Investigating Temperature Effects on Both Aerobic and Anaerobic Batch Growth of E.coli

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Experiment Overview

Technology

Bioreactors are vessels that host chemical reactions involving living organisms. They also allow for mass production processes.

Applications

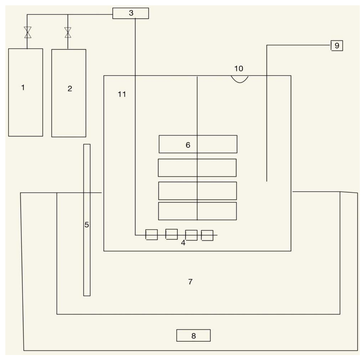
Climbing diabetic's demand for insulin introduced two new alternate methods of administration: inhalation and oral route which require a much higher dose since they do not enter the blood stream directly. Since insulin is a protein, it requires a host organism to produce it. The main hosts for human insulin has been predominantly *E.coli* and *Saccharomyces cerevisiae.* Mass production of insulin is needed to accommodate increasing diabetics' demands. Thus, insulin producing E.coli is best cultivated in bioreactors.

Objectives

Study the differences between aerobic and anaerobic cell growth of E.coli. Additionally, investigate optimal temperature conditions by testing above and below the optimal temperature, 37 °C.

Hypotheses

The best temperature for E.coli growth is 37 °C as they exist naturally at body temperature in the intestines of humans and animals. Therefore, as temperature deviates from 37 °C, growth rates and doubling times will deteriorate. Aerobic conditions will demonstrate better growth rates and lower doubling times since it can extract more energy from its environment and grow faster.



1. Air Tank (Aerobic)
2. Nitrogen Tank (Anaerobic)
3. Pressure meter (25 PSI)
4. Aerator (2 SCFM)
5. Thermometer
6. Stirrer (290 RPM)
7. Water bath
8. Temperature control
9. Sample collecting valve
10. Inlet for LB broth and E.coli feed
11. Bioreactor

Theory

$$substrates + cells \rightarrow extracellular\ products + more\ cells$$

$$\Sigma S + X \rightarrow \Sigma P + nX$$

$\mu_R \equiv \frac{1}{N}\frac{dN}{dt}$

μ_R : net replication rate (h^{-1})

N : Cell number Concentration

$\frac{dN}{dt} = \mu RN, \quad N = N0 \text{ at } t = 0$

$\ln\left(\frac{N}{N_0}\right) = \mu_R t \text{ or } N = N_0 \exp(\mu_R t)$

$\tau d = \frac{ln2}{\mu_R}$

τd : Doubling time

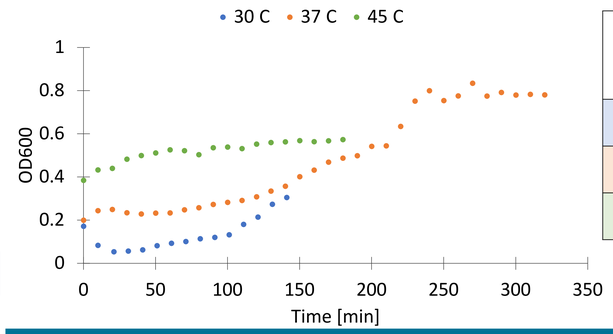
Summary of Trials and Procedure

(1) Aerobic, 30°C	(4) Anaerobic, 30°C
(2) Aerobic, 37°C	(5) Anaerobic, 37°C
(3) Aerobic, 45°C	(6) Anaerobic, 45°C

Fill bioreactor with 1.25 L of broth. Heat water bath to desired temperature. Turn on stirrer to 290 rpm. Introduce gas set at 2 SCFH and 25 psi. Add 125 mL seed culture to reactor to begin experiment. Sample 5 mL every 10 minutes starting at t=0. Blank spectrophotometer with LB medium. Transfer 3 mL of sample to cuvette and obtain OD measurements as each experiment progresses. Rinse and repeat for different temperatures and whether aerobic or anaerobic.

