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EXAMINATION OF INNOVATIVE HIGH-THROUGHPUT FERMENTATIONS

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During the investigation of fermentations, issues such as the need for numerous parallel experiments with regard to strain improvement or screening were often met, or in the case of media optimization the need for online measurements to avoid a lack of night-samples was also required. Therefore, several new instruments were introduced to solve one or more of these problems: impedimetric- and reverse-spin-technologies (RST) were compared *via* fermentation of a well-known species of yeast, *Saccharomyces cerevisiae,* under both aerobic and anaerobic conditions, resulting in a diauxic growth curve. To identify the most accurate method, a well-known mathematical description was fitted to the measured data. Since the initial parameters were considered reliable as they originated from real experiments, during model fitting, the parameters were further fine-tuned, and the less modifications reported the better the system since it produces a growth curve that is more similar to standard bioreactors. According to our study, the impedimetric equipment was more efficient, and could run 40 parallel experiments, but the RST was more flexible.

Keywords: fermentation, high-throughput, scale-down, online measurement, mathematical modelling

1. Introduction

Developments in fermentations face numerous challenges which may require expensive analytics, media components or special tools to facilitate aseptic work and sampling. Furthermore, these biological processes vary significantly. To overcome these difficulties, the process should be scaled-down in combination with high-throughput methods, resulting in many parallel, small-scale experiments. Such experiments are used in terms of strain and technological improvements as well as media optimization.

A good solution may be the consideration of micro-bioreactors. However, because of their high investment and operational costs, they have not become widespread in Hungary. While each can provide almost every service required for bioreactors, for example, aeration, agitation and sampling in addition to pH and temperature control, they possess considerable limitations, namely non-standard conformations resulting in scale-up difficulties, or special measurement techniques that are incompatible with standard methods.

A readily available alternative, to be more precise, Microtiter-Plates (MTP), for microscale high-throughput fermentations has already been presented and reported [1]. The basic principle is to use sterile ’96-Well’ microtiter plates with a special “sandwich cover” that facilitate sufficient aeration but reduce the likelihood of cross-infection. This system requires an adapter to be able to mount microtiter plates into a commercial rotary incubator shaker. The next issue is to analyse and follow the processes in the wells since their volumes are so small (*ca.* 100 μL) that sampling is impossible. Therefore, either a microplate reader is required or a simple office scanner to produce a grey-scale photo taken from the bottom of the plate. The colour of high cell-densities is close to white, but empty broths have a black background. In the case of species that produce high levels of acid, like *Lactobac*illus, even a pH indicator can be applied and besides a grey-scale photo a coloured one has to be taken as well; alternatively, $CaCO_3$ should be added at the start but this can disturb the scanner-based “photometry”.

Our partner (enzyscreen.com) even offers microtiter plates for fed-batch fermentations. To achieve this, the feed components are adsorbed onto the material of the MTP, and are programmed to slowly release the fresh substrate during cultivation. However, another innovative solution has been developed for small-scale fermentations using online monitoring: Biosan Ltd. (Lithuania) applies Reverse-Spin Technology in the equipment of their Personal Bioreactor (RTS-1). This cost-effective equipment rotates a standard Falcon tube, filled with *ca.* 10 ml of fermentation media, at different rotation speeds in several directions at various controlled temperatures using a variety of aeration holes on the cap. This instrument also involves a photometer to facilitate the programming of measuring frequencies at a given wavelength (λ = 850 nm). For calibrated and reproducible measurements, a constant film layer is necessary, therefore, the instruments increase the rate of

