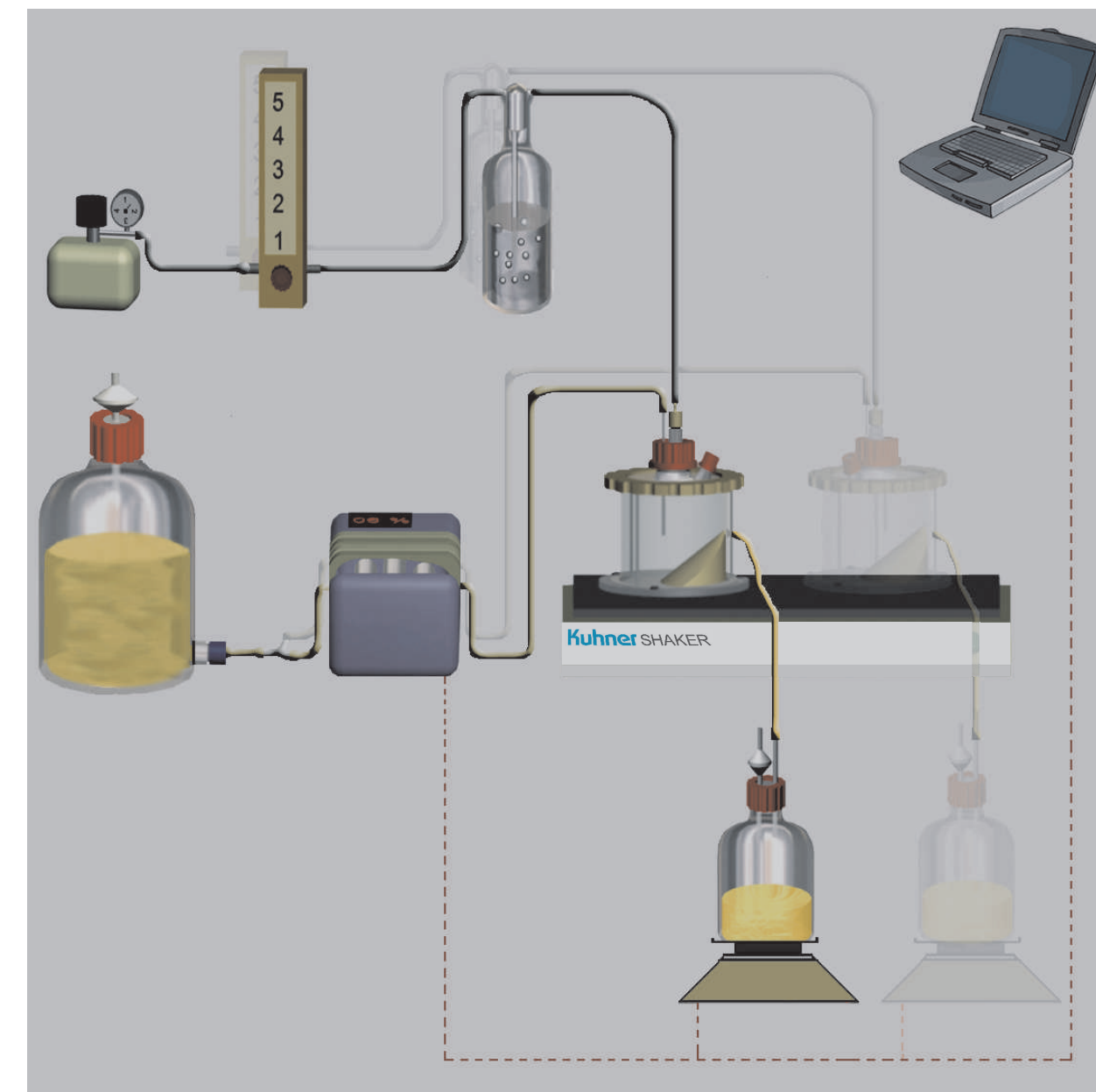


Goal

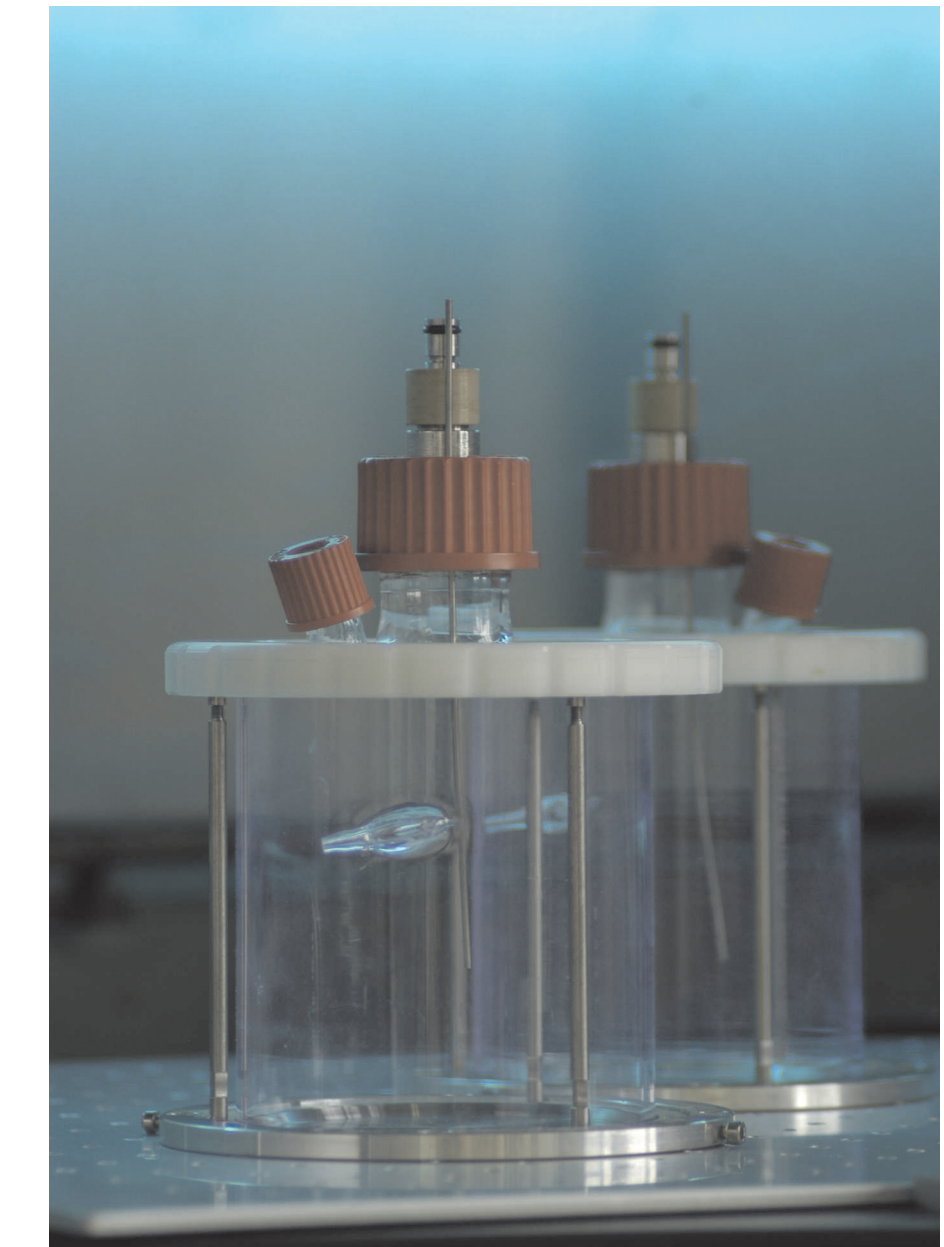
Easy and fast procedure for testing plasmid stability of strains

Method/Tools

COSBIOS mainly consists of a specially designed flask (picture 2) with two inlets, one for gas (air) supply and one for medium supply, and a combined outlet on the side of the flask for exhaust gas and the fermentation broth. As a result of the circulating motion of the fermenter on a shaker, the fermentation broth reaches the outlet port and leaves the glass flask.



