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ECOSYSTEM-LEVEL WET TEST OF CAFETERIA DRAINAGE USING MICROCOSM SYSTEM

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ABSTRACT: In Japan, the introduction of the WET (Whole Effluent Toxicity) test is under processing in consideration, but this test may be different from the actual ecosystem phenomena because it is a single species test in spite of the natural ecosystem consists of multi species biota. In this study, we developed the WET test using microcosm (M-WET) which is a simulated ecosystem model and consisted of several species of producer, consumer and decomposer. Both the number of organisms as structural parameter and DO as functional parameter after cafeteria drainage addition were analyzed by branched-type ANOVA, and the microcosm no observed effect concentration (m-NOEC) and toxicity unit (TU) were calculated. As result, the m-NOEC of cafeteria drainage was calculated as 5% and the TU was as 10, and assessed that the cafeteria drainage was discharged safely. It is important to assess the complex influences of chemical agents, such as surfactants, medicines and so on, to the environment. To conduct such comprehensive assessments, M-WET tests should be used to examine drainage with collecting total toxicity test data through single-species tests of chemical contaminants in environmental water.

Keywords: Microcosm, Whole effluent toxicity (WET) test, Branched-type ANOVA, Cafeteria drainage, m-NOEC

1. INTRODUCTION

Currently, introduction of WET (Whole Effluent Toxicity) test is being considered in Japan, but it is difficult to believe that the actual ecosystem can be represented by the test method by current single species as typified by small crustaceans *Daphnia magna* as primary consumer and small fish *Danio rerio* as secondary consumer [1,2]. Therefore, by introducing microcosms, which is a simulated ecosystem including producers, predators, and decomposers, into the WET test, more realistic evaluation can be considered possible [3].

In this study, we conducted WET test, which is a method of managing the influence on ecological systems for actual drainage, with a microcosm, which is a model ecosystem that imitates natural ecosystem to calculate the microcosm no observed effect concentration (m-NOEC) and toxicity unit (TU) from the experimental results.

2. NECESSITY OF MICROCOSM TEST FOR WET

Impact assessments at the ecosystem level utilizing aquatic model ecosystems, including a microcosm, are well understood, but focus on the WET test method utilizing a microcosm as a wastewater management method is needed. In parallel with a single-species test using species belonging to different niches, such as fish, crustaceans, and algae, microcosm tests are positioned as multiple-species tests. They may be correlated with natural ecosystems, and it is essential to construct these systems as comprehensive test methods based on ecological impact assessment.

The WET test is a method that evaluates toxicity, including multiple effects, because it is targeted at test waters containing multiple chemical substances rather than being used for evaluations of the toxicity of individual chemical substances [1,2]. However, although we use individual species (fish, crustaceans, and algae) located in different niches in the food chain for the assay, it is a single-species test only. It is not a test method that accounts for material cycles, energy flow, or biological interactions, which are the basic elements of natural ecosystems [4]. The microcosm is a system that includes multiple species in coexistence and contains material circulation, energy flow, and biological interactions [5,6]. Therefore, the microcosm makes it possible to evaluate the risks associated with added chemical substances at the ecosystem level. The safety factors for the ecological effects of chemical substances obtained by the microcosm test were supported by the high correlation observed between the microcosm test and mesocosm (natural ecosystem) test obtained from the Environmental Research Comprehensive Promotion Fund (2009–2011) and the New LRI (2012–2014) [4]. Therefore, we determined that it is possible to use the microcosm test to evaluate the effects of chemical substances in an ecosystem with higher precision and with less dispersion than in the conventional single-species tests, such as the HC5 method. Consequently, it is possible to calculate more realistic values of the PNOEC for natural ecosystems using the microcosm.

