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Characterisation of operation conditions and online monitoring of physiological culture parameters in shaken 24-well microtiter plates

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Abstract:

A new online monitoring technique to measure the physiological parameters, dissolved oxygen (DO) and pH of microbial cultures in continuously shaken 24-well microtiter plates (MTP) is introduced. The new technology is based on immobilised fluorophores at the bottom of standard 24-well MTPs. The sensor MTP is installed in a sensor dish reader, which can be fixed on an orbital shaker. This approach allows real online measurements of physiological parameters during continuous shaking of cultures without interrupting mixing and mass transfer like currently available technologies do. The oxygen transfer conditions at one constant shaking frequency (250 l/min) and diameter (25 mm) was examined with the chemical sulphite oxidation method. Varied filling volumes (600–1,200 µL) of *Escherichia coli* cultures demonstrated the importance of sufficient oxygen transfer to the culture. Cultures with higher filling volumes were subjected to an oxygen limitation, which influenced the cell metabolism and prolonged the cultivation time. The effects could be clearly monitored by online DO and pH measurements. A further study of different media in an *E. coli* fermentation elucidated the different growth behaviour in response to the medium composition. The MTP fermentations correlated very well with parallel fermentations in shake flasks. The new technique gives valuable new insights into biological processes at a very small scale, thus enabling parallel experimentation and shorter development times in bioprocessing.

Key-words: Optode, oxygen sensor, pH sensor, oxygen transfer, microbial growth