Dynamic Flux Balance Analysis

LEARNING OBJECTIVES

Each student should be able to:

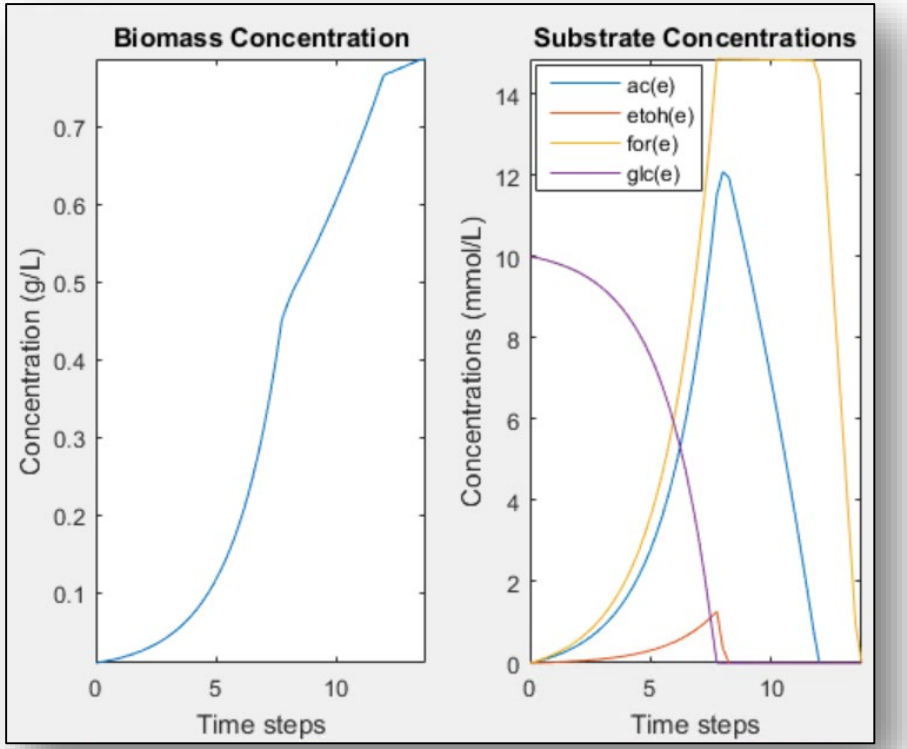
- Explain dynamic flux balance analysis.
- Describe the strengths and limitations of dynamic flux balance analysis.
- Describe the difference between the regular flux balance analysis and the dynamic flux balance analysis analysis.
- Describe the capabilities of the Matlab Property Editor.
- Explain how minimal media is modeled.
- Explain the difference between minimal and K-12 media.

Lesson Outline

- Dynamic Flux Balance Analysis (dynamicFBA) Overview
- "dynamicFBA" Basic Operation
- Minimal Media Examples
- Limiting Media Examples

Dynamic Flux Balance Analysis

- FBA can be used to examine dynamic processes such as microbial growth in batch cultures by combining FBA with an iterative approach based on a quasi-steady-state assumption (static optimization–based dynamic FBA).
  - ✓ At each time step, FBA is used to predict growth, nutrient uptake and by-product secretion rates.
  - ✓ These rates are then used to calculate biomass and nutrient concentrations in the culture at the end of the time step.
  - ✓ The concentrations can, in turn, be used to calculate maximum uptake rates of nutrients for the next time step.
  - ✓ Using this iterative procedure, dynamic FBA has allowed the simulation of batch experiments.
- This function will perform dynamic FBA to predict the outcomes of growth in batch culture conditions.



Becker, S. A., A. M. Feist, et al. (2007). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox." Nature protocols 2(3): 727-738.

Dynamic Flux Balance Analysis

- The substrate concentration (S_c) $(mmol/L)$ is determined from the substrate concentration predicted for the previous step (S_{co}) or from the initial substrate concentration if it is the first time step:

$$S_c = S_{co}$$

- The substrate concentration is scaled to define the amount of substrate available per unit of biomass per unit of time $(mmol\ gDW^{-1}h^{-1})$:

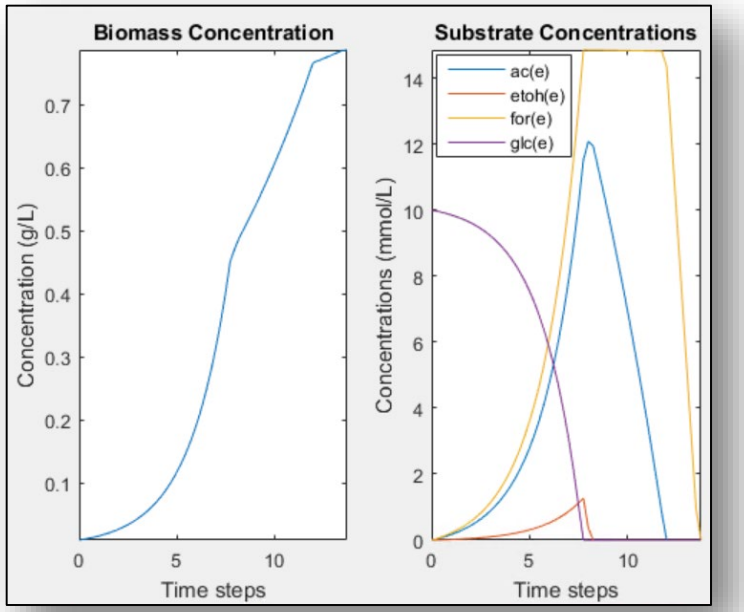
$$Substrate\ available = \frac{S_c}{X \cdot \Delta t}$$

where X (gDW/L) is the current cell density and X_o is the cell density from the previous step.

- FBA is then used to calculate the substrate uptake, S_u $(mmol/L)$ and the growth rate μ (h^{-1}).
- Concentrations for the next time step are calculated from the standard differential equations:

$$\frac{dX}{dt} = \mu X \rightarrow X = X_o \cdot e^{\mu \Delta t}$$

$$\frac{dS_c}{dt} = -S_u \cdot X \rightarrow S_c = S_{co} + \frac{S_u}{\mu} X_o \cdot (1 - e^{\mu \Delta t})$$



- The output of dynamic FBA is two graphs: one showing the flux through the objective reaction over time, and one showing the flux through the exchange reactions for the selected metabolites over time.

Varma, A. and B. O. Palsson (1994). "Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type Escherichia coli W3110." Applied and Environmental Microbiology 60(10): 3724-3731.

