ChE 170B.
Midterm Examination 1
6 March 2009

1. *Growth Models*

a) Using a Monod growth model, a line was fit to *E. coli* growth data as shown below in Figure 1. It was found that μ_{max} was 1.2. The same strain of *E. coli* was grown in a similar experiment using 20% more glucose. What would the new growth curve look like? Draw your estimate on the plot below given that μ_{max} was 2.

SHAPE * MERGEFORMAT

Answer: Higher final growth rate (not quite double), and steeper slope during exponential phase since reaches half max growth at a lower substrate concentration than the original. (3 points)

b) Below is a typical growth curve for an organism undergoing substrate inhibition. Set up mass and substrate balances based on this data. You do not need to solve.
Hint: The substrate concentration that yields the highest growth rate is $S_{critical}$ = QUOTE . What are $\mu_{max,}$ K_i, and K_s?

Mass Balance (3 points):

Values (4 points):
$\mu_{max,}$ is 0.38
Ks is about 30 (many things will do so long as this is shown)

Scritical = QUOTE . = 180

Ki is about 1080

2. *Protein Properties and Structure*

a) A peptide has the sequence Glu-His-Trp-Ser-Gly-Leu-Arg-Pro-Gly
What is the net charge of the molecule at pH 11?

Answer: -1 (5 points). Note the pH.
Some shown work will get partial credit (3 points)
No work, "negative" (2 points)

b)

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Ile-Ala-His-Thr-Tyr-Gly-Pro-Phe-Glu-Ala-Ala-Met-Cys-Lys-Trp-Glu-Ala-Gln-Pro-
 1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19

Asp-Gly-Met-Glu-Cys-Ala-Phe-His-Arg
20  21  22  23  24  25  26  27  28
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In the amino acid sequence above, where would you predict that bends or beta-turns would occur? Where might intra-chain disulfide cross-linkages be formed? Give the residue numbers.

Answer: Turns/Beta sheets at residues 7 and 19 (prolines are good for turns due to their cyclic structure) Disulfides at the cysteines residues 13 and 24. (5 points)

c) Many proteins catalyze biochemical reactions. Which one of the following statements are true of enzyme catalysts?

They bind to substrates, but are never covalently attached to substrate or product
They increase the equilibrium constant for a reaction, thus favoring product formation
They increase the stability of the product of a desired reaction by allowing ionizations, resonance, and isomerizations not normally available to substrates
They lower the activation energy for the conversion of substrate to product
To be effective, they must be present in the same concentration as their substrates.

D. (5 points). Needs to be selected to get any credit.
Partial credit was given if any others were selected in addition.

3. *Cell line development*. Your company has generated a hybridoma cell line that produces the beta-globulin antibody at 3 pg/cell/day. How might you generate a cell line with improved production of your protein?

Two possible answers, each requiring a sentence or two of explanation:
DHFR, stringent selection (5 points)

Once you have generated your improved cell line, how might you determine where in the genome it has inserted?

Sequence out using a primer designed from your gene of interest. Sequence in both directions and identify genome elements closely associated. (5 points)

Design a way to direct recombinant genes for future cell lines to integrate at this site. Describe similarities and differences of this process to knocking out a gene in bacteria. Diagrams are necessary.

The sequence you determined in the previous section is important here. This is similar to making a gene knockout in bacteria in that the gene you want is embedded in sequence homologous to the region you want to insert into. Instead of a marker gene (e.g. kan resistance) you are inserting your gene of interest. (10 points: 4 for diagram, 3 for the use of flanking DNA, 3 for describing similarities)

4. Microbial Coexistence in a Chemostat

EMBED Equation.3

where:

X_1= Biomass of species 1

X_2 = Biomass of species 2

S= Substrate concentration

μ_1 = specific growth rate of species 1

μ_2 = specific growth rate of species 2

D = dilution rate

Solve the mass balance equations for the steady state substrate concentration S_{ss}, and D_c the dilution rate for coexistence. Show all steps! What major assumption do you have to make?

