Special Issue on

Industrial Biotechnology-Made in Germany: The path from policies to sustainable energy, commodity and specialty products

Edited by:

Dr. Thomas Brück

Professor of Industrial Biocatalysis, Dept. of Chemistry, Technische Universität München (TUM), Germany
**Abstract**

Imagine a future world with a significantly grown influence of biotechnology into all parts of society. Let us assume that the majority of products in a consumer’s world, including food & beverage, furniture and clothes, plastics, buildings, streets and fuel are produced with the help of modern biotechnology. Meaning that at least parts of finished products are produced in an industrial environment with outcome from a biotechnological procedure.

There might be many reasons for a biotechnological revolution like exhaustible raw materials, minimizing ecological pollution, the consumer’s political will or just quality improvements.

Whatever the final drivers are, a transformation like this will open new fields in laboratory work and create new requirements for laboratory devices.

Without predicting the future world in details, it will be essential that biotechnological production processes are competitive to industrial productions. Main industrial challenges are 1st optimal economic parameter definitions in laboratory scale, 2nd a quick and reliable scale-up to minimize time to market and 3rd a maximal yield assurance in production. To overcome all these requirements for a successful biotechnological process development, a highly efficient screening tool, as the 2mag bioREACTOR, is urgently needed.

**INTRODUCTION**

The industrial landscape is no longer imaginable without biotechnological processes (Figure 1). Whether at the creation of new transgene plants e.g. resistant corn (green biotechnology) [1] for the food and feed industry, the development of new drugs in the pharmaceutical industry (red biotechnology) [2,3], the use of aquatic resources e.g. deep sea bacteria or other marine organisms (blue biotechnology) [4], the protection of the environment (brown biotechnology) or the waste industry (bacteria for treatment of waste water, grey biotechnology), biotechnological processes displace more and more the classical processes or bring off some new production methods for difficultly accessible substances.

Also in other classical industrial branches, as the production of washing or cleaning agents, cosmetics or plastics (white biotechnology) [5], biotechnological production methods are already permanent features, because they are more environmentally friendly and more resource saving than comparable industrial chemical processes.

In many of the industrial sectors relying on petroleum based resources, such as the chemical industry, biotechnology is still in its infancy. It is suggested that fossil oil and gas reserves will run dry in the next decades resulting in price fluctuations for fossil resources, which urgently calls for alternative action [6].

To develop a competitive biotechnological production, some critical points have to be addressed: the selection of the most cost-effective raw materials, the most efficient production strain which is able to metabolize different sugars with high yields, the optimal process parameters to reach high production rates and to ensure the reconditioning of the product out of the fermentation broth [7].

Identification of the most efficient production strain and development of such new biotechnological processes is time and cost intensive, due to the high amount and combination possibilities of parameters. As a result, mass screening for the identification of optimal parameters and therewith an efficient screening tool is urgently needed for biotechnological process development to have a shorter time to market and therewith to become competitive to already existing petrol based production processes [8].

**Requirements of minibioreactors/minibioreactor systems**

Minibioreactors and minibioreactor systems have to fulfill some requirements to be useful screening tools for biotechnological processes: These systems should be highly parallelized to generate a lot of data with only one run.

Moreover the working volume of the reactors/reaction vessels has to be in a useful range: as small as possible to minimize costs for media components, antibiotic or inducing agents but big enough to allow multiple taking of samples for other more detailed characterizing experiments.

Parameters like pH, dissolved oxygen, and temperature should be precisely adjustable to represent a real fermentation
process in laboratory fermenters and should be monitored over the whole run. Therefore precise measuring methods must be implemented for the small volumes and concentrations in minibioreactors.

Moreover the microbial metabolism, growth and production kinetics drastically change depending on the cultivation strategy. This demonstrates the importance of an automated feeding (with glucose or another carbon source) and titration of acid or base for pH control to realize meaningful screening experiments also in parallelized minibioreactors [9,10].