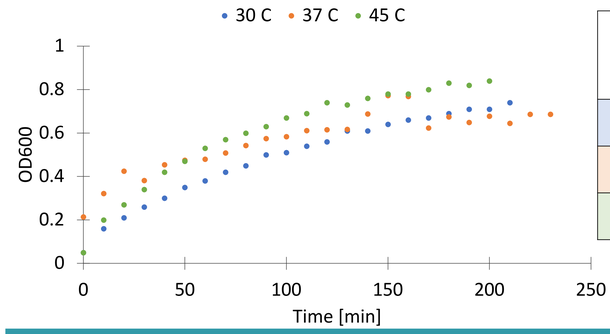
Results

Aerobic Data



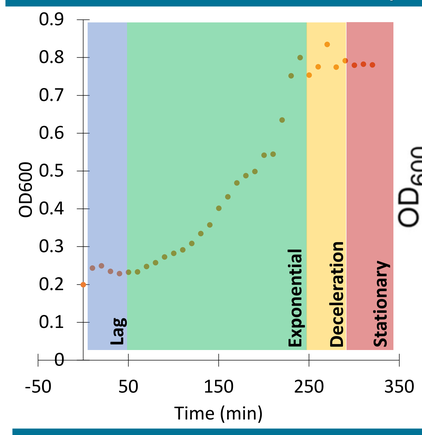
μ_R [1/h]	τ_d [min]
0.894	46.5
0.402	103.5
0.078	533.2

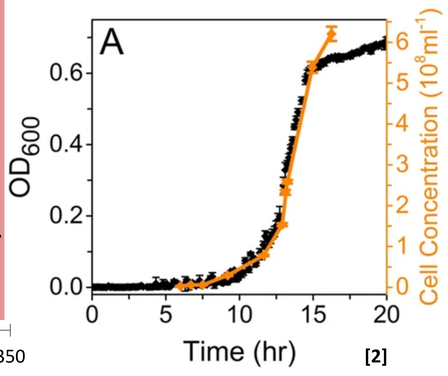
Anaerobic Data



μ_R [1/h]	τ_d [min]
0.738	56.4
0.300	138.6
0.636	65.4

Growth Phases: Experimental vs Literature





Discussion

The basis of growth rate and doubling time values hinges on choosing when exponential growth phase occurs. Considering numerous published experiments were conducted over long periods of time, sometimes days, there is no solid basis for deciding whether these trials have reached exponential growth phase at all; one can argue that many of these experiments only reached an extended lag phase. Ultimately, the results obtained do not disprove the hypotheses. In order to make these claims, a single experiment must be run for the entire lifetime of a cell culture in order to rationally identify the exponential growth phase of each experiment.

Conclusion

Both hypothesis were not proven right or wrong. The results obtained suggests that growth rate/doubling time improves at lower temperatures; however, the reliability of these data sets need further investigation to make definitive conclusions about optimal growth conditions. Furthermore, future work includes longer reruns of aerobic and anaerobic experiments at 30 C and 37 C, respectively. Additionally need to verify cell number density with cell mass concentration by purifying cells and obtaining reliable dry cell masses for every sample taken at different conditions.

Acknowledgements

We would like to thank Professor Daniel Knight, Mr. Steve, and the chemical engineering class of 2022!

Design Extension

How many batch bioreactors are needed to satisfy the demand of Insulin per day in Irvine?

Design Constraints:

Diabetic Population in Irvine [2]	37,096
Insulin Consumption [4]	2.45 mg / person / day
Insulin Demand	90.9 g/day

Assumptions:

Conditions	Aerobic @ 37°C
Conversion Factor [5]	$2.4 * 10^9$ cells / mL / unit OD600
Cell Dry Weight [1]	$3 * 10^{-13}$ g / cell
Bioreactor Volume	1000 L (90 % Capacity)
# of Bioreactors	8 (parallel)
Lysogeny Broth: Seed Culture	873:27
Trials per Reactor	4 / day
Delta OD (37 C aerobic)	0.600
Time per trial	240 min

Design Approach:

Time of Four Trials	16 hours
Time for Disinfecting	6 hours
Total Time	**22 operation hours/day**

References

[1] *ECMDB: ECMDB Statistics*. (n.d.). Ecmdb.Ca. Retrieved March 1, 2022, from https://ecmdb.ca/e_coli_stats
[2] Baeshen, N. A., Baeshen, M. N., Sheikh, A., Bora, R. S., Ahmed, M. M. M., Ramadan, H. A. I., Saini, K. S., & Redwan, E. M. (2014). Cell factories for insulin production. *Microbial Cell Factories*, *13*(1), 141. https://doi.org/10.1186/s12934-014-0141-0
[3] *Irvine Population*. (n.d.). Populationu.Com. Retrieved March 1, 2022, from https://www.populationu.com/cities/irvine-ca-population
[4] Lassalle, L. (2019, November 13). *How many diabetics could be served by a small-scale insulin production system?* Open Insulin Project. https://openinsulin.org/our-blog/how-many-people-could-be-served-by-a-small-scale-production-system-something-that-could-fit-on-top-of-a-lab-bench-or-table/
[5] Yap, P. Y., & Trau, D. (2019, April). *Tip Biosystems.* Retrieved from Direct E.Coli Cell Count at OD600: https://tipbiosystems.com/wp-content/uploads/2020/05/AN102-E.coli-Cell-Count_2019_04_25.pdf