Chapter 1
Introduction

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Abstract The importance of the current conditions of environmental pollution from chemical substances and their environmental impact statement are provided here. The problems of the current test methods (i.e., examinations of single species) and the need for having many kinds of organisms examined are expounded. With this in mind, the summary and effectiveness of the microcosm test are shown and are the main purpose of this book.

1.1 Background

Testing methods for assessing the impact of chemical substances on an ecosystem are divided into single-species and multiple-species tests. Single-species testing has hitherto been widely used for assessing the environmental impact of chemical substances, and various standardized methods have been developed (Beyers and Odum 1993; Graney et al. 1994). However, it is predictable in natural environments that the presence or absence of interactions among different species may give rise to the onset of different toxicity mechanisms caused by chemical substances. The testing of model ecosystems with multiple species accounts for the interactions among different species; hence, it is a robust approach for conducting more realistic risk assessments of ecosystems. Although the need for risk assessment has been acknowledged globally, the development of a standardized testing method has been delayed. Therefore, the promotion of an official, standardized, and generalizable method of testing model ecosystems, with the aim of global applicability, holds great importance.

In Europe and the United States, model ecosystem tests are used in high-risk assessment processes, such as for exposure to pesticides. Test guidelines developed by the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) of the US Environmental Protection Agency (EPA) recommend model ecosystem tests, such as the "OPPTS 850.1900 Generic freshwater microcosm test, laboratory," as methods aimed at understanding the dynamics of chemicals and at measuring their

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impact on generic freshwater ecosystems. Additional examples include the "OPPTS 850.1925 Site-specific aquatic microcosm test, laboratory," in which testing is performed by reproducing a specific aquatic ecosystem, and the "OPPTS 835.3180 Sediment/water microcosm biodegradation test," which measures biodegradation in the bottom sediments at a given study site. However, the problem with these approaches is that they lack standardized methods for creating a model ecosystem. Furthermore, the reason model ecosystem testing is less frequently used in the process of evaluating high risks, despite its utility, is that model ecosystem testing is generally costlier than single-species testing (U.S. Environmental Protection Agency 1980, 1981, 1982).

It is important to note that microbial ecosystems, consisting primarily of producers (algae), low-level consumers (microanimals), and decomposers (bacteria), constitute the foundation of aquatic ecosystems. High-level predators such as fish, together with the microbial ecosystem, play an especially important role in water purification and material cycling in aquatic ecosystems. The microbial ecosystem is composed of algae (as photosynthetic primary producers), microscopic animals (acting as consumers), and heterotrophic bacteria (acting as decomposers). It is important to consider the variation in parameters of the ecosystem due to contamination from chemical substances, such as nitrogen, phosphorus, pesticides, and heavy metals. The microcosm method described in this manual is a test that utilizes a flask-scaled model ecosystem that is sampled to form a microcosm, which is equipped with the requirements necessary to solve the aforementioned challenges. An important characteristic of this test is that by designating production (P) and respiration (R) as endpoints in the aquatic microbial ecosystem, which can be easily measured, analyzed, and assessed using a dissolved oxygen (DO) meter, it allows researchers to resolve the issues of complexity and high costs associated with traditional model ecosystem testing.

In assessing and analyzing an ecosystem, it is effective to utilize a complementary dynamic analysis of a microbial community that uses a microcosm (i.e., a stable model ecosystem), which has been established based upon aquatic monitoring data and constitutes the core of the microcosm testing. Currently, traditional methods that utilize a single species do not include the performance of ecosystem risk assessments that examine the effects of chemical substances on ecosystem functions. In the single-species techniques that have been used, ecosystem risk evaluations that include the influence of chemical substances on ecosystem functioning have not conventionally been performed. Furthermore, current ecological studies that utilize a microcosm also emphasize the need for official methods (i.e., international standardization) to assess environmental impacts, which can thus be generalized and used to ameliorate the complexity and high costs of assessment methods involved in model ecosystem testing. The Organisation for Economic Co-operation and Development (OECD) test guidelines also discuss the importance of ecosystem assessments. From such a point of view, the development of an ecosystem-scale evaluation of a microcosm model system can be deemed essential.