Lesson Outline

- Dynamic Flux Balance Analysis (dynamicFBA)
- "dynamicFBA" Basic Operation
- Minimal Media Examples
- Limiting Media Examples

Dynamic Flux Balance Analysis

- The dynamic flux balance analysis function is called as:

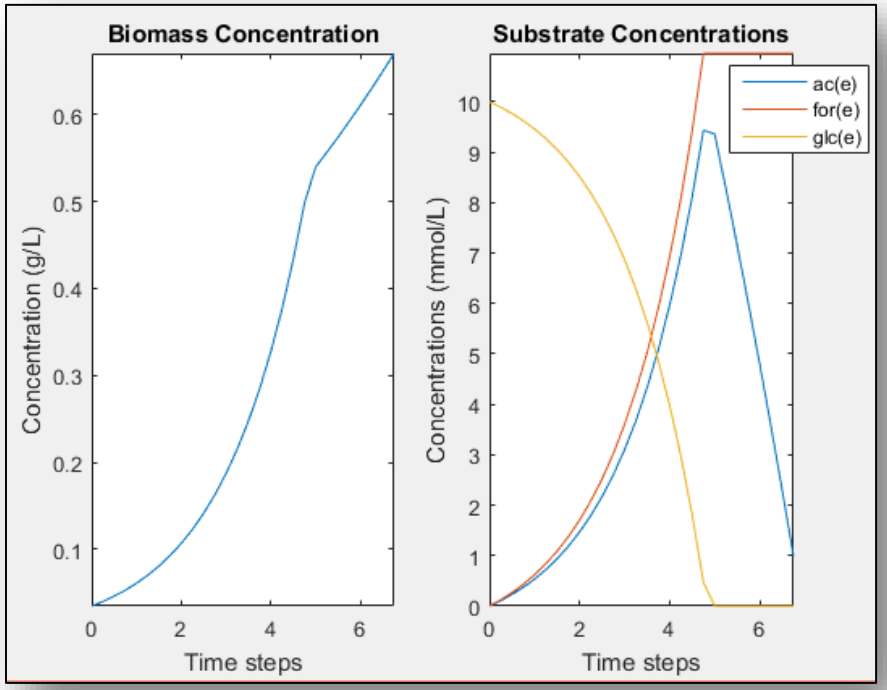
  dynamicFBA(model, substrateRxns, initConcentrations, initBiomass, tStep, nSteps,plotRxns)

- The list of exchange reactions corresponding to the substrates that are initially in the media (e.g., glucose, ammonia, phosphate) is described in substrateRxns.
- The initConcentrations variable sets the initial concentrations of substrates (mmol/L) in the substrateRxns vector.
- The initBiomass variable is needed to specify the initial amount of biomass (g/L) in the simulation.
- The tStep variable sets the time step size interval (h) and the nSteps variable designates the maximum number of time steps for the analysis.
- The plotRxns variable is optional and contains the names of the exchange reactions for the metabolites whose time-dependent concentrations should be plotted graphically.

Varma, A. and B. O. Palsson (1994). "Stoichiometric flux balance models quantitatively predict growth and metabolic by-product secretion in wild-type Escherichia coli W3110." Applied and Environmental Microbiology 60(10): 3724-3731.

Dynamic Glucose, Low Aerobic

```
% dynamicGlucoseAnoxic_Core.m
clear;

model = readCbModel('ecoli_core_model.mat');

model = changeRxnBounds(model, 'EX_glc(e)',-10, 'l');
model = changeRxnBounds(model, 'EX_o2(e)',-10, 'l');
model = changeObjective(model,'Biomass_Ecoli_core_N(w/GAM)-Nmet2');

% Set-up variables for dynamicFBA
substrateRxns = {'EX_glc(e)'};
initConcentrations = [10]; initBiomass = .035;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ac(e)','EX_etoh(e)','EX_for(e)','EX_fru(e)','EX_fum(e)','EX_glc(e)','EX_gln_L(e)',...
    'EX_glu_L(e)','EX_lac_D(e)','EX_mal_L(e)','EX_succ(e)','EX_acald(e)'};

 [concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
     dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, timeStep, nSteps, plotRxns);

 % Plot labels
subplot(1,2,1); title('Biomass Concentration'); xlabel('Time steps'); ylabel('Concentration (g/L)');
subplot(1,2,2); title('Substrate Concentrations'); xlabel('Time steps'); ylabel('Concentrations (mmol/L)');
```



Dynamic FBA Programming Hints

To plot the concentration of a desired metabolite in extracellular space using dynamicFBA, you cannot set the boundary conditions for either the exchange or demand reactions associated with that metabolite. Thus, if you want to plot the extracellular concentration of a metabolite you must set the desired constraints on the reaction producing the metabolite to the fixed value, e.g.

As an example, you should change either of the following from

```
model = changeRxnBounds(model,'EX_metabolite[c]',0.00xxx,'l');
model = changeRxnBounds(model,'DM_metabolite[c]',0.00xxx,'l');
```

To

```
model = changeRxnBounds(model,'ProducingReaction',0.00xxx,'l');
```

DynamicFBA Hints

Exhausted Nutrients

Finding exhausted nutrients in dFBA

1. The first step is to look at the workspace and click the cell called excRxnNames. It has the names of all the metabolites that dFBA is tracking. You can see that on the graphic to the right.

2. The next thing you need to look at is the metabolite concentrations over time that dFBA is saving. This information is saved in the cell called concentrationMatrix, unfortunately it is saved as a sparse matrix which is difficult to read. You can save that sparse matrix to a full matrix by using the following function

