Characteristics and Comparison of Microorganism Cultivation Technologies

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Abstract. Microorganisms are of vital importance in marine ecosystems. However, the cultivation of marine microorganisms faces various difficulties. In this paper, various cultural technologies and techniques marine biologists usually are analyzed, and comparison is made based on their principles, pros and cons and application. Batch culture, fed-batch culture and chemostats are selected to represent traditional culture technologies. High-throughput cultivation is a revolutionary technology that greatly improves the efficiency of culturing microorganisms. Co-culture helps cultivation by partly replicating the natural context of microorganisms. Diffusion chambers feature in situ culture. The microfluidic system provides the ability to precisely control nutrients and environmental parameters. Genome-enabled cultivation brings insights from omics approaches to guide microorganism cultivation. The combination of various technologies and techniques offers specialized functions towards various research goals, especially the application of edge-cutting omics and computing tools which is novel to marine biology. Also, constant development in technology should help improve the cost-efficiency of new techniques and complicated systems, leading to more employment and finally more novel findings.

Keywords: Microorganisms cultivation, High-throughput cultivation, Microfluidic system, Genome-enabled cultivation.

1. Introduction

Microorganisms play an important role in the entire marine ecosystem. Therefore, marine biologists keep trying to isolate and culture various kinds of marine microbes in lab environments. However, the cultivation of marine microbes turned out to encounter a sea of troubles. Back in 1985, *Great Plate Count Anomaly* was discovered, which indicated that species that can be isolated on plates are just the tip of the iceberg, leaving enormous unculturable microbes still unidentified [1]. Later on, with the development of molecular biology methods, the divergence of microorganisms in the marine ecosystem was verified, reiterating the urgency of efficiently cultivating nonculturable microorganisms.

In addition, the food and pharmaceuticals industry also need the culture of microorganisms, since microorganisms provide various organics useful to them. By now it's still an ongoing effort to unveil these mysterious tiny beings. In this paper, multiple common technologies and techniques in culturing microorganisms are sketched and compared, starting with traditional ones like batch, and fed-batch, and to more recent ones like high-throughput cultivation (HTC) and genome-enabled cultivation. It's worth noticing that combining various technologies to reach the apex in outcome has already been recognized as highly effective.

2. Traditional microorganism cultivation technologies

Traditional culture techniques can be roughly divided into batch culture, continuous culture and semi-continuous culture. Here the most common technology of each group is presented.

2.1. Batch Culture

Batch culture is one of the simplest and oldest methods of cultivating microorganisms. In a batch culture system, microorganisms are grown in a closed vessel (e.g., a flask or bioreactor) containing a fixed volume of growth medium. The culture begins with an initial inoculation of microorganisms,

and then it proceeds without further additions or removals of nutrients or culture volume until it reaches a stationary phase, at which point growth ceases [2]. This technique is often used for laboratory-scale experiments and is particularly suitable for studies of microbial physiology, genetics, and basic metabolism.

2.2. Fed-Batch Culture

Fed-batch culture represents an extension of batch culture and was developed to address some of its limitations. In fed-batch culture, microorganisms are initially cultivated in a vessel with a fixed volume of growth medium, similar to batch culture. However, as the culture progresses, additional nutrients or substrates are continuously or intermittently added to the system while waste products may be removed. This controlled nutrient feeding allows for longer cultivation times, higher cell densities, and the accumulation of specific products [3]. Fed-batch culture is commonly used in industrial applications, especially in the production of biofuels, pharmaceuticals, and high-value bioproducts.

2.3. Chemostat Culture

The chemostat, short for "chemical environment is stationary", is a continuous culture system that provides precise control over microbial growth conditions. In a chemostat, microorganisms are grown in a closed vessel with a continuous inflow of fresh growth medium and simultaneous removal of an equal volume of culture. This maintains a stable environment where the growth rate can be controlled by adjusting the inflow rate of nutrients [4]. The chemostat is particularly valuable for studying microbial physiology, population dynamics, and microbial interactions. It has applications in environmental microbiology, wastewater treatment, and research on microbial ecosystems.