1.2 Outline of the Microcosm

The term microcosm is derived from the Greek words for “small” (mikrós) and “universe” (kósmos); it denotes a system in which a population of a single species or a community, a group of populations of two or more species, is cultured in a container under controlled conditions. A large number of microcosms have hitherto been created to elucidate microbial interactions and their mechanisms, as well as to assess the effects of hazardous chemical substances and foreign microbes on ecosystems from the perspectives of microbial ecology and environmental science. These microcosms are classified into three types by size: (1) real-world scale (mesocosm), (2) pilot plant (pilot site) scale, and (3) flask scale. Furthermore, they can be divided into three types according to their population compositions: (1) gnotobiotic, in which the species composition is fully known, the population sizes of each species can be measured, and the traits of each species can be analyzed in isolation; (2) stress-selected, in which a natural community is cultured under specific conditions to promote natural selection in an effort to maintain and develop a specific biological community; and (3) naturally derived, in which a real-world community is maintained without varying any conditions. Among these, the concept of the gnotobiotic and the stress-selected types is used in the same sense as a standardized aquatic microcosm and abstract model ecosystem, respectively. Because the abstract model ecosystem (i.e., stress-selected microcosm) can be steadily sustained with repeated subculturing, it is well-suited to repetitive experiments and has been used in both theoretical ecology and applied ecology. It has also recently begun to be used as a test for assessing environmental impacts. The microcosm in our research model consists of producers, consumers, and decomposers. It can be considered to fall under the abstract model ecosystem category with respect to its properties and under the standardized aquatic microcosm category with respect to its structure.

The microcosm in this experiment is not merely a system for microbial cultures. The system is characterized by its ability to replicate the target phenomena at an ecosystem level, as it includes the physical, chemical, and biological factors of an ecosystem and some of their interactions, which substantiate four relationships: proliferation, consumption, production, and inhibition. Therefore, when applying outcomes obtained through a simple experimental system in a laboratory to the interpretation of real-world phenomena, the phenomena observed in a microcosm are expected to play an intermediary role, linking laboratory and natural conditions and responses.

This manual presents microcosms (N-systems) that were originally developed from water in a natural environment at Tohoku University, Japan, by Prof. Yasushi Kurihara (1926–2005) through the process of natural selection and that were subsequently followed and modified by the National Institute for Environmental Studies (NIES), Japan. They were subcultured as stable ecosystems at the Bio-Eco Engineering Research Institute in the Foundation for Advancement of International Science (FAIS), the Chiba Institute of Technology, and the Yokohama National University, Japan. These microcosms are aquatic, microbial model ecosystems that are composed of at least four bacterial species normally observed in natural ecosystems as decomposers, including *Pseudomonas putida*, *Bacillus cereus*, *Acinetobacter* sp., and coryneform bacteria. The consumers include a protozoan ciliate (*Cyclidium glaucoma*), metazoan rotifers (*Philodina erythrophthalma* and *Lecane* sp.), and a metazoan oligochaete (*Aeolosoma hemprichi*), and the producers include green algae (*Chlorella* sp. and *Scenedesmus quadricauda*) and filamentous cyanobacteria (*Tolypothrix* sp.). These microcosms are considered highly reproducible and stable aquatic model ecosystems. When the microcosms are transferred to a new medium during their stable phase, similar proliferation curves are observed, and, once the system reaches a steady state, it will endure for an extended period of time with the same amount of biomass. Therefore, unlike model ecosystems (mesocosms) established from environmental water, this microcosm will not result in the loss of species—the producers, consumers, and decomposers that constitute an ecosystem—and their impact can be properly assessed from the perspectives of function and structure. Thus, this microcosm is an abstract model ecosystem consisting of producers, consumers, and decomposers. Additionally, it has been demonstrated that the system still endures even if a small fish, such as a guppy (*Poecilia reticulata*), is introduced as a high-level predator. Moreover, despite variations in the number of days required to reach a steady state, this microcosm developed into systems with similar species compositions and similar amounts of biomass at various culture temperatures, ranging from 10, 20, 25, and 30 °C. For this reason, using microcosms with differing culture temperatures allows researchers to evaluate the effects of variations in water temperature on ecosystems. Furthermore, with regard to the effects of cesium radiation on ecosystems, in our joint research with the National Institute of Radiological Sciences (NIRS), Japan, we have reported the novel finding that bacteria, algae, protozoans, and metazoans that underlie the food chain are not affected by even high doses of cesium, which supports the feasibility of assessing the impact of different chemical substances.

