

# Chapter 9

## Application to the Whole Effluent Toxicity Test



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**Abstract** In Japan, evaluation methods have been developed for the introduction of the WET test. However, the WET test is a test for a single species, as typified by *Daphnia magna* and *Danio rerio*. For this reason, biological interactions are not considered, and it is possible that this will cause impact assessments to differ from the conditions in natural ecosystems. Therefore, we aimed to further develop and verify the WET test using the microcosm. In addition to the single-species test using species belonging to different niches (ecological roles), such as fish, crustaceans, and algae, the microcosm test was conducted as a multiple-species test correlated with a natural ecosystem. It was necessary to construct a comprehensive test method based upon the concepts of ecological impact assessments. The microcosm test can also be incorporated as an expanded test along with a single-species test if necessary.

### 9.1 Necessity of the 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.

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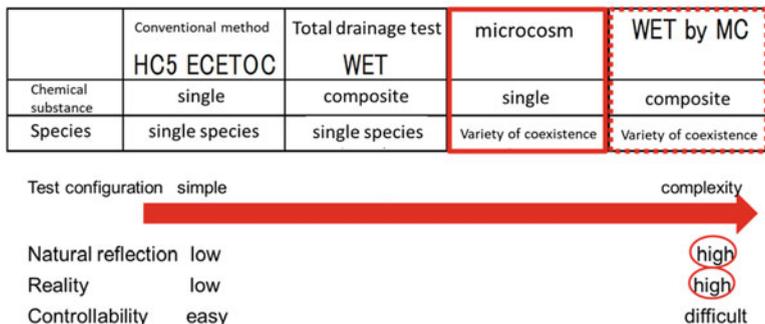
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The WET test is a method that evaluates toxicity, including multiple effects, because it is targeted at test waters containing multiple chemical substances rather than being used for evaluations of the toxicity of individual chemical substances. However, 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. The microcosm is a system that includes multiple species in coexistence and contains material circulation, energy flow, and biological interactions. 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). 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. 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. 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.

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. 9.1. 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



©Compared with the conventional method, it is superior in natural reflection and reality, making possible risk assessment analysis at the ecosystem level. In the future, establishment as a more advanced test method is also expected by fusion with the WET test.

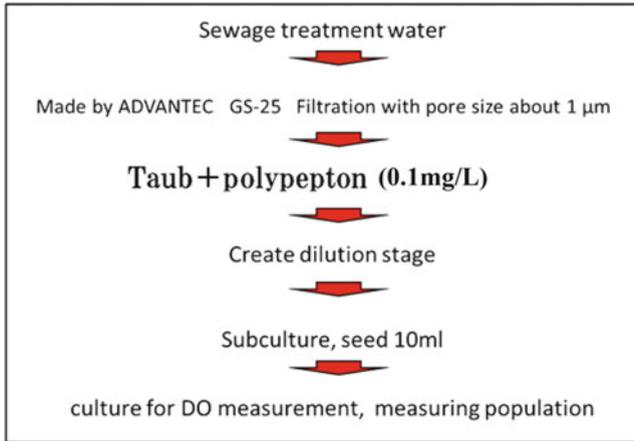
**Fig. 9.1** Placement of microcosm test for WET

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.

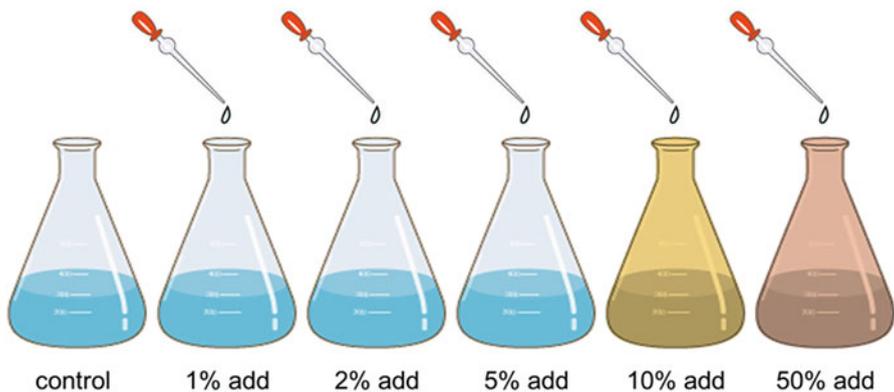
## 9.2 Preparation and Addition of Test Effluent

### 9.2.1 Preparation of Test Effluent

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), 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  $\mu\text{m}$  size mesh filter, and add the filtrate to the microcosm. The time of addition is at the beginning of the growth phase (i.e., day 0 of cultivation) or 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 Figs. 9.2 and 9.3.



**Fig. 9.2** Procedure of microcosm-WET test



**Fig. 9.3** Addition procedure of test effluent

### **9.2.2** *Addition of Test Effluent*

Addition of test effluent to the microcosm is conducted on day 0, when cultivation of the microcosm begins, or on the 16th day, when the microcosm has reached a stationary phase. For experiments with day 0 additions of chemical substances, the effect of the test effluent on the microcosm and the constituent microbiota may or may not allow a stationary phase to be reached (i.e., a stable ecosystem may or may not be established). 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). For both addition methods, 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 nonadditive system (control), so an appropriate medium was developed with reference to the amount of organic matter added at the time of wastewater addition.

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 mg/L, 5 mg/L, 10 mg/L, 20 mg/L, 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 (Murakami et al. 2017). 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) as shown in Fig. 9.4. 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  $\pm 34.13\%$  for each, it was considered that there was no influence. Since the range of  $\pm 34.13\%$  of the slope of the DO was  $0.0028 \leq a \leq 0.0056$ , it was considered as having no influence on the medium containing only 5 mg/L. Additionally, since the range of  $\pm 34.13\%$  of the coefficient of variation of the DO was  $0.10 \leq cv \leq 0.20$ , it was considered as having no effect on the medium containing 0 mg/L to 20 mg/L. Therefore, a medium containing 5 mg/L of peptone was considered appropriate for adding wastewater in the microcosm-WET test (Fig. 9.4).