Microcosms are highly correlated with natural ecosystems and can be regarded as models containing the basic components and characterized by the basic principles of natural ecosystems [7]. From this fact, it is considered that a more realistic estimate of the NOEC can be obtained using a microcosm as compared with the current single species test that does not account for material cycling, energy flow, or biological interactions [8]. In the ecosystem impact assessment of wastewater containing various chemical substances, comprehensive tests were conducted to introduce the ecosystem model, a multiple-species test with a food chain and energy flow, simultaneously with the single-species test. A series of test methods were constructed, making it possible to evaluate the degradability and persistence of chemical substances contained in wastewater and the collapse and restoration of ecosystem functions [9]. The microcosm test can be performed at a lower cost than the WET test with commonly used single species (fishes, crustaceans, and algae).

The microcosm has already been subcultured for nearly 40 years and is easy to use by researchers accustomed to environmental microorganism testing who can master the microbial culturing procedures. It is also easy to use after short-term training. Additionally, a series of tests are possible without using expensive culturing tools or measurement instruments. The position of the microcosm test within the WET test is shown in Fig.1. The foundation of a certain ECETOC method and the HC5 method conventionally includes the evaluation of independent chemical substances in toxicity tests using single species, and the current WET test also uses single species for toxicity tests on the composition of chemical substances. The interactions between species constitute the ecosystem and drive the cycling of materials and energy flow; no single species is able to exist alone. It is therefore insufficient to evaluate the influence of added chemical substances at the ecosystem level using a single-species test. However, the model ecosystem consists of combinations of species, and multiple microcosm tests contain the basic elements of ecosystems, such as biological interactions, cycling of materials, and energy flow between species.

Using the microcosm, it is possible for impact assessments at the ecosystem level to reflect the natural ecosystem, advancing the WET test as well evaluating single chemical substances.

3. MATERIALS AND METHODS

3.1 Microcosm

A gnotobiotic-type flask-size microcosm system (N-system) [3,5,6] applied in this study consisted of combination of several species of four dominant bacteria as degraders, *Bacillus cereus*, *Pseudomonas putida*, *Acinetobacter* sp., coryneform bacteria, two green algae *Chlorella* sp., *Scenedesmus quadricauda* and one filamentous alga *Tolypothrix* sp as producer, and one species of protozoan ciliates *Cyclidium glaucoma*, two metazoan rotifer *Lecane* sp., *Philodina erythrophthalma* and one metazoan oligochaeta *Aeolosoma hemprichi* as consumer. Since this microcosm is a model microbial ecosystem with high stability and reproducibility, it is suitable for experiment [3]. The biomass (individual number) of each microorganism in the stationary phase are maintained about 1.1×10^6 N/ml in bacteria, 1.1×10^5 N/ml in *Chlorella* sp., 5.3×10^3 N/ml in *Scenedesmus quadricauda*, 8.0×10^2 cm/ml in *Tolypothrix* sp., 6.0×10^1 N/ml in *Cyclidium glaucoma*, 1.3×10^2 N/ml in *Lecane* sp., 1.1×10^1 N/ml in *Philodina erythrophthalma*, and 6.0 N/ml in *Aeolosoma hemprichi*, respectively.

3.2 Preparation of Test Effluent

	HC5 ECETOC (conventional)	WET (whole effluent toxicity)	microcosm	WET by MC (microcosm-WET)
Chemicals	single	complex	single	complex
Species	single	single	multiple	multiple

Test configuration	simple	complexity
Nature reflection	low	high
Reality	low	high
Controllability	easy	difficult

Fig.1 Placement of microcosm test for WET

According to the testing procedure described in the 1997 Sewage Examination Method (Japan Sewage Works Association, Volume III Biological Examination, Chap.1 Biological Examination, Sect.10, Ecosystem Impact Assessment Testing) [10], the ratio of TP medium and test effluent should be set to 0, 25, 50, 75, or 100 %. Remove contaminants by filtering with a 0.45 μm size mesh filter, and add the filtrate to the microcosm. The time of addition is during the stationary phase (16th day of cultivation). The water quality of the test effluent should be analyzed if necessary. The procedures of microcosm-WET test are shown in Fig.2.

3.3 Addition of Test Effluent

Addition of test effluent to the microcosm is conducted on the 16th day, when the microcosm has reached a stationary phase. For experiments in which chemical substances were added on the 16th day, the effect of the test effluent on the microcosm and the constituent microbiota may or may not allow for the retention of species diversity (*i.e.*, a stable ecosystem may or may not be recovered). The structural parameter (microbial population) and the functional parameter (P/R ratio) should be measured to evaluate the effects of the test effluent.

