL min⁻¹. A standard gas mixture (Air Liquids Ltd., Singapore) composed of 49.95% methane, 30.05% carbon dioxide in nitrogen was used for calibration. A 2.5 mL gas-tight Samplelock® syringe (Hamilton, USA) was used for gas sampling.

2.4 C/N Ratio Calculation in Co-Substrate Feedstocks

The C/N ratio was determined by dividing the total organic carbon content by the total nitrogen content, according to the following equation [8].

$$\frac{C}{N} = \frac{W_1 C_2 + W_2 C_2}{W_1 C_1 + W_2 C_2} \tag{1}$$

Where W_1 and W_2 were the VS weight in a single substrate in the mixture, C_1 and C_2 were the organic carbon content (g kg-VS⁻¹) in each substrate and N_1 , and N_2 were the nitrogen content (g kg-VS⁻¹) in each substrate.

2.5 Data Processing

All the data were tested for variance homogeneity prior to statistical analysis. One-way ANOVA and Duncan post-hoc tests were conducted for multiple biogas yield comparisons among treatments. An alpha (α) level of 0.05 was used to determine the statistical significance of all analyses. The analysis was performed using the statistical software IBM SPSS (version 22.0 for Windows). The results were presented in tabular form and graphs were plotted using SigmaPlot software version 14.0.

3. RESULTS

3.1 Biogas Production

Fig. 1. shows daily biogas production recorded for the five different biomass feedstock treatments (A, B, C, D and E). The biogas production of all reactors rapidly increased in the first 7 days and reached peaks from 4.02 to $6.10 \text{ L kg-VS}^{-1} \text{ day}^{-1}$ within 16 days of digestion. In which, the highest peak was found in reactor B (RS + SS), while reactor A (only SS) showed the lowest production peak. Thereafter, although volume of daily biogas production varied among reactors, a stable level of o bio-gasification remained until the end of the experiment. As can be seen from Fig. 1, almost all reactors displayed a similar trend in the acceleration of biogas production with the exception of reactor E. On the other hand, co-digestion of DC and SS in reactor E obviously limited biogas production from day 14 onwards. Here, the limited volume of daily biogas production was not measurable. While the mean values of daily biogas production in reactors A, B, C, and D reached a stable output period (from 16 to 45 days) measured at 2.29, 2.43, 2.78, and 3.51 L kg-VS_{added}⁻¹ day⁻¹. Co-digestion of BA and SS in reactor D was

higher than that of reactors A, B, and C. This difference was estimated at 53.1%, 44.1% and 25.8%, respectively.

It can be observed that the co-digestion treatments accelerated biogas production at the beginning of the experiment when compared to solely the shrimp sludge substrate (treatment A). Specifically, co-digestion treatments including B, C, and D produced biogas from day 7 - 9 onwards, with initial volumes varying between $2.99 - 3.84 \text{ L kg-VS}^{-1} \text{ day}^{-1}$, while the biogas production from the shrimp sludge (A) was only $0.92 \text{ L kg-VS}^{-1} \text{ day}^{-1}$. At the end of the experiment, reactors were still producing biogas, but the volume was lower than that of previous stages. Among the four co-digestion treatments, the mixture of SS and BA (treatment D) achieved the highest biogas production values, suggesting the potential of bagasse biomass for biogas production.



Figure 1. Daily biogas production in reactors A, B, C D, and E.



Figure 2. Cumulative biogas yield in reactors A, B, C D, and E.

The cumulative biogas production for the treatments A, B, C, D, and E are shown in Fig. 2. Out of all co-digestion reactors, treatment D showed the highest biogas yield over the whole digestion period (p<0.05). This is followed by treatments B and C, which share a similar tendency (p>0.05), while treatment E yielded the worst cumulative biogas production. It was found that the biogas yield of the co-digestion reactors B, C, and D was 26.40%, 29.21%, and 53.70% higher than that of reactor A (reactor control; biogas yield was 82.48 L kg-VS_{added}⁻¹), respectively. By comparison, the methane yield

for the co-digestion reactors B, C, and D was 81.40%, 85.06%, and 107.7% higher than that of reactor A (control reactor; biogas yield was $25.5 \text{ L kg-VS}_{added}^{-1}$). Moreover, Table 3 depicts that the initial pH and redox potential were appropriate for biogas production and the co-digestion substrates increased CH₄ and CO₂ concentration in the biogas composition compared with mono-digestion (reactor A), yet did not decrease H₂S.

Doromotors	Unit	Reactors					
Farameters	Ullit	А	В	С	D	Е	
Initial pH	-	6.84	6.73	6.69	6.69	6.65	
Initial redox	mV	-229	-280	-296	-278	-221	
Retention time	day	45	45	45	45	45	
Biogas yield	L kg-VS _{added} ⁻¹	82.48	104.3	106.6	126.8	16.40	
CH ₄ yield	L kg-VS _{added} ⁻¹	25.50	46.25	47.18	52.96	2.12	
Average CH ₄	%	30.91	44.36	44.27	41.77	12.92	
Average CO ₂	%	29.83	31.58	33.47	37.61	43.07	
Average H ₂ S	ppm	7,811	7,026	6,499	10,203	7,787	

Table 3. Experimental results from the different reactors A, B, C D, and E.