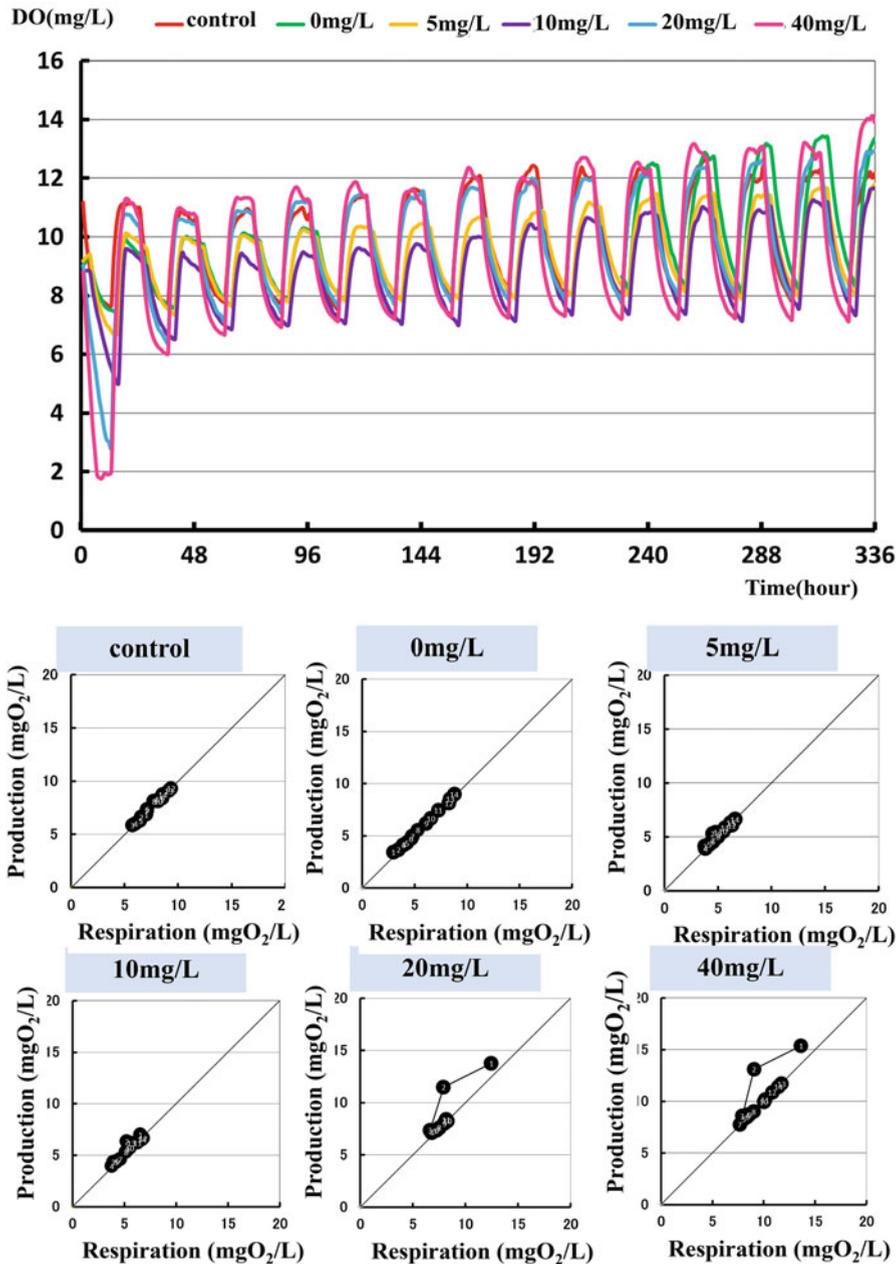


Fig. 9.4 Investigation of proper concentration of peptone in TP medium added to microcosm

### 9.3 Example of the Microcosm-WET Test

Examples of WET tests using a microcosm are presented below for sewage-treated domestic and plant drainage water, rice-washed effluent, paddy field effluent, golf course effluent, factory effluent, and landfill leachate.

#### 9.3.1 Sewage-Treated Domestic Drainage

Sewage-treated water from domestic drainage that was suction filtered using a 1  $\mu\text{m}$  diameter membrane (ADVANTEC, GS-25) was added at the initial stage of microcosm cultivation (day 0), and the addition concentrations were set to 0% and 80% in the microcosm medium. By comparing the 80% addition of the sewage-treated domestic drainage with the control system (0% addition), the ecological effects were assessed relative to the total toxicity. As a result, in the sewage-treated domestic drainage, the extinction of *Aeolosoma hemprichi* was observed, whereas the decreases observed in *Lecane* sp., *Philodina erythropthalma*, *Tolypothrix* sp., and *Scenedesmus quadricauda* tended to recover. Even in the evaluation using P and R, the initial P and R values tended to decrease in the sewage-treated domestic drainage more than in the control system, and it can be inferred that respiration and photosynthesis were inhibited. With respect to the classification patterns of ecosystem influence (i.e., maintenance, recovery, metastasis, and disintegration of the system), the sewage-treated domestic drainage (80%) was classified as a metastasized system.