In the conventional microcosm experiments, chemical addition is usually performed on the 16th day after the cultivation began, when the system stabilizes. In the WET test using the microcosm, the medium in the flask must be exchanged for wastewater, which is then added. At that time, it is necessary to separate the organisms and the culture medium, and a fresh medium containing wastewater is added instead of the extracted medium. The addition of the attached microcosm standard medium increases the number of individuals in the steady state. This is because organic substances that feed microorganisms are already consumed. In this case, it is impossible to compare it with the non-additive system (control), so an appropriate medium was developed with reference to the amount of organic matter added at the time of wastewater addition.

3.4 Substance Concentration

Addition of a substrate was conducted by replacing the culture medium in the flask with wastewater. A fresh medium containing wastewater was added as a substitute for the extracted medium. However, when the standard microcosm medium was added as a new medium, the number of individuals in the steady state increased. Therefore, as a new medium, the amount of peptone in the medium was adjusted to 0, 5, 10, 20, and 40 mg/l and then added into the medium. The impact of each culture medium was assessed from both the P/R ratio as the functional parameter and the abundance of microbiota as the structural parameter in the microcosm. The environmental impact and ecological risk were estimated by comparing the treated microcosms with the no-addition system (control) in both assessment methods. With respect to the structural parameters, the abundance of *Cyclidium glaucoma*, a ciliate primary predator, was greatly increased by increasing the amount of peptone in the medium; there was no major change in the abundance of other species in the microcosm. From the P/R ratio, which is the functional parameter, no change was observed when compared to the no-addition system (control). From this, the system was considered stable unless wastewater was added. Statistical analysis of the DO concentration was performed. The slope (a) and the coefficient of variation (cv) were obtained assuming that the microcosm was normally distributed. If they fell within the range of ±34.13 % for each, it was considered that there was no influence. Since the range of ±34.13 % of the slope of the DO was $0.0028 \leqq a \leqq 0.0056$, it was considered as having no influence on the medium containing only 2 mg/l. Additionally, since the range of ±34.13 % of the coefficient of variation of the DO was $0.10 \leqq cv \leqq 0.20$, it was considered as having no effect on the medium containing 0 to 20 mg/l. Therefore, a medium containing 2 mg/l of peptone was considered appropriate for adding wastewater in the microcosm-WET test [9,11].

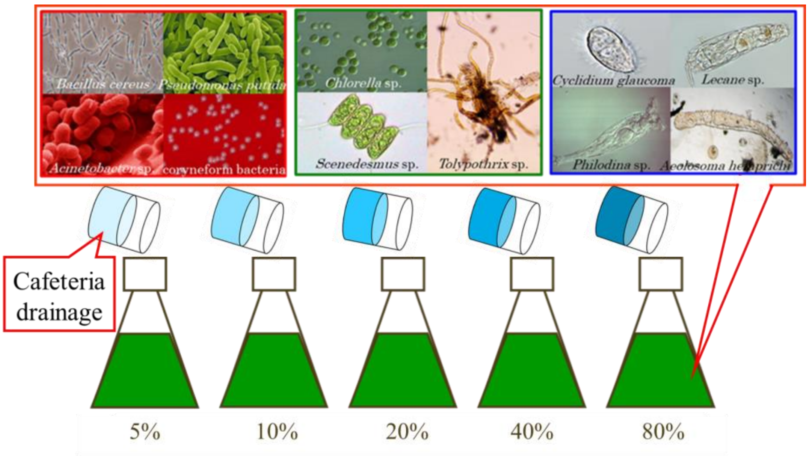
3.5 Microcosm WET Test

As shown in Fig.3, for microcosm culture, 200 ml of TP medium adjusted to a concentration of 100 mg/l of polypeptone was placed in a 300 ml volume Erlenmeyer flask, inoculated with 10 ml of subcultured microcosm as a seed, 25 °C, 2,400 lux (bright 12 hr./dark 12 hr.) and without shaking. Treated water of cafeteria drainage from the wastewater treatment facility in the university campus filtered using a membrane filter with a caliber of 0.45 μm. Thereafter, on the 16th day, which is the stable period of the microcosm, it was added to have concentrations of 0, 5, 10, 20, 40 and 80%.