   ```
   CM = full(concentrationMatrix);
   ```

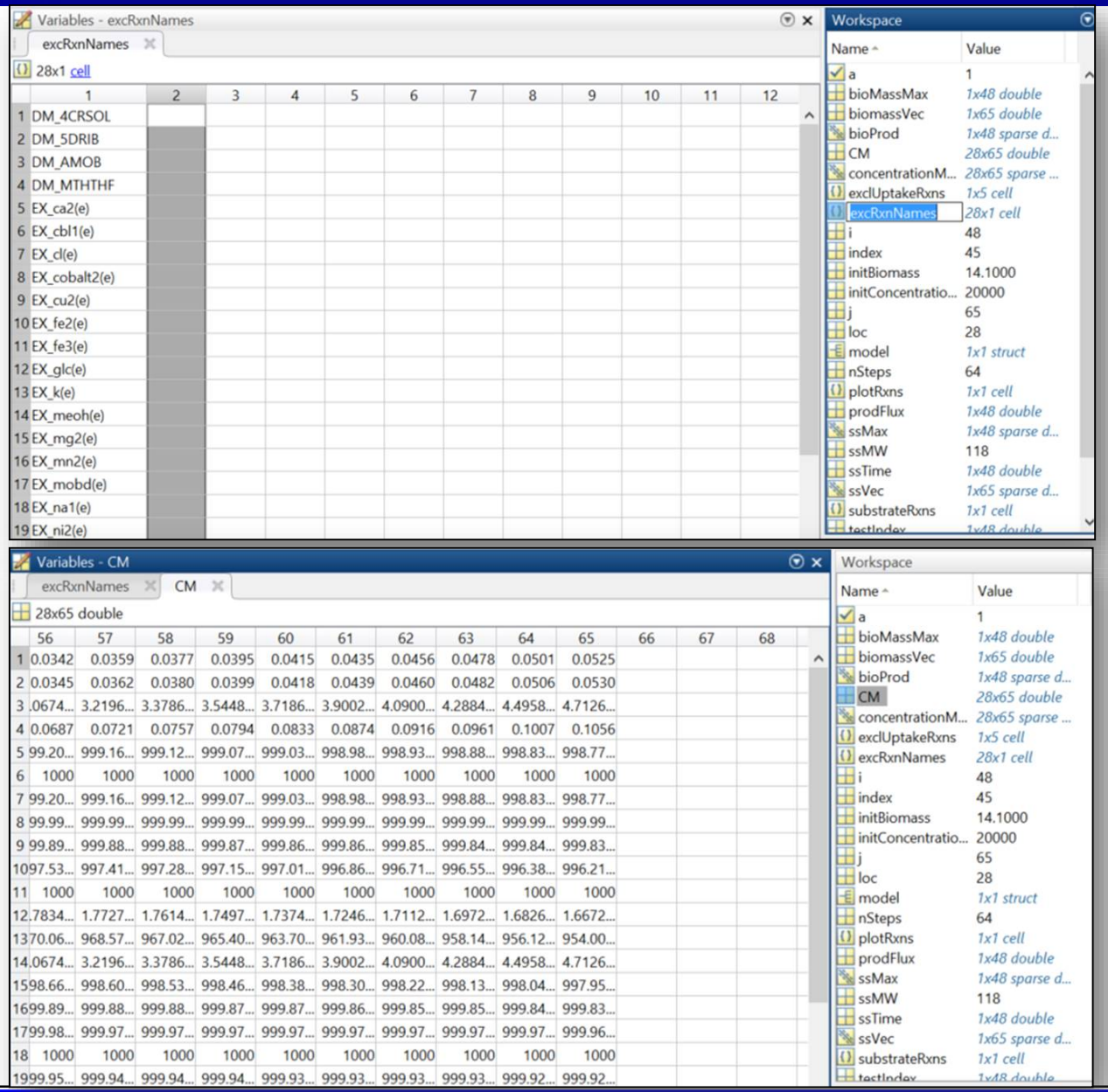
3. You can click on this matrix and it will show the concentrations of each metabolite (the names are at the same rows as they are in exclUptakeRxns). See graphic to the right.

4. If you look in the final column you should be able to find a 0 or looking at the previous columns you should be able to find one of the metabolites that is approaching zero. That should be the nutrient that is exhausted.

5. Now you go back to dFBA set-up code and add a new exchange reaction, associated with the exhausted metabolite, and set a new initial concentration, such as

   ```
   substrateRxns = {'EX_glc(e)', 'EX_nh4(e)'};
   initConcentrations = [2000 2000];
   ```

6. You might need to do this several times if there are several metabolites that are close to being exhausted.



DynamicFBA Hints (II)

Exhausted Nutrients

5. Now you go back to dFBA set-up code and add a new exchange reaction, associated with the exhausted metabolite, and set a new initial concentration, such as

```
substrateRxns = {'EX_glc(e)', 'EX_nh4(e)'};
initConcentrations = [2000 2000];
```

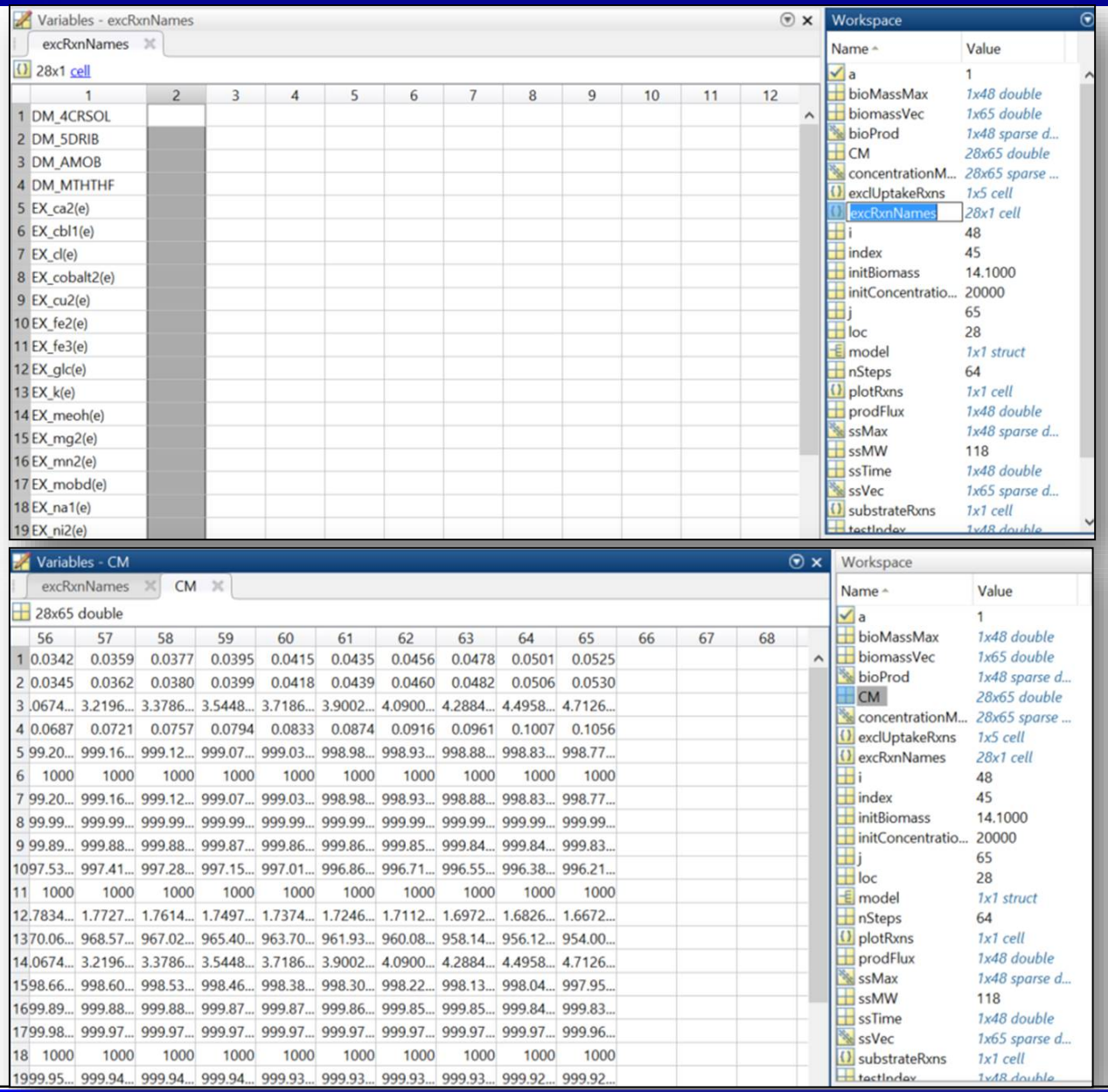
6. You might need to do this several times if there are several metabolites that are close to being exhausted.

