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Residual of 17β-estradiol in digestion liquid generated from a biogas plant using livestock waste

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ABSTRACT

A biogas plant using livestock waste in which a methane fermentation process is applied is a useful facility for generating energy. The digestion liquid generated from the biogas plant as a residue has high potential for use as a crop fertilizer. However, high-density estrogens such as 17β-estradiol (E2) are included in livestock waste, and there is little information on the behavior of E2 in the digestion liquid. In this study, a survey of E2 concentration at each process in a biogas plant using livestock waste was carried out. In addition, the efficiencies of E2 removal from the digestion liquid by activated carbon adsorption and soil infiltration were examined. The total concentration of E2 in raw livestock waste was reduced to 2 μg/l after treatment, and the removal efficiency of E2 was about 80% for the plant. The methane fermentation process is important not only for the generation of methane but also for the removal of E2. The proportion of E2 conjugates comprising the total E2 concentration was 10% or less in all treated samples. In the plant, there is no likelihood of an increase in estrogen activity by the cleaving of E2 conjugates. By carrying out activated carbon adsorption to remove E2 from the digestion liquid, a large portion of E2 was removed from the digestion liquid, but an E2 concentration of 0.5 μg/l still remained in the treated digestion liquid. In contrast, it was possible to purify the digestion liquid to an E2 concentration of less than 0.002–0.011 μg/l by soil infiltration. It is thus possible to utilize the digestion liquid as a fertilizer without causing aquatic environmental pollution, but factors such as application rate, soil characteristics, and the E2 concentration of digestion liquid should be considered first.

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1. Introduction

Endocrine-disrupting compounds (EDCs) act on organisms even at extremely low concentrations. Aquatic ecosystems may be affected by estrogenic activity caused by the micropollution of EDCs. According to the results of surveys on EDCs in wastewater [1] and aquatic environments [2], bisphenol A, a resinous raw material used to make resins, nonylphenol, a degradation product of non-ionic surfactants, and 17β-estradiol (E2), derived from human and livestock waste, were concluded to be the most prevalent EDCs. Judging from the results of estrogenic activity obtained by both yeast [3] and fish assays [4,5], out of these materials, E2 exhibits markedly higher activity than the other two materials. It has been estimated that over 90% of estrogenic activity in aquatic environments is attributable to the presence of E2 [6,7]. Since considerable quantities of estrogens are included in livestock manure, livestock waste also contains a high concentration of estrogens such as E2 [8,9]. Estrone (E1), which was not included as a target compound in this study, is the most important estrogen in waste from livestock, similarly to E2 [9]. Furthermore, the amount of livestock waste generated as urine is estimated to be about 30 million t/year in Japan. Therefore, livestock waste is a major potential source of E2 in the aquatic environment [10]. It is mandatory that livestock waste from livestock facilities be managed properly according to a law from the Ministry of Agriculture, Forestry and Fisheries, Japan. Reading the processing technology, however, there are many problems such as cost and convenience.

Since livestock waste has a high value as a biomass resource for biogas plants and as an agricultural fertilizer, the effective utilization of livestock waste has been investigated. Hybrid facilities with the two functions of power generation using methane formed by an anaerobic digestion process using livestock waste and the utilization of the remaining digestion liquid as a crop fertilizer has considerable appeal. However, when the digestion liquid containing

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E2 is applied to farmland or a paddy field, aquatic environmental pollution such as that of groundwater by osmosis and that of surface water through runoff will occur. At present, there is little information on the behavior of E2 in biogas plants. It is necessary to understand such behavior to enable the utilization of livestock waste as a resource. In this study, a survey on the concentration of E2 at each process in the biogas plant using livestock waste was carried out. In addition, the efficiencies of removing residual E2 from digestion liquid by the activated carbon adsorption and soil infiltration were examined.