2.4. Comparison of Batch, Fed-Batch, and Chemostat Cultures

2.4.1. Control Over Growth Conditions

Batch culture has limited control over growth conditions as nutrients are not replenished during the culture period. Fed-batch culture offers better control by allowing nutrient feeding during cultivation. Chemostat culture provides precise control over growth conditions, allowing continuous adjustment of nutrient supply to maintain a specific growth rate.

2.4.2. Growth Profile

Batch culture typically exhibits a sigmoidal growth curve with distinct lag, exponential, and stationary phases. Fed-batch culture can extend the exponential growth phase and achieve higher cell densities compared to batch culture. Chemostat culture maintains cells in a continuous exponential growth phase, providing a steady-state culture.

2.4.3. Applications

Batch culture is primarily used for laboratory-scale research and studies of microbial physiology. Fed-batch culture is commonly employed in industrial bioprocesses for product accumulation. Chemostat culture is valuable for ecological and environmental microbiology research and wastewater treatment.

2.4.4. Nutrient Efficiency

Batch culture is an inefficient use of nutrients, often resulting in depletion and accumulation of waste products. Fed-batch culture is more efficient in nutrient utilization compared to batch culture. Chemostat culture has maximized nutrient utilization efficiency, making it suitable for steady-state microbial cultures.

In summary, batch culture, fed-batch culture, and chemostat culture are essential techniques in microbiology, each offering distinct advantages and applications. The choice of cultivation method depends on the specific research or industrial objectives, including the need for control over growth conditions, nutrient efficiency, and the desired growth profile.

3. New microorganism cultivation technologies

3.1. Co-culture

A substantial fraction of microorganisms from diverse environments, such as soils, oceans, and the human microbiome, defy laboratory isolation. Co-culture represents an innovative strategy to bridge this gap by harnessing the interactions and dependencies that exist between microorganisms in their natural habitats.

The concept of co-culturing microorganisms has a long history in microbiology, dating back to early experiments by scientists like Sergei Winogradsky [5] in the late 19th and early 20th centuries. Winogradsky's work on nitrogen-fixing bacteria and sulfur bacteria laid the foundation for understanding microbial interactions. Over the years, co-culture techniques evolved as researchers recognized the importance of studying microorganisms in their natural context.

Co-culture capitalizes on the intricate web of microbial interactions, which may involve mutualism, competition, predation, or syntrophy [6]. By co-culturing previously unculturable microorganisms with known species that provide essential factors or metabolic support, researchers create a conducive environment for growth. This technique leverages the fact that microorganisms can influence each other's physiology, releasing inhibitory factors or essential nutrients.

Co-culture techniques encompass a spectrum of approaches tailored to specific microbial systems and objectives:

(1) Symbiotic co-culture: In mutualistic relationships, microorganisms mutually benefit from each other's metabolic activities. For example, the co-culture of nitrogen-fixing bacteria with legume roots exemplifies this approach, where plants provide organic compounds in exchange for fixed nitrogen.

(2) Syntrophic co-culture: In syntrophic associations, microorganisms cooperate to perform metabolic reactions that would be thermodynamically unfavorable for an individual. Anaerobic digestion consortia, where fermentative bacteria cooperate with methanogens, exemplify this mutualistic interaction.

(3) Predator-prey co-culture: Predatory bacteria, such as Bdellovibrio spp., prey on other bacteria. Co-culturing predators with prey allows for the isolation of specific strains, as predators can control the population of non-target microorganisms.

As a long-established technique, co-culture works well when combined with nearly all types of cultivation technologies. Microfluidic systems, microchambers, and 3D-printed devices enable precise control of microenvironments, facilitating the study of intricate interactions. Additionally, high-throughput co-culturing approaches, coupled with metagenomics and multi-omics analyses, have expanded our ability to explore complex microbial communities.

Despite the promise of co-culture techniques, there are still some challenges. Applying co-culture requires the knowledge of specialized conditions for target microorganisms. Yet some microorganisms may have highly specialized requirements that are difficult to replicate in the laboratory. Furthermore, the interpretation of co-culture data can be complex, requiring careful consideration of community dynamics.

3.2. Diffusion Chambers

Since the cultivation of microorganisms under lab conditions faces difficulties, researchers came up with the idea of in situ culture. Diffusion chambers are permeable devices that allow for the exchange of nutrients, metabolites, and signalling molecules between the external environment and confined culture space. They create a unique microenvironment that can support the growth of microorganisms that are otherwise difficult to cultivate in the laboratory.