picture 1



picture 2

Material

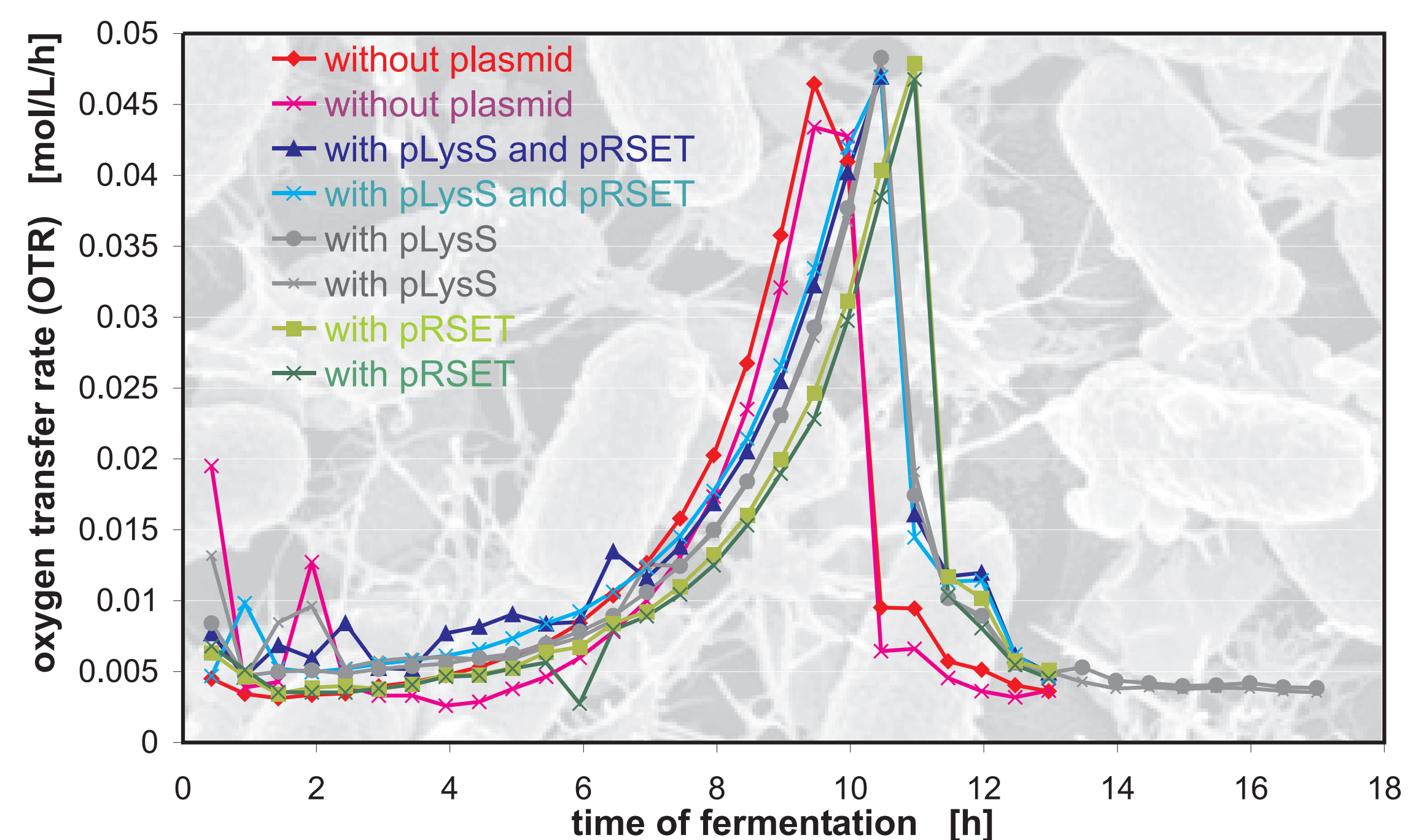
Strain: *Escherichia coli* BL21 (DE3)

Plasmids: pLysS (chloramphenicol resistance)

pRSET (Ampicillin resistance, YFP-Interleukin-6 fusionprotein)

Picture 3 displays the online measured oxygen transfer rates (OTR) by RAMOS. From the OTR-curves the max. growth rate can be calculated.

no plasmid > pLysS = pRSET > pLysS+pRSET
0.63 > 0.55 = 0.55 > 0.48 max [1/h]