2. Materials and methods

2.1. Sampling

A pilot biogas plant using swine waste consisting of a waste stock tank (4.0 m^3), a solid-liquid separation unit, a raw material tank (1.0 m^3), an anaerobic methane fermentation tank (2.5 m^3), and a digestion liquid stock tank was used in this study (Fig. 1). In addition, the digestion liquid was sterilized by heating before applying it as a fertilizer. Swine waste from a swine farm was placed in the waste stock tank every week. The waste collection system in the swine farm is of the solid-liquid mixed type. The conditions of the fermentation process are as follows: anaerobic closing condition; load rate of raw material, 0.12 m^3/day; hydraulic retention time in the tank, 21 days; and tank temperature, 36 °C. The general parameters in each process are summarized in Table 1. Each parameter was monitored every 2–3 weeks from May to December in 2007. About 50% volatile that was input into the plant was consumed during fermentation with the stable generation of methane gas throughout the investigation period. Although biodegradable organic substances such as BOD were markedly consumed during fermentation, the stable organic substances remained in the digestion liquid as COD(Cr). The removal of ammonia nitrogen cannot be expected in a plant uses anaerobic fermentation.

Samples were collected from the raw material tank, the fermentation tank, the digestion liquid stock tank, and the sterilized digestion liquid tank. The biogas plant was surveyed three times during periods when the plant was operating well (Survey 1, 20th November 2007; Survey 2, 16th January 2008; Survey 3, 22nd January 2008). In each survey, about 1000 ml of sample was collected in triplicate from each tank, and the composite sample prepared by mixing the three samples were analyzed.

2.2. Preparation and extraction for E2 analysis

The preparation and extraction of samples for E2 analysis by enzyme-linked immunosorbent assay (ELISA) were in accordance with the E2 ELISA kit manual (Ecologiena, Tokiwa Chemicals, Japan), although some modifications were made to their method [11]. Each 500 μl sample was mixed with 100 ml of Milli-Q water (Millipore). The mixture was filtered using a glass fiber filter (GF/C type, Whatman). The filter, to which suspended solids adhered, was soaked in methanol (4 ml) and homogenized using a homogenizer. After filtration of the filter extract, the extract was mixed with the filtrate, and the pH was adjusted to 5 by adding a buffer of 1 M acetic acid. After passing the samples through a C18 cartridge (Bakerbond, J.B. Baker), the cartridge was washed sequentially with 10 ml of distilled water and 5 ml of *n*-hexane (HPLC grade, Wako) at a flow rate of 20 ml/min. Analytes were extracted from the C18 cartridge

