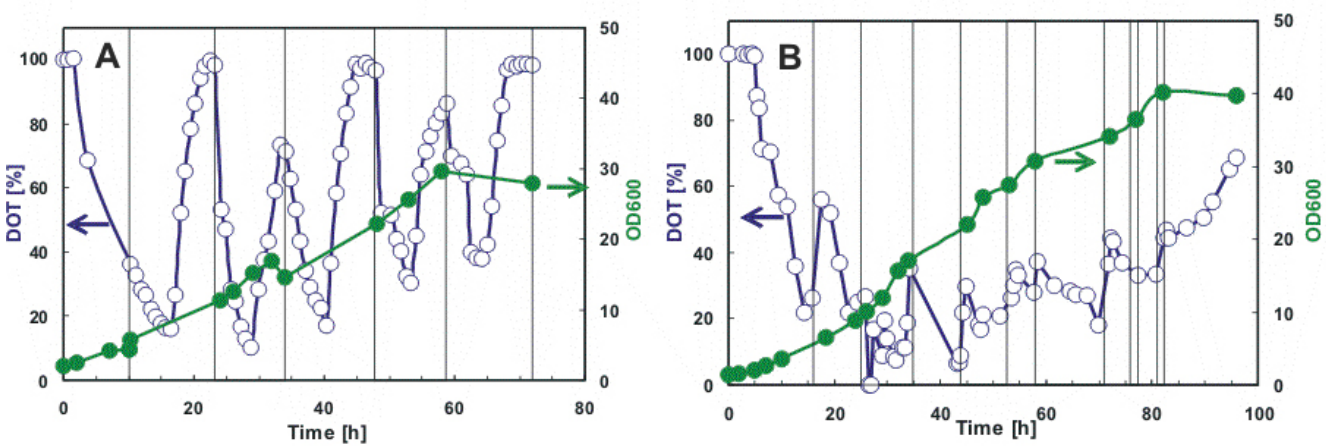

Figure 3
Optimisation of substrate feeding in *Pichia* pastoris cultures. *Pichia* uses methanol as a carbon/energy source. Methanol, however, is toxic at high concentrations. A recombinant strain of *Pichia pastoris* GS115 was cultivated in 1 l baffled shake flasks with three side necks containing 200 ml of BMM medium at 30°C with 200 rpm. To maintain the expression of the product, 100 % of methanol was added to a final concentration of 0.5 % (v/v) either twice a day (a) or alternatively, at the time when the DOT level increased (b). Times for substrate feeding are indicated with vertical lines.

wireless platform is also a versatile tool for other measurements in industrial and environmental applications. It has a better mobility than wired systems and new measurements can be set up fast, mostly without changes at the target site. The possibility for such at-time measurements outperforms the use of data-loggers.

Methods

Sensors and shake flasks

pH was measured with an autoclavable electrochemical pH sensor (EGV150, Sensortechnik Meinsberg, Germany). Dissolved oxygen was monitoried with a 6 mm diameter polarographic Clark-electrode manufactured by Medorex (Nörten-Hardenberg, Germany). Both sensors were sterilized by autoclavation at 121°C for 20 min before use. 1000 ml 3-baffled shake flasks with three side necks for placement of the electrodes were obtained from Glasgerätebau Ochs (Bovenden, Germany).

Escherichia coli *cultivation in mineral salt medium (MSM)*

Escherichia coli K-12 strain RV308 was cultivated at 37°C in 1 l shake flasks with four vertical baffles containing 200 ml mineral salt medium [26] with 5 gl^{-1} of glucose as carbon source, 100 mg l^{-1} thiamine and a starting pH of about 7.0. Overnight cultures were prepared in 100 ml shake flasks with 10 ml of the same medium. For shake flask cultivation the preculture was diluted (OD_{600} = 0.1) into fresh medium.

Pichia pastoris *cultivation with methanol feeding*

A recombinant strain of *Pichia pastoris* GS115 containing a plasmid for expression of type II human collagen gene cloned under control of the AOX1 promoter (kindly obtained from FibroGen Europe Ltd.) together with propyl-4-hydroxylase encoding gene was cultivated according to Pichia Expression Kit (Invitrogen) instructions [25]. It was first cultivated in a 1 l shake flask with 100 ml buffered minimal medium (BMG) containing 10 g l^{-1} glycerol. Cells were grown for 14 h at 30°C on a shaker with 200 rpm. When the OD_{600} reached a value between 2 and 6, the cells were centrifuged at 3500 rpm for 5 min, and washed with methanol medium by further centrifugation. The supernatant was decanted and the cell pellet was resuspended to obtain an initial OD_{600} around 1.5 in a methanol containing medium (buffered minimal methanol medium, BMM). Baffled shake flasks with three side necks containing 200 ml of BMM medium and a pH and an oxygen sensors was incubated at 30°C on a shaker (Certomat® MO, B. Braun Biotech International) with 200 rpm. To maintain the expression of the product, 100% of methanol was added to a final concentration of 0.5% (v/v) twice a day, every 10 to 14 hours, or alternatively, at the time when the DOT level increased

Authors' contributions

JP carried out *E. coli* cultivations and assisted in all experiments which were monitored with SENBIT® system by setting up and supervising measurements and data analysis. Monika Bollók designed and performed *P. pastoris* cultivation experiments. AV was responsible for software