The diauxic growth curve – Homework assignment

Next session we will try to reproduce the famous diauxic growth curve experiment first performed by Jacques Monod in the 1940's during his PhD work. Subsequent work by Monod led him to decipher the basic mechanisms of gene regulation and propose the lac operon model of gene expression, for which he received the Nobel prize together with Jacob and Lwoff in 1965. The idea behind the diauxic growth experiment (literally meaning double growth) is very simple – we put E. coli in a medium with two types of sugars, one that it can directly metabolize (for example glucose) and a second sugar (lactose for example) that needs to be further processed in order to be metabolized by the cell. What Monod discovered was that the cell prefers to first digest glucose, which endows the cell with a higher growth rate (making it more fit), and only when glucose is exhausted from the medium will it start to synthesize the machinery needed to transport and digest and ultimately grow, albeit more slowly, on the second sugar. Thus growth on a mixture of sugars therefore exhibits the following phases: (1) fast growth on glucose (2) lag period (3) slower growth on lactose (4) saturation. See for example Fig. 1d taken from Monod's Nobel lecture. The following is Monod's own account of his findings.

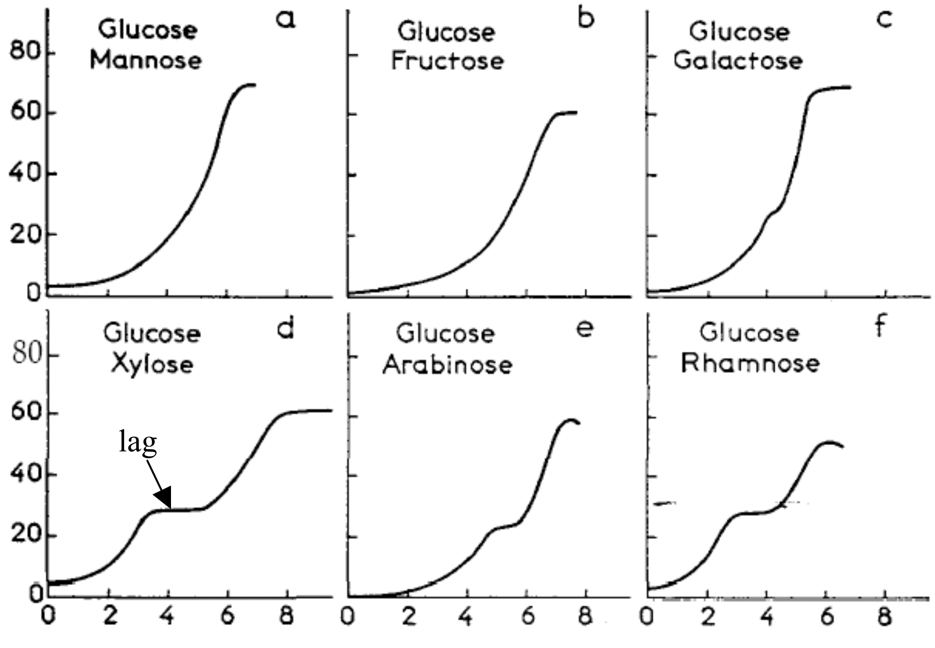

Fig.1. Growth of *Esherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[50].

> *"I was working then at the old Sorbonne, in an ancient laboratory that opened on a gallery full of stuffed monkeys. Demobilized in August in the Free Zone after the disaster of 1940, I had succeeded in locating my family living in the Northern Zone and had resumed my work with desperate eagerness. I interrupted work from time to time only to help circulate the first clandestine tracts. I wanted to complete as quickly as possible my doctoral dissertation, which, under the strongly biometric influence of Georges Teissier, I had devoted to the study of the kinetics of bacterial growth. Having determined the constants of growth in the presence of different carbohydrates, it occurred to me that it would be interesting to determine the same constants in paired mixtures of carbohydrates. From the first experiment on, I noticed that, whereas the growth was kinetically normal in the presence of certain mixtures (that is, it exhibited a single exponential phase), two complete growth cycles could be observed in other carbohydrate mixtures, these cycles consisting of two exponential phases separated by a-complete cessation of growth (Fig.1)."* – 1965 Nobel lecture by Monod

The following is a simple exercise in gross estimation. Monod concluded that when glucose is exhausted by the cell the diauxic shift occurs. Can you predict based on simple arguments when this will occur?

Here is the experimental setup.