As discussed above, the microcosm, with its high reproducibility and stability, allows for different approaches in assessing ecosystems from a functional perspective. When viewed as a standardized model for multi-species testing of the impacts of chemical substances and microorganisms on an ecosystem, it is a very effective model. Additionally, it holds a great value as an ecosystem impact test that assesses the effects of chemical substances and microorganisms on the stability of a system in which material cycles and energy flows exist, which are the foundations of any ecosystem (Fig. 1.1).

1.3 Purpose

The aim of the testing method presented in this book is to perform an aquatic ecosystem risk assessment using a microcosm. Various interactions in the ecosystem were exposed to a chemical substance, which served as a pollutant, and these are shown in Fig. 1.2. The microcosm is a model ecosystem that simulates, at a reduced

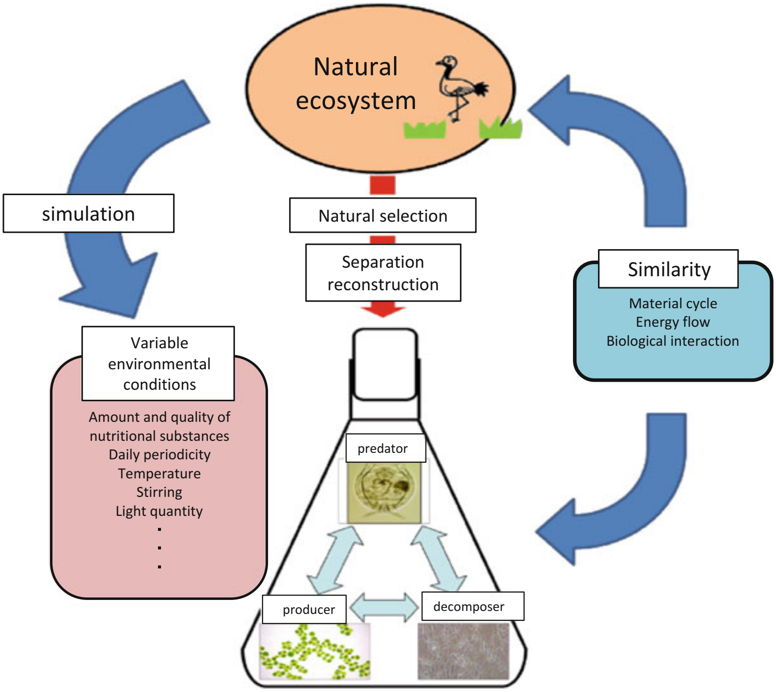

Fig. 1.1 Relationship between natural ecosystem and microcosm system

scale, an aquatic ecosystem that is spatially controllable, and joins laboratory testing and outdoor monitoring (Fig. 1.3). Material circulation and the flow of energy among a variety of organisms are thought to be important in performing an impact assessment of a given ecosystem, which is why there is a limit to rating systems that consist of single species, such as the alga, fish, and water fleas used for ecosystem assessments in the current Organisation for Economic Co-operation and Development (OECD) test. The microcosm (N-system) in question is based upon microbial samples collected from rivers and lakes around Japan by the late Yasushi Kurihara, a Professor Emeritus at Tohoku University. He subcultured samples in a Taub-Peptone (TP) medium (described below) over an extended period of time and confirmed the formation of a stable ecosystem before isolating single microbes and recombining them again as a model system for stable, aquatic ecosystems. Following transfer to the NIES, it was named the "N-system" after the acronym "NIES." In the microcosm, producers (algae), predators (microscopic animals), and decomposers (bacteria) exist as tools for assessing the impact of chemical substances on an aquatic ecosystem. The system encompasses the rules and principles of a functional ecosystem, such as microbial interactions, material cycles, and energy flows, which are not found in single-species culture systems.