#### 9.3.2 Sewage-Treated Plant Drainage

Sewage-treated water from plant drainage that was suction filtered using a 1  $\mu\text{m}$  diameter membrane (ADVANTEC, GS-25) was added at the initial stage of microcosm cultivation (day 0), and the addition concentrations were set to 0%, 20%, 40%, and 80% in the microcosm medium. The ecological effects were evaluated from the total toxicity by adding a dilution stage (0%, 20%, 40%, and 80%) of sewage-treated plant drainage, which was highly toxic. As a result, at 80%, all micro-animals and *Tolypothrix* sp. went extinct, and, at 40%, only *Cyclidium glaucoma* maintained its abundance, while the abundance of *Tolypothrix* sp. decreased. At 20%, *Aeolosoma hemprichi* became extinct, and the populations of other species, except for *Chlorella* sp., decreased. Even in the evaluation using P and R, as shown in Fig. 9.5, P and R transitions were similar to those in the control system, and the P/R ratio was observed to gradually decline. From the results obtained when the sewage-treated plant drainage was diluted in a stepwise manner, when the influence patterns were

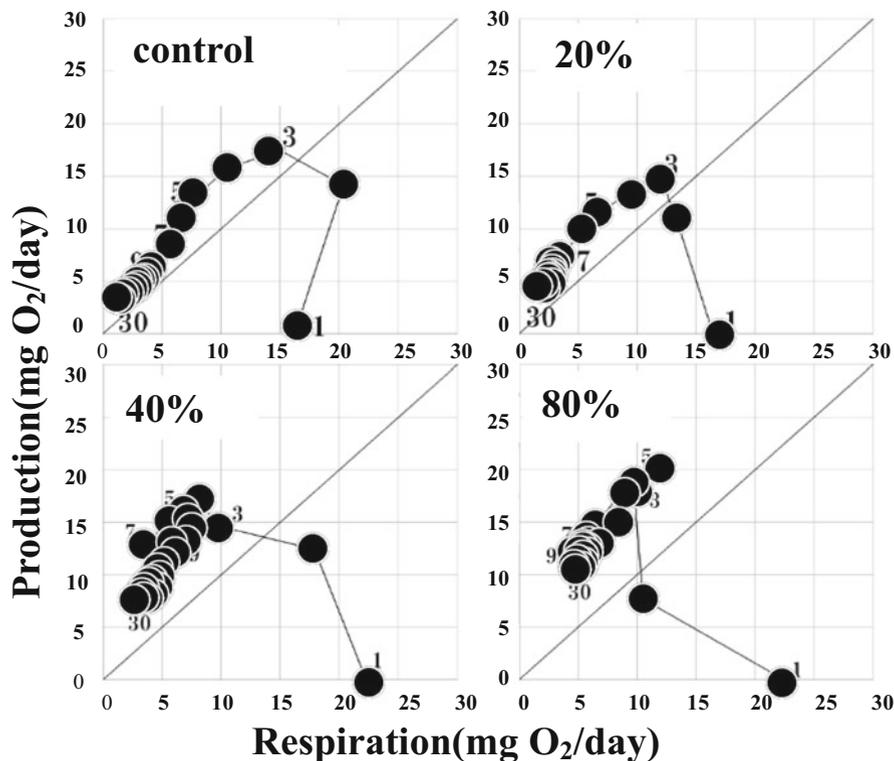


Fig. 9.5 Time course of P/R ratio in sewage-treated plant drainage added microcosm

classified, it was classified as being in a state of system collapse with 80% sewage-treated plant drainage and a system transition with 40% and 20% sewage-treated plant drainage. Additionally, when diluted to 20%, it was nearly identical to the control system. The sewage derived from plant drainage was demonstrated to have a substantial effect on the microcosm. The influence of sewage-treated water from domestic or plant drainage on the microcosm was also clearly confirmed from the appearance of the microcosm during the culturing process. For sewage-treated water, the substances that may be influenced by precise values of heavy metals and other chemicals were analyzed, and it was suggested that copper, nickel, and chlorothalonil may influence the added substances (Fig. 9.5).

### 9.3.3 Rice-Washed Effluent

A large amount of nitrogen and phosphorus are included in cleansed rice drainage. These are causative agents of eutrophy and lead to red tides and blue tides—

outbreaks of algae—if discharged carelessly. In this section, the WET test was used to investigate the influence of rice-washed effluent on the microcosm (i.e., the model of the natural ecosystem). Additionally, the influence of rice-washed effluent on the ecosystem in which it was discharged was determined based on the toxicity units (TUs) of the m-NOEC.

Rice-washed wastewater that was suction filtered using a 0.45  $\mu\text{m}$  diameter membrane (ADVANTEC, GS-45) was added at a stable stage 16 days after microcosm cultivation began, and the addition concentrations were set to 0%, 5%, 10%, 20%, 40%, and 80% of the microcosm medium. The impact of rice-washed effluent was estimated from both the P/R ratio (Sugiura 2010) as the functional parameter and the abundance of microbiota (Murakami et al. 2016) as the structural parameter in the microcosm. The environmental impact and ecological risk were estimated by comparing the microcosm with the no-addition system (i.e., the control system) using both assessment methods.

As the addition concentration increased in comparison with the control system, the amplitude of the DO increased, and the rice-washed effluent raised the bioactivity in the microcosm. The time series of the P/R ratio was stable, and both phytoplankton (producers) and zooplankton (consumers) were assumed to multiply together with all addition concentrations (i.e., 0%, 5%, 10%, 20%, 40%, and 80%) in the microcosm. Because  $\pm 34.13\%$  of the control system values of the slope  $a$  in the regression equation were  $0.0025 \leq a \leq 0.0051$ , it was determined that there was no influence in the 10% addition system. Because  $\pm 34.13\%$  of the control system values of the coefficient of variation  $cv$  exhibited amplitudes wherein  $0.099 \leq cv \leq 0.20$ , it was determined that all addition concentrations of rice-washed effluent had an impact on the ecosystem. Based on the functional parameter (P/R ratio), all addition concentrations also had an influence on the ecosystem. However, there was no influence noted for any addition concentrations based upon the structural parameter because no population declines or extinctions were observed.