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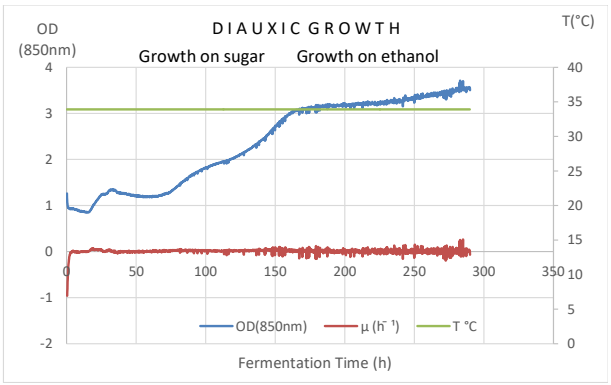
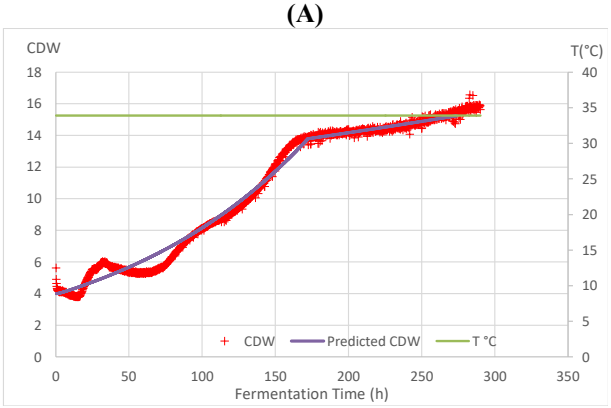
rotation until 2000 rpm. The changes in parameters effect shear forces as well as levels of aeration.

Finally, this comparative study used an impedimetric system by SY-LAB (Austria) which is called BacTrac 4100 [2]. This equipment possesses a block thermostat composed of 40 measurement cells, each containing 4 electrodes. One pair of them follows the changes in the impedance of the media, M%, caused by the secreted acids and metabolites. In the case of microorganisms that exhibit high levels of ionic strength in the media, it is hard to detect M%, therefore, with the application of a different frequency the changes in impedance on the other electrode surface (E%) can be followed. In direct measurements, these electrodes are immersed directly into the culture, but in the case of indirect measurements, they are rinsed with KOH which can adsorb the formed CO_2 released by the culture. While this system does not possess mixer/aerator solutions, this result can be transferred carefully to the known systems, namely benchtop fermenters or shaking flasks. However, it is able to follow forty different cultures.

In this study, a well-known model organism (*S. cerevisiae*) was chosen that exhibits special biochemical behaviour. It was used to test the compare the ability of the three systems introduced above. What is special about *S. cerevisiae* is that it can change from aerobic to anaerobic cultivation according to Pasteur and Crabtree effects; i.e. under lack of oxygen or excess to sugar, respectively. After changing to anaerobic metabolism, it produces mostly alcohol but later this can be consumed by yeast as well resulting in a stepwise growth curve, also referred to as a diauxic growth profile. Thus, the question was whether such a system could show and follow this diauxic growth.



(A)



(B)

Figure 1. The measured parameters (temperature, μ, OD_{850}) and calculated data (CDW from OD_{850}) along with the data of the predicted (i.e. fitted) model. (A) green line: temperature; blue line: measured turbidity at 850nm; brown line: measured specific growth rate. (B) green line: temperature; red crosses: calculated cell dry weight from the measured turbidity at 850nm; purple line: fitted model-based prediction for CDW.

2. Experimental

Commercial *S. cerevisiae*, i.e. baking yeast produced by Lesaffre, was cultured on a media of molasses that were diluted by a factor of 10 resulting in a saccharose concentration of ca. 75 g dm^{-3} and a 20:1 volume of molasses to NH_4OH ratio at 34°C. The 100 μL of inoculum possessed a cell-dry-weight (CDW) content of 10 g/dm^3. RTS-1 collected the data in a Microsoft Excel database. BacTrac only provided the data collected on screen plots, but with the help of Digitizelt v.2.3 software the measurement data was transported into Microsoft Excel. To compare the data in Microsoft Excel, the structured model of Blanch et al. [3] was adopted and programmed in Berkeley Madonna for Windows 8.1. This model can describe both anaerobic cell growth on excess sugar with the formation of alcohol and aerobic cell growth on alcohol as a substrate. It divides cells into two main compartments, i.e. substructures: one is responsible for metabolism (both aerobic and anaerobic), and the other is responsible for cell division. The parameters, for example reciprocal yields and stoichiometric coefficients of the model, were partly determined experimentally, but others were determined by non-linear model fitting, i.e. model calibration on real samples.