3.2 Biogas Composition

Fig. 3 illustrates the methane concentration (v/v) in the biogas composition of reactors A, B, C, D, and E. During the first 7 days, there was insufficient biogas for detecting the composition. At this time in reactors B, C and E, the methane content was only about 6.3 - 7.0%, while reactor D showed the highest concentration with 18.3%. The mono-digester A with only the shrimp sludge displayed the lowest methane concentration at 4.5%. For reactors B, C and D, the methane concentrations increased drastically and obtained 45% on day 18 and then remained at a stable level until the experimental period concluded. Whereas, treatment A (shrimp sludge) showed a methane concentration of 40% after 20 days. The biogas production inhibition of treatment E led to the lowest methane concentration (<25%). The maximum biogas content was obtained from reactor D which contained a mixture between shrimp sludge and bagasse, while suppression was found in the desiccated coconut material.

Fig. 3 shows that the CO₂ concentration was highest within the first 15 days of biogas generation. The CO₂ content in biogas reached the highest concentration in reactor A accounting for 60%, while the cosubstrate reactors B, C, D, and E showed CO₂ concentrations of 57.1%, 45.1%, 31.7% and 36.6%, respectively. Thereafter, CO₂ concentrations reduced and reached a stable level (30.4 - 39.2%). Furthermore, it is apparent that the H₂S concentration was seen at a very high in biogas composition. However, the highest concentration was observed in the first week of biogas production (maximum recorded values ranged from 23,710 - 65,040 ppm). Thereafter, H₂S concentrations rapidly decreased and remained lower (5,873 - 9,155 ppm).



Figure 3. Biogas compositions in reactors.

3.3. pH and Redox

During the digestion process, pH values showed a decreasing tendency in the first 10 days (Fig. 4(a)). The pH value in co-digestion reactors (B, C, D, and E) quickly dropped compared to the pH in the shrimp sludge reactor (A). Specifically, the pH values of the treatment of D and E significantly decreased on day 1 of the experiment to 5.5, while the other reactors (A, B, and C) sustained a higher pH value of 6.0. However, over time reactor D gradually recovered a stable pH condition suitable for biogas production. However, reactor E showed little improvement and maintained pH values between 5.10 and 5.27 in the hydrolysis and acidogenesis phases. Notably, the pH of co-digestion reactors illustrates a

reverse relationship with daily biogas production if treatment E is excluded. In particular, the average pH value of the treatments A, B, C, and D for the whole anaerobic digestion process were 6.67, 6.57, 6.47, and 6.21, respectively; whereas the daily biogas production were 2.29, 2.75, 2.86, and 3.43 L kg- VS_{added}^{-1} day⁻¹, respectively.



Figure 4. pH value (a) and redox potential (b) in reactors.

Fig. 4(b) shows that the redox potential (Eh) rapidly decreased in the first five days of the experiment and remained stable for the entire fermentation period. In particular, the Eh value varied between -316 and -196 mV on the first day, in which the reactors B and C recorded their lowest values, while treatment B and D persisted at higher values as compared to reactor A. Thereafter, the Eh varied between -300 mV and -350 mV, which indicates that this period transformed to a stable phase and the metabolic status for an anaerobic digestion system was obtained. However, reactor E showed higher fluctuations in Eh values which suggests the ineffectiveness of biogas production. Interestingly the interaction between pH and Redox potential showed an inverse correlation. Noticeably, the reactors A, B, C, and D with high pH values showed Eh potentials of -341 mV, -334 mV, -328 mV, and -310 mV, respectively.

4. DISCUSSION

In this study, we used locally available biomass as a supplementation feedstock for producing biogas. Bio-gasification was seen to start after 7 - 9 days of digestion for co-digestion reactors of biomass and shrimp sludge, whereas in solely shrimp sludge, bio-gasification was firstly recorded on the 9th day. This indicates that hydrolysis and acidogenic microorganism activities were ongoing in the earlier period and also that co-digestion accelerated the onset of biogas production. Among potential biomass sources, our results showed that co-digestion of DC+SS produces biogas ineffectively, even though C/N was adjusted to an optimal value by 25 for the consumption of anaerobic microbial population as suggested by [10,17]. This is because a sharp pH reduction also led to a dramatical accumulation of volatile fatty acids (VFAs), inhibition of methanogenesis bacteria and biogas production suppression [11,18]. For instance, the pH optimum for methanogenesis is roughly 7.0, while the hydrolysis and acidogenesis periods require pH values from 5.5 - 6.5 [1]. Previous studies have suggested that pH should be maintained between 6.8 and 7.2 for single-stage anaerobic systems to avoid the accumulation of VFA concentration [19,20,21,22]. Previous studies were revealed a pH reduction in the early stage of co-digestion reactors

and then gradually recovered [15,23,24], which was a similar trend in our findings. However, several co-digestion reactors displayed a failed pH recovery after hydrolysis and acidogenesis stages, although the co-digestion maintained an optical C/N ratio for the reactor [25,26]. Two possible solutions to overcome this problem could be (i) applying two-stage anaerobic digestion to effectively use the different microbiomes in each stage [27], and (ii) measuring pH in the liquid phase and resultantly adjusting pH to a favorable range for bio-gasification [28,29]. However, it is not feasible to adjust pH in reactors with co-substrates derived from biomass due to the high-cost and hi-tech requirements. In addition, the use of oxidation-reduction potential is potentially a great indicator for controlling anaerobic digesters because it reflects the oxidoreduction state. Therefore, redox potential values should be maintained between -100 and -350 mV, which are satisfactory conditions for anaerobic digestion [30].

