STANDARD OPERATING PROCEDURES FOR A SINGLE-USE FERMENTER

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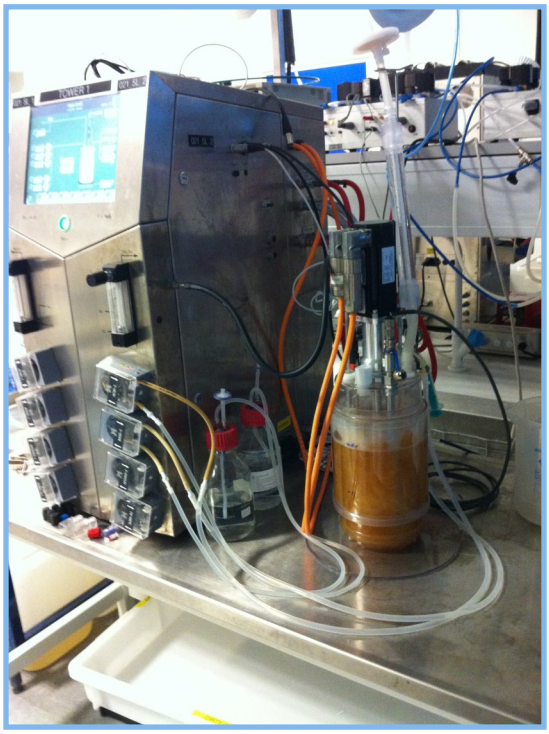
Introduction

Operating Procedures are fundamental in every fermentation experiment. Single-Use fermenters are getting of higher demand and knowing the operating procedure differences between a Single-Use fermenter and a conventional fermenter is necessary in order to experience a succesful fermentation and hence be able to use a Single-Use fermenter from CerCell ApS.

This Application Note describes a step-by-step procedure for growing E. *coli* in a 3 Liter Single-Use benchtop fermenter from CerCell ApS. The Single-Use fermenter are of the BactoVessel™ series meant for microbial applications. They are unique and fully configurable fermenters in a scalable platform. The BactoVessel family is in size of 2.1 Liters and up to 30 Liters.



3 Liter Single-Use fermenter (SUF) running a fermentation with E. coli.

Materials and Methods

The fermenter

A 3 liter BactoVessel™ Single-Use fermenter (SUF) was prepared in a LAF bench. Clamps were applied to every tube on the fermenter and 2 liter LB media was added to the fermenter. Temperature-, pH-, and DO sensors were sterilized and applied to the fermenter. The Fermenter was then put on top of the working bench next to its control station. Wires and tubes were fixed, acid– and base flasks were connected and a Kollmorgen motor was applied. The exhaust gas was connected to the gas analyzer. Clamps were removedand the control conditions were set.

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Materials and Methods (continued)

Control Conditions

The conditions were set at the touch screen on the control station before the inoculation

- Temperature (°Celcius)……...37
- Agitation (RPM)……..……..…..750-1000
- pH……..…7
- Airflow (vvm)…..……0.5-1

After the control conditons were the fermenter was running. The day before the fermentation was started a preculture with 100 ml LB media and a single colony of E. *coli* was mixed in a shakeflask at 150 RPM overnight (18-20 hours).

LB Media Composition (LB Miller)

- 10 g/L NaCl
- 10 g/L Tryptone
- 5 g/L Yeast Extract
- 5 µL/L antifoam

The media was autoclaved at 121 °Celcius before use.

Inoculum size

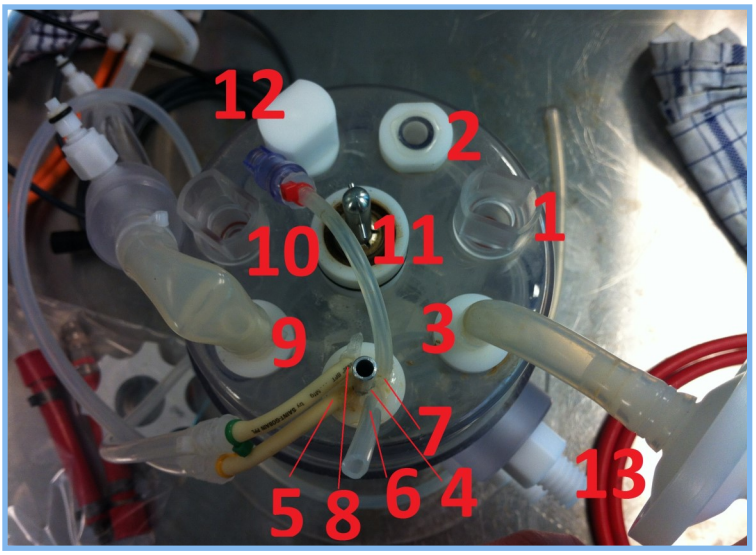
The inoculum size for the fermenter inoculation was calculated with this formula:

$$V_2 = \frac{V_1 \cdot C_1}{C_2}$$

Where V_2 is the inocululum size in ml, C_1 is the wanted starting OD of the fermenter (0.1-3) C_2 is the observed OD of the inoculum and V_1 is the working volume of the fermenter (2000 ml)

Sampling

After the inoculum size was calculated and added to the fermenter the fermentation started. Samples were taken every 45 minutes the first 3 hours and then every 30 minutes for the duration of the fermentation. OD measurements were taken for every sample while dry weight measurements were taken every hour after the first 3 hours.