Diffusion chambers can take various forms, from simple bags with permeable membranes to more complex microfluidic systems. Researchers design chambers to match the specific requirements of their target microorganisms and environmental samples. The composition of the diffusion chamber could be adjusted to encourage the growth of specific microorganisms by providing selective nutrients

or conditions. Diffusion chambers can remain in the field or situ for extended periods, allowing researchers to monitor microbial growth and community dynamics over time [7].

Diffusion chambers have a more recent history, with their development gaining momentum in the late 20th and early 21st centuries. The concept of diffusion-based cultivation chambers was introduced as a means to mimic natural microenvironments and promote the growth of unculturable microorganisms. As microfabrication and microfluidics technologies advanced, diffusion chambers evolved into precise and versatile tools for microbial cultivation and study, offering a bridge between traditional laboratory techniques and the complexities of natural environments. Integration with microfluidic systems allows precise control of culture conditions within the chamber, enabling the study of microorganisms in highly controlled microenvironments. Despite their advantages, diffusion chambers have limitations, including challenges in scaling up for large-scale applications and potential biases in microbial capture.

3.3. High-Throughput Cultivation (HTC)

Microbial cultivation has traditionally been a labor-intensive process, limiting our ability to explore the vast microbial diversity present in various environments. The advent of HTC has revolutionized this aspect of microbiology by allowing researchers to culture microorganisms from complex samples at an unprecedented pace.

The advent of automation, robotics, and advanced culture techniques paved the way for HTC. HTC is grounded in the concept of parallelization, where multiple culture conditions are simultaneously tested to optimize growth. By using automation, robotics, and specialized microplates, HTC expedites the screening of a multitude of growth conditions.

HTC methodologies include:

(1) Automated liquid cultivation: Automated liquid handling systems can inoculate, monitor, and analyze multiple liquid cultures simultaneously, enabling the rapid screening of various growth conditions, such as temperature, pH, and nutrient availability.

(2) Solid media arrays: HTC can employ high-density agar plates containing different nutrients, supplements, or growth factors. Robotic systems can spot samples onto these plates, creating diverse culture conditions in a single experiment.

(3) Combinatorial approaches: HTC often employs combinatorial strategies to explore a wide parameter space. For example, the utilization of a factorial design approach allows for the simultaneous evaluation of multiple factors influencing microbial growth [8].

HTC is a relatively recent innovation, emerging in the late 20th century and gaining prominence in the 21st century. Therefore, it's often seen in a joint system with other edge-cutting technologies. The integration of microfluidic systems with HTC platforms has enabled precise control of culture conditions at the microscale. Microfluidic devices offer the advantage of miniaturization, reducing reagent consumption and facilitating high-resolution experimentation. Combining HTC with metagenomics, metatranscriptomics, and other omics approaches has allowed for the in-depth characterization of cultivated microorganisms.

While HTC has revolutionized microbial cultivation, challenges remain. Some microorganisms may still evade cultivation, and the scalability of HTC for large-scale industrial applications needs further optimization. Future research should focus on refining HTC techniques, integrating multi-omics analyses, and exploring the vast biotechnological potential of cultivated microorganisms.

3.4. Microfluidic System

The history of microfluidic systems is rooted in the microfabrication revolution of the 20th century. Microfluidics emerged in the 1980s and 1990s as researchers began developing miniaturized devices for fluid manipulation at the microscale. Initially, microfluidics found applications in chemistry and biotechnology, but their potential in microbiology became apparent in the early 2000s. Since then, microfluidic systems have rapidly evolved, enabling precise control over the growth and manipulation of microorganisms, single-cell analysis, and the study of microbial interactions in highly controlled

microenvironments. Their integration with microbiology has revolutionized our ability to explore microbial ecosystems and their interactions with the environment.

Microfluidics leverages the behavior of fluids at the microscale, where laminar flow, reduced volumes, and rapid mixing prevail. These principles enable precise control of culture conditions, making microfluidic systems invaluable for microbial cultivation and manipulation.