Another significant requirement is, that new identified or optimized production processes developed with minibioreactors should be easily scaled up into laboratory scale stirred tank reactors [11].

Minibioreactor systems based on microtiter plates (Applicon Biotechnology, m2p-labs GmbH, Pall GmbH) rely on a strong minimization (a few µl up to a few ml) and a very high parallelization of up to 96. The high parallelization is an optimal way to obtain much data with only one run.

Due to their simple construction, these systems are easy to handle and therewith cost and time saving, as well as spacesaving. Moreover automation with laboratory robots (e.g. the BioLector, m2p-labs GmbH) is easily possible, which allows the realization of feeding processes and pH control [9]. A disadvantage is the small working volume which allows only a very small number of samples. Usually only an end-point measurement is realized [19]. Moreover, stopping of the shaking is needed for taking samples, which disturbs the respiration profile. Last but not least, control of pH, dissolved oxygen or oxygen transfer rate is difficult [12] and the oxygen capacity is lower than in stirred miniature bioreactors [20].

Stirred minibioreactors systems

Sequential mass screening can be realized with classical laboratory fermenters (e.g. Infors AG, Switzerland) or with parallelized stirred tank reactor systems in 60-250 milliliter scale (e.g. DASbox, DASGIP, Germany) which are based on multiple reactors (usually 4, parallelization up to 24 is possible by using more units).

The identical geometry and function of these reactors compared to industrial scale fermenters allow an easy down- and up-scaling of production processes. pH, temperature and dissolved oxygen can be controlled and therewith stirred minibioreactors can offer the flexibility and controllability of conventional bench-scale reactors [19]. The higher working volume enables multiple sampling and therewith a good characterization of the cultivation process.

A disadvantage is that not all of these systems can be used fully automated with pipetting robots. Moreover they have a higher space and material demand compared to microtiter plate systems and parallelization is limited due to the more complex setup (cables, tubes, peripheral units).

There is only one stirred minibioreactor system, which allows a simple and detailed miniaturization of biotechnological production processes into the milliliter scale and a high parallelization at the same time – the 2mag bioREACTOR 48 (see Figure 2). This minibioreactor system is the modern and simple answer to the existing demand for a screening tool for efficient biotechnological process development.

The bioREACTOR 48 is a space-saving and user-friendly
fermentation system with 48 miniaturized reaction vessels, which is especially designed for the scaling down of biotechnological processes (aerobic and anaerobic) [21,22]. High material and cost savings in process development can be achieved due to the miniaturization (8-15 ml).

Experiments in the miniaturized reaction vessels can displace approaches in shake flasks and microtiter plates due to the high parallelization of up to 48 units. Moreover, the bioREACTOR generates more detailed experimental data comparable to laboratory stirred tank reactors especially due to the non-invasive pH and dissolved oxygen measurement at all 48 positions via special sensor systems from PreSens GmbH, Germany.

pH can be measured in a range from 6.0 to 8.0 with a maximal standard deviation of pH 0.2 and with a complex medium at pH values around the threshold of the measurement. Lower deviations of maximal pH 0.1 are standard for pH 7.0 and a defined medium. Standard deviation of dissolved oxygen is about 5% air saturation independent of the medium components [23].

The power input is comparable to conventional stirred tank reactors and also analogous $K_a$-values up to 0.3 s$^{-1}$ can be achieved [17]. Based on different production processes, e.g., cultivation of mycelium forming Streptomyces tendae or parallel studies of enzymatic biomass hydrolysis, the reliable scale-up of experimental data from the bioREACTOR into the liter-scale could be demonstrated [24-26].

Moreover, the bioREACTOR can be used as stand-alone unit for batch-processes or fully integrable into pipetting robots [21,27]. This automation allows pH control, taking samples, or realization of fed-batch processes.

Therewith the development of new biotechnological processes can be sped up or already existing processes, e.g., production of riboflavin, can be optimized [28].