3. Results and Analysis

3.1. Reverse-Spin Technology vs. Personal Bioreactor (RTS-1)

Fig.1 presents the results of RTS-1. While optical density (OD), i.e. turbidity at λ = 850nm, changed slowly, the specific growth rate calculated online only reflected the uncertainty of the OD measurements, but the temperature remained constant as expected. Additionally diauxic growth was also detected but over a very long period of time. The model fitting was quite difficult because a satisfactory fit was only achieved after remarkable changes to basic constants, for example maximum specific growth rates on both substrates, etc., had been applied.

3.2. Impedimetric System: BacTrac

In the case of the impedimetric experiments, three different arrangements were tested: an anaerobic cell with

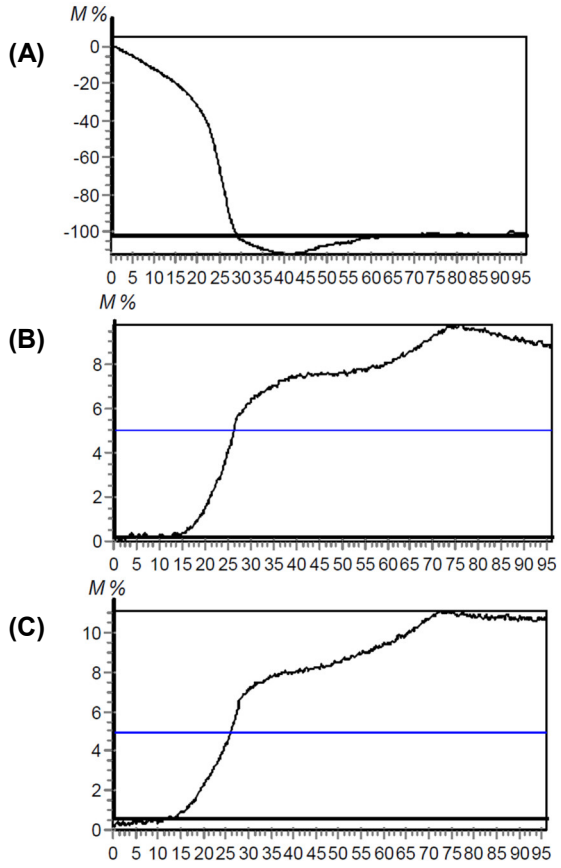


Figure 2. The results of three BacTrac measurements: relative changes in impedancy in the M% of media *vs.* time (h) - (A) indirect-; (B) aerobic-direct-; (C) anaerobic-direct measurements.

an incorporated valve for gas release, an aerobic one, and an indirect one (*Fig.2*). Only M% values yielded explainable curves. Indirect measurements yielded an inverse growth curve (decreasing) as expected, but did not exhibit a two-step decrease, i.e. diauxic growth, therefore, M% values of direct measurements were evaluated. The two curves of aerobic and anaerobic M% values were very similar to each other, but perhaps the anaerobic example is more relevant as in the case of high sugar content, the metabolism of yeast shifted in the anaerobic direction. *Fig.3* shows the fits of the model in which less constants had to be changed and diauxic growth was detected.

4. Conclusion

Both tested systems – Personal Bioreactor (RTS-1, Biosan) and BacTrac (SY-LAB) – detected diauxic cell growth of baking yeast. RTS-1 seemed to be a little bit more flexible, but BacTrac gave faster results, was able to make 40 measurements at the same time and offered three options in terms of evaluation. Maybe in the near future a solution to regular automatic sampling from

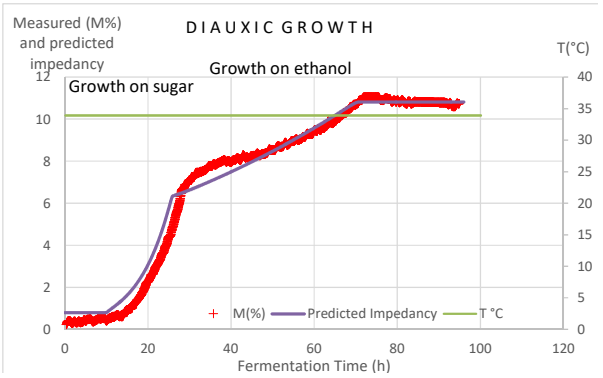


Figure 3. Model fitting to the anaerobic BacTrac curve (M%): red crosses: measured data; purple line: model-predicted values; green line: temperature (°C).

larger-scale fermenters will be found and then the results can be compared with the ones presented here.

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