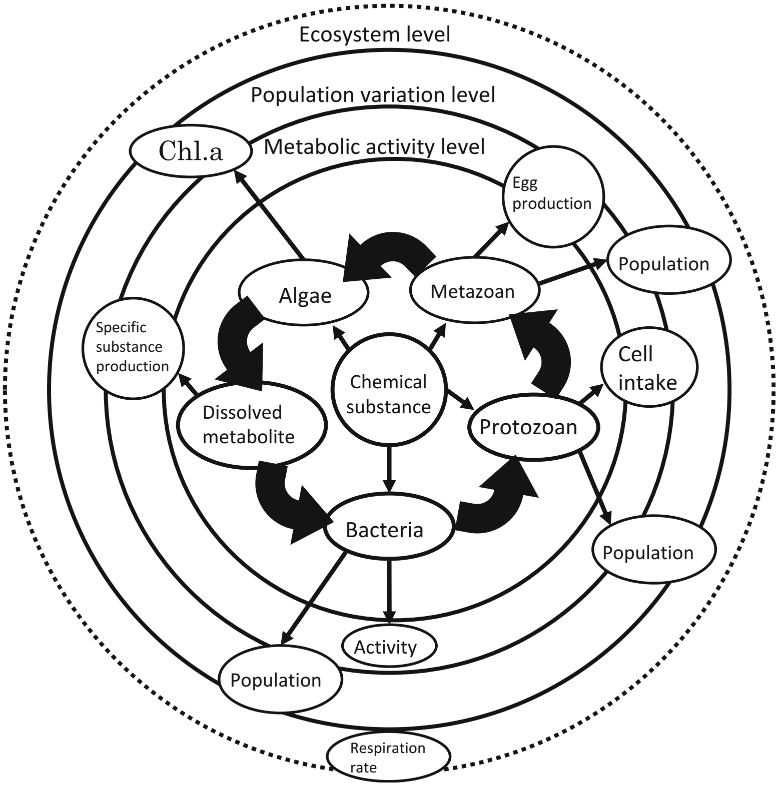

Fig. 1.2 Interaction of pollutants on ecosystem

The N-system is a microcosm that consists of a combination of at least four dominant bacterial species acting as decomposers, four microanimal species acting as predators, and three photoautotrophic species acting as producers. The N-system is a model microbial ecosystem with high stability and reproducibility that, when transferred to a new medium as a seed, during its stable phase, will repeatedly yield a coexisting and coevolving system with a similar proliferation curve to the original microcosm. With over 40 years of stable transfers, it is highly effective as a unified standard for comparing and analyzing data (Fig. 1.4). Furthermore, operation under various conditions is possible while retaining the basic elements of ecosystem functions. Given these characteristics, this model is intended to assess ecosystem risks using production volume/respiration volume (P/R) ratios concomitant with shifts in the entire ecosystem as an indicator. Using the flask microcosm N-system as a model of ecosystem functions, our aim is to offer researchers internationally applicable guidelines for this general microcosm testing method developed in Japan.

Because it is expected that the microcosm is highly correlated with the corresponding natural ecosystem, it is assumed that a more realistic and predictable