7. The reactions located in "substrateRxns" and "plotRxns" need to be in the order that they appear in the model.

8. If no initial concentration is given for a substrate that has an open uptake in the model (i.e. model.lb < 0) the concentration is assumed to be high enough to not be limiting (e.g. 1000).

```
model = changeRxnBounds(model, 'EX_xxx_e', 0.1, 'l');
```

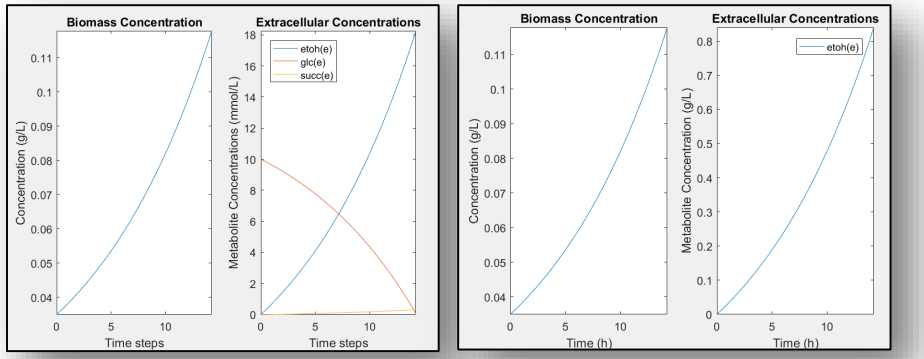
9. If the uptake rate for a nutrient is calculated to exceed the maximum uptake rate for that nutrient specified in the model and the max uptake rate specified is > 0, the maximum uptake rate specified in the model is used instead of the calculated uptake rate.