A microcosm-WET test for rice-washed effluent was conducted, and the influence of the rice-washed effluent on the m-NOEC was less than 5% in both the functional and the structural parameters. Furthermore, the toxicity unit (TU) was determined to be 20, so dilution by more than 20 times was necessary. It is also necessary to examine the consistency using statistical analyses, and a branching-type analysis of variance was used to validate these findings; this simple statistical technique is described in the microcosm test method manual.

### 9.3.4 Paddy Field Effluent

Paddy field effluent, after pesticide spraying, was suction filtered using a 0.45  $\mu\text{m}$  diameter membrane (ADVANTEC, GS-45) and added at a stable stage on the 16th day after microcosm cultivation began. The addition concentrations were set to 0%, 25%, 50%, and 100% of the microcosm medium. The abundance of *Cyclidium glaucoma* decreased in the 50% addition system, as shown in Figs. 9.6 and 9.7, and became extinct in the 100% addition system. Therefore, it was determined that a

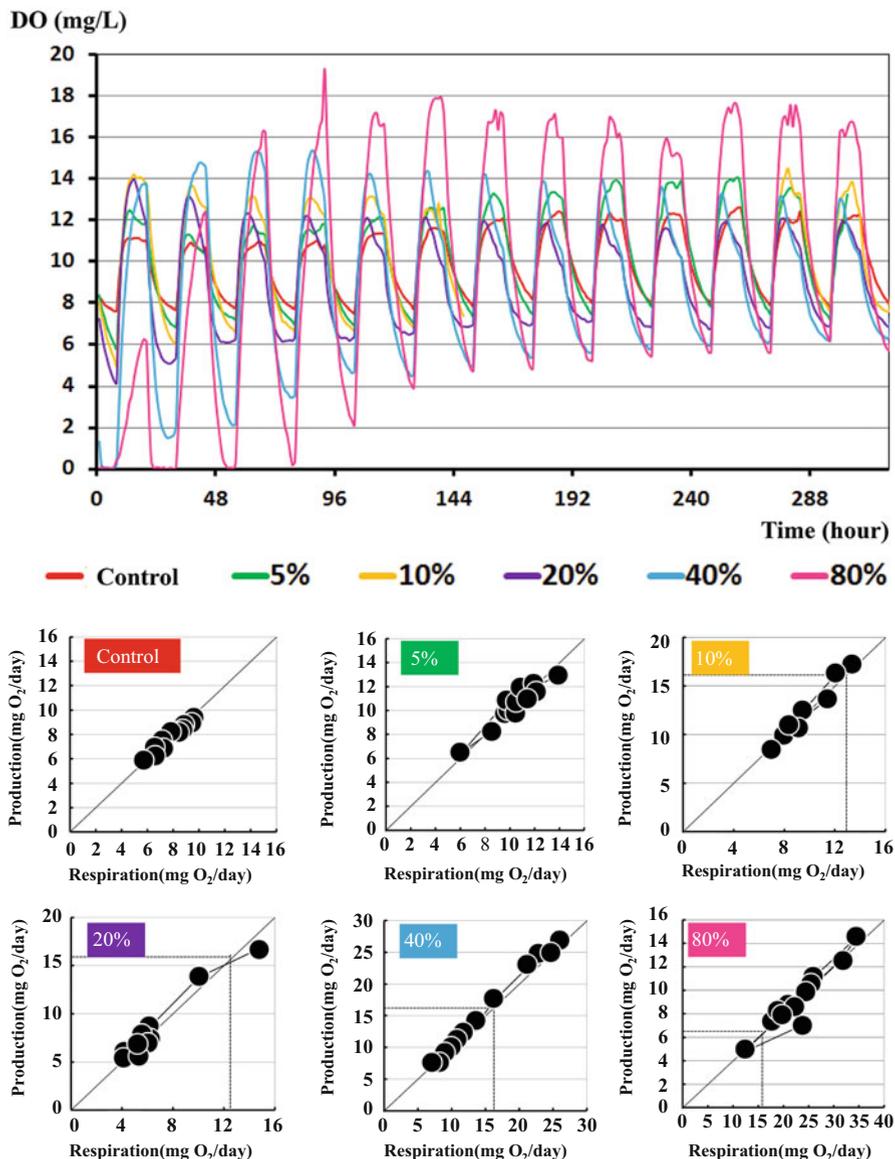


Fig. 9.6 Time course of DO and P/R ratio in rice-washed effluent added microcosm-WET test

dilution of more than double the concentration was necessary to maintain the system. The m-NOEC of the structural parameter was estimated as 1–10%; more specifically, it was found that there would be some influence on the ecosystem at any addition concentration without being diluted more than 10 times (Fig. 9.7).

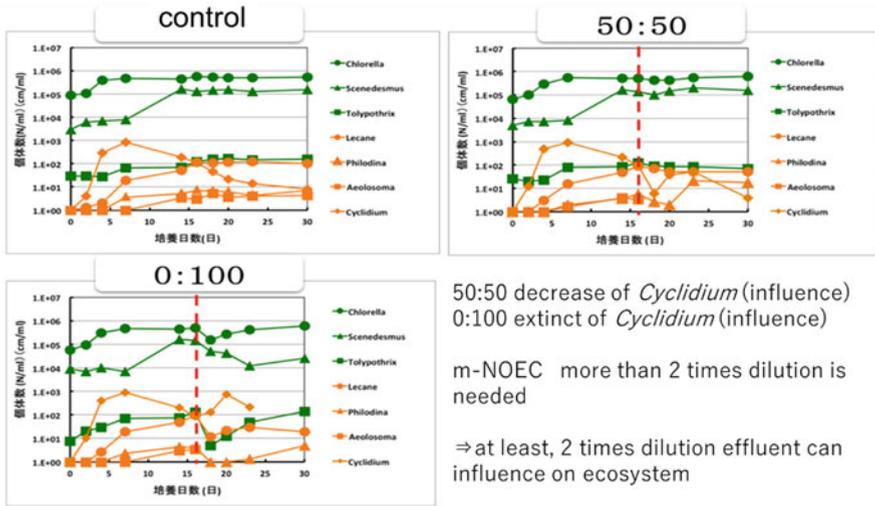


Fig. 9.7 Time course of population in paddy field effluent added microcosm-WET test