The different compartments of the SUF. 1) sensor port 2) thermowell 3) ingoing airflow tube 4) gas exhaust holder port 5) acid/base tube 6) harvest tube 7) harvest tube 8) acid/base tube 9) gas exhaust tube 10) sensor port 11) motor holder 12) media port 13) water jacket inlets.

Ending fermentation

After the last sample has been taken the fermenter is run ideally for a total of 36-48 hours in order to let the cells use up all the substrate and to get useful graphs for CO_2.

all the tubes and wires are detached expect for the acid– and base flasks which are replaced with water flasks and run in order the cleanse the tubes of acid and base. The control station is shut off, The biowaste in the fermenter is poured into a biowaste container.

Setup and handeling of SUF

Here is a list of setup procedures necessary to pay attention to:

- The fermenter has to be fastened in a steady position so nothing is shaking during fermentation.
- Place the fermenter so the media port and harvesting tube are easy accessable.
- Put aside the acid and base flasks so that they are not interveining your working area.
- Place a cloth on the working bench but under the water cooling tubes against leak.

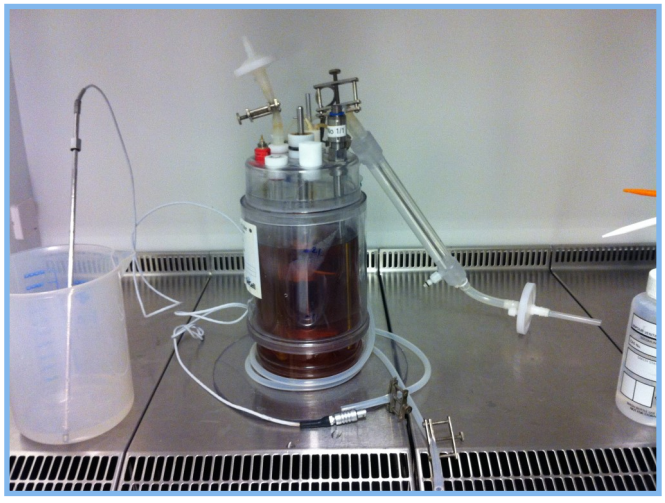
Materials and Methods (continued)

- Fit every sensor tightly with your own hands and not any tool. The material cannot hold against the forces of powerful tools.
- Never autoclave the SUF before use. It is made of rigid plastics and cannot withstand heat over 50° Celcius
- Make sure tubes are laid out properly in order to avoid tangeling
- Write everything down during fermentation for controlling conditions and afterwards for examining things that went wrong and things to pay attention to.

Sterility

In order to keep the fermentation steril it is necessary to:

- Wear a Labcoat in and only in the working area of the SUF. This is to avoid being exposed to cells and also not carrying cells out of the area.
- Always work in a LAF bench when preparing the SUF
- Wear gloves whenever in contact with SUF culture
- Use 70% ethanol for elmination of cells and for cleaning when exposed to cell culture, but never use it on compartments involved directly with the current fermentation.
- Only autoclave the media you need. Contamination risks of media are very high



Preparation of SUF in LAF bench.

Results

Five E. *coli* fermentations were carried out each between 7 and 10 hours. The controlling conditions were the same for all five of them.

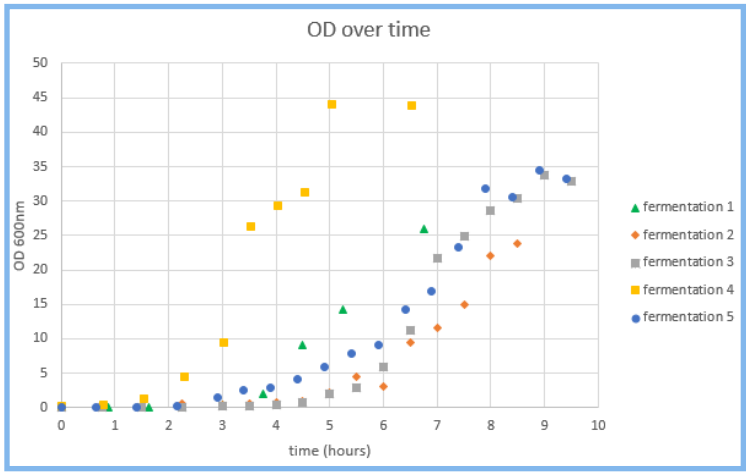
The first results display the OD measurements over time. It can be seen that fermentation 4 is a bit out of range from the others. This is due to the fermentation media being precontaminated before autoclavation. The media was filled with dead biomass material.