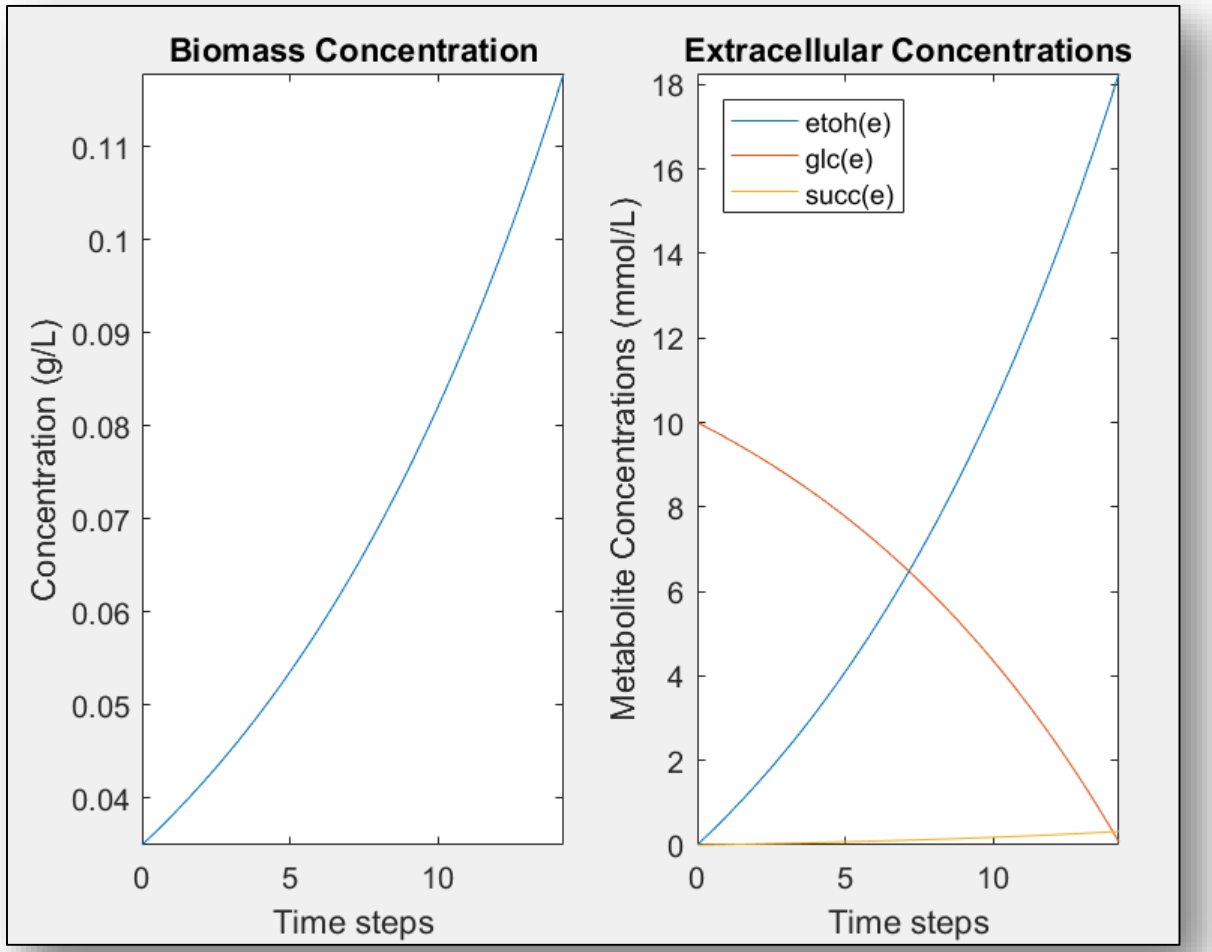
Plotting Production in g/L

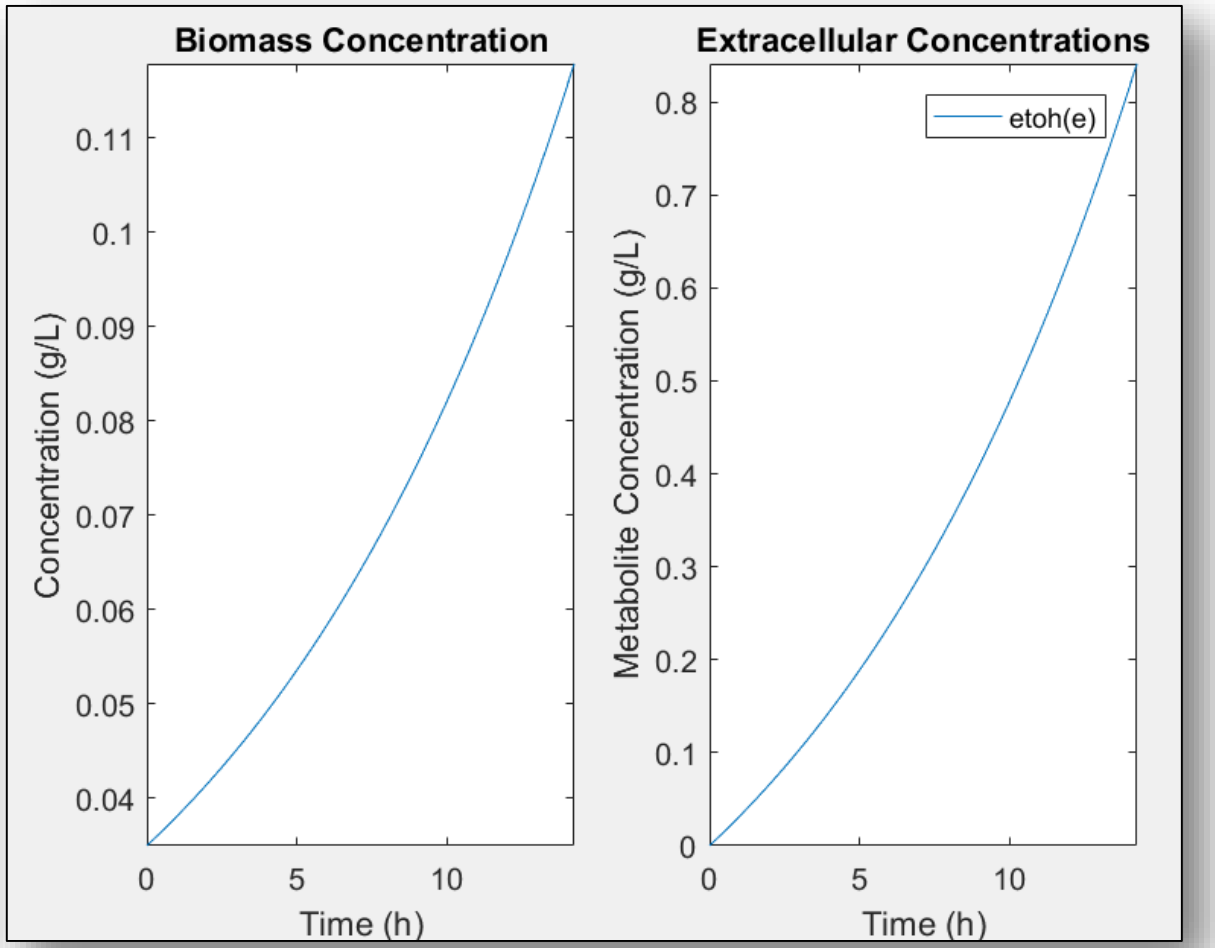
```
% dynamicEthanolProduction.m
clear;

% Load the E.coli core model
model = readCbModel('ecoli_core_model.mat');
model = changeRxnBounds(model,'EX_glc(e)',-10,'l');
model = changeRxnBounds(model,'EX_o2(e)',-0,'l');

% 3 Knockout reactions ( Growth-rate > 0.05 )
model = changeRxnBounds(model,'PTAr',0,'b');
model = changeRxnBounds(model,'GLUDy',0,'b');
model = changeRxnBounds(model,'PYK',0,'b');

% Set-up variables for dynamicFBA
substrateRxns = {'EX_glc(e)'};
initConcentrations = [10]; initBiomass = .035;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ac(e)','EX_etoh(e)','EX_for(e)','EX_glc(e)','EX_succ(e)'};

 [concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
     dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, timeStep, nSteps, plotRxns);
```

```
 % Plot labels
subplot(1,2,1); title('Biomass Concentration'); xlabel('Time steps'); ylabel('Concentration (g/L)');
subplot(1,2,2); title('Extracellular Concentrations'); xlabel('Time steps');
ylabel('Metabolite Concentrations (mmol/L)');

% Plot ethanol production in g/L
CM = full(concentrationMatrix);
[a,loc] = ismember({'EX_etoh(e)'},excRxnNames);
ethanolMW = 0.04607; % ethanol molecular weight (g/mmol)

figure(2)
clf;
subplot(1,2,1);
plot(timeVec,biomassVec);
title('Biomass Concentration'); xlabel('Time (h)'); ylabel('Concentration (g/L)');
axis tight
subplot(1,2,2);
plot(timeVec,ethanolMW*concentrationMatrix(loc,:));
title('Extracellular Concentrations'); xlabel('Time (h)'); ylabel('Metabolite Concentration (g/L)');
axis tight
legend(strrep(excRxnNames(loc),'EX_',''));
```



Plotting Production in g/L (II)





dynamicEthanolProduction.m

Lesson Outline

- Dynamic Flux Balance Analysis (dynamicFBA)
- "dynamicFBA" Basic Operation
- Minimal Media Examples
- Limiting Media Examples

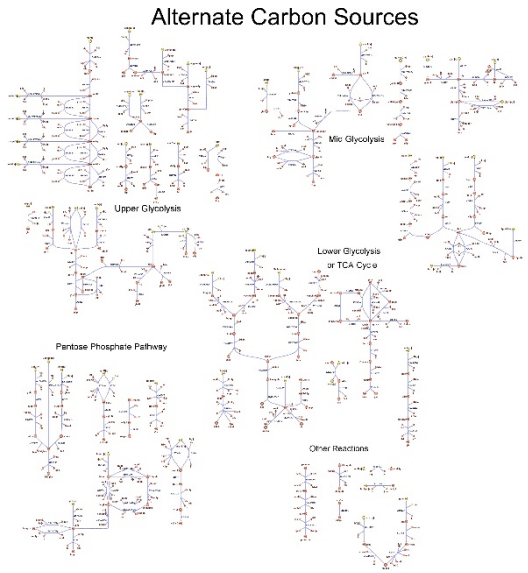
Media Impact

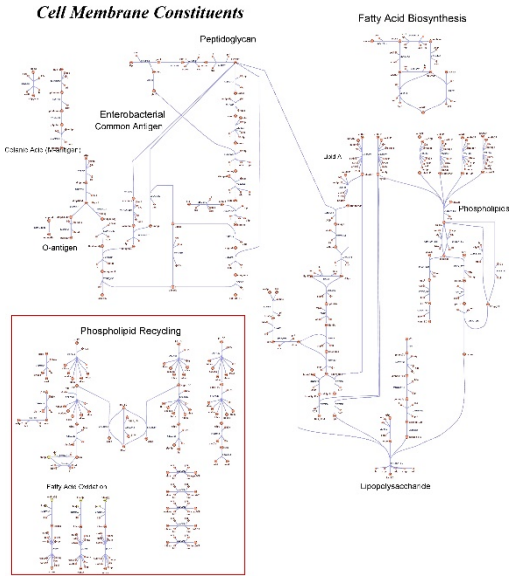
- The media can significantly alter the growth rate and bioproduct production
- Some amino acids and other metabolites can act as carbon sources
- Some produced bioproducts could require increased minerals uptake
- Adapting the composition of media to meet the needs of the engineered cells can significantly improve both the growth rate and bioproduct production.
- More complex models than the *E.coli* core model will be required to simulate media effects.