3.6 Assessment Method

Impact risk assessment of each concentration was estimated from the DO change in the microcosm was continuously measured sequentially by the DO meter, and the transition (functional parameter) of P (production), R (respiration) and P/R ratio was obtained.

Simultaneously, sampling was started from the microcosm on 0, 2, 4, 7, 14, 16, 18, 20, 23 and 30 days after the start of culture, and plankton was



Fig,2 Addition procedure of test effluent

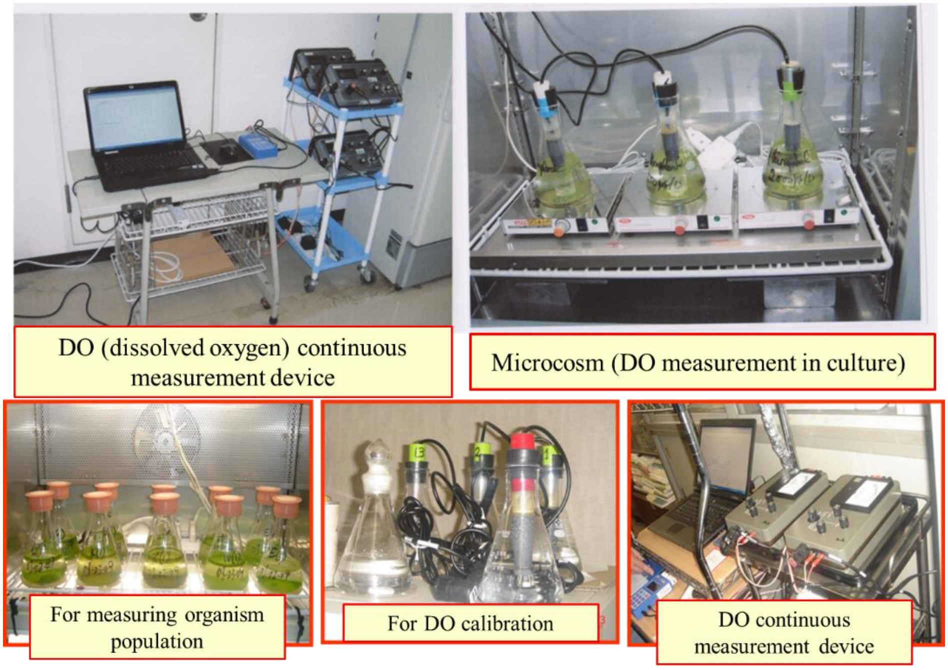


Fig.3 Microcosm N-system apparatus

sampled under a light microscope using a plankton counter and the transition (structural parameter) of the number of organisms constituting microorganisms was observed. Also, "Branched-type ANOVA" was performed on the change over time of functional parameters and structural parameters. As shown in Fig.4, in the branched-type ANOVA evaluation was performed from two items, "group" which is the variance analysis by all the values of each system and "interaction" which is an analysis of variance over time [3].

In the functional/structural parameter, the highest concentration among the concentrations not affected as a result of the branched-type ANOVA was taken as the maximum no-effect concentration (m-NOEC), and the toxicity unit (TU) was calculated from the following Eq.(1). The safety factor is proposed as 200 by the former investigation [3,4,12].

$$\text{Toxicity unit (TU)} = 100 / (\text{m-NOEC} \times 200) \quad (1)$$

4. RESULTS AND DISCUSSION

4.1 Water Quality of Cafeteria Drainage

The water quality of the treated water from the treatment facility as tested drainage effluent in the university campus was pH: 7.18-7.21, COD: 15.2-17.2 mg/l, T-N: 2.68-12.4 mg/l, T-P: 0.13-0.98 mg/l, SS: 9.40-18.5 mg/l, Cl^-: 14.2-15.7 mg/l, respectively.