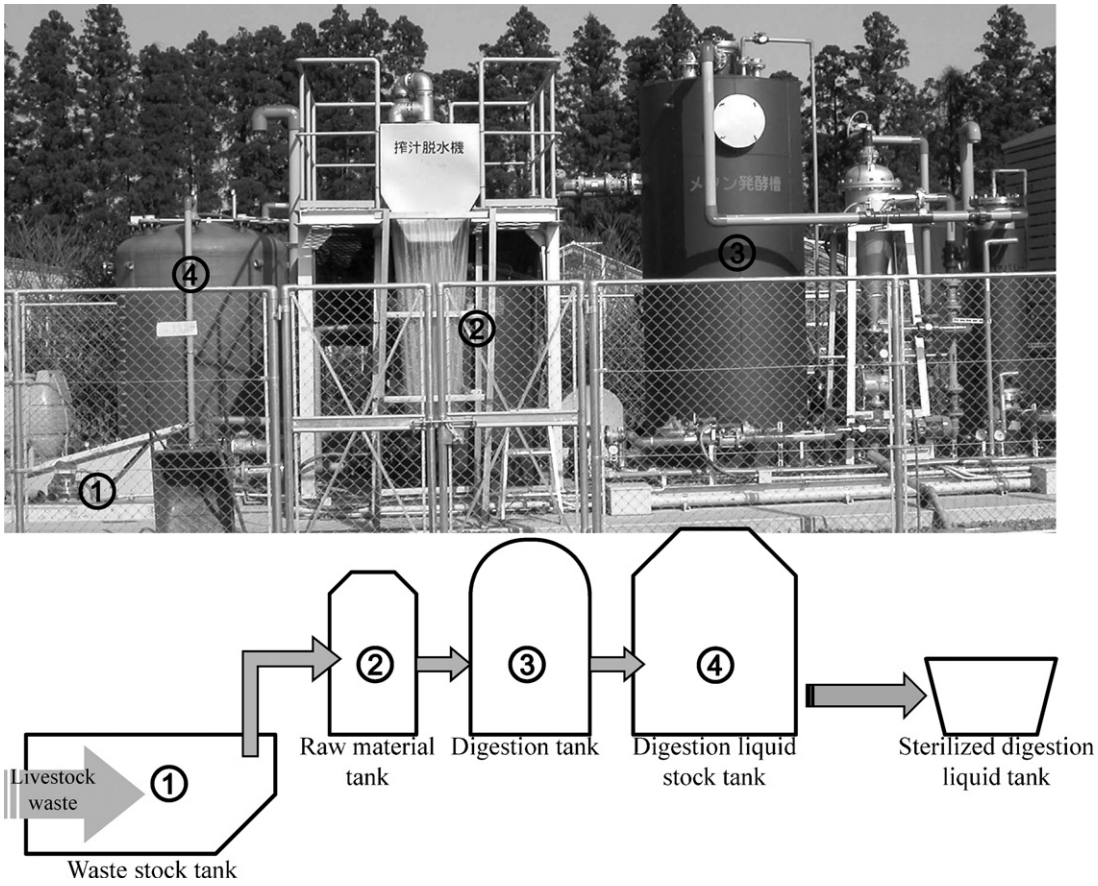

Fig. 1. A pilot type biogas plant for swine waste and processing flow in the plant.

Table 1
Operational condition of the biogas plant (each parameter was monitored every 2–3 weeks from May to December in 2007).

Parameter	Raw material tank			Fermentation tank			Digestion liquid stock tank		
	Mean	S.D.[a]	n[b]	Mean	S.D.	n	Mean	S.D.	n
Temp. (°C)	42.4	6.2	18	36.0	0.4	18	28.5	3.8	18
pH	7.11	0.4	18	7.83	0.05	18	8.22	0.06	18
ORP (mV)				−515.2	3.8	18			
Total solids (mg/l)	33,878	8,950	18	26,800	2794	18	18,588	5051	18
Volatile solids (mg/l)	22,947	5,831	18	16,226	1706	18	10,756	3171	18
Suspended solids (mg/l)				22,339	2544	18			
Volatile suspended solids (mg/l)				14,606	1642	16			
Total nitrogen (mg/l)	3,729	714	17	4,100	549	18	3,156	618	17
Ammonia nitrogen (mg/l)	2,582	666	17	2,975	447	18	2,385	439	17
COD(Cr) (mg/l)	34,333	13,317	3	18,750	6718	2	13,700	6393	3
BOD (mg/l)	16,000	5,196	3	2,775	247	2	1,443	829	3

[a] Standard deviation.
[b] Sample number.

by elution with 5 ml of dichloromethane. The eluate was mixed with the same volume of *n*-hexane. The mixture was then passed through a florisil cartridge (Sep-Pak Plus, Waters), and the cartridge was washed sequentially with 10 ml of *n*-hexane/dichloromethane (1:1). Analytes were re-extracted from the florisil cartridge by eluting 6 ml of acetone/dichloromethane (1:9). The eluate was then dried under a nitrogen stream. After drying, the residue was redissolved in dimethylsulfoxide (DMSO) and methanol, and the analytes were diluted with distilled water (DMSO:methanol:Milli-Q water = 1:10:89, v/v/v) to 2 ml. Finally, the sample prepared for ELISA was diluted to a volume fourfold (2 ml/0.5 ml) that of the liquid sample.

2.3. E2 analyses by ELISA

An E2 ELISA kit (Ecologiena) was used to determine the E2 concentration. A calibration curve was generated using standard E2 solutions (DMSO:methanol:Milli-Q water = 1:10:89, v/v/v) with concentrations between 0.05 and 0.5 μg/l. Procedures were in accordance with the instructions of the manufacturer. The detection limit of E2 was 0.2 μg/l, as determined from the quantitative analysis range and sample dilution.