### 9.3.5 River Water Containing Germicides and Pesticides

River water that flowed after dispersion and was sampled for a day was targeted for evaluation before spraying the insecticide, DEP 50%, and the sterilizer, tricyclazole 20%, over the rice field. An inorganic salt solution and 5 mg/L of polypeptone and Taub’s basal medium was added to the river water, which was then filtered and sterilized. An inorganic salt solution and 50 mg/L of polypeptone and Taub’s basal medium were poured by 20 mL amounts into the experimental system. Finally, a TP<sub>50</sub> nutrient medium was used for the control system, and the microcosm was subcultured in a TP<sub>50</sub> nutrient medium with a microcosm seed at 2800 lux (L/D = 12 h/12 h), 25 °C, without stirring.

In the river water before pesticide dispersion, an increase was seen in the bacteria as in the control system until microcosm culturing began on the fourth day, but it tended to decrease sharply after the seventh day. Additionally, with respect to the producer, a notable increase was seen in the abundance of *Chlorella* sp. However, in *Tolypothrix* sp., an increase in abundance was controlled, and, for the predators *Cyclidium glaucoma*, *Lecane* sp., *Philodina erythrophthalma*, and *Aeolosoma hemprichi*, survival was possible, but the increase in *Aeolosoma hemprichi* was half that in the control system. Therefore, there were many things that dispersed, and a patchy cluster was characteristic of this microcosm in which high predation activity of *Philodina erythrophthalma* on *Chlorella* sp. was observed. The possibility that even the river water before the pesticide dispersion had an influence on the microcosm ecosystem was thus suggested.

When microcosm culturing with the river water began soon after pesticide dispersion, an increase was seen in the bacteria, as in the control system. Additionally, an increase was also seen in the abundance of the producer, *Chlorella* sp., as was observed before pesticide dispersion, but the increase in *Tolypothrix* sp. was controlled, and the survival of *Lecane* sp. and *Philodina erythrophthalma* was confirmed. *Cyclidium glaucoma*, which predominated early in the culture of the control system, and *Aeolosoma hemprichi* were not observed again. As for the patchy structure, the formation of clusters decreased after pesticide dispersion relative to before pesticide dispersion, and abundant, distributed *Chlorella* sp. was recognized. From this, it was suggested that, after pesticide dispersion in the river water, the species composition of the microcosm ecosystem came to be more affected by an increase in river water volume.

### 9.3.6 River Water Containing Pesticides and Herbicides

River water was targeted for evaluation by the raising of a seedling box with insecticide (Gazette granules) and a weedkiller (precutting emulsion, crushing 1 kg of granules, Mamet SM granules) in a rice field after dispersion, before dispersion, and with the passage of water, and it was sampled for a day. Additionally, the rice field consisted of upper reaches, where it is thought that there is an inflow of pesticide, areas in which it is thought that pesticide does not flow into the lower reaches, and areas in which it is thought that there is an inflow of pesticide.

An inorganic salt solution and 5 mg/L of polypeptone Taub's basal medium were added to the river water, which was then filtered and sterilized. An inorganic salt solution and 5 mg/L of polypeptone Taub's basal medium were added to the experimental system and Milli-Q water and then poured by 200 mL. Finally, a TP<sub>50</sub> nutrient medium was used in the control system, and the microcosm was subcultured in a TP<sub>50</sub> nutrient medium with a microcosm seed at 2800 lux (L/D = 12 h/12 h), 25 °C, without stirring. The species composition of the microcosm did not exhibit any remarkable changes. When compared with the control system, at the location where it was assumed that there was no inflow of pesticide, no substantial difference was observed before and after weedkiller dispersion.

At the upper reaches of the farm, no change was observed in the abundance of *Chlorella* sp., the threadlike blue-green alga, *Tolypothrix* sp., or in the rotifer, *Philodina erythrophthalma*, in the microcosm using river water sampled on the day when herbicidal dispersion was completed at the farm. However, a decrease was observed in the abundance of the rotifer, *Lecane* sp., the oligochaete, *Aeolosoma hemprichi*, and in a bacterium. Meanwhile, an increase was observed in the abundance of the protozoan, *Cyclidium glaucoma*. Moreover, a decrease in the abundance of *Lecane* sp. was remarkable, and their presence was not able to be confirmed by the 21st day of the experiment.