At t=0 you inoculate a 1L growth medium containing 0.1g/L of glucose with 10mL of saturated E. coli culture (@ $1.5 \cdot 10^8$ cells/mL) and allow the cells to grow aerobically at 37degC. Assume that the cells are growing exponentially with a doubling time of 20 min, and that at this growth rate an average cell has about $6 \cdot 10^6$ proteins per cell (on average 300 aa in length each). You may also assume that the medium has been supplemented with amino acids so that the cell doesn't need to synthesize these building blocks for itself. Under these conditions it is known that most of the energy consumed by the cell is required for amino acid polymerization (i.e. making proteins). Finally you will need to know that under aerobic growth conditions the cell can generate 30 ATP molecules from each glucose molecule, and that 4 of these ATP molecules are required to elongate a single peptide bond. *Hint: estimate the total number of glucose molecules in the medium and compare that with the number of glucose molecules being consumed by the exponentially growing population of cells.*

Please also read up a little on the lac operon (for example in Alberts, Essential cell biology pp. 271- 275 or PBoC pp. 138- 140, section 4.4.3)

Some references

Diauxic growth

http://nobelprize.org/nobel_prizes/medicine/laureates/1965/monod-lecture.html
http://en.wikipedia.org/wiki/Diauxie
http://en.wikipedia.org/wiki/Lac_operon
http://bioinfo.bact.wisc.edu/themicrobialworld/regulation.html
Brock, Biology of microorganisms, 2006, pp. 216-218
“The Growth of Bacterial Cultures” – Monod, *Ann Rev Micro Biol* **3**: 371, 1949.
“The phenomenon of enzymatic adaptation” – Monod, GROWTH 11 (4): 223-289, 1947

Growth phases

Brock, Biology of microorganisms, 2006, pp. 142-144
Microbe, Schaechter, Ingraham and Neidhardt, 2006, pp. 55-58, 259-262.

The lac operon

http://en.wikipedia.org/wiki/Lac_operon
Microbe, Schaechter, Ingraham and Neidhardt, 2006, pp. 229-232.
Little Alberts, pp. 271- 275
PBoC pp. 138- 140, section 4.4.3

Other:

“Bacterial Growth: Constant Obession with dN/dt” – Neidhardt, *J Bact* **181**: 7405, 1999.

When does the diauxic shift occur?

Let's calculated how long can 1L of glucose at 0.1g/L can in principle sustain cell growth:

1. For doubling time of 20 min there are $6 \cdot 10^6$ proteins per cell
2. ATP needed to construct 1 cell is mostly due to amino acid polymerization (media is supplemented with casamino acids).
   Rough number of ATPs required to build one cell:
   (300 aa/protein) * ($6 \cdot 10^6$ proteins per cell) * (4 ATP per aa) = $7.2 \cdot 10^9$ ATP per cell
3. 1 glucose = 30 ATP
   Number of glucose molec required to produce 1 cell: $7.2 \cdot 10^9/30$ = **$2.4 \cdot 10^8$ glucose molec/cell**
4. Number of cells in culture at time *t*:
   $N_{cells}(t) = N_0 2^{\mu t}$
5. Total number of glucose molec recquired to produce $N_{cells}(t)$ cells:
   $N_{\text{glucose consumed}}(t) = (2.4 \cdot 10^8 \text{ glucose per cell}) \cdot N_{cells}(t)$
   Inoculum is 10mL of saturated medium (measure to be OD=1.5):
   $N_0 \approx (10mL)(1.5 \cdot 10^9 cells/mL) \approx 1.5 \cdot 10^{10} cells$
   Thus
   $N_{\text{glucose consumed}}(t) = (2.4 \cdot 10^8 \text{ glucose}) \cdot (1.5 \cdot 10^{10} cells) 2^{\mu t} = 3.6 \cdot 10^{18} 2^{\mu t}$
6. Total number of glucose molecules in medium that we prepared:
   $N_{\text{glucose in medium}}$=(0.1 g glucose/L) * (1 L) / (180 g/mole of glucose) *$6 \cdot 10^{23}$ = $3.4 \cdot 10^{20}$ glucose molecules
7. Time required to exhaust all of the glucose in the medium (equating results from 5 & 6):
   $N_{\text{glucose consumed}}(t) \equiv N_{\text{glucose in medium}}$
   $N_{\text{glucose consumed}}(t) = 3.6 \cdot 10^{18} 2^{\mu t} \equiv 3.4 \cdot 10^{20} \text{ glucose molec}$
   $2^{\mu t} \approx 10^2$
   $\mu t = \log_2(10^2) = 6.6$
   $t/21 \min = 6.6$
   $T \approx 140 \min$

Shift occurs after 200 min.