Major assumption: Set all rates to 0 because you are at steady state. (5 points)

Solve this with the quadratic formula

where
a = QUOTE ; b = QUOTE ; c = 0

There are two roots (5 points, no 0 is OK):

Thus (5 points):

5. *Protein folding.* Your company has scaled up a new process to manufacture a therapeutic protein for clinical trials. Refractile bodies from a 1,000 L *E. coli* fermentation are dissolved in 8M urea, 20mM dithiothreitol; and then refolded by dilution into a 10,000 L tank, 2M urea, 4mM glutathione, pH 9. After refolding, the solution is held in the tank awaiting chromatography for final purification. Because of limitations in the capacity of the first chromatography column, only $^1/_5$ of the tank can be processed at one time, so the material is processed in 5 batches which are purified on succeeding days. Analysis of material purified on the first and fifth days shows the following differences:

Reversed phase HPLC – at day 5, an additional peak is present (about 5% as large as the main product peak).
SDS-PAGE – no differences day 1 to 5.
Isoelectric focusing – at day 5, an additional band is present with more acidic isoelectric point.
Peptide map – at day 5, a small additional peak is present.
Mass spec – at day 5, an additional species with a mass increase of 43da is present.

What is the contaminant which is present at day 5? How do you know?

The storage conditions and analytical results are consistent with carbamylation of the desired product. Carbamylation results in an increase in mass of 43da (detected by mass spec, but too small a change to be noticed on SDS-PAGE) and makes the protein more acidic (IEF shift). Carbamylation should have been anticipated as an adverse result of prolonged exposure to urea at high pH. (5 points)

While maintaining the same production schedule (refolding at 10,000 L scale, and purifying 5 batches over 5 days) suggest at least two changes to minimize formation of the contaminant.

Carbamylation can be slowed by a) reducing the pH during storage to minimize formation of reactive cyanate, and by b) including ethylenediamine or another cyanate scavenger. (5 points for 2 reasons)

6. *Cloning and molecular biology*. Below are the reactions, nucleotide sequences and translations of two enzymes catalyzing steps in the lignin biodegradation pathway:

