

Monitoring bio-processes using cellular autofluorescence

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The monitoring of bio-processes is an important topic in optimising these processes for higher product levels or improved product quality. Of special interest there are methods that allow an online monitoring without the need of taking samples out of the processes and the risk of contaminating it. Important parameters for the assessment of bio-processes are pO_2 , pCO_2 , pH and concentrations of glucose, lactate, methanol or ethanol. The instrumentation used process monitoring comprises probes (e.g. electrodes) that have to be sterilisable or flow injection analysis systems.

Another basic parameter for the assessment of bio-processes is the **metabolic state of the organisms**. This parameter yields information about the metabolic activity of the cells, the biomass development or changes in the composition of the nutrition medium.

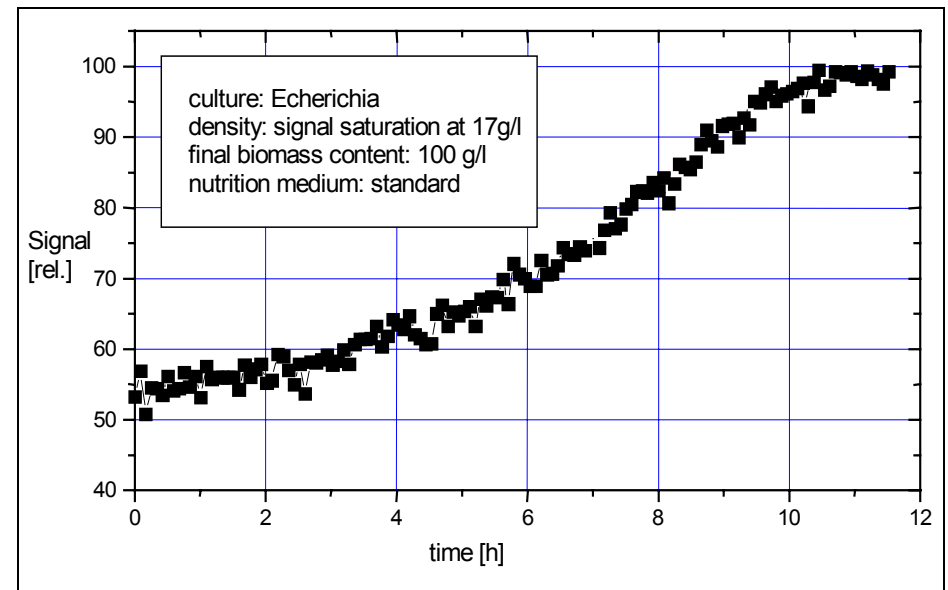
A non-invasive way of monitoring cellular metabolism is given by using the cellular fluorescence properties. Cells contain different fluorescing bio-molecules, among others Nicotinamide-Adenine-Dinucleotide (**NADH**) and several **Flavines**. The (semi-) quantitative measurement of the intracellular concentration of these molecules provides direct information about the metabolic state of the organism. Possible meanings of changes of the NADH content are summarised by the following table:

<i>increasing NADH content</i>	<i>decreasing NADH content</i>
<ul style="list-style-type: none">- superior cell activity- substrate uptake- reductive biosynthesis- respiration- growth- bacterial infection	<ul style="list-style-type: none">- reduced activity- cell death

The instrument **LF 402 Metabolic** is an excellent tool for the in-situ assessment of cellular fluorescence signals. It is equipped with fibre-optic probe that easily can be sterilised and fit into large volume or miniaturised bioreactors. The instrument is optimised for a high sensitive online measurement of the

NADH and the Flavine fluorescence in two channels. It exploits the **different specific fluorescence lifetimes** of the fluorophores in order to improve the selectivity of the signal detection. This results in an improved signal quality.

The graph shows the growth of a bacterial culture (Echerichia Coli) in a standard fermenter monitored with the LF 402 Metabolic during the initial 11 hours of the bioprocess.



Already at the beginning of the process the instrument showed a signal level of app. 50 rfu (relative fluorescence units) resulting from the fluorescence of the nutrition medium and the initial cell concentration. Then the signal increases following an exponential function indicating the increase of biomass due to cell division. At a cell density of 17 g/l the signal reached the instrumental saturation level and the final density was estimated to be 100 g/l.

Summary: The online monitoring of cellular cultures (bacteria, hybridoma, insect cells, ..) is possible using the **LF 402 Metabolic**. It provides basic information about the metabolic state of the organisms.