4.2 Structural Parameter Analysis

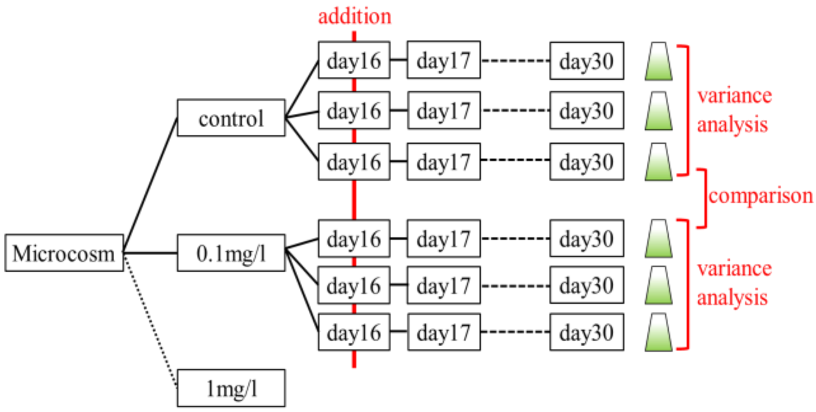


Fig.4 Outline of branched-type ANOVA

Time course of individual number of plankton (structural parameter) in microcosm under each test condition were shown in Fig.5. Two zooplankton species (*Lecane* sp. and *Aeolosoma hemprichi*) decreased in 20, 40, 80% addition system in the evaluation by the number of individuals (structural parameters), but did not exterminate. It was considered that these were influenced by chemical substances remaining in effluent as well as functional parameters. However, since all the microorganisms did not exterminate but coexisted, the ecosystem in the microcosm was maintained. There was no influence in 5 and 10 % addition system.

4.3 Functional Parameter Analysis

Time course of DO and P/R ratio (functional parameter) in microcosm under each test condition were shown in Fig.6. In the evaluation by DO and P/R ratio (functional parameter), the amplitude of DO decreased as the added concentration became higher, and the activity in the microcosm was lower. It is thought that residual chemical substances are contained in the cafeteria drainage. However, the P/R ratio was stable around 1 at all added concentration. This is probably because the activities ratio was stable around 1 at all added concentration. This is probably because the activities of phytoplankton and zooplankton have both decreased. In functional parameters, the P/R ratio, respiration, and production were tested by branched-type ANOVA and the presence or absence of influence was determined. As a result, m-NOEC was evaluated to be less than 5 %.

4.4 Statistical Analysis for Ecotoxicological Assessment

In the evaluation by DO and P/R ratio (production/respiration ratio) as the functional parameter, the amplitude of DO decreased as the added concentration became higher, and the activity in the microcosm became lower. It is thought that residual chemical substances were contained in the drainage. However, the P/R ratio was stable around 1 at all added concentration. This is probably because the activities of phytoplankton as producer and zooplankton as consumer have both decreased. For the functional parameters, the P/R ratio was significant-tested by branched-type ANOVA, and the influence was determined. Based on the statistical processing result of the functional parameters, m-NOEC which is the maximum no-effect concentration was evaluated to be present in a range lower than 5% as shown in Table 1. The toxicity unit (TU) was calculated as 100 / (0.05 × 200) = 10. Here, 200 is the safety factor for microcosm test. This value means that 10 times dilution is necessary to discharge safety to the environment, but 10 times dilution is expected after discharge by natural purification. Considering the above, the cafeteria drainage in the university campus was evaluated as being properly treated.

It is important to assess the complex influences of chemical agents, such as surfactants and medicines, on the environment. To conduct such comprehensive assessments, microcosm-WET tests should be used to examine drainage by collecting total toxicity test data through single-species surveys of chemical contaminants in environmental water. It is thought that more details for high-risk materials can be analyzed by the microcosm-WET test with a general comparison analysis. Additionally, because the microcosm-WET test offers high precision at a low cost, and it is expected that the correlation between the natural ecosystem and the microcosm is high, the estimated NOEC is more realistic than that derived from single-species tests, and the prediction of the behavior of the natural ecosystem is more practically enabled.