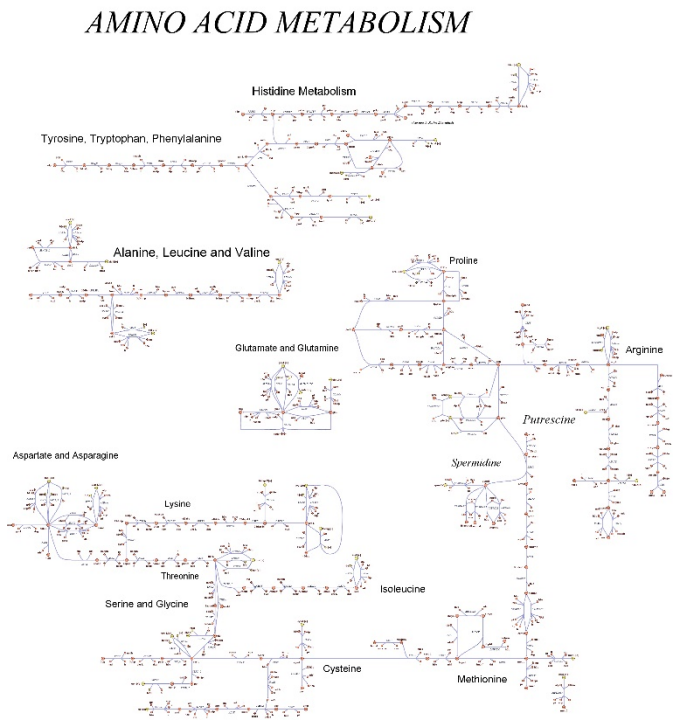
iJO1366

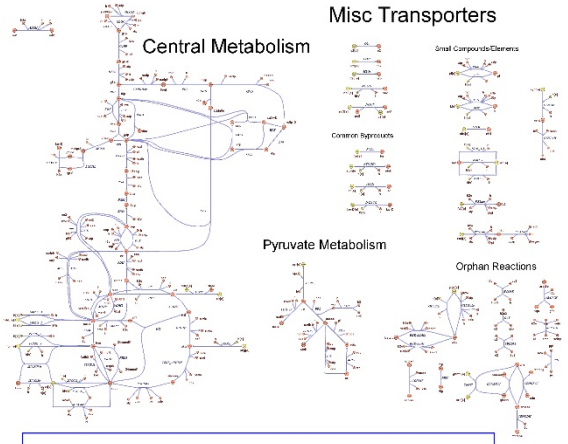
A Escherichia coli K-12 MG1655 Model

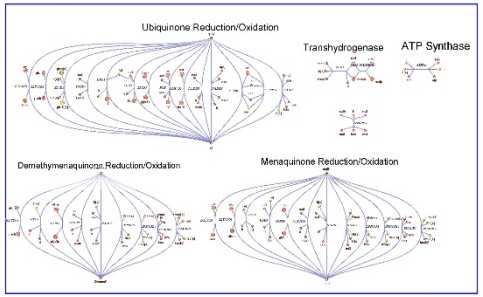
- 1366 Genes
- 2583 Reactions
- 1805 Metabolites