At a downstream location at the farm, no change was observed in the abundance of *Chlorella* sp., *Tolypothrix* sp., *Philodina erythrophthalma*, or *Aeolosoma*

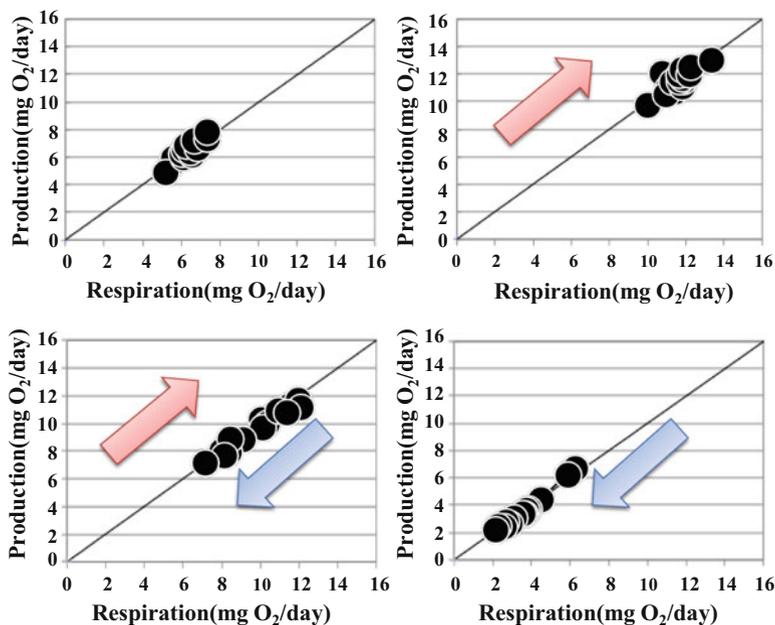
*hemprichi* in the microcosm using river water sampled on the day when herbicidal dispersion was completed at the farm. However, a decrease was observed in the abundance of *Lecane* sp. and a bacterium, and an increase was observed in the abundance of *Cyclidium glaucoma*. Additionally, the decrease in the abundance of *Lecane* sp. and in bacteria were remarkable, and the presence of *Lecane* sp. was not able to be confirmed by the 21st day of the experiment.

No difference was observed after culturing began in the upper reaches or in the downstream area of the farm until the seventh day, when a difference between these areas became noticeable. A decrease was seen in the downstream abundance of *Lecane* sp. and in a bacterium in comparison with the upper reaches on the 14th day after culturing began, and an increase in abundance was observed in *Cyclidium glaucoma*. Additionally, at the location of inflow, where it was assumed that there was no influence from the dispersion of pesticide because there was no control system, remarkable differences in the species composition of the microcosm were confirmed.

### 9.3.7 Golf Course Effluent

The investigation of golf course effluent was conducted for underdrainage after pesticide dispersion at a golf course in Narita City, Chiba. The pesticides used were all herbicides, namely, Double Up DG, Scoly Tex (SDS), Ajiran, Mecoprop (MCP), and Dictran. Additionally, field measurements of water temperature, pH, and DO and laboratory measurements of T-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, NH<sub>4</sub>-N, T-P, PO<sub>4</sub>-P, COD, BOD, and SS were taken. The ratios of the test drainage water were set as 0% (control/no-addition system), 25%, 50%, 75%, and 100%. After 16 days of microcosm cultivation, test drainage water, which was suction filtered using a 0.45 μm diameter membrane (ADVANTEC, GS-45), was added to the microcosm.

As the addition ratio of the environmental water increased to 25%, 50%, and 100%, the structural parameters (abundance) of *Chlorella* sp. (a producer), *Scenedesmus quadricauda* (a producer), and *Aeolosoma hemprichi* (a top predator) were influenced. In the 100% addition system, *Aeolosoma hemprichi* perished. From these results, the m-NOEC of the structural parameter was estimated as less than 25% because some influence was observed at all addition ratios of in the golf course effluent. As shown in Fig. 9.8, system bioactivity increased in the 25% addition system for the functional parameter (P/R ratio); it then decreased before rising again in the 50% addition system. Furthermore, bioactivity decreased remarkably and immediately in the 100% addition system. From these results, the m-NOEC of the functional parameter was estimated as 25% of the golf course effluent. Dilution of more than four times was considered necessary to discharge this drainage safely (Fig. 9.8).



**Fig. 9.8** Time course of P/R ratio in golf course effluent added microcosm-WET test

➡ Activity of microcosm system increased in time course

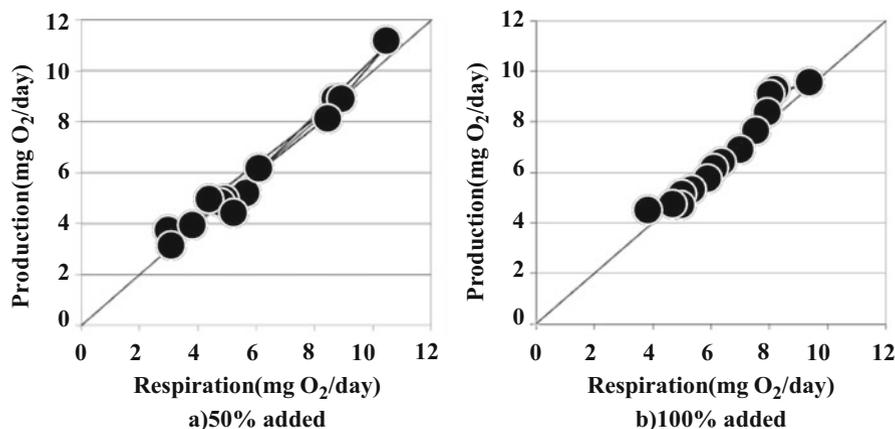
➡ Activity of microcosm system decreased in time course

\*The activity in each concentration was increased in 25% addition with influence to phytoplankton, increased initially and then decreased in 50% addition with influence to both phyto- and zooplankton, and decreased in 100% addition with zooplankton perish

### 9.3.8 Brewing Plant Effluent

The drainage from an industrial site located in a northwestern area of Chiba Prefecture that was near a brewing factory was tested. Water temperature, pH, and DO were measured in the field, and T-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, NH<sub>4</sub>-N, T-P, PO<sub>4</sub>-P, COD, BOD, and SS were measured in the laboratory. The ratios of the test drainage water were set as 0% (control/no-addition system), 25%, 50%, 75%, and 100%. After 16 days of microcosm cultivation, test drainage water, which was suction filtered using a 0.45 μm diameter membrane (ADVANTEC, GS-45), was added to the microcosm. The species composition on the 30th day (N30) was contrasted with that of the control system. Whether the species abundance ratio to the control system in each microcosm for each microorganisms in the addition systems increased or decreased was evaluated.