2.4. Examination of ELISA by standard addition method

It has been suggested that the strict quantitative analysis for E2 in sewage and river water is difficult by the ELISA method since it is affected by cross-reactivity and inhibition due to coexisting materials [12]. However, it has also been verified that the use of appropriate solvents and an integrated cleanup operation improves the correlation of analysis values for ELISA and high-performance liquid chromatography/mass spectrometry. In addition, to monitor the changes in the E2 concentration of sewage, ELISA measurement can provide analytical precision from the coefficient of variance and the recovery rate. Therefore, the quantitative determination of E2 for manure samples was carried out using the ELISA kit and the above preparation and extraction methods using a standard E2 solution.

The standard E2 solution was added to 100 ml of unknown digestion liquid to set the added E2 concentration from 1.0 to 7.5 μg/l, and the E2 concentration was determined by ELISA. The E2 concentration determined by ELISA and the added E2 concentration exhibited a high correlation ($n = 3$, $r = 0.985$, Fig. 2). The measured concentration (2.35 μg/l) of the unknown sample without E2 addition agreed with the intercept value (2.35) of the regression line. The recovery efficiency of E2 obtained from the relationship between the measured E2 value and the added E2 concentration was 24.1%. The determination of E2 concentration using the ELISA kit had high accuracy, although the recovery efficiency was low. The recovery efficiency of E2 for the digestion liquid was about 1/3 lower than that for municipal wastewater using the same procedure. The high density and various types of organic substance contained in the digestion liquid seemed to obstruct adsorption and recovery for E2 during the preparation and extraction. As the result of the examination, we judged that the measurement of the E2 concentration using the ELISA kit could be used to, at least semiquantitatively, monitor the changes in the E2 concentration in samples collected from the biogas plant. In this study, the E2 concentration obtained by ELISA indicates not E2 absolute concentration but E2-equivalent concentration.

2.5. Deconjugation

To determine the total E2 concentration, E2 conjugates were cleaved under high-temperature and high-acidity conditions in accordance with a provisional manual prepared by the Environment Agency, Japan [14]. After drying the eluate from the florisil cartridge, the residue was redissolved in 1 ml of 1 M hydrochloric acid and methanol, and the analytes were sealed and heated at 80 °C for 20 min before drying under a nitrogen stream. The residue was neutralized with 0.1 ml of methanol and one drop of 1 M trimethylamine/methanol solution, then dried under a nitrogen stream. After drying, the residue was redissolved in DMSO and methanol and the analytes were diluted with distilled water. The concentration of detectable E2 with post-heating and acid hydrolysis was then determined by ELISA as the total E2 concentration.

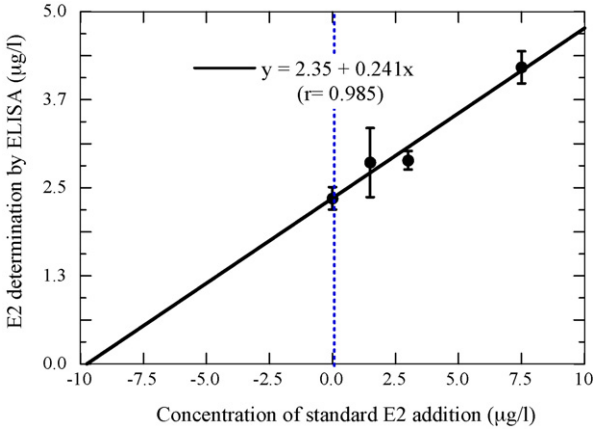

Fig. 2. Relationship between adding E2 concentrations and the E2 determination values determined by ELISA. Data are mean ± S.D., $n = 3$.

Table 2
Characteristics of activated carbon.

