

Exhaust gas monitoring of fermentation processes

Exhaust gas analysis provides a powerful tool for process control and troubleshooting at all scales of fermentation.

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When fermentation is carried out on a batch basis, the proportions of carbon dioxide and oxygen are reliable indicators of the efficiency of the production process and the status of the bioreactor. While traditional instrumentation on fermenters provides data for control loops, thereby ensuring constant conditions, it is not well suited for the analysis of fermentation metabolism. Production staff soon realised that a better indication of the emissions of CO₂ and O₂ could help them determine the respiration rate in the fermenter and thereby increase the efficiency of production.

Exhaust gas monitoring is a powerful tool for process control and troubleshooting at all scales of fermentation, as fermentation activity is reflected in the consumption of O₂ and the production of CO₂. Any deviation from the optimal metabolism of the micro-organism will be reflected in the ratio between production of carbon dioxide and consumption of oxygen - the so-called respiratory quotient (RQ). Exhaust gas analysis of fermentation

processes based on integrated photo-acoustic spectroscopy and magneto-acoustics (PAS/MA) offers a cost-effective method of measurement with minimal maintenance costs and a high level of measurement quality.

Background

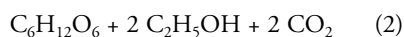
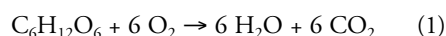
In the operation of fermentation-based processes, it is important to be able to monitor the status of the process in the fermenter. Standard fermenters are equipped with instrumentation used in control loops, which keep the controllable conditions of the fermentation constant - for example, flow rates of feed and exhaust gas, pressure in the fermenter, pH level, dissolved oxygen and temperature in the broth, and agitator power input.

Since microbial activity is the most important parameter in the fermenter, it would be of great value to monitor this parameter. Monitoring of the amount of biomass will include living as well as dead cells, whereas monitoring of the consumption of oxygen (oxygen uptake ratio, OUR) and the

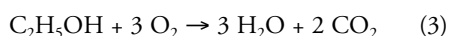
production of carbon dioxide (carbon dioxide evolution rate, CER) will directly reveal the activity of the biological culture. The analysis of fermenter off-gases may thus be used to obtain important knowledge about microbial activity.

As an example of the potential of off-gas analysis in monitoring fermentation processes, the states of operation of *S. cerevisiae* will be described; the principles may, however, be equally well applied to other cases, for example, the consumption of biomass instead of glucose.

In the desired aerobic fermentation, carbon dioxide and water is produced from glucose with a high production of energy through (1), and in anaerobic fermentation, ethanol is produced from glucose with less energy production through (2).



If excess glucose is present, then ethanol may be produced through (2) - even under aerobic conditions. Finally, an aerobic state of the fermentation exists where ethanol is consumed producing water and carbon dioxide through (3), but this state is only active in the absence of glucose. In a continuous culture, the ethanol produced would typically be washed out before being oxidised.



Production of ethanol is unwanted since less energy is produced compared with complete oxidation and, in addition, the presence of ethanol may inhibit growth of micro-organisms and thus production of the protein desired from the fermentation. To avoid anaerobic production of ethanol, traditional control of fermentation processes has included monitoring of dissolved oxygen; an absence of dissolved oxygen would indicate a situation where fermentation conditions were anaerobic, and production of ethanol would occur. Monitoring of dissolved oxygen is, however, insufficient for identifying ethanol production since ethanol may also be produced under aerobic conditions with excess glucose; furthermore, the broth concentration of ethanol will be an integrated parameter, changing slowly compared with the immediate change in exhaust gas. Instead, exhaust gas analysis has been employed. If (1) is the overall reaction taking place in a fermenter, the consumption of oxygen matches the production of carbon dioxide, and the respiratory quotient (RQ) is unity. Similarly, (2) will result in no consumption of oxygen parallel to production of carbon dioxide (with RQ approaching infinity), and (3) in an RQ of 2/3 (Table 1).

Reactions	CER and OUR	RQ	Description
(1), (2)	CER>OUR	RQ>1	Aerobic ethanol production
(1)	CER~OUR	RQ~1	Desired aerobic state
(3)	CER~2/3OUR	RQ~2/3	Aerobic ethanol consumption (glucose exhaustion)
(2)	OUR~0	RQ~∞	Anaerobic fermentation

Table 1. Four possible states of fermentation. States I-III are not likely to be distinguished by measurement of dissolved oxygen. State II is the desired state of the fermentation.

Monitoring of fermentation exhaust gases

One method for monitoring exhaust gases is integrated photo-acoustic spectroscopy and magneto-acoustic (PAS/MA) monitoring; PAS technology is used to measure carbon dioxide and hydrocarbons, and oxygen is measured by means of MA. The method was described by Christensen *et al* (1) and is employed worldwide in both laboratory-scale, pilot project-scale and full-scale fermenters.