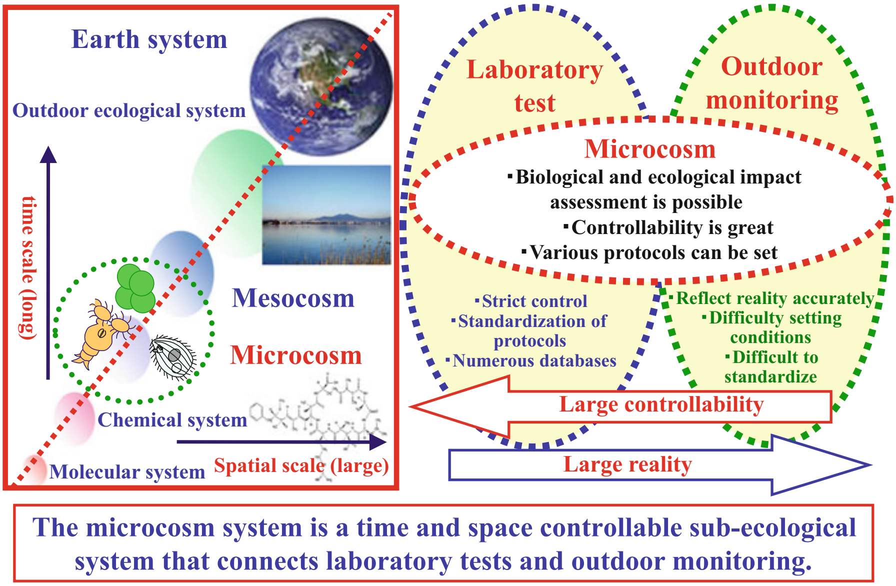

Fig. 1.3 Certainty of microcosm test method from hierarchy of nature

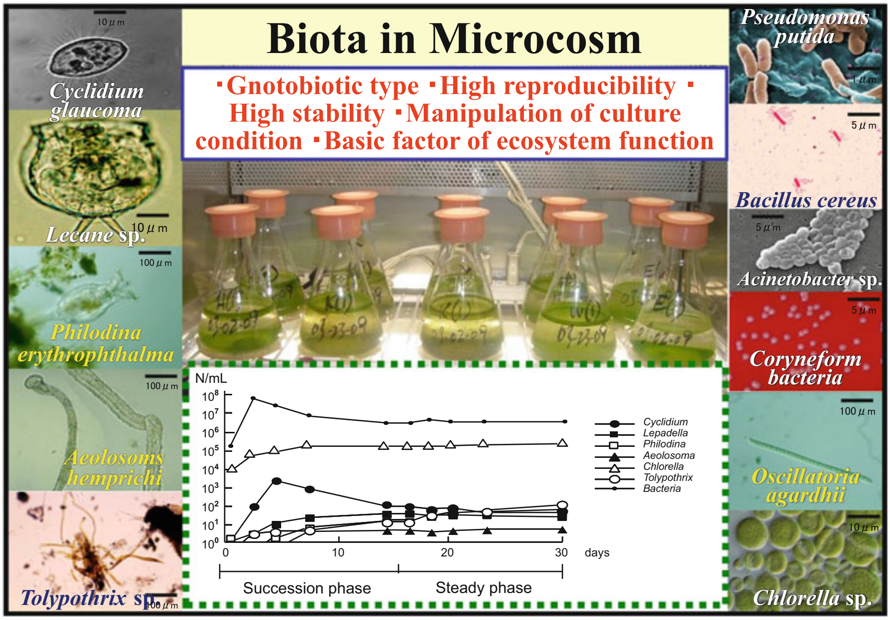

Fig. 1.4 Microorganisms in microcosm and their growth curve

no-effect concentration can be obtained, as compared to the methods currently available (i.e., assessment based on a single species). In assessing the effects of chemical substances on ecosystems, it is difficult to avoid the current testing methods for algae, crustaceans, and fish, which are exemplified in the Whole Effluent Toxicity (WET) test. Using an ecosystem model that includes parallel food chains and energy flows allows us to accumulate knowledge on the decomposition and persistence of chemical substances and on the recovery and disruption of associated ecosystem functions. In short, it is expected that the advantage of microcosms will be appreciated when establishing an approach that numerically assesses ecosystem impacts. The WET test assesses toxicity, including complex effects, by testing water that contains multiple chemical substances rather than assessing the toxicity of each chemical substance in isolation. Although species located in different niches within a food chain are used for the assay, it is a single-species test, and, as previously reported, a drawback of WET testing is that it is conducted under conditions in which material cycles, energy flows, and interactions among different species—the basic components of an ecosystem—are all absent. Microcosms are systems in which multiple species coexist, allowing researchers to assess the risk of chemical substances at the ecosystem level, and the safety coefficients obtained are considered different from those obtained from conventional approaches, as shown in previous studies on the correlations in both mesocosm and microcosm tests. It is expected that further accumulation of data will allow for the calculation of realistic levels of no-effect concentrations predicted for natural ecosystems. The similarity of the P/R ratio between the natural ecosystem and the microcosm is shown in Fig. 1.5. Additionally, the idea underlying the development