It can also be expected that wastewater management can greatly contribute to the remediation of the environmental impacts of effluents by introducing the microcosm-WET test of the Ministry of the Environment, Japan.

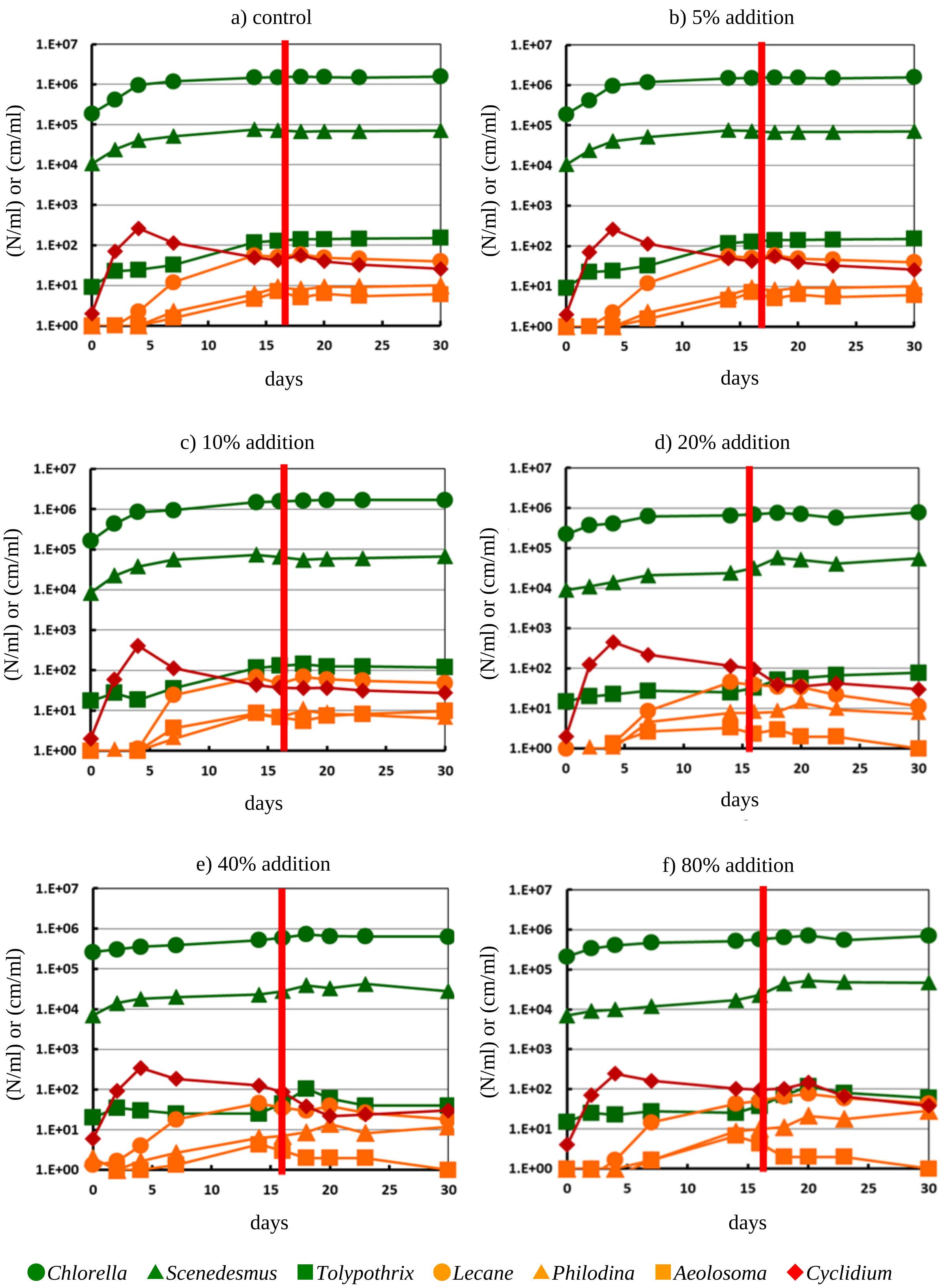


Fig.5 Time course of individual number of microorganism (structural parameter) in microcosm under each test condition

