Application note

Biomanufacturing

In support of a sustainable future:

How online process analytics are assisting the biofoods industry

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Keywords

Biotechnology, food, enzymes, alternative protein, mass spectrometry, scanning magnetic sector, off-gas analysis, respiratory quotient (RQ)

Introduction

To quote the Office of the President of the United States of America, "The world is on the cusp of an industrial revolution fueled by biotechnology and biomanufacturing."[1] These are the opening lines in a document outlining the purpose of Executive Order 14081 on advancing biotechnology and biomanufacturing innovation for a sustainable, safe, and secure American bioeconomy.[2]

Biotechnology and bioprocesses are present in many aspects of our daily lives, most prominent is the development and production of pharmaceutical products, many people would have learned of the importance of this sector during the COVID-19 pandemic when billions of doses of vaccines were produced by the global biopharma industry, but the reach of biotechnology extends beyond medicine into fuels, plastics and other materials, and food products.

While it is difficult to accurately define the value of this sector, many reports state that biotechnology accounts for more than a trillion dollars of global revenue and at annual growth rates exceeding 14% this value is forecast to reach around 3 trillion dollars by 2030.

The subject of this application note is bio-based food products and how online analytics are implemented to support this part of the biotechnology industry.

This application note will describe how online process mass spectrometry (MS) is used as an effective process analytical tool in biofood production, reporting in real-time critical process parameters that have a positive impact on quality attributes of fermentation processes.

Biofood products

A good example of a biofood product is enzymes; in the chemical industry, the most important component is surely the catalyst after all 90% of all chemicals and fuels produced involve a form of catalysis and these products account for around 30% of global domestic product.[3] Enzymes are a form of catalyst, in the past enzymes used in the food industry would have derived from animal offal and plant extracts, today these are produced primarily by microbial fermentation.[4]

Enzymes used in food production effect a surprisingly wide range of attributes in the food, a staple such as flour can be altered (quite naturally) by an enzyme called α-amylase to produce bread that has better volume, texture, flavour, and longevity; another example is the enzyme Protease which weakens the gluten making the resulting dough softer, easier the shape and shortens dough mixing times.

Increasingly prominent on supermarket shelves are alternative proteins[5] including those to displace meat, seafood, and dairy products. Among the range of alternative proteins are those derived from fermentation such as mycoprotein or to give it its full title *Fusarium venenatum* is a thread-like fungus which can be used as a food product or as an ingredient blended into food. Biomass fermentation is the process by which these protein-rich microorganisms are quickly grown until that biomass is "harvested."

Then there is precision fermentation where gene sequences of animal proteins are introduced into organisms such as yeast, the fermentation of this yeast then generates the necessary quantities of the target protein[5] (egg, dairy, and meat proteins).

Overcoming the objections of consumers to alternative (non-animal) proteins will be necessary for them to be adopted widely. Fermentation processes can produce proteins with fibrous textures similar to animal meat, precision fermentation is a process used to create a protein called myoglobin which is found naturally in meat and gives the product its unique smell and taste. As production volumes of fermentation-based proteins are still relatively small this sector will need to follow the biopharmaceutical industry in improving efficiency in development and production to make products more cost effective and therefore available at the right price point for consumers, this is where effective process analytical technology (PAT) can play an important role.

Implementation of a PAT strategy can be described by three steps, when a critical quality attribute (CQA) is identified as having an impact on process and/or product quality, firstly determine which critical process parameters (CPP) impact that CQA, next continuously monitor those CPPs and finally control CPPs to effect the desired improvements. Refer to Figure 1 for the components of the PAT process.

One place where PAT has a proven track record is upstream bioprocess; monitoring of fermentation and cell cultures with online process analyzers pre-dates the FDA guidance and has become the principal method by which these processes are tracked in real time.

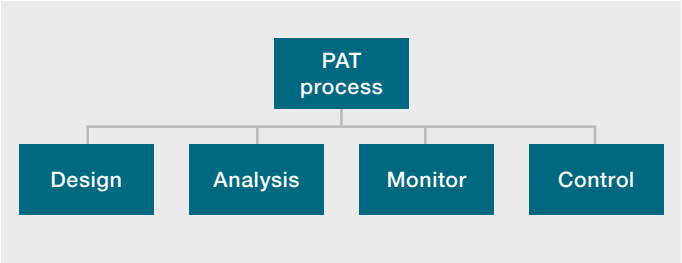

Figure 1: The steps of the PAT process.