Other special devices

Other special devices for screening experiments are cuvette-based microreactors [29] or bubble-column mini bioreactors [30].

Their construction is simple but the mass transfer is limited to $K_a$-values up to 0.15 s$^{-1}$ due to the restricted power input.

Advantages of minibioreactors/minibioreactors systems

Minibioreactors allow mass screening experiments for the development of new biotechnological production processes or the optimization of already known production processes.

Due to their high parallelization and miniaturization, they are cost, material and time saving and can reduce time to market. This allows the fast and economic development of new biotechnological processes as the production of 2-hydroxyisobutyric acid as precursor of polymethyl methacrylate (Plexiglas®) [5] or the production and esterification of biosuccinic acid as building block chemical for chemical, pharmaceutical, food and cosmetic industry branches [31].

Moreover the optimization of already known biotechnological production processes leads to more efficient and therewith also more competitive processes compared to the fossil based productions, as shown on a riboflavin production process with Bacillus subtilis [28].

Minibioreactors are time and cost saving analytical screening tools for the identification of robust strains and process conditions and provide the possibility to evaluate how a process will behave in the final laboratory and production scale [32].

Moreover, there is a second point regarding the behavior of biotechnological production processes. Biotechnological processes are always subject to natural variances, because the basis is a living microorganism which reacts sensitively on any influence, e.g., small variations in the raw material or other process parameters. To avoid the loss of a whole batch, it is also important to have a precise process monitoring. With a miniaturized parallel fermentation system, as the bioREACTOR, it is possible to model the production process in a small approach and therefore quicker response time. Unwanted influences can be shown earlier than in the parallel mass production and quality can be assured. This allows a greater range for correcting the real process or for stopping it in an early stage to reduce the costs and maximize the yield.

CONCLUSION

Comparing the different types of minibioreactors, microtiter plate based systems are the screening systems of choice to overcome the huge amount of parameters of biotechnological strain (wild-types and molecular developed new strains) and media optimization. An intense parallelization of up to 96 positions and automation is feasible. However, due to the small working volume and the limited oxygen transfer into the media the generated data is not sufficient to guarantee an easy upscaling into laboratory stirred tank reactors.

Stirred mini bioreactors/mini bioreactor systems, such as the 2mag bioREACTOR, are certainly more suitable to provide subsequent process up-scaling. The identical parameters (high $K_a$-values, power input) compared to conventional stirred-tank
reactors allow an easy down- and up-scaling of biotechnological processes. Moreover, high parallelization up to 48 is possible. pH and dissolved oxygen can be monitored by using the bioREACTOR as a stand-alone unit with the PreSenS sensor systems. Integration into a pipetting robot enables control of pH, realization of fed-batch experiments and automated taking of samples.

In summary, microtiter plates are a first good step for the pre-selection of the parameters (strains, media compositions) of a biotechnological production process.

For detailed strain characterization and process optimization (feeding, inducing…) a more instrumentally equipped screening tool, as the 2mag bioREACTOR, is required, that provides pH control, automation, sample taking and an easy up-scaling into the laboratory liter-scale of stirred tank-reactors.

OUTLOOK

Minibioreactors and minibioreactor systems are efficient tools for the identification of new production strains and the optimization of media and process conditions. Nevertheless, in order to demonstrate a complete biotechnological production process, the product has to be extracted from the fermentation broth after the fermentation and purified from other components in the next steps (downstream processing). Downstream processing technologies, as for example a prototype miniature microfiltration, are rarely available [33]. There is an urgent requirement for the down-sizing of common downstream procedures into the milliliter scale.

Moreover, additional analytical features for these small volumes would be desirable to characterize strains and conditions in more detail. In this respect exhaust gas analysis to close nitrogen and carbon balances would be a great benefit. Further, on-line GC (gas chromatography) or HPLC (high pressure liquid chromatography) measurement methods would provide detection of special metabolites and target products.

REFERENCES