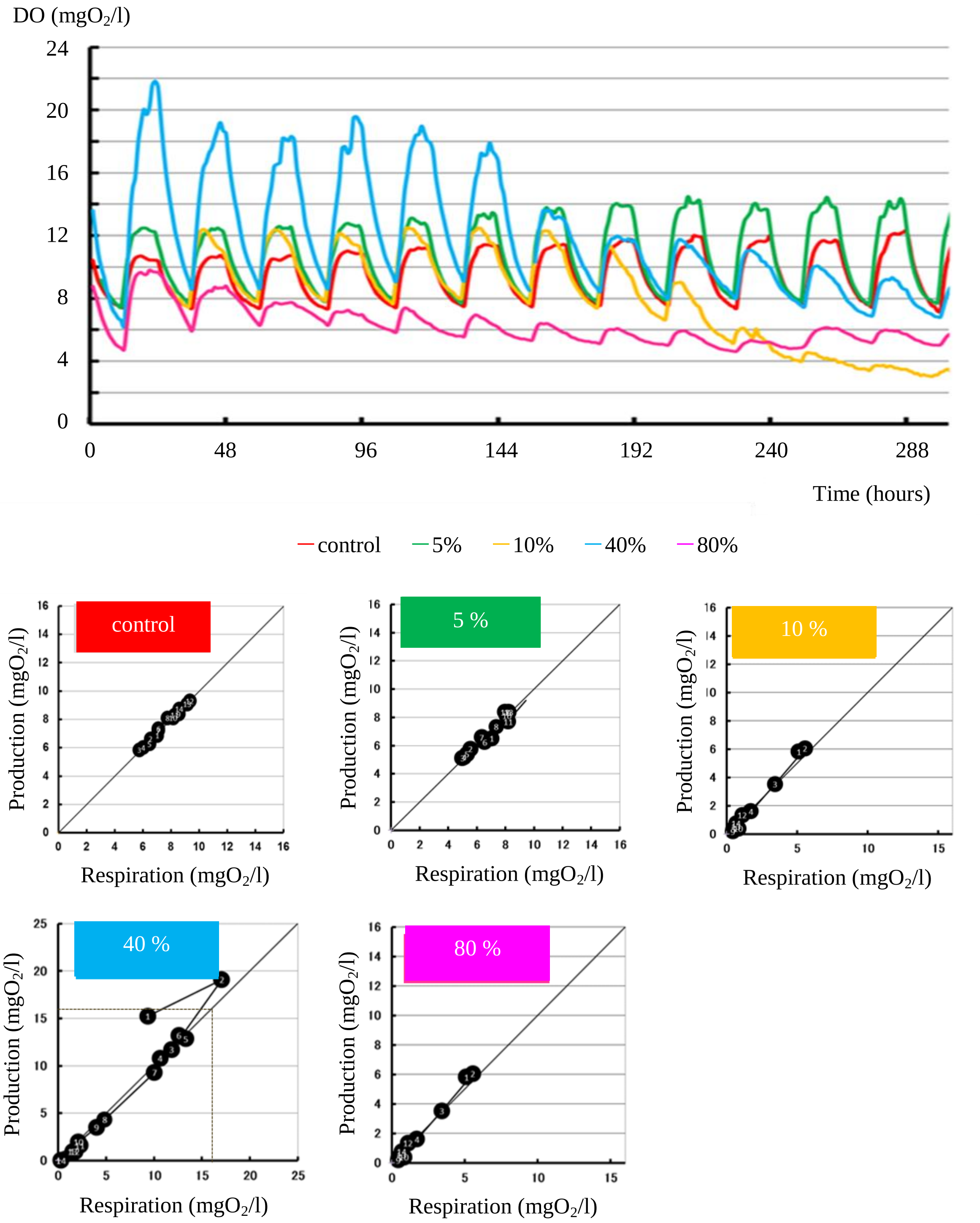


Fig.6 Time course of DO and P/R ratio (functional parameter) in microcosm under each test condition

5. CONCLUSIONS

A gnotobiotic-type flask-size microcosm system (N-system) was applied to assess the whole effluent toxicity (WET) of cafeteria drainage, which consisted of several species of four dominant bacteria as decomposer, two green algae and one filamentous alga as producer, and one species of protozoan ciliates, two metazoan rotifer and one metazoan oligochaeta as consumer.