Microfluidic devices can create microenvironments with controlled nutrient gradients, temperature, and gas concentrations, allowing for the growth and study of microorganisms under precisely defined conditions [9]. Microfluidics enables the isolation and analysis of individual microbial cells, facilitating studies on microbial heterogeneity, gene expression, and responses to stimuli. Microfluidic platforms can mimic ecological niches and interactions between microorganisms, shedding light on microbial consortia, competition, and symbiosis.

Microfluidics can perfectly adapt to all kinds of cultivation systems as long as it's needed, thus bringing new possibilities to traditional cultural technologies, of which progress has been brought up previously. While microfluidic systems offer remarkable advantages, challenges remain, including the need for standardization, scalability, and cost-effectiveness. Future research should focus on addressing these challenges and exploring novel applications in microbiology and related fields.

3.5. Genome-Enabled Cultivation

Genome-enabled cultivation is a more recent development in microbiology, closely tied to advancements in genomics and molecular biology in the late 20th and early 21st centuries. It involves using genomic data to guide cultivation strategies, particularly for the isolation of previously unculturable microorganisms.

Genome sequencing provides insights into the metabolic capabilities and nutrient requirements of microorganisms. Researchers can analyze genomic data to predict the necessary growth conditions and culture media components for specific microbes [10]. This approach has been crucial in devising innovative cultivation strategies, such as the use of defined media with customized nutrients, vitamins, and cofactors tailored to the specific needs of target microorganisms. Genome-enabled cultivation has opened new avenues for isolating and studying microorganisms that were once challenging to culture using traditional methods [11].

Genome-enabled cultivation encompasses several key techniques:

(1) Genomic sequencing: Advances in high-throughput sequencing technologies have made it possible to obtain complete or draft genomes of unculturable microorganisms directly from environmental samples.

(2) Metagenomics: Metagenomic data analysis allows for the reconstruction of metabolic pathways, identification of potential growth substrates, and prediction of metabolic requirements.

(3) Defined media design: Researchers design culture media based on genomic information, tailoring nutrient composition, pH, and environmental conditions to mimic the microorganism's natural habitat.

(4) Co-culture strategies: Genome-based predictions can guide the selection of companion microorganisms that provide essential nutrients for target unculturable microorganisms.

Genome-enabled cultivation can be combined with single-cell techniques allowing for the study of individual cells from complex microbial communities, even if they cannot be isolated in pure culture. Also, advanced computational tools and machine learning algorithms are aiding in the prediction of growth conditions and metabolic pathways from genomic data. Challenges in genome-enabled cultivation include the complexity of microbial interactions, the need for validation of predictions, and the scalability of techniques.

4. Conclusion

In this paper, the principle, pros and cons, and application of multiple microorganism cultivation technologies such as batch culture, fed-batch culture, chemostats, HTC, co-culture, diffusion

chambers, microfluid system and genome-enabled cultivation, are summarized and some advice on further development is given.

Batch culture, fed-batch culture and chemostats are still among the most frequently applied cultivation technologies under lab conditions. Researchers can choose from them based on their specific research or industrial objectives. Their convenience is their advantage.

HTC focuses on efficiency, gaining an unshakable position in research these years. HTC also paves the way for omics approaches in biology fields, thus revolutionizing microorganism culture. Co-culture has long been an effective tool, and will still be. The only difficulty lies in finding the special condition the target microorganism requires. Diffusion chambers make in situ culture much easier, partly solving the problem of lab conditions. It works better with a microfluidic system to precisely control its microenvironment. Therefore, it's a rather expensive technique to function. Microfluidic system upgrades every traditional cultivation technology by providing accurate control over nutrients and environmental parameters. Microfluids therefore can mimic the natural environment, offering another approach to in situ culture. However, it also faces the problem of cost-effectiveness. Genome-enable cultivation brings brand new insights into the culture of microorganisms. By analyzing genetic data, researchers can predict necessary growth conditions and culture media components, which guide their experiments in an unprecedented way. The future of genome-enabled cultivation combined with advanced machine learning is promising.

With new technologies like HTC and genome-enabled cultivation producing plenty of important outcomes, traditional culture technologies still keep their vitality with the help of various powerful techniques. Future research should focus more on combining different technologies to build a complicated culturing system, realizing precise control and efficiency at the same time.

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