As a structural parameter, all constituent species for the microcosm existed in all addition systems. With respect to increases and decreases in the abundance of each microorganism in comparison with the control system, there was no significant



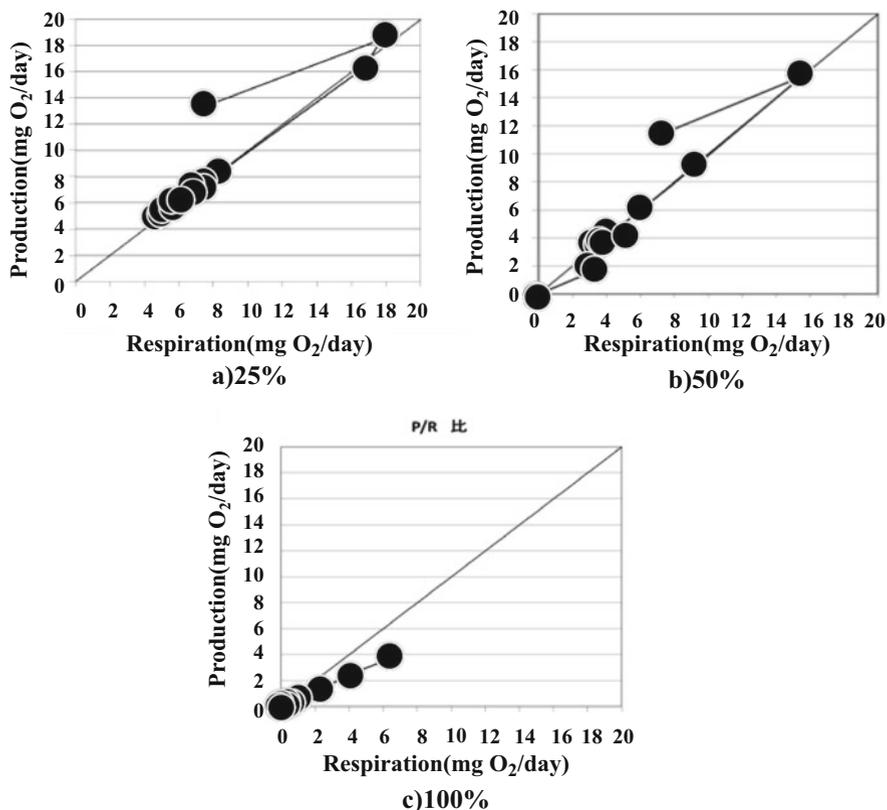
**Fig. 9.9** Time course of P/R ratio in brewing plant effluent added microcosm-WET test. (a) 50% added (b) 100% added

change in the population in the 50% addition system. Conversely, *Chlorella* sp. (a producer), *Scenedesmus quadricauda* (a producer), and *Tolypothrix* sp. (a producer) decreased in their abundance, and the abundance of *Philodina erythrophthalma* (a predator) increased in the 100% addition system. From these results, the m-NOEC of the structural parameter was estimated as 50%. Comparison of the DO and P/R ratio of each system revealed that the P/R ratio was close to one in all microcosm systems. The system bioactivity increased and then decreased in the 50% addition system and significantly decreased immediately in the 100% addition system. Consequently, the m-NOEC of the functional parameter was estimated as less than 50% because some influences were recognized in all addition systems. Dilution of more than two times was judged as necessary to discharge this drainage safely (Fig. 9.9).

### 9.3.9 Metal Processing Plant Effluent

The drainage from an industrial site located in a central area of Chiba Prefecture and near a metal processing factory was tested. Water temperature, pH, and DO were measured in the field, and T-N, NO<sub>2</sub>-N, NO<sub>3</sub>-N, NH<sub>4</sub>-N, T-P, PO<sub>4</sub>-P, COD, BOD, and SS were measured in the laboratory. The ratios of the test drainage water were set as 0% (control/no-addition system), 25%, 50%, 75%, and 100%. After 16 days of microcosm cultivation, test drainage water, which was suction filtered using a 0.45 μm diameter membrane (ADVANTEC, GS-45), was added to the microcosm.

For the structural parameter, no significant decrease in the abundance of each microorganism in the microcosm was observed in the 25% addition system when



**Fig. 9.10** Time course of P/R ratio in metal processing plant effluent added microcosm-WET test. (a) 25% (b) 50% (c) 100%

compared with the 50% and 100% addition systems. The abundance of predatory micro-animals decreased in the 50% addition system, more than in the 25% addition system. The abundance of the producers, *Chlorella* sp., *Scenedesmus quadricauda*, and *Tolypothrix* sp., decreased in the microcosm, and the top predator, *Philodina erythrophthalma*, became extinct in the 100% addition system. From these results, the m-NOEC of the structural parameter was estimated as 25%. As a functional parameter, the P/R ratio was close to 1 in all microcosm systems. In comparison with the control system, the system bioactivity (i.e., the value of DO) of the 25% addition system rose and then decreased before finally stabilizing; that of the 50% addition system rose and then decreased to 0, and that of the 100% addition system decreased to 0 immediately after the addition of drainage water. Put succinctly, the bioactivity of the microcosm increases to maintain itself with low drainage input but cannot rise, and it collapses with high drainage input. From these findings, the m-NOEC of the functional parameter was estimated as 25%. Dilution of more than four times was judged as necessary to discharge this drainage safely (Fig. 9.10).

