25-31.8 Continuous production and gc analysis

TUESDAY, 8/25

Started testing the strain E. coli BL21(DE3)ΔyjgB ΔyqhD and its propane production capasity. Did the overnight culture with 20 mL in TB, with incubation at 38 °C and 230 rpm. OD600 was 1,4246 at this point. Inoculated with 10 % (v/v) of the previous culture by adding 200 ml of TB media containing appropriate antibiotics (amp 100 ug/ml + spec 50 ug/ml + str 20 ug/ml + chloramphenicol 34 µg/ml). Incubated 1 h 10 min at 37 °C and 230 rpm until OD600 was 0,5212. Because of the OD samples taken out, the volume of cultivation was 199 ml at this point in a 250 ml baffled flask. Added 47,43 µl IPTG (500 mg/ml) for the consentration of 0,5 mM and induced the culture with 2 h incubation at 30 °C, 200 rpm. At this point the cultivation volume seemed to be too big for the flask so the media was divided into four 250 ml baffled flask after 2 hour induction, each flask containing 50 ml of cultivated media. Contiunued induction with the same temperature and 250 rpm for 3 hours, so overall induction time was 5 hours with 200 and 250 rpm shaking in 30C. Pelleted the cells with centrifuge (3214xg, 21°C, 10 min) and resuspended with 50 ml of TB-media. Transfered 8 ml of culture for two 22 ml GC-vials with a gas tigh syringe and incubated in RT 17 h with 50 rpm shaking. Started analysis which generated followed results:

propane ug/L sample
26.8.2015 14:16 751_l1_260815.D 111
26.8.2015 15:13 751_c1_260815.D 88

Continuous production which GC-analysis methods and growth, pH and gas curves available at http://2015.igem.org/Team:Aalto-Helsinki/Results:

25.8.2015

Started production by incubating 50 ml of E. coli BL21(DE3)ΔyjgB ΔyqhD pET-TPC4 + pCDF-cAD (P. marinus) + pACYC-Fdx-Fp) o/n 16 hours at 37C 230 RPM in TB-media. After the incubation, the OD600 of culture was 1,9300 with 1ml cuvettes. Started batch phase with 50 ml inoculation (1:10) in 500 ml TB-media without IPTG at 10:15 in 37C with 350 rpm shaking. Started aeration with 0,5 l/h of air stream to create 1 ppm air concentration.

At 16:20 started continuous production by connecting feedstock containing 2500 ml of TB-media and set the flow rate of 44 ml/h. Some foaming detected in the reactor. At this point OD600 of liquid was 8,3800 when measuring 1ml samples. Glucose concentration was also analysed with Liquid Chromatography. Glucose concentration was 2,668 g/L at this point.

At 17:07 started 1,0 l/h aeration creating 2 ppm air concentration, and added 2,5 ml Struktol to the feed stock and 1 ml to the reactor to prevent foaming. Left the operation to run o/n and started weighting the outline stream.

26.8.2015

At 10.39 some contamination in the feedstock line detected, which didn't seem to be effects into growth rates. After 10 hours, the weight of removed liquid was 0,618 kg at this point, so assumed and calculated the flowrate to be 36,35 ml/h with D=0,072. At 12:00 measured OD600=18,532. Glucose concentration was 0,025 g/l at this point. Left the operation to run o/n.

At 18:25 glucose sample taken which concentration was 0,0235 g/l.

27.8.2015

At 11.15 OD600 was 31,4976 and glucose concentration was 0,3206 g/l. At 13:36 OD600 measured cell mass with try weight of 4ml sample. At this point cell mass was 9,875 mg/ml. At 14:17 the first steady state was observed so the induction of propane production was started by switching the feed stock to 2500 ml of TB-media containing 2 mM of IPTG. At the same time IPTG was added into reactor through the septum to get same concentration. Glucose concentration of 20,8656 g/l was measured from the first feed stock without IPTG. Thus, the feed stock container didn't contain any significant contaminations because glucose wasn't consumed. Left the operation to run o/n.

28.8.2015

At 17.15 cell mass was 8,250 mg/ml. The growth curve had slightly decreased. However, propane samples weren't taken due to the time limit. Changed the feed stock into larger container and left the cultivation to run over the weekend.

31.8.2015

11:33 Started weighting again the outflow mass and calculated 24,17 ml/h flow rate. Glucose concentration of the reactor was 0 g/L at this point. Decreasing rate may be resulted by cell mass plugging outflow lines. Growth curve had also decreased significantly. At 11:37 Took the gas samples from one outlet of reactor's head space by connecting the needle into line and injecting it through a septum into 22ml GC-vial. The septum had also another needle flushing the air from the vial head space. First sample's flushing time was 10 min and second 20 min. Ran GC-analysis. Following propane amounts were obtained:

Sample	ug/vial
31.8.2015 11:46 754_10minK_310815	0,5
31.8.2015 12:34 754_20minK_310815	0,1

Masses were so close to standard limits that the accuracy may have suffered. The propane concentration per both vials for the samples was calculated to be 22,72 ug/L and 4,54 ug/L. More information available from analysis at http://2015.igem.org/Team:Aalto-Helsinki/Results.

At 11:47 measured the mass of cells which was 2,625 mg/ml, and 0 g/l glucose concentration. Stopped the cultivation.

Dry sample measurements for 4 ml of sample media:

<u>27.8.2015</u> 13:36
5a 37mg
5b 42mg
AV: 39,5 mg 9,875 mg/ml

<u>28.8.2015</u> 17:15
6a 35mg
6b 31mg
AV: 33,0 mg 8,250 mg/ml

<u>31.8.2015</u> 11:47
7a 10mg
7b 11mg
AV: 10,5 mg 2,625 mg/ml