Type	Specific surface (m^2/g)	Mean pore diameter (nm)	Pore volume (cm^3/g)
Commercial	1319.1	0.89	0.585
Original	1394.5	0.99	0.693

2.6. Activated carbon adsorption

The removal of E2 from the digestion liquid was examined using two types of activated carbon, a commercially available activated carbon (chemical grade, Wako) and an original active carbon. The original active carbon was made of cedar bark and was obtained by an activation process using chemicals (under patent application, 2008). The powdered activated carbon was sieved through a 75 μm screen and dried for 2 h at 110 °C. The physical properties of the active carbon are shown in Table 2. The activated carbon in powder form was suspended in Milli-Q water as a stock solution with a concentration of 10,000 mg/l. The stock solution of activated carbon was added to 20 ml of the digestion liquid to form a concentration of 100 mg/l, then was mixed for 1 h at 200 rpm. After mixing, the supernatant was allowed to stand for 1 h, then collected, and the E2 concentration was determined.

2.7. Soil infiltration

Soil (water content, 30.0%; ignition loss, 190.8 mg/g; ignition residue, 109.2 mg/g) collected from the farm was placed in a glass column (diameter, 32 mm; height, 25 cm), and the digestion liquid (100 ml) was passed through the column. The rate of liquid passing through the column was about 8–10 ml/h. The sample prepared for ELISA was concentrated to 25-fold (50 ml/2 ml) that of the liquid sample (50 ml), and the detection limit of E2 was 0.002 μg/l. In addition, the turbidity and chromaticity of the sample were determined in accordance with the Japanese Industrial Standard [13].

3. Results and discussion

3.1. Changes in E2 concentration in the biogas plant

The concentrations of total E2 and free E2 in the samples collected from the raw material tank, the fermentation tank, the digestion liquid stock tank, and the sterilized digestion liquid tank are shown in Fig. 3. The average total E2 concentration in the raw waste in the three surveys was 16.0 μg/l (minimum–maximum, 13.8–18.6 μg/l). The fluctuation of E2 concentration in the raw waste was small in the plant. When the concentrations of total E2 and free E2 were compared by a *t*-test ($n = 3$) for the raw waste collected in each survey, the free E2 concentration was significantly lower in two surveys. The difference in the total E2 and free E2 concentrations is assumed to be due to the presence of E2 conjugates. The proportions of E2 conjugates in these samples were 10.2% and 2.2%, respectively. E2 decomposed in the fermentation tank; thus, the concentrations of both total and free E2 decreased. There was no significant difference between the total and free E2 concentrations in each sample collected from the fermentation tank. It is assumed that a small proportion of the E2 conjugates in the waste were cleaved, and the released free E2 was decomposed in the fermentation process. The total E2 concentration in the digestion liquid, which was a residue of methane fermentation, was markedly less than that in the raw waste, and the average total concentration of E2 in the digestion liquid was 2.4 μg/l. The removal efficiencies for total E2 for the plant ranged from 75% to 88% in the surveys. There was one sample (Survey 1) in which a significantly lower concentration of free E2 than that of the total E2 was obtained for the three samples. However, the average concentration of free E2 was 2.3 μg/l, and the difference in the concentrations of total E2 and free E2 was small. It was clear that the proportion of E2 conjugates existing in the digestion liquid was negligible. In addition, E2 was stable in the liquid after heat sterilization at 65 °C for 30 min. In the biogas plant, the digestion liquid, which is continuously generated by fermentation, is stored once in the digestion liquid stock tank. Then the digestion liquid is sterilized in the batch system. The sterilized digestion liquid collected from the sterilization tank is different from the digestion liquid (nonsterilized). Therefore, the E2 concentration of the sterilized digestion liquid is also different from that of the nonsterilized digestion liquid.