Data obtained from this study showed that the biogas and methane yield of co-digestion between bagasse and shrimp sludge were approximately 20% higher than that of co-digestion of rice straw and shrimp sludge, as well as steamed lemongrass and shrimp sludge which indicates its potential use for biogas digestion. Rice straw and steamed lemongrass materials are also two feasible sources of biomass feedstocks that produced 26 - 29% higher biogas yields than single shrimp sludge digestion. On the other hand, this study confirms that co-digestion of biomass and shrimp sludge at a C/N ratio of 25/1 significantly increases the effectiveness of biogas production compared to mono-digestion of solely shrimp sludge which is consistent with co-digestion of biomass materials and various waste sources from the previous works [31,32,33,34]. In one aspect, the results of this study show that although cosubstrates were adjusted to an appropriated C/N ratio, biogas production from the mixture was tentatively lower than that of previous studies [12,14]. This could be explained by the effects of salinity in the initial sludge $(10.16 \text{ mg L}^{-1})$ and a shorter retention time within 45 days. It has been reported that the microbial communities of the anaerobic digestion process are stressed by high salinity [7], inhibiting methane production and the degradation of organic compounds [35]. It has been demonstrated that the supplementation of 0.5 - 2.0 g-NaCl L⁻¹ offers beneficial conditions for methane formation, yet methane yield reduces from 36% to 41% when NaCl was added in concentrations of 5 - 10 g L^{-1} [36]. Moreover, the extension of the fermentation period obviously increases biogas generation due to the degradation of recalcitrant polymers/lignocellulose within the biomass. At the end of the experiment, biogas production still continued indicates that the prolongation of retention time instead of 45 days would significantly increase the yield. It can be seen that biogas yield reached in this study was less than 115 L kg-VS_{added}⁻¹ (45-day digestion), while biogas yields in a 120-day long experiment with a mixture of biomass feedstocks (shrimp sludge, and bagasse, or molasses) reached more than 400 L kg-VS_{added}⁻¹ [12]. The findings show that the co-digestion of shrimp sludge and local biomass substrates increased the biogas yield compared with feeding only shrimp sludge. Notably, this study shows that the treatment mixture of shrimp sludge and bagasse obtained highest biogas production. This is consistent with results obtained by [14].

In this study, co-digestion reactors produced CH₄ from day seven and obtained a stable level after 18 days of digestion, greater than 45% (reactors B, C, D), while reactor A obtained 40% CH₄ after 20 days. CH_4 generated from reactor E was insignificant. The finding drew up a low CH_4 formation pace and low CH₄ concentration compared with the previous study [12,14], although it could be acceptable for biogasto-energy. This could be partly explained by the inhibitory factor of the above-discussed salinity which obtains lower the popular range of methane content in biogas (50 - 75%) [37,38]. CO₂ concentration was similar to the CH₄ generation trend in the stable stage. However, higher concentration was found in hydrolysis and acidogenesis [38,39], but it was then generally ranged from 24 - 45% [40]. H₂S content reached very high levels. In the start-up phase (within the first 15 days), the H₂S concentration varied between 2.37 - 6.50% over the reactors. Although the H_2S concentration reduced 0.58 – 0.92% in the stable biogas-production phase, it was still high compared to that of common H₂S content level in biogas compositions (0.1 - 0.3%) [37,38,40]. The high H₂S concentration indicates that obtained biogas would be hard to effectively use for many purposes (cooking, heating, or running electrical machinery). H_2S is generally known as the most dangerous, toxic and corrosive contaminant in biogas compositions. It is normally recommended to remove it for improving energy efficiency [41]. The high H_2S is undesirable in energy consumption, even at low concentrations. H₂S concentrations of 150 - 500 ppm are required for internal combustion engines [42], [43]. Thus, H₂S removal in biogas is necessary to use biogas devices without corrosion and unpleasant smells effectively.

5. CONCLUSION

The results obtained in this study suggested that the co-digestion of super-intensive shrimp sludge and biomass feedstock is a promising approach for biogas production. The biogas yields increased by 26.40%, 29.21%, and 53.70% compared to that from solely shrimp sludge with the co-digestion of SS+RS, SS+SL, and SS+BA, respectively. The co-digestion of SS+DC failed to create a suitable environment for biogas production due to rapid pH reduction. Irrespective of the input materials, the concentration of H₂S in biogas compositions is identical at high levels. Thus, the continuation of the work should focus on (i) circumventing pH inhibition for improving the stability of the digester systems when DC is added as a co-substrate and (ii) eliminating H₂S out of biogas before use.

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