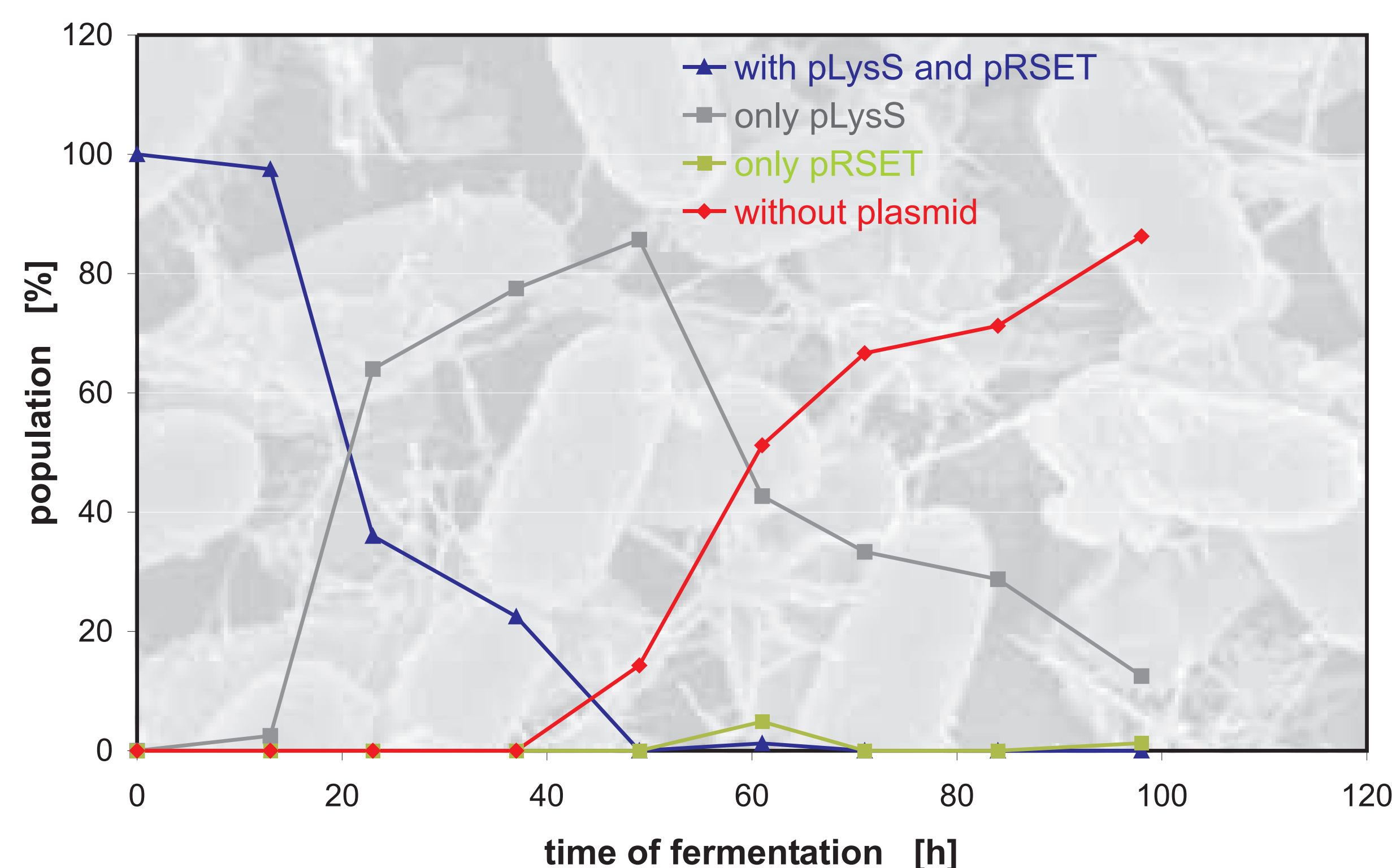


picture 3: fermentation conditions: 30°C, shaking speed 300 rpm, shaking diameter 50mm, filling volume 12.5mL

Results

Picture 4 illustrates the fermentation results from a continuous cultivation in COSBIOS.

In the beginning of the experiment only a population containing both plasmids (pLysS and pRSET) was present. After 15 hours a second population without the pRSET-plasmid and after 38 h a third population without both plasmids started to grow. The strain containing only the pRSET plasmid built up nearly no population, although it had the same growth rate as the strain with the pLysS plasmid. These results show that COSBIOS is a very good and easy to handle tool to test the plasmid stability of strains.



picture 4: fermentation conditions: 30°C, shaking speed 300 rpm, shaking diameter 50mm, filling volume 20mL, dilution rate 0.2751/h

Advantages of COSBIOS

- + low investment costs
- + six parallel bioreactors on one shaker
- + low liquid volume (tracer experiments)
- + high time saving in comparison to an stirred bioreactor
- + six different states of equilibrium at one time

further applications:

- + substrate inhibited systems
- + testing foamy systems
- + metabolic flux analysis (tracer experiments)
- + and more

Adolf Kühner AG
Dinkelbergstr. 1
CH-4127 Birsfelden (Basel)
Switzerland
www.kuhner.com

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Postfach 17 05 - 49007 Osnabrück