The need for gas analysis

Fermentation processes require continuous monitoring to ensure good outcomes. Online monitoring provides real-time insight to the health of the culture, how nutrients are metabolised, whether any unwanted by-products are present, and vigilance to the risk of poisoning. There are a range-of-analysis tools available to the fermentation laboratory, some are inserted into the fermentor (such as dissolved oxygen probes or probes for pH) while others are outside the vessel (and outside the sterile environment), these include gas analyzers for monitoring respiratory activity, from this gas analysis cell growth kinetics, substrate consumption and preferred end point for maximum yield are derived.

Why use mass spectrometry for gas analysis?

The rapid analysis speeds of mass spectrometry make it an ideal candidate for respiratory gas analysis, especially where the monitoring of multiple fermentors is required, but mass spectrometers are not only fast, they can also be very precise at the same time. This precision is an important characteristic as during critical stages of fermentations, changes in oxygen and carbon dioxide concentrations can be very small, and poor precision in gas analysis risks missing important process transitions. Figure 2 shows the typical installation of a fermentation gas analysis setup.

The highest performing MS in this field is the scanning magnetic sector type, this MS will report the percentage level oxygen and carbon dioxide concentrations with a precision of just a few parts per million (ppm), while still achieving measurement cycle times of ~10 seconds per measurement point.

By coupling the fast and ultra-stable magnetic sector MS with a reliable multi-stream inlet selector it's possible to apply a single MS to as many as 50 fermentors while maintaining a cycle time of just a few minutes. The MS is also very versatile, it offers more than just the measurement of oxygen and carbon dioxide but can also measure inert gases such as nitrogen and argon as well as a range of volatiles including methanol, ethanol, and others. Figure 3 shows the Thermo Scientific™ Prima BT scanning magnetic sector MS.

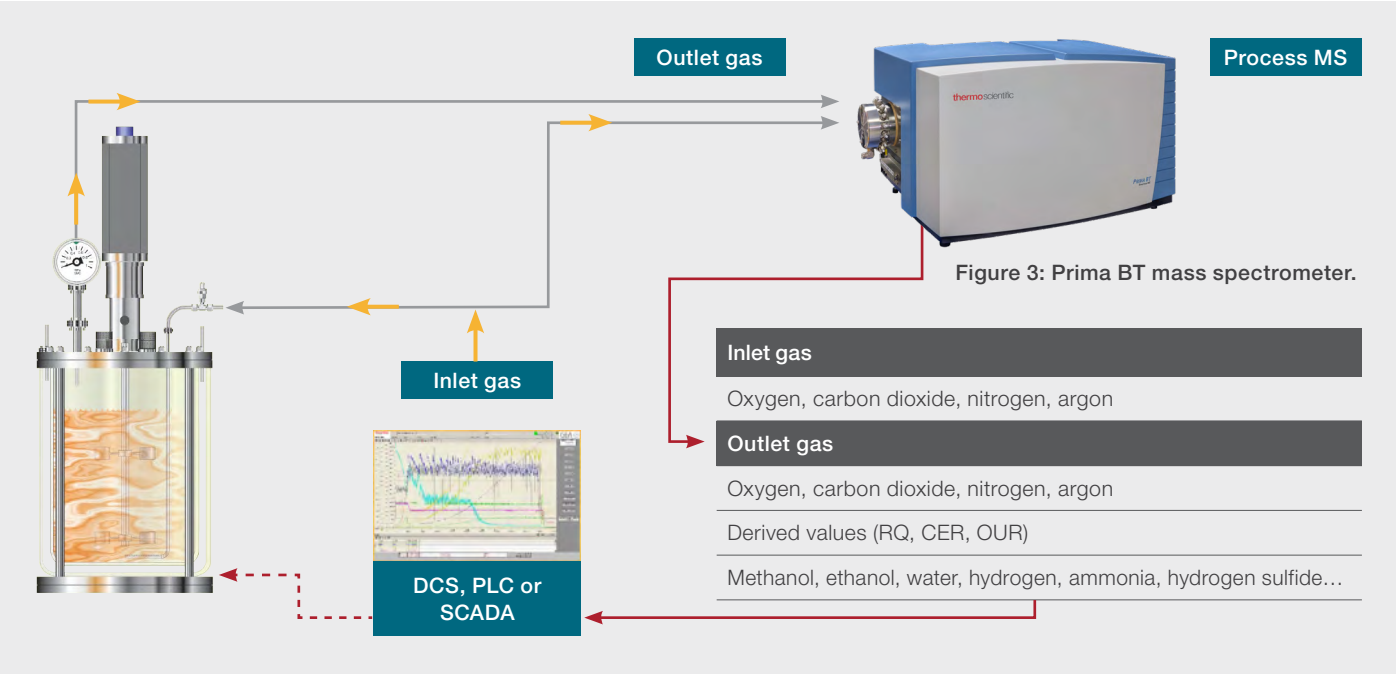

Figure 3: Prima BT mass spectrometer.

Figure 2: A typical fermentation gas analysis setup.

Respiratory quotient

The process where cells oxidize food to produce energy is respiration. During aerobic fermentation cells consume oxygen, referred to as the oxygen uptake rate (OUR), and produce carbon dioxide referred to as the carbon dioxide evolution rate (CER), a comparison of the rate of change in these values yields the parameter Respiratory Quotient (RQ). The full calculation of RQ is shown in Table 1.

CER (CO_2 evolution rate)	=	(%Volume of CO_2 out x flow out)	-	(%Volume CO_2 in x flow in)
OUR (O_2 uptake rate)	=	(%Volume of O_2 in x flow in)	-	(%Volume O_2 out x flow out)
RQ (respiratory quotient)	=	CER/OUR		

Table 1: Respiratory quotient for fermentation off-gas analysis.

Highly efficient metabolism resulting in high levels of carbon dioxide being produced yields a high RQ value, the RQ number also enables the identification of the carbon source being metabolized, and ultimately control of the RQ based on respiratory gas analysis can be implemented. Where glucose (or other carbohydrates) is the carbon source, then RQ will theoretically be 1. However, this equation only relates to combustion, it does not cover the production of biomass or product formation. So for example, the RQ for glucose is unlikely to be 1.00, it may be something like 1.04.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

$$RQ = \frac{6CO_2}{6O_2} = 1$$

Example of a fed-batch fermentation

Figure 4 displays data from a fermentation of S. cerevisiae (yeast). In the beginning, the carbon source being consumed is ethanol, later this is exchanged for glucose which prompts the cells to produce the desired product. Note the period up to ~10 hours cell count and the corresponding consumption of oxygen is very low (lag phase), this results in a relatively unstable RQ measurement. When organisms multiply more rapidly we observe an acceleration in oxygen consumption (OUR) and carbon dioxide evolution (CER), from this point the RQ value becomes very stable and the value ~1 corresponds with the metabolism of glucose. The mass spectrometer measures the concentrations of oxygen, nitrogen, carbon dioxide, and argon on the inlet (sparge) gas stream and in the outlet stream the same gases are measured as well as ethanol which is representative of the amount of ethanol in the fermentor headspace.

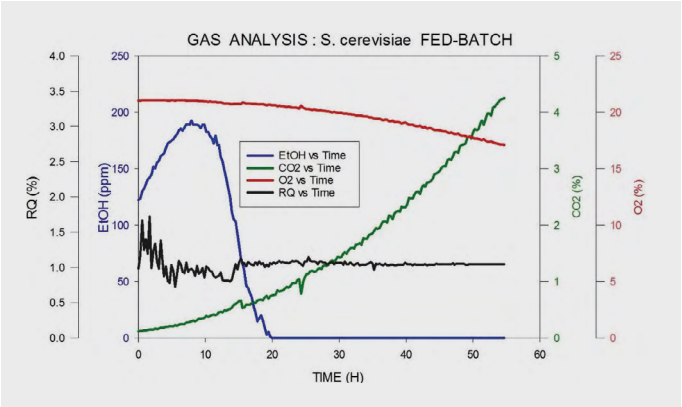

Figure 4: Off-gas and RQ data generated by MS from S. cerevisiae fed-batch fermentation.

The choice of MS technology is important as there are many different types and they each have their unique characteristics. The type that has been proven over many decades to have superior analytical performance, as well as longer periods between calibration and maintenance, is the scanning magnetic sector MS. The scanning magnetic sector MS separates positively charged ions generated from the sample gas molecules in a variable magnetic field prior to measuring the current generated by ions of each mass at a Faraday detector. The spectral peaks produced in the magnetic field have a very symmetrical shape with a flat top, the height of a peak is directly proportional to component concentration and its flat top ensures consistent height measurement while being very tolerant to small variations in peak position. Figure 5 shows the design of a scanning magnetic sector MS.

MS performance for fermentation monitoring

The excellent long-term stability provided by magnetic sector MS is illustrated in Table 2. A Prima BT benchtop gas analysis mass spectrometer was configured to analyze nitrogen, oxygen, argon, and carbon dioxide in a cylinder of compressed air continuously, without interruption or recalibration, for seven days. The analysis cycle time was 5 seconds to measure these four components. Day-to-day mean values for nitrogen and oxygen varied by 0.005 %mol or less, and day-to-day mean values for carbon dioxide varied by 1 ppm or less.

Summary

Biotechnology applied to pharmaceutical production has sustained ongoing improvements to the health of the human population for many years, the biotechnology industry also supports the development and manufacture of many food products and ingredients and concern over the sustainability of food production and agriculture grows the worlds largest economies will increase their utilisation of biofood products.

Efficient biotechnology depends on analytical systems to elevate process knowledge and improve the outcomes of fermentation processes. Within this field of PAT, the use of gas analysis mass spectrometers enables an insight into cell metabolism enabling good decision-making during processes and the potential for automation of fermentation control.

The magnetic sector MS is the most precise of all process MS while inherently fast; the versatility of this technique allows users to analyze many gas components including air gases and other volatiles in the inlet and outlet streams of multiple fermentors while remaining outside of the sterile environment. Process MS is a cost effective, low risk and highly dependable PAT tool which is already in place at many hundreds of biotechnology facilities globally.

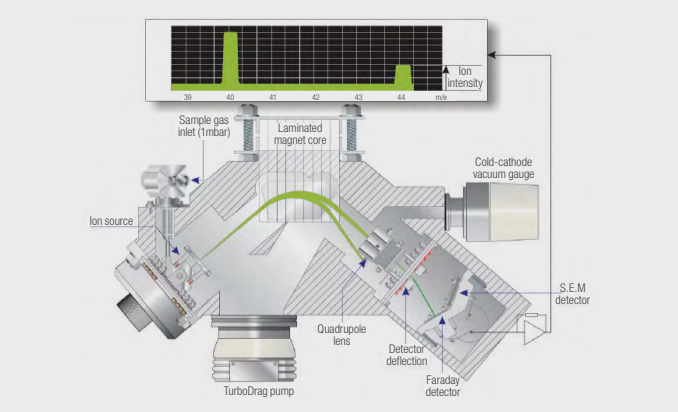

Figure 5: Scanning Magnetic Sector analyzer of the Prima PRO MS.

	Nitrogen %mol		Oxygen %mol		Argon %mol		Carbon Dioxide ppm	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Day 1	78.0807	0.0028	20.9459	0.0026	0.9337	0.0003	396.84	1.31
Day 2	78.0767	0.0023	20.9494	0.0023	0.9342	0.0003	397.46	1.25
Day 3	78.0761	0.0024	20.9500	0.0023	0.9342	0.0003	397.34	1.28
Day 4	78.0798	0.0023	20.9469	0.0023	0.9337	0.0003	396.31	1.31
Day 5	78.0777	0.0030	20.9487	0.0028	0.9339	0.0003	396.76	1.34
Day 6	78.0741	0.0023	20.9518	0.0022	0.9344	0.0003	397.47	1.27
Day 7	78.0750	0.0023	20.9512	0.0022	0.9342	0.0003	397.23	1.30

Table 2: Long-term stability by magnetic sector MS.

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