You are interested in expressing them in the bacterium *E. coli* with the plasmid pUC19(bb), a pUC19 vector modified with the following multi-cloning site.

```
                                  EcoRI    BglII    BamHI      XhoI
                                  ~~~~~~   ~~~~~~   ~~~~~~~   ~~~~~~~
AAGCTTGCAT GCCTGCAGGT CGACTTCTAG AGAATTCATG AGATCTATAG GATCCTAACT CGAGACTGGC CGTCGTTTTA
CAACGTCGTG A
TTCGAACGTA CGGACGTCCA GCTGAAGATC TCTTAAGTAC TCTAGATATC CTAGGATTGA GCTCTGACCG GCAGCAAAAT
GTTGCAGCAC T
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a. In addition to the multi-cloning site in *lacZ*, what two other aspects of pUC19 make it a good cloning vector?

Antibiotic resistance marker, independent origin of replication (5 points). Partial credit was given for mentioning "broad host range" plasmid. pUC19 is NOT a broad host plasmid (in fact it is *E. coli* specific) but shows some indication of thinking of independent origins of replication

b. Design primers using BioBricks and SLIC to insert the two pieces into the vector. Diagrams, rationale, and special considerations in primer design are required. Use the back of this page.

This one was graded on a case-by case basis. No primers at all, only explanations: minus 6 points. Attempts of only one BB or SLIC process: minus 3 points.

BioBricks primers (5 points):

First, remove the BglII site in PhoA, e.g. (worth 2 of 5 points)
CCGAGCTGAGGTCTGGCCTC and complement (need silent mutation)

And the EcoRI site in PnpA, e.g.
GCGAG AACGAGTTCC TGCTG and complement (need silent mutation)

Then design some BioBricks primers using the canonical primer ends:
phoAF: TTG GAATTC ATG GGATCC ATGATCAATA AGAC (change Lys codon to avoid to many A's)
phoAR: TTC CTCGAG CTA AGATCT TCAGAC AGCTTCGGCA (Lys codon again)

pnpAF: TTG GAATTC ATG GGATCC ATGACCATCA CCAAA
pnpAR: TTC CTCGAG CTA AGATCT TCACT TGATCAGCGG
Although the question prompt put BglII in front of BamHI, the reverse will be accepted for full credit as the diagram in the notes put the BioBricks sites in that order.

SLIC Primers (5 points):
Easiest way to do this is just make a long version of the final sequence you want and select primers from that. Special considerations include making sure there are not too many overlapping regions. **(2 points for some explanation)**

CGACTTCTAG AGAATTCATG AGATCT ATGATCAATA AAGCGTACGA... ...TCGAT TGCCAAGGCT GTCTGA GGAGG

phoA *intervening*

ATAAT ATGACCATCA CCCAAGAAGCT... ...GCGTG CCGTGATCA AGTGA ATAG GATCCTAACT CGAGACTGGC

sequence pnpA

CGTCGTTTTA

Thus, primers might be: **(3 points)**

phoA-fwd: AGAATTCATG AGATCT ATGATCAATA AAGCGTACGA
phoA-rev: TTGGGTGATGGTCAT ATTAT GGTCC TCAGACAGCCTTGGCA

pnpA-fwd: TGCCAAGGCT GTCTGA GGAGG ATAAT ATGACCATCACCCAA
pnpA-rev: AGTTAGGAT CCTAT TCACT TGATCACGG

vector-fwd: CCGTGATCA AGTGA ATAG GATCCTAACT

vector-rev: TCGTACGCTT TATTGATCAT AGATCT CATGAATTCT

c. A paper you just read has suggested that the active site is the catalytic triad of PnpA is Arg24, Asn38, Glu71. You are interested in improving catalytic efficiency of the enzyme. Describe a method to do this. Include clear, labeled diagrams and the sequences of any primers you might order.

Site directed mutagenesis. Diagrams necessary (1 point)

Arg24: CAGGAAGGCNNNTACGTAAA and TTTACGTANNNGCCTTCCTG
Asn38: CTGGTGGCCNNNTACCTGGG and CCCAGGTANNNGGCCACCAG
Glu71: GCGAGAACNNNTTCCTGCTG and CAGCAGGAANNNGTTCTCGC
(4 points)

d. How might you screen for improvements to the enzyme? Using the method you described above, how many colonies will you have to screen?

Since your enzyme converts a colored (PNP) substrate to a colorless (beta-ketoadipate) substrate, a colorometric assay is ideal. One way might be to express the enzyme in cells in the presence of PNP and look for the colonies that convert PNP to beta-ketoadipate more rapidly than the non-engineered control. (3 points)

If you were to screen ALL of the possible mutants, you would have to screen 3*(20*20*20) = 24,000 cfu (will also accept 60*60*60 =216,000). However, if you screen for each mutation separately and only select ONE best fit each sequentially, you can screen 60 + 60 + 60 = 180-colonies. However, you may miss combinatorial improvements (e.g. Glu24 might work better in combination with Asn38, but in combination with Arg38 it might not). (2 points)

Sufficient dilutions and spreading across several plates may make this possible in a single experiment. With this many items to screen, a selection method would be most efficient.

e. A strain of *B. subtilis* has been known to use PNP as growth substrate. How might you use this strain to improve your screen or select for improvements in your enzyme? Include diagrams of any plasmids necessary.

Transform genes of interest into a plasmid that will replicate in *B. subtilis* (not pUC19)
Perform mutagenesis, express genes
Grow cells on PNPP as sole carbon source
Cells that grow faster than the wild-type contain improvements. (5 points)

Growth rate μ

[Substrate]

lacZ