In the evaluation by DO and P/R ratio (production/respiration ratio) as the functional parameter, the amplitude of DO decreased as the added concentration became higher, and the activity

Table 1 Summary of evaluation results

	5%	10%	20%	40%	80%
Functional parameter	×	×	×	×	×
Structural parameter	○	○	×	×	×
Evaluation	×	×	×	×	×

○ : no effect × : effected

in the microcosm became lower. It is thought that residual chemical substances were contained in the drainage. However, the P/R ratio was stable around 1 at all added concentration. This is probably because the activities of phytoplankton and zooplankton have both decreased.

In the functional parameters, the P/R ratio was significant-tested by branched-type ANOVA, and the influence was determined. Based on the statistical processing result of the functional parameters, m-NOEC (microcosm No Observed Effect Concentration) was evaluated to be in the range lower than 5 %.

The toxicity unit (TU) was calculated as 100 / (0.05×200) = 10, and the cafeteria drainage from the university treatment facility was evaluated as being properly treated.

From above, microcosm WET test is considered to be essential for ecosystem-level environmental impact risk assessment of various drainage.

6. ACKNOWLEDGEMENT

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7. REFERENCES

[1] United States Environmental Protection Agency, Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing, 40 CFR Part 136, EP 821-B-00-004, 2000.

[2] Tatarazako N., The Newest Domestic and Overseas Trend of Assessment and Regulation of Effluent Water using Bioassay –Overseas Operation Case to Japanese WET Test Introduction", Tokyo, NTS, 2014. 322pp. (in Japanese)

[3] Inamori Y., ed., Microcosm Manual for Environmental Impact Risk Assessment: From Chemicals to Whole Effluent Toxicity (WET), New York, Springer, 2019, 258pp.

[4] Inamori Y., Murakami K., Kakazu K., Inamori R., Kanzo Y., Shibata K., Amemiya T. and Sugiura K., Development of Environmental Impact Risk Assessment Method using Microcosm for OECD Standard Test, Proceedings of 20th Symposium on Japan Society of environmental Toxicology in Toyama, 2014. (in Japanese)

[5] Kurihara Y., Finite Ecology –A System of Stability and Coexistence-, Tokyo, Iwanami Shoten, 1975, 187pp. (in Japanese)

[6] Kurihara Y., Stability in Ecological Microcosm, Proceedings of Japan Academy, Series B, Physical and Biological Sciences, Vol.80, No.7, 2004, pp.327-335.

[7] Beyers R. J., and Odum H. T., Experimental Microcosms, New York, Springer-Verlag, 1993, 545pp.

[8] Graney R. L., Kennedy, J. H. and Rodjers, J. H. Jr., Aquatic Mesocosm Studies in Ecological Risk Assessment, Boca Raton, CRC Press, Lewis Publishers, 1994, 736pp.

[9] Tsuchiya T. and Murakami K., Ecosystem Level Whole Effluent Toxicity Test using Microcosm System, Papers on Environmental Information Science, Vol.33, 2019, pp.31-36. (in Japanese)

[10] Japan Sewerage Works Association, Wastewater Examination Method -FY2012-, Tokyo, 2012, 819pp. (in Japanese)

[11] Tsuchiya T., Murakami K., Inamori R. and Inamori Y., Development and Verification of Microcosm-WET Test on Basal Medium Concentration, Proceedings of IWA World Water Congress & Exhibition 2018, Tokyo, 2018, online.

[12] Sugiura K., Effects of Chemicals on Microcosms: Comparison with the NOECs in Experimental and Natural Ecosystems, Japanese Journal of Environmental Toxicology, Vol.13, 2010, pp.13-22.