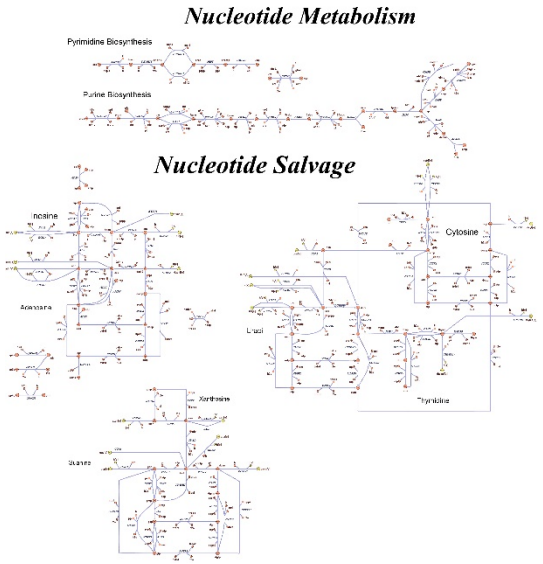


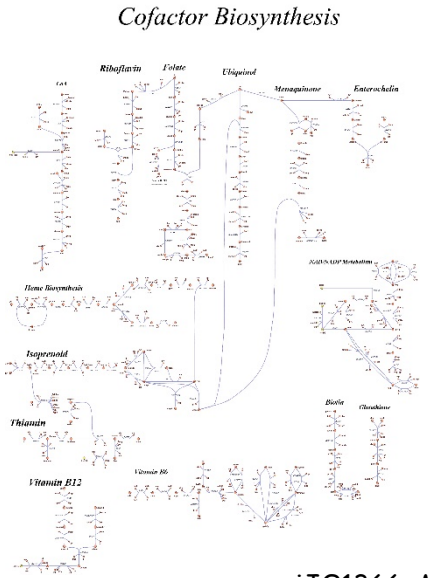










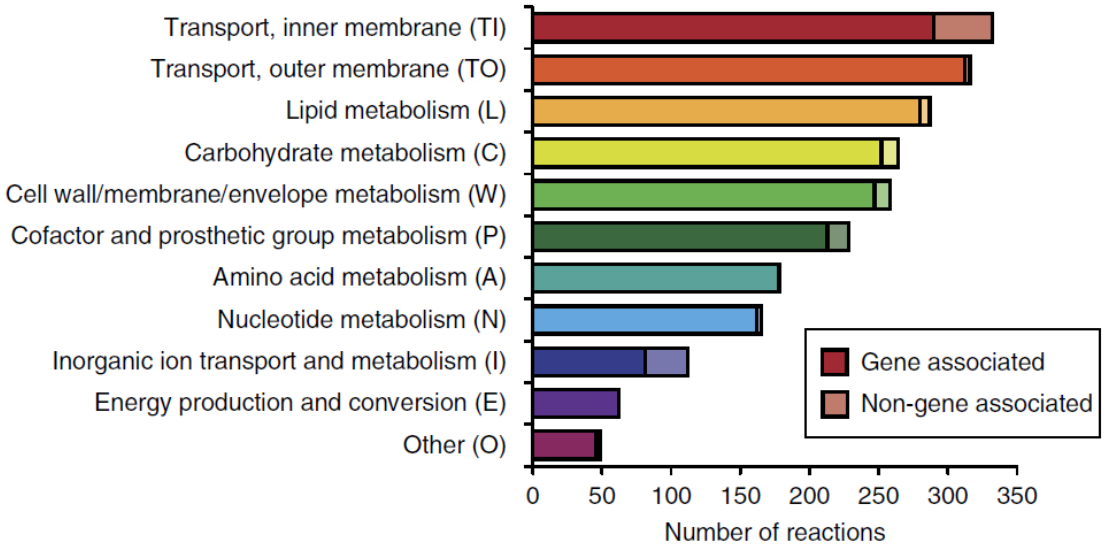


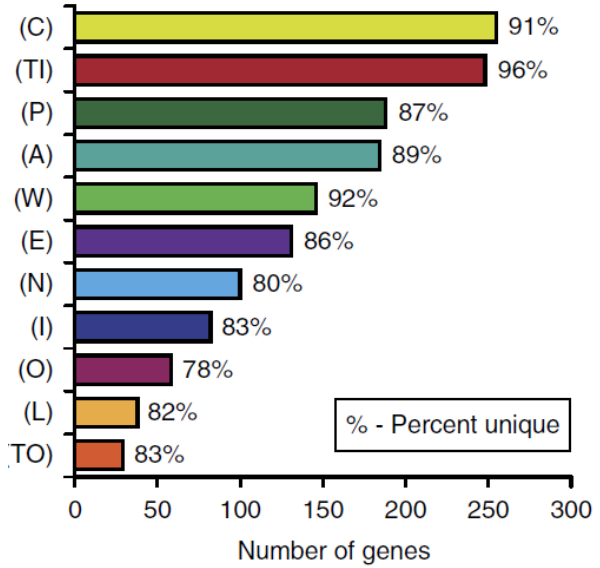
Orth, J. D. and B. O. Palsson (2012). "Gap-filling analysis of the iJO1366 Escherichia coli metabolic network reconstruction for discovery of metabolic functions." BMC systems biology 6(1): 30.

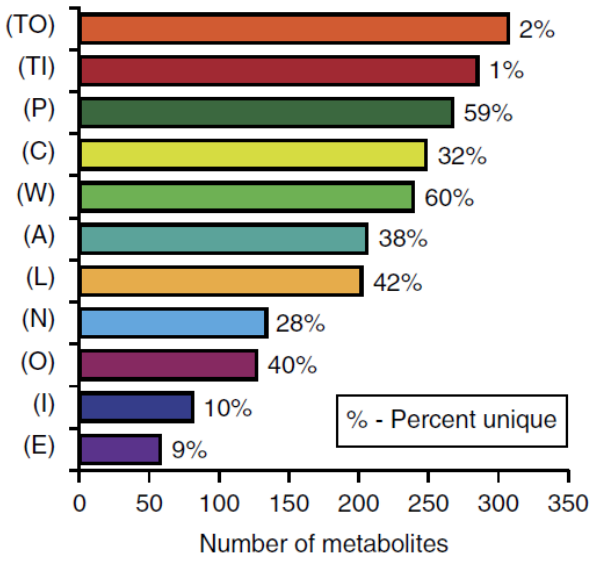
iJO1366_Map.pdf

iJO1366: *A Escherichia coli* K-12 MG1655 Model

Orth, J. D. and B. O. Palsson (2012). "Gap-filling analysis of the iJO1366 Escherichia coli metabolic network reconstruction for discovery of metabolic functions." BMC systems biology 6(1): 30.







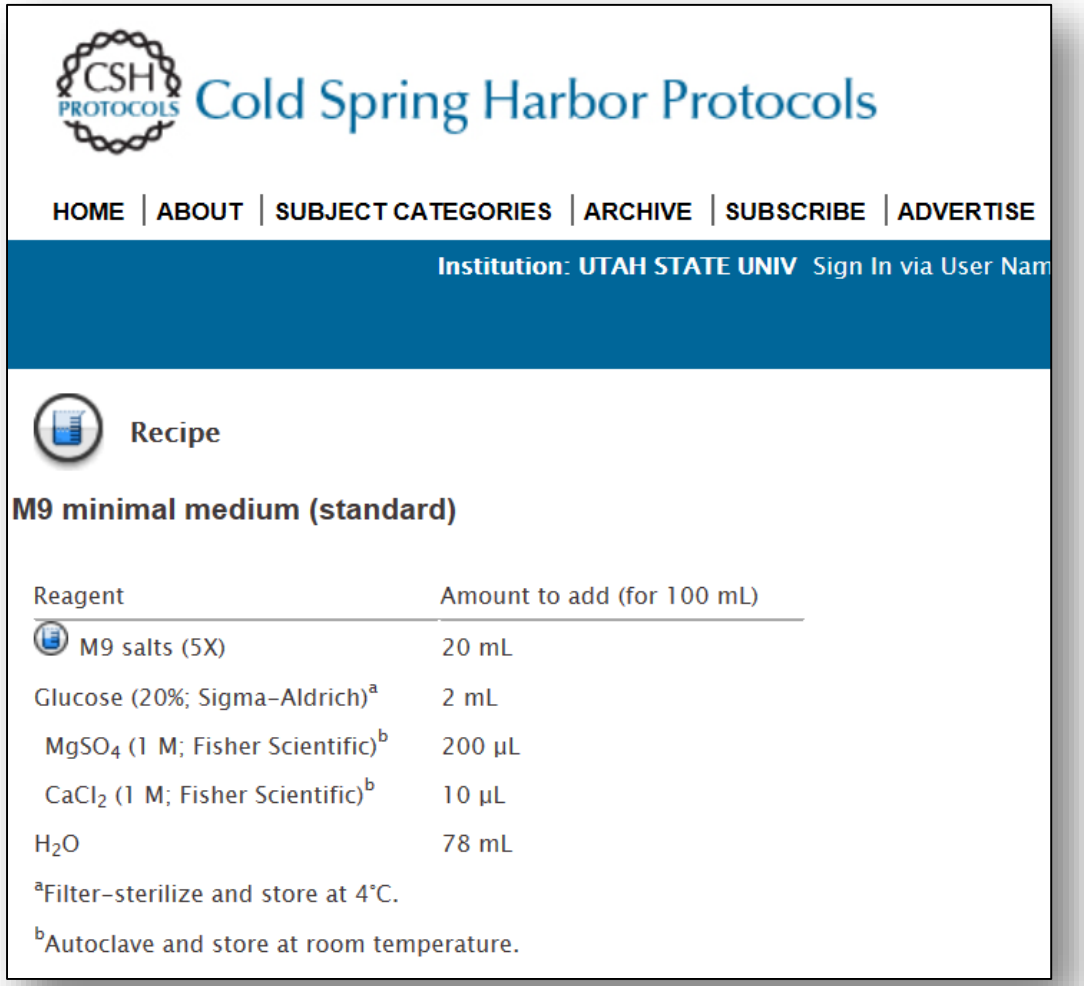
	*i*JO1366	
	Potential substrates	Growth supporting
Carbon	285	180
Nitrogen	178	94
Phosphorus	64	49
Sulfur	28	11