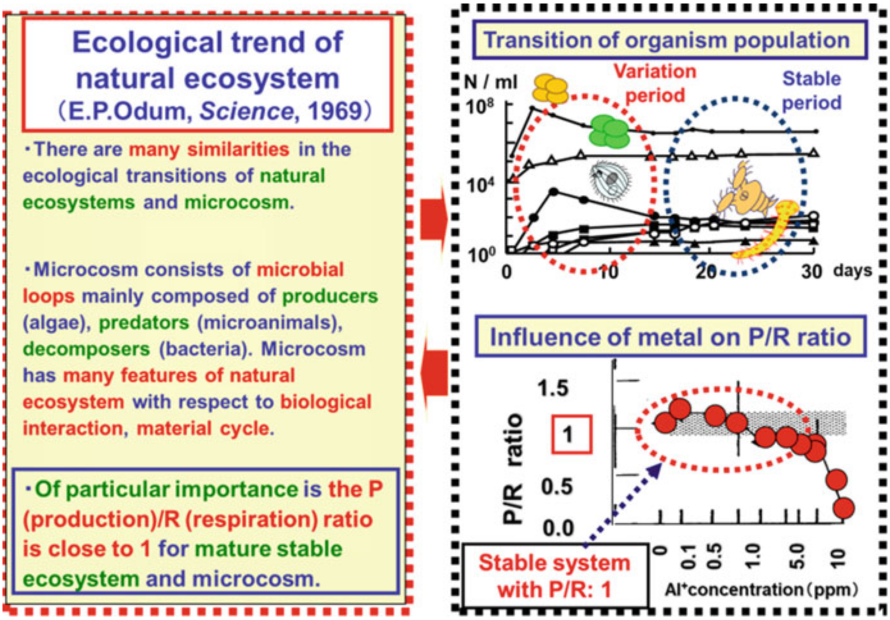

Fig. 1.5 Similarity of P/R ratio between natural ecosystem and microcosm

of the environment risk evaluation technique using the microcosm is illustrated in Fig. 1.6, which also shows the principles and experimental and analytical methods of measuring the P/R ratio in a microcosm. The aim of development of environmental impact risk assessment method using microcosm system is shown in Fig. 1.7.

