Accelerating Microorganism Strain Selection for Enhanced Productivity: A Review of Microdroplet Technology Solutions for Screening Mutant and GMO Strains

Kriss SPALVINS, Svetlana RAITA, Zane KUSNERE, Indra BERZINA, Linda FELDMANE

Riga Technical University, Institute of Energy Systems and Environment

RIGA TECHNICAL UNIVERSITY

Contact information:

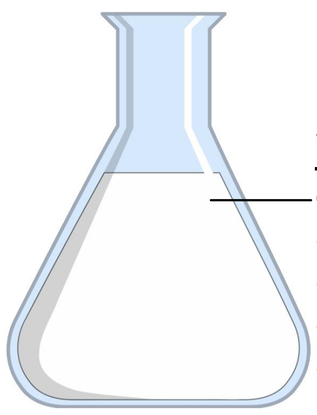
Azenes 12/1, Riga, Latvia

+371 26854884,

kriss.spalvins@rtu.lv

Introduction

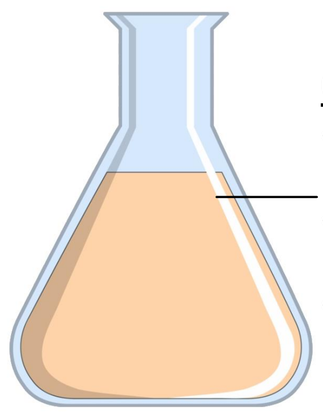
This article reviews state-of-the-art microdroplet technological solutions for screening microorganism mutant and GMO strains. Microorganisms used in the production of various products - single-cell protein, single-cell oil, enzymes, pigments and other bioactive compounds - can always be improved and their properties enhanced to increase the production of products of interest, to simplify microbial cultivation process, improve efficiency or adapted strains to use cheaper raw materials such as agroindustrial by-products. Microorganisms can be improved using either classical mutagenesis techniques or genetic engineering methods. Regardless of the selected method for mutant or GMO creation, during the process most promising microorganism strains must be selected, which is usually a slow and labor-intensive process. The use of microdroplets is a promising technological solution to speed up strain selection. This review looks at the latest developments in microdroplet technology, compares their variations, and identifies future prospects.



Water-based medium

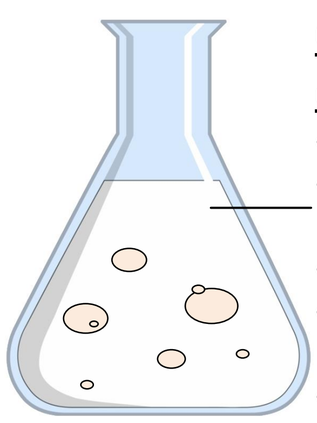
Can be supplemented with:

- Microorganism cells;
- Micro and macro nutrients;
- Gelation agents;
- Specific reagents for assays.



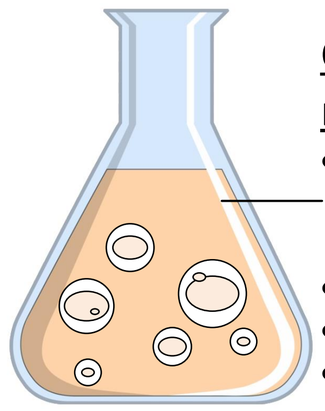
Oil-based phase

- Prevent cross-talk between individual microdroplets;
- Surround water-based microdroplets;
- Surfactant can be used to stabilize microdroplet surface tension.



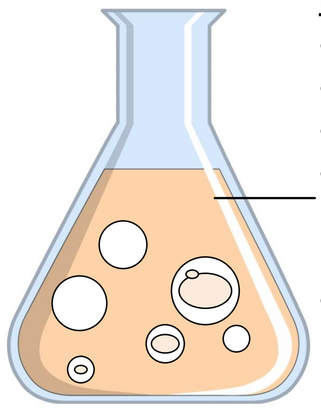
Conventional non-compartmentalization

- One batch culture;
- Different mutants share a single cultivation vessel;
- Mutants affect each other;
- Difficult to select individual mutant strains;
- Labour intensive, slow.



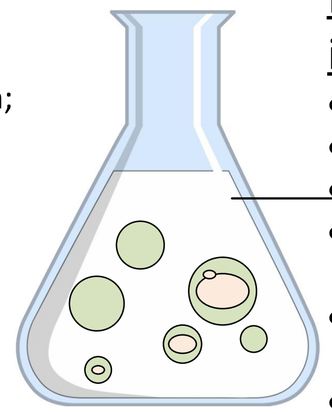
Compartmentalization in microdroplets

- Individual mutants separated in micro-vessels (droplets) surrounded by oil;
- Each droplet is a single batch culture;
- Mutants do not affect each other;
- High screening rates ($1*10^8$/h).



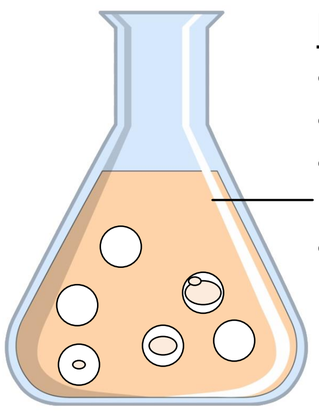
Polydisperse water in oil

- Inexpensive set up, easy to use;
- High production rate: 10^6-10^{10}/h;
- Different droplet volumes;
- Affect absolute product amount and product concentrations in droplets;
- Difficult to select moderately improved mutant strains.



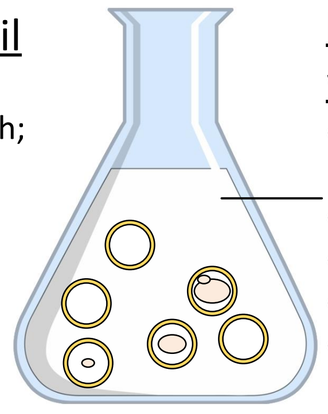
Polydisperse hydrogel beads in medium

- Inexpensive set up, easy to use;
- Production rate: 10^6/h;
- Wider droplet size range;
- Affect absolute product amount and product concentrations in droplets;
- Difficult to select moderately improved mutant strains;
- Gives option to choose if cell products are capable of affecting each other.



Monodisperse water in oil

- Expensive set up;
- Lower production rate: 10^5-10^8/h;
- Low droplet volume variation (~3%);
- Accurate comparison between absolute product amount and product concentrations in different droplets;
- Easier selection of moderately improved mutant strains.



Monodisperse water in oil in water

- Prevents cross-talk of oil soluble compounds;
- Expensive set up;
- Lower production rate: 10^5-10^8/h;
- Low droplet volume variation (~3%);
- Accurate comparison between absolute product amount and product concentrations in different droplets;
- Easier selection of moderately improved mutant strains.

Conclusions

- Various types of microdroplet generation techniques can be used to produce and utilize microdroplets for screening of mutant and GMO strains.
- These microdroplets can be used in various assays to generate both fluorescence and cell concentration derived signals, which can be sorted using: Fluorescence based, Cell number based, Weight based and Scatter based compartmentalization methods.
- Based on set up and available options on chip, particle sorter, serial propagation can be selected as sorting methods.
- Current assays require significant fine-tuning before they can be applied effectively, thus wider comparison with other screening methods e.g. microtiter-plates etc. needs to be assessed.

The work has been developed by the Fundamental and Applied Research Project «Herbicides as tool for selection of edible protein-rich mutants», project No. Lzp-2022/1-0126, funded by the Latvian Council of Science.