Growth supporting carbon, nitrogen, phosphorus, and sulfur sources

	Experimental	
	Essential	Non-essential
	Growth on glucose	
Computational		
Essential	168 (12.3%)	39 (2.8%)
Non-essential	80 (5.9%)	1079 (79.0%)

Gene essentiality predictions on glucose minimal media

M9 Minimal Media



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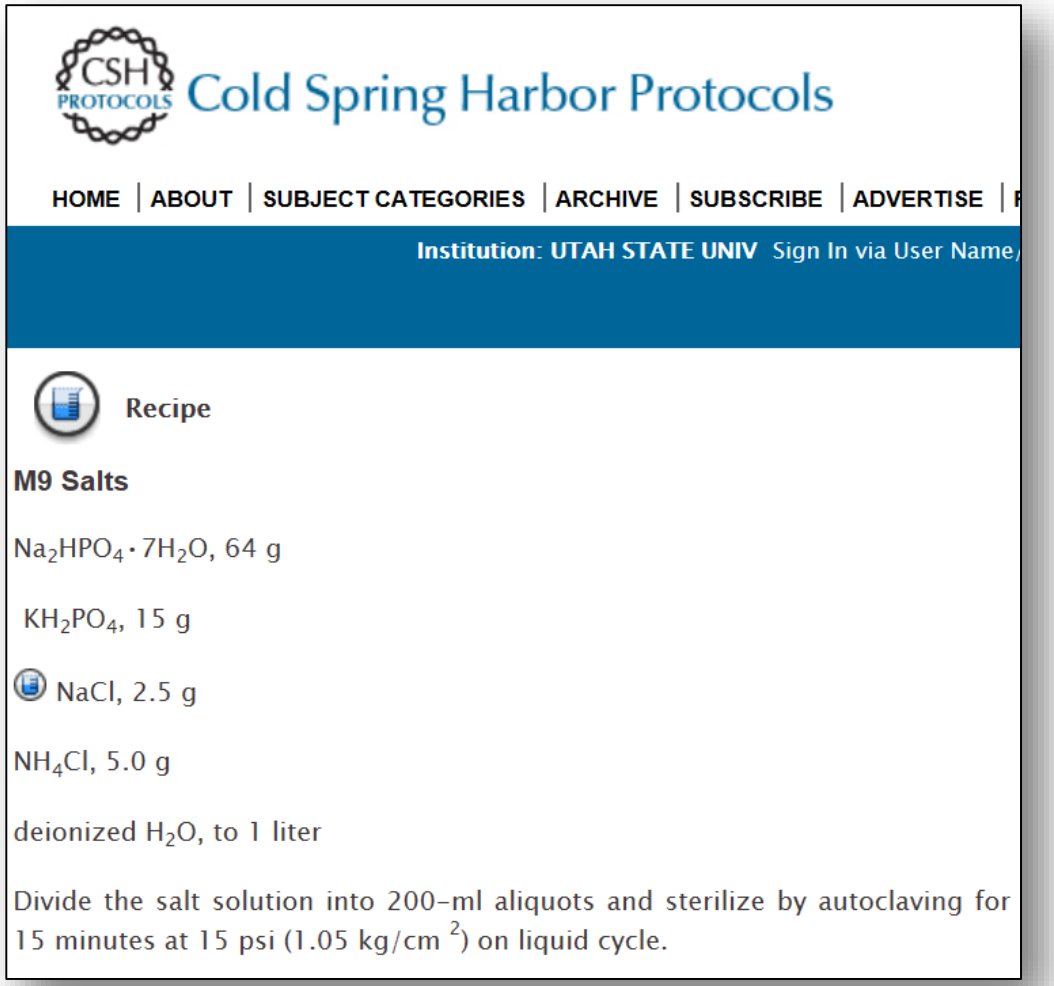
Recipe

M9 minimal medium (standard)

Reagent	Amount to add (for 100 mL)
M9 salts (5X)	20 mL
Glucose (20%; Sigma–Aldrich)[a]	2 mL
$MgSO_4$ (1 M; Fisher Scientific)[b]	200 µL
$CaCl_2$ (1 M; Fisher Scientific)[b]	10 µL
H_2O	78 mL

[a]Filter–sterilize and store at 4°C.

[b]Autoclave and store at room temperature.



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Recipe

M9 Salts

$Na_2HPO_4 \cdot 7H_2O$, 64 g

KH_2PO_4, 15 g

NaCl, 2.5 g

NH_4Cl, 5.0 g

deionized H_2O, to 1 liter

Divide the salt solution into 200–ml aliquots and sterilize by autoclaving for 15 minutes at 15 psi (1.05 kg/cm ^{2}) on liquid cycle.

http://cshprotocols.cshlp.org/content/2010/8/pdb.rec12295.short

in silico M9 Minimal Media

This *in silico* media assumes the cell can uptake all the minerals wanted/needed from the media. It does not allow amino acid uptake.

Reaction Abbreviation	Reaction Name	Formula	Lower Bound	Upper Bound
EX_ca2(e)	Calcium exchange	ca2[e] <=>	-1000	1000
EX_cl(e)	Chloride exchange	cl[e] <=>	-1000	1000
EX_co2(e)	CO2 exchange	co2[e] <=>	-1000	1000
EX_cobalt2(e)	Co2+ exchange	cobalt2[e] <=>	-1000	1000
EX_cu2(e)	Cu2+ exchange	cu2[e] <=>	-1000	1000
EX_fe2(e)	Fe2+ exchange	fe2[e] <=>	-1000	1000
EX_fe3(e)	Fe3+ exchange	fe3[e] <=>	-1000	1000
EX_h(e)	H+ exchange	h[e] <=>	-1000	1000
EX_h2o(e)	H2O exchange	h2o[e] <=>	-1000	1000
EX_k(e)	K+ exchange	k[e] <=>	-1000	1000
EX_mg2(e)	Mg exchange	mg2[e] <=>	-1000	1000
EX_mn2(e)	Mn2+ exchