Bioreactor characterization at a new level

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The pharmaceutical industry needs powerful systems for efficient production of preparations, and against this background the demands on bioreactors have become ever more exacting over the past few years. Bioprocessing is a complex topic: numerous factors have an impact on cell growth but are difficult to be determined and interpreted reliably. Optimal growth conditions are crucial for a satisfying high quality product yield: optimum temperature and pH, efficient nutrient supply, discharge of metabolites and carbon dioxide stripping capacity are essential parameters. Process engineers make huge efforts to provide the cells with optimal process conditions and to guarantee reproducibility and consistent product quality. Detailed knowledge about performance parameters and their interdependencies support the plant design and allow a more precise prediction of scaling effects.

One of the most relevant performance parameters is the volumetric mass transfer coefficient (kLa). It describes the efficiency of gas transfer (e.g. oxygen) from gaseous to the liquid phase, because only dissolved oxygen can be metabolized by living cells. To allow cell metabolism to function at its fastest, the dissolved oxygen concentration has to be above a critical concentration level at any point of the bioreactor.

The k_La value is not easy to predict, because it is influenced by a number of different parameters: process parameters (agitator speed, temperature, pH, gassing rate), physiochemical characteristics of the medium (viscosity, density, salt content, surface tension, coalescence behaviour) and geometries (vessel, agitator, sparger) are only some of them. This is why a reliable measuring method for the constant monitoring of k_La is an essential requirement. The kLa value determination in a timely manner at different stages of a process represent a reliable PAT tool that makes bioprocesses more transparent.

Difficulties of exact kLa measurements

In order to predict the kLa value despite numerous influencing hydrodynamic conditions, experimental measurements are combined with mathematical models that describe the systems kinetics. Garcia-Calvo et al.[1] propose an adjusted version of the standard equation, which considers the delay time (time of dissolved oxygen to reach the sensor) as well as the response time of the electrode.

Traditional k_La measurements use standard oxygen sensors that measure dissolved oxygen at only one point in the bioreactor: at the probe belt near the bottom of the reactor. So far, these sensors had to be as robust as possible in order to stand recurrent CIP/SIP cycles. That's why membranes of these sensors have a high layer thickness, although this means a slower diffusion of oxygen molecules and long response time, which effects measurement accuracy. Since measurements were taken at only one point in the bioreactor so far, gradients of dissolved oxygen within the bioreactor were not considered and data were not valid throughout the whole vessel. Additionally, gas bubbles gave wrong signals.

[1] Garcia-Calvo 2000 – Thomas?

New method brings improvement

ZETA Biopharma – who is specialized on tailor made solutions for the design and construction of bioreactors – picked up this shortcoming and developed a new method for the measurement of k_La: A mobile measurement tool with an oxygen sensor was developed in a way that it is compatible to any nozzle of the bioreactor for sampling (in-line). The sampling unit is easily adaptable to any vessel geometry or agitator design and enables to sample also in higher regions of the bioreactor. Furthermore, it includes a filter that eliminates gas bubbles, and a peristaltic pump guides the sample to an optical high-speed sensor that measures dissolved oxygen within tenths of a second. The device is easily movable and twistable, thus, from now on, measurements for the kLa value determination are possible at any point in a bioreactor, resulting in a comprehensive picture of actual production conditions within different vessel zones.

For the kLa determination, ZETA decided for the dynamic startup method (DSM)[2]. A big advantage of the method is the availability of all technical requirements at commercial scale, such as sterile aeration and nitrogen supply. In a first step, dissolved oxygen is stripped out with the help of nitrogen. Thus, the oxygen content is 0%, when the measurement starts. Subsequently, by starting the agitator and gassing system, the oxygen concentration starts to increase. By integration of the dO_2 curve the precise kLa value can be calculated.

In a final step, the mathematic model and the actual data from measurements are iteratively adapted for the determination of the kLa value (Figure 1). The measurements at different areas in the bioreactor allow a correlation for each point. The closer these measurement points are defined the more reliable is the characterization of the whole bioreactor system.

Opportunities for the optimization of existing bioreactors

The new possibility of reliable k_La measurement enable process operators to get more information on the possible oxygen supply in the medium and indicates, how agitation speed and gassing rate influence the value. Knowing a precise kLa value facilitates the identification of risks during bioreactor design and it helps to generate representative scale-down models. Users can define their process parameters more precisely, they can compare different gassing systems or media, and the Ne-value, expressing the agitator power uptake, can also be determined.

A special benefit of ZETA's mobile measurement device is the applicability to existing plants without the need for any structural changes. In a specific application, a commercial scale bioreactor with a working volume of up to 12 m^3 and with three Rushton like impellers had to be characterized. For the measurement, three high speed pO_2 sensors were placed at defined positions (top, middle, bottom area of the bioreactor). Using the DSM method for kLa determination, the degassing with nitrogen was perfomed for 10 minutes followed by subsequent gassing with compressed air through the sparger. In more than 20 trials measurements were taken in order to explore different agitator speed, gassing rates, head pressure conditions and the effects of salt and antifoam in fermentation media.

ZETA's process engineers observed a clear zoning effect and the middle zone of the bioreactor was identified to provide the best growth conditions for the living cells: The highest kLa value was shown there, followed by the top and the bottom part – independently of gassing rate (figure 2). The oxygen dissolution from the head space has a significant impact on the kLa value within this zone (sensor pO_2A). Obviously, also the filling level of the cultivation broth is influencing values of this area.

[2] Linek V et al.: Analysis of differences in kLa values determined by steady-state and dynamic methods in stirred tanks. Chem Engin J 1982, 25, p77-88

The study showed that at higher agitator speed this zoning effect was reduced and a gassing optimum was observed at 1,6 vvm at maximum stirrer speed. Furthermore, the agitator speed was shown to become more important at higher gassing rates in order to finely disperse the gas bubbles. In other words, the agitator speed was the limiting factor for reaching a high kLa value.

By increasing the operating temperature from 24°C to 30°C the average kLa value was increased 40% at given agitator speed. A significant negative impact was shown by salts and antifoam, which led to a decrease of 54%.

The formation of different zones is strongly dependent on geometric vessel dimensions and process parameters, thus, very specific. Anaerobe zones, where oxygen is depleted, significantly reduce productivity and also quality, since undesired side products are formed. The new method for kLa determination allows a quantitative picture of these zones and supports the indication of appropriate process adaptations (e.g. agitator speed, gassing rate).

Further application fields

Up- and down-scaling

Another big challenge is the up- and down-scaling of fermentation systems. The ideal scaling process has to be defined based on numerous variables and characteristics, but some parameters, such as mixing times cannot be transferred in a linear manner – this always demands for compromises. The kLa value is an appropriate scaling indicator which allows the calculation of further quantities (e.g. combustion energy, heat development). These values represent a sound basis for the bioreactor and agitator design.

Nowadays, a lot of strain improvements as well as feed- and process optimizations are executed in technical scale-down models, which simulate production conditions in commercial scale. Again, the accurate characterization of performance parameters such as kLa and mixing time are decisive for a successful approach. They are used for the definition of the design space, which gives a frame for the optimization experiments in lab and pilot scale. A well-defined design space enables process engineers to a smooth process transfer from test equipment to the commercial scale.

Optimization of feed strategies

The primary carbon source for animal cell cultures is glucose, which is fed according to sporadic off-line glucose measurements. A disadvantage of the system is a permantently strong variation of glucose concentration, which has a negative impact on productivity and quality: a low glucose level inhibits growth, a high level leads to toxic metabolites and unusual glycosylation patterns[3].

The optimum level is reached by constant sampling in short intervals but this is time consuming and includes a high contamination risk during manual sampling. Thus, there is a demand for a non-invasive online tool for the glucose measurement. The application of an advanced process control strategy (APC) regulates the glucose feed by correlating glucose uptake rate with the oxygen transfer rate (OTR).[4] The integration of this model based control strategy into the GMP environment of industrial plants is challenging and demands for a comprehensive automation know-how in DCS systems. The model allows an online

[3] Wong DCF et al.: Impact of dynamic online fed-batch Strategies on metabolism, Productivity and N-Glycosylation quality in CHO Cell cultures. Biotechnol Bioeng. 2005, 89(2) p 164-177

[4] Goldrick S et al: Online Control of Glucose Concentration in High Yielding Mammalian Cell Cultures enabled through oxygen transfer rate measurements. Biotechnol J, 2018, 13, 1700607

prediction of the glucose concentration during the whole cultivation time – the major requirement is again a precise kLa value! The implementation of this control strategy is easy: the linear correlation between cumulative OTR and glucose concentration serves as a soft sensor without the need for any additional sensors or changes in standard operating procedures.

Benefits at a glance

The importance of an accurate process validation is also emphasized by FDA (Food and Drug Administration). According to the general FDA guidance laboratory and pilot-scale models need to be representative and assist in the prediction of an industrial process. Therefore, it needs to be demonstrated that small and industrial bioreactor scales are comparable. The new kLa measuring method is a valid PAT tool that significantly improves bioreactor design and bioreactor characterization as well as process understanding.

It allows:

- Accurate kLa measurement at any defined position in the bioreactor
- Comparison and optimisation of different process conditions and parameters
- Revolutionary impact on QbD (quality by design) approach
- Comparison of bioreactor geometries, agitator and sparger design
- Improved flexibility since measurements are adaptable to different cultivation systems
- Bioreactor design comparison with a wide range of different analysed systems due to ZETA's long term experience as an engineering specialist and equipment manufacturer

The output is a higher product yield and quality, an increased product purity and safety and optimized processing times.

Figure 1: iterative approach for the determination of kLa value. The slope between 20 and 80% pO2 saturation was used to calculate the kLa value.

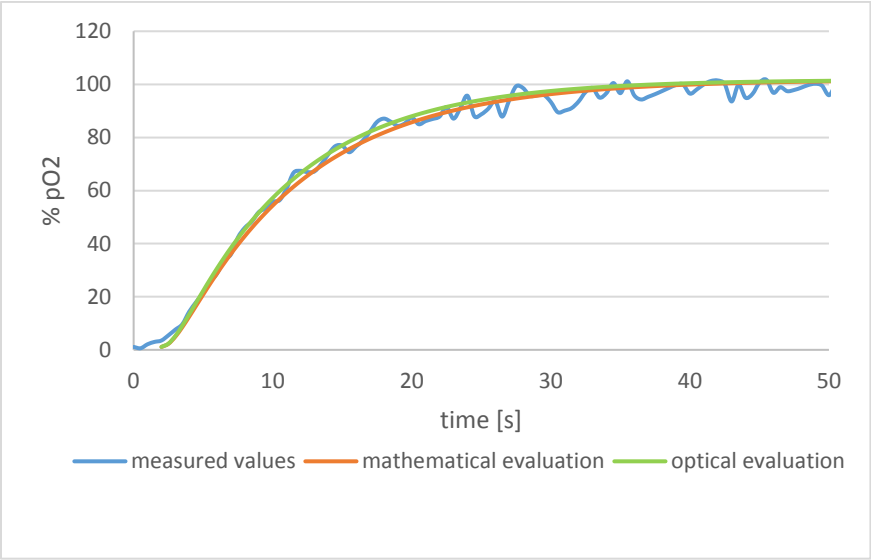

Figure 2: Correlation example: kLa vs aeration at 60 rpm