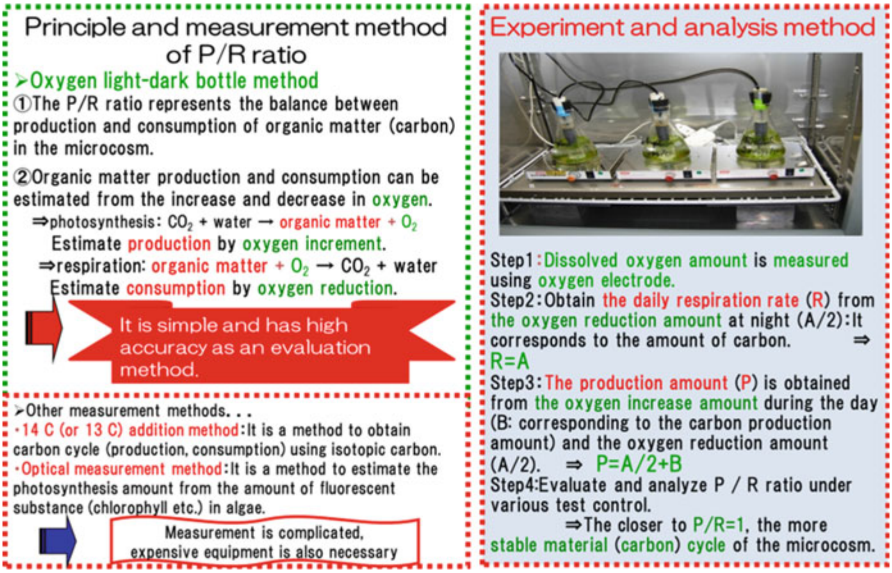

Fig. 1.6 Principle, experiment, and analytical method of P/R ratio in microcosm

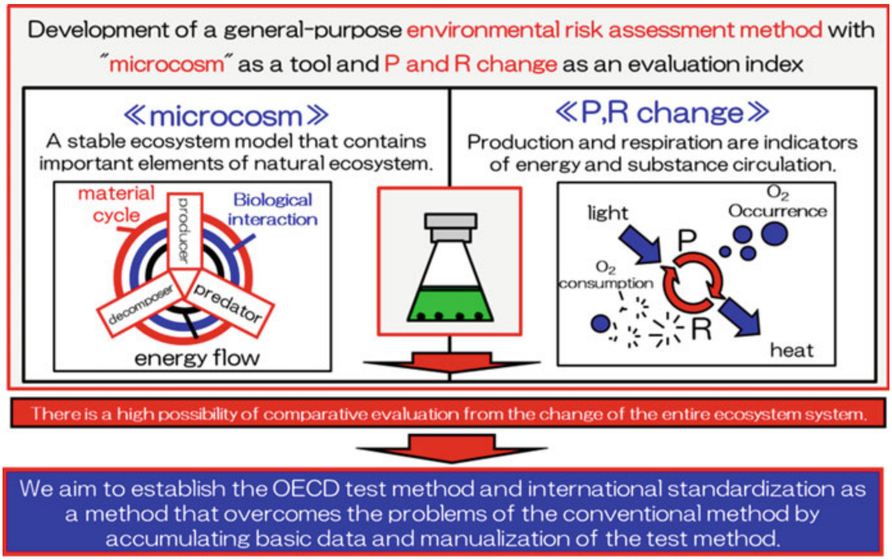

Fig. 1.7 Development of environmental impact risk assessment method using microcosm system

The concept of microcosm testing was discussed in a study entitled “Basic Examination for Discussion on an Outdoor Testing Approach Associated with Usage of Recombinants in Open Systems,” which was commissioned by the Planning and Coordination Bureau, Ministry of the Environment of Japan, in the 1989 fiscal year (FY), and also in a study conducted from 1992 to 1993 entitled “Research on the Development of a New Approach for Water Quality Assessment Using Microbial Ecosystem Microcosm (04650505)” supported by the Grants-in-Aid for Scientific Research on Priority Areas (General Research C). On these bases, microcosm testing was listed in the 1997 Sewage Examination Method (Japan Sewage Works Association, Volume III: Biological Examination, Chapter 1: Biological Examination, Section 10: Ecosystem Impact Assessment Testing), and the testing procedures were described in the study conducted during the 2009–2011 FYs in the project entitled “Development of an Ecosystem Risk Impact Assessment System Using Microcosm (S2-09)” supported by the Environment Research and Technology Development Fund. Furthermore, the new Long-Range Research Initiative (LRI) of the Japan Chemical Industry Association (JCIA) in the 2012–2014 FYs, entitled “Development of an Ecosystem Risk Impact Assessment System Against Chemical Substances Using Microcosm (2012PT4-02),” assessed a broader range of substances and conducted correlation analyses with natural ecosystems. The JCIA highlighted the importance of the relationship of microcosm tests with the existing toxicity data and created a database, which sought to define optimum values for determining safety coefficients. Moreover, during the course of testing across different facilities for the purpose of generalization, a ring test that is in line with OECD test guidelines was established and has been subsequently enhanced and revised.

Literature Cited

Beyers RJ, Odum HT. Ecological microcosms. New York: Springer; 1993. 557pp.

Graney RL, Kennedy JH, Rodjers JH Jr. Aquatic mesocosm studies in ecological risk assessment. Boca Raton: Lewis Publishers; 1994. 723pp.

U.S. Environmental Protection Agency. Microcosms as potential screening tools for evaluating transport and effects of toxic substances; Athens, Athens, U.S. Environmental Protection Agency; 1980. 370pp.

U.S. Environmental Protection Agency. Microcosms as test systems for the ecological effects of toxic substances; an appraisal with cadmium, Athens, U.S. Environmental Protection Agency; 1981. 171pp.

U.S. Environmental Protection Agency. Community structure, nutrient dynamics, and the degradation of diethyl phthalate in aquatic laboratory microcosms; Athens, U.S. Environmental Protection Agency; 1982. 135pp.