However the max OD for the other fermentations varied but were around 24 for number 1 and 2 and around 34 for fermentation 3 and 5.

It can be seen that with the controlling conditions chosen the exponential phase starts approximately after 3 hours for everyone except number 4 which starts after 1½ hours.

The stationary phase can be seen reached for fermentation 3 and 5 at around 9½ hours.

When making a plot like this it is possible to calculate the maximum growth rate of the fermentation called μMax. When taking a linear regression of the logarithm of the exponential phase the slobe will be the value of the μMax.



Results of OD measurements over time for 5 E. coli fermentations

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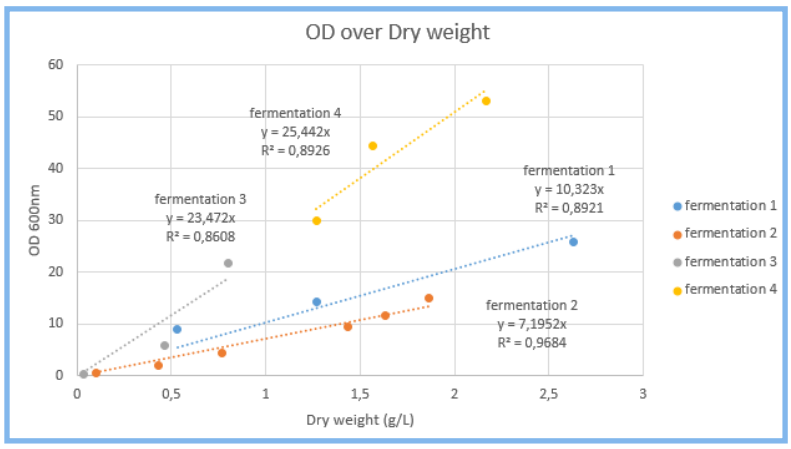
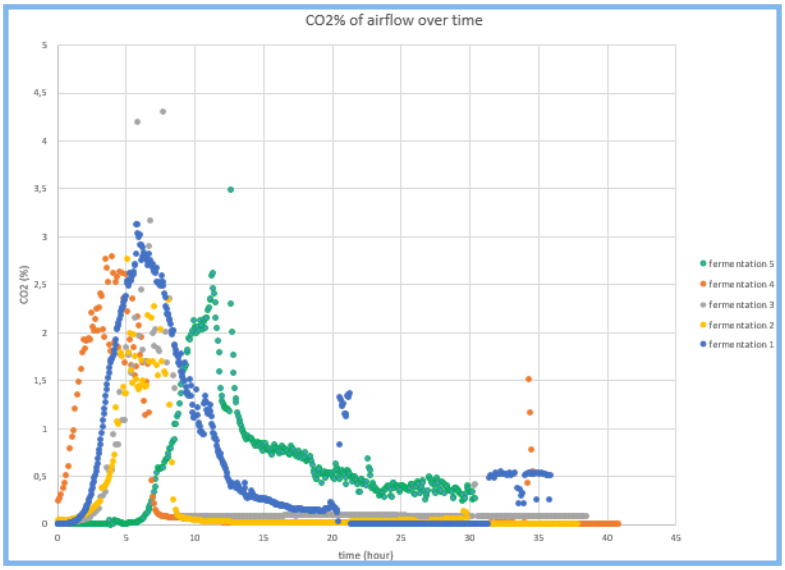
Results (continued)



Results of the linearity between OD and dry weight for 5 E. coli fermentations.



Results of the CO_2 % profiles of the exhaust airflow over time for 5 E. coli fermentations

Results next to be displayed is the correlation between dry weight measurements and the OD measurements for fermentation 1-4. According to the theory of Lambert-Beer's law the concentration (dry weight) and the absorbance (OD) are in a linear relationship. This is shown to a certain extent with the results but dry weight measurements are quite difficult to handle without multiple sources of error so usually many samples will be invalid. Compared to the fact that the fermentations were run with the same conditions it is odd to see that the correlations are varying so much from each other.

Last to be displayed is the results for the % CO_2 profiles for the 5 fermentations. It can be seen how most of the fermentations peak at around 5 to 10 hours only with fermentation 4 being a little earlier (as expected) and fermentation 5 to be a little later. These results can be intepreted as when the cells are most active and when they begin to die. When no more CO_2 is produced then no more cells are expected to be alive.

For further information read the B.Sc. Project Thesis "Standard Operating Procedures for a Single-Use Fermenter" by Kristian Krakau or visit cercell.com

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