The PAS/MA technology is very accurate and incredibly stable over long periods of time (instrument calibration is only required every 6 or 12 months). In addition, the instruments are designed to be optimally sensitive under the conditions of a fermenter. The principle of PAS/MA is characterised by the synchronisation of measurements and simplicity of use. An important aspect of instrumentation, especially in relation to production, is down-time and maintenance costs. The PAS/MA measurement principle is known for its high degree of stability, and associated down-time and other maintenance costs are minimal. Compared with other technologies (mass spectrometers, gas chromatographs and traditional IR transmission spectroscopy), PAS/MA is almost maintenance-free and offers a considerably more cost-effective solution.

In production with fermenters, it is of course important to be aware of the rate of production. During the initial stages of a new culture, growth is typically exponential and should be identical to that observed previously; any deviation from this must be determined. The parallel use of CER and OUR ensures that independent measurements of microbial activity are available, and the use of RQ may provide an explanation for deviations from expected levels. If an error in the feed to a cultivation is observed at an early stage, it is possible to correct the feed without having to stop the culture: the early warnings provided by the rapid response of gas analysis are clearly to be preferred for this purpose. In addition to troubleshooting, the results from exhaust gas analysis may be used in an active control scheme,

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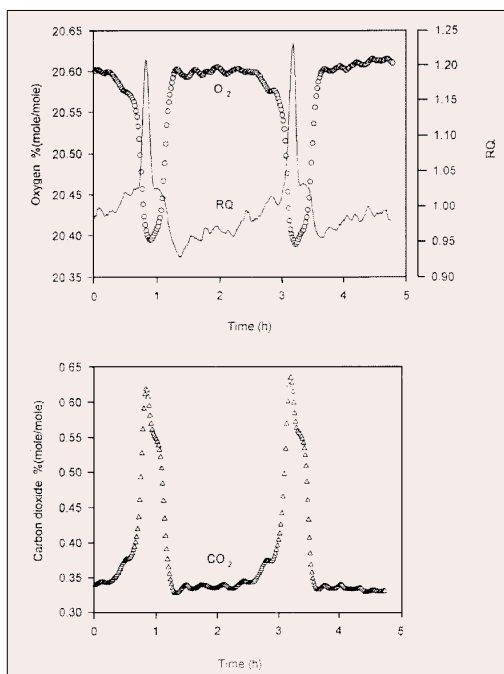


Figure 1. Exhaust gas analysis from an oscillating continuous culture of *S. cerevisiae*. (From Christensen *et al.* (1)).

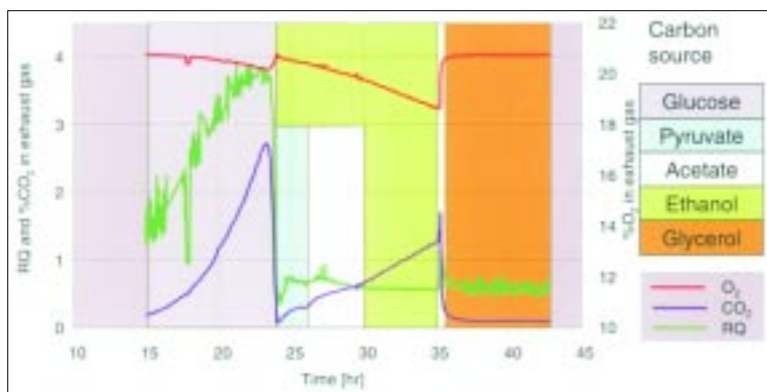


Figure 2. Exhaust gas analysis may be used to identify the carbon source in batch fermentation. Small variations in O_2 , CO_2 and RQ were used to classify the carbon source as indicated by colours. The classifications correspond perfectly with HPLC measurements. (Data courtesy of Frede Lei, Technical University of Denmark).

which may in turn ensure higher productivity levels for the plant.

Laboratory-scale case study

In a laboratory study, Christensen *et al* (1) studied the oscillation of *S. cerevisiae* in a continuous culture, and found that the oscillations in the process were well characterised using a PAS/MA analyser (Figure 1). The reproducible oscillations shown in the figure are not only a fascinating

phenomenon, they also have the practical implication that ethanol is produced in periods of the fermentation, which may reduce the productivity of, for example, a recombinant culture genetically engineered for the production of insulin. The authors concluded that the high time-resolution and synchronicity of the O_2 and CO_2 signals provided by PAS/MA were important for the study of the oscillations.

Figure 2 demonstrates the power of exhaust gas analysis. Here, the oxidation of different intermediates is identified through analysis of exhaust gases and, at the same time, the activity of the yeast culture is reflected in the exhaust concentrations of oxygen and carbon dioxide.

During the development of new biological cultures, the rapid feed-back from off-gas analysis makes it easy to see the effects of variations in operating conditions, both to identify optimum conditions for operation and to analyse unexpected observations in the laboratory

Instrumentation

The instrument described by Christensen *et al* has been optimised for on-line use in the monitoring of fermenters, and is marketed by Innova AirTech Instruments as Fermentation Monitor 1313. The monitor may be equipped with a multiplexer for sampling and process control of several different fermenters, as shown in Figure 3, or it may be used for pilot project or laboratory-scale monitoring.