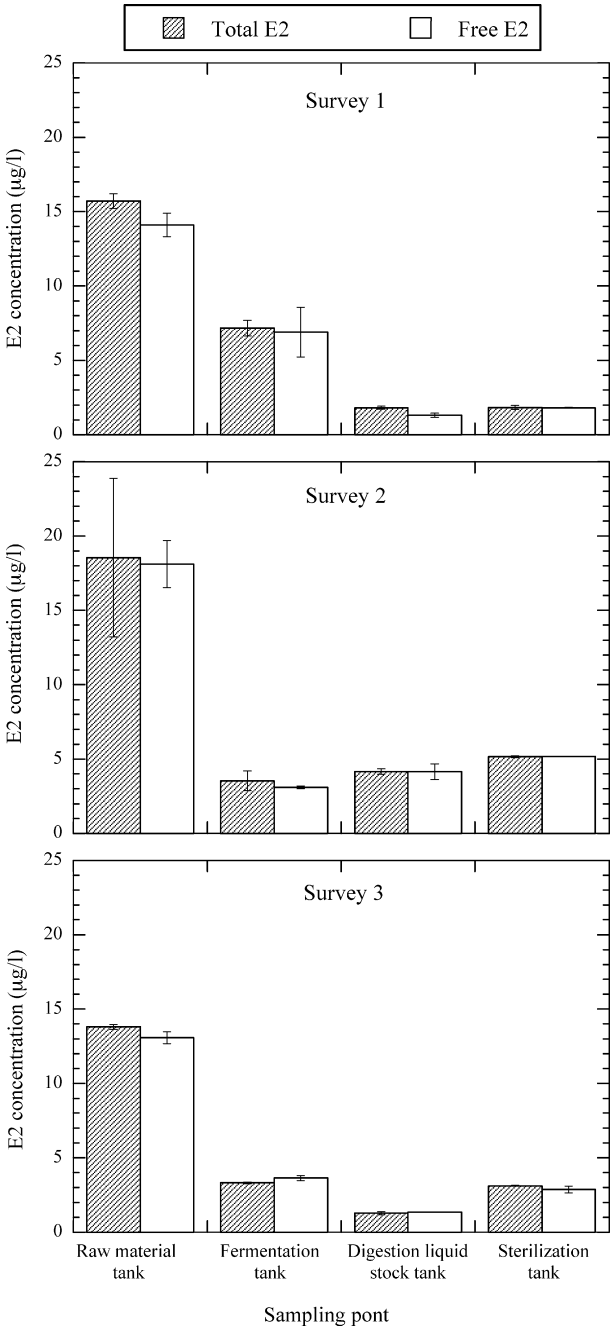

Fig. 3. Concentrations of total E2 and free E2 in the samples collected from the raw material tank, the fermentation tank, digestion liquid stock tank, and the sterilized digestion liquid tank. Data are mean ± S.D., $n = 3$.

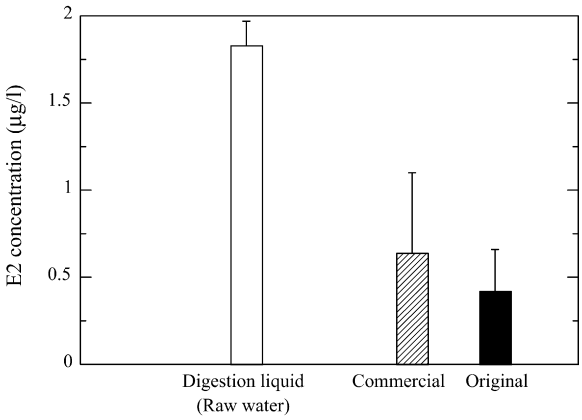

Fig. 4. Removal efficiency of total E2 from digestion liquid by activated carbon adsorption. Data are mean ± S.D., $n = 3$.

In the aerobic biological process used for the treatment of municipal wastewater, it has been reported that the behavior of free E2 is affected by the cleavage of E2 conjugates such as glucuronides [14]. Therefore, it is important to monitor trace E2 conjugates in the treatment of municipal wastewater [15]. In contrast, the monitoring of E2 conjugates is unnecessary for the biogas plant, because the presence of E2 conjugates is negligibly small in both the raw waste and the digestion liquid. We assume that the cleaving reaction of E2 conjugates is completed in the swine manure under anaerobic conditions with a high density of organic substances and ammonia before the waste is treated in the plant. To discuss the conjugates of estrogens in livestock waste, information on E1 and its conjugates is necessary.