### 9.3.10 Landfill Leachate

Jeong et al. (1995, 1996) examined landfill leachate as a very minor pollutant component of drainage and evaluated its influence on *Daphnia magna* in both a single-species culture test and in a multiple-species microcosm test. Landfill leachate (untreated), ozone-treated leachate, fluidized bed-treated leachate, and ozone + fluidized bed-treated leachate were applied to the microcosm-WET test. The ratios of the test drainage waters were set as 0 (control/no-addition system), 5, 10, 25, 50, and 100%. At the beginning of microcosm cultivation, test drainage water, which suction filtered using a 0.45  $\mu\text{m}$  diameter membrane (ADVANTEC, GS-45), was added to microcosm.

As a result of the untreated landfill leachate, the NOEC of the swimming inhibition of *Daphnia magna* was 2.5%, and the half-influence concentration ( $\text{EC}_{50}$ ) was 6.0%. The NOEC for the populations of the constituent species in the microcosm test were evaluated for the 1–5% addition of untreated landfill leachate. All species in the microcosm survived and were coexisting on the 28th day in the 1% addition system, and almost no influence of landfill leachate was observed. Meanwhile, for the 5% addition system, microbial species, except for bacteria and *Chlorella* sp., became extinct after the seventh day, and biological interactions among producers, consumers, and decomposers had collapsed. From these outcomes, it was estimated that there exists a boundary concentration for system stability between 1% and 5% of landfill leachate. *Cyclidium glaucoma*, the most sensitive species, exhibited growth similar to that in the control system until the fourth day, and it was clearly influenced by the landfill leachate during the first 7 days.

### 9.3.11 Ozone-Treated Landfill Leachate

From the addition of ozone-treated landfill leachate, all microbial species except for *Aeolosoma hemprichi* survived and coexisted, and almost no influence was observed in the 1% addition system. Meanwhile, all species except for bacteria and *Chlorella* sp. became extinct after the seventh day, and biological interaction collapsed in systems with more than 5% addition. These results were very similar to those for the addition of untreated landfill leachate. Two interpretations of these results were considered, one in which hazardous materials in landfill leachate still remained and influenced the microcosm in spite of ozone treatment and another in which new hazardous materials were generated by ozone treatment. It has been reported that humic in landfill leachate converts to aldehyde or carboxylic acid, which are toxic mutagens, after ozone treatment. From this, the possibility of generating new hazardous materials may be assumed. Furthermore, it was estimated that there exists a boundary concentration for the stability of the ecosystem between 1% and 5% of ozone-treated landfill leachate. *Cyclidium glaucoma* exhibited similar growth as in

the control system until the fourth day, and it was clearly influenced by the ozone-treated landfill leachate during the first 7 days.

### **9.3.12 Fluidized Bed-Treated Landfill Leachate**

From the addition of fluidized bed-treated landfill leachate, all microbial species except for *Lecane* sp. and *Aeolosoma hemprichi* survived and were coexisting on the 28th day in the 100% addition system, and it was estimated that the influence of fluidized bed-treated landfill leachate was minimal because the microbial interactions among producers, consumers, and decomposers were maintained. This tendency did not change in the lower addition systems until 10% addition, and the concentration at which all microbial species, including *Lecane* sp. and *Aeolosoma hemprichi*, could coexist was estimated as less than 10%. This is due to the fact that *Lecane* sp. and *Aeolosoma hemprichi* lack resistance to fluidized bed-treated landfill leachate. In comparison with untreated landfill leachate and ozone-treated landfill leachate, in which almost all microbial species became extinct at concentrations of more than 5%, the influence of fluidized bed-treated landfill leachate on the ecosystem was clearly reduced. Namely, it was clear that landfill leachate that may greatly influence ecosystems can be changed to treated water, which has a lesser influence on the ecosystem through the use of fluidized bed treatment.

### **9.3.13 Ozone + Fluidized Bed-Treated Landfill Leachate**

The results of the addition of ozone + fluidized bed-treated landfill leachate were similar to those from the addition of fluidized bed-treated landfill leachate; all microbial species, except for *Lecane* sp. and *Aeolosoma hemprichi*, survived and were coexisting on the 28th day in the 100% addition system. It was estimated that the influence of ozone + fluidized bed-treated landfill leachate was also minimal because the microbial interactions were maintained, and this tendency did not change in lower addition systems until 10% addition. The concentration at which all microbial species, including *Lecane* sp. and *Aeolosoma hemprichi*, could coexist was estimated as less than 10%. This is due to the fact that neither *Lecane* sp. nor *Aeolosoma hemprichi* are resistant to ozone + fluidized bed-treated landfill leachate. In comparison with the ozone-treated landfill leachate, in which almost all microbial species became extinct at concentrations of more than 5%, the influence of ozone + fluidized bed-treated landfill leachate on the ecosystem was clearly reduced. It is thought that this is because the aldehyde or carboxylic acid, which are by-products of ozone treatment, was decomposed and mineralized by fluidized bed treatment. Put succinctly, it was made clear that the ozone-treated landfill leachate, which greatly influences the ecosystem, can be converted to treated water, with a reduced influence on the ecosystem by combination with fluidized bed treatment. From these results,

the microcosm test can be assumed to be representative of the swimming inhibition test of *Daphnia magna*, which is a single-species test regulated by the OECD. Therefore, the utility of the microcosm test as a multiple-species culture system that is a closer approximation of real ecosystems was made clear. After assessing the impacts of landfill leachate from the oxygen demand that followed a 100-day oxygen consumption principle of the landfill leachates, namely, the Ultimate BOD, the NOEC for the 100-day oxygen consumption agreed with the m-NOEC. From this finding, the effectiveness of the microcosm test was supported.

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.

## Literature Cited

- Japan Sewage Works Association. Wastewater examination method -FY2012-. Tokyo; 2012.
- 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.