Using PAS/MA technologies, it is possible to construct a measurement system with a measurement chamber with a volume of 0.15 cm^3 . This means that the instrument is "satisfied" with a continuous gas flow of 130 ml/min and, in spite of this, reacts very quickly. The PAS/MA measuring system is integrated so that CO_2 /hydrocarbons and O_2 are detected in real-time by the same detector. Therefore, the correct time correlation between the gases is obtained. The 1313 is controlled by PC software which is used to set up measurements and collect data. Analogue outputs for each gas concentration are present at the rear plate of the



Figure 3. Innova AirTech Instruments' Fermentation Monitor 1313 with Multipoint Sampler 1309.

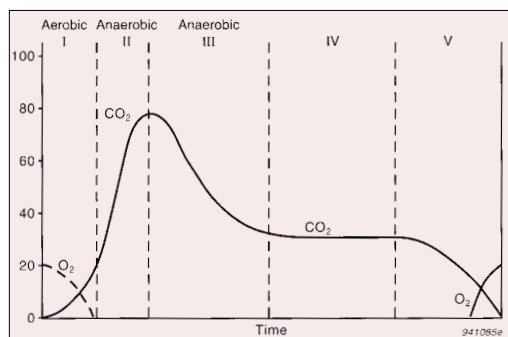


Figure 4. Online monitoring of gases using the Application Software.

monitor. The monitor has a built-in pump system that draws gas samples continuously.

A gas multiplexer (the Multipoint Sampler Type 1309) makes it possible to monitor up to 12 different fermentation processes with the same fermentation monitor. The monitor can be connected to three multipoint samplers, expanding the monitoring capabilities to 36 channels. The multipoint sampler has a pneumatic valve system with 12 inlet channels; each inlet channel is fitted with a tube-mounting stub for 3mm tubing. All inlet channels are joined together inside the sampler by a manifold. The manifold and valve system are optimised with regard to "dead volume", so that fast response times can be obtained when changing from one gas channel to another. The 1309 sampler is constructed of stainless steel and PTFE (polytetrafluoroethylene) to ensure minimum adsorption of gases onto the internal components.

The Multipoint Sampler 1309 is controlled from the same PC as the Fermentation Monitor 1313; the Application Software controls both the monitor and the sampler. Using the software, it is possible to select active sample channels, sampling sequences and purge times to ensure that a "fresh" gas sample is always available (Figure 4).

Small- and large-scale applications

Innova Airtech Instruments supplies fermentation monitoring systems for applications such as small-scale fermentation processes in research and development and pilot plant-scale facilities, as well as systems for large scale production lines. Such application areas often require the following:

- *Small sample volume due to the limited amount of sample gas in small fermentation vessels* Due to the very small measuring cell in the fermentation monitor, and the optimised valve/manifold system in the multiplexer, the sample size can be kept to a minimum.

- *High humidity in gas samples can interfere with other gases* Interference from water vapour in the gas sample is electronically subtracted to ensure correct reading of CO₂ and hydrocarbons.
- *Fast response time to ensure all changes regarding CO₂ and O₂ in the fermentation process are registered* The PAS/MA measuring system in the 1313 has a sample rate of up to five measurements per second, which ensures that fast fluctuations in the metabolism of the microbial cultures can be observed.
- *Low maintenance frequency ensures high stability* The maintenance cycle in PAS/MA measuring systems is very low. Check calibrations are only necessary every 6 to 12 months. In addition, the 1313 does not need any kind of additional gases or other consumables in order to function.
- *Built to withstand an industrial environment* The fermentation systems can be installed in industrial graded racks which include all the necessary gas conditioning (water/foam traps and special Nafion® tubing to equalise the content of water vapour in the gas flow before it reaches the monitor).

Conclusion

At present, the biotechnology industry uses a varying level of instrumentation for their fermentation processes. While the industry agrees that there is a need to ensure that fermentation conditions are constant, exhaust gas analysis has only recently started to become more widely used. Process descriptors such as the respiratory quotient (RQ) are very important, since this reveals process information which is not available through traditional fermenter instrumentation. In addition, knowledge of oxygen uptake rate (OUR) and carbon dioxide evolution rate (CER) provides a measure of the production in the fermenter.



Anders Broe Bendtsen holds a PhD in Chemical Engineering. His experience in the analytical sciences is widespread, working with analysis of combustion gases, milk, beer and fermentation; he also has a strong interest in the field of chemometrics.

Reference

1. Christensen LH, Schulze U, Nielsen J and Villadsen J (1995). Acoustic off-gas analyser for bioreactors: precision, accuracy and dynamics of detection. *Chem Eng Sci* 50 (16) 2601-2610.

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