3.2. Removal of E2 from digestion liquid

The removing efficiency of E2 from the digestion liquid by adsorption on activated carbon is shown in Fig. 4. The E2 concentrations in water treated using the commercially available carbon and the original carbon decreased to 0.64 and 0.46 μg/l, respectively. The removal efficiency of E2 for the original carbon was higher than that for commercially available carbon. This is because the original carbon has a high specific surface area. However, the residual E2 concentration was also two orders higher than the critical concentration that adversely affects aquatic organisms such as fish [4,5]. When considering the amount of activated carbon required and its cost, the adsorption using activated carbon does not seem to be practical for removing E2 from the digestion liquid.

The changes in parameters such as total E2, turbidity, and chromaticity, for the digestion liquid upon soil infiltration are summarized in Table 3. The total E2 concentration in the water treated by passing it through the soil column was markedly reduced to approximately 0.005 μg/l. Upon soil infiltration, E2 in the digestion liquid was adsorbed onto the soil, and the removal efficiency was over 99%. The turbidity and chromaticity of the treated water were equal to those of tap water (Fig. 5). In addition, although the digestion liquid has a strong odor of ammonia, the treated water was almost odorless. The contaminants in the digestion liquid such as E2, ammonia, suspended solids, and colloids were effectively removed by physical filtration and adsorption onto the soil. In this study, we could not address different soil types with different capacities, loading rates, and concentrations of loading for soil infiltration. Soil capacity varies very widely, and E2 concentration has also been shown to vary with soil type.

Table 3
Removal of E2, suspended solids and chromaticity matter by soil adsorption.

	E2 (μg/l)	Turbidity[a]	Chromaticity[b]
Run 1			
Digestion liquid	1.49	4,900	780
Treated water	0.005	2.3	3.0
Run 2			
Digestion liquid	1.83	16,000	4000
Treated water	nd[c]	1.6	6.1
Run 3			
Digestion liquid	6.2	15,000	2000
Treated water	0.011	2.8	3.3

[a] Unit as a kaolin suspension standard.
[b] Unit as a platinum cobalt solution standard.
[c] Not detected.

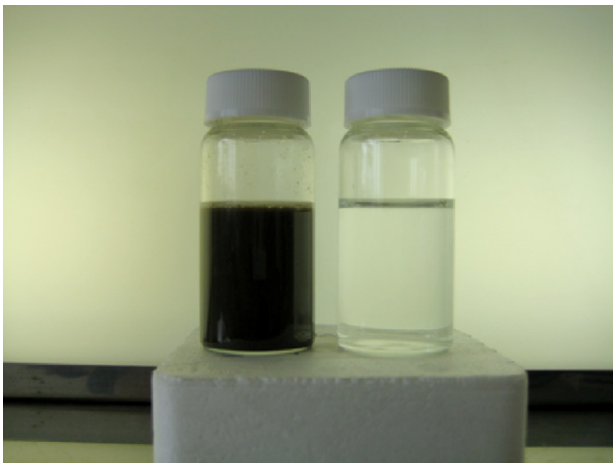

Fig. 5. Digestion liquid and treated water by soil infiltration (left, digestion liquid; right, treated water).

4. Conclusions

In a biogas plant, the total E2 concentration in the treated raw material was reduced to 2 μg/l, and the removal efficiency for E2 was about 80%. The methane fermentation process is important not only for the generation of methane but also for the removal of E2. However, the E2 concentration remaining in the digestion liquid is much higher than the threshold at which it affects aquatic organisms, and its direct runoff into the aquatic environment should be prevented. The proportion of E2 conjugates in the E2 was 10% or less in all the samples. In the plant, there is no likelihood of an increase in estrogen activity due to the cleaving of E2 conjugates. Although the E2 was effectively removed from the digestion liquid by activated carbon adsorption, 0.5 μg/l still remained in the treated digestion liquid. In contrast, it was possible to purify the digestion liquid to an E2 concentration of a few nanograms per liter level by soil infiltration. The adsorption capacity of different soils for E2 may enable the application of the digestion liquid as a fertilizer in fields, without risk of groundwater contamination, but factors such as application rate, soil characteristics, and the E2 concentration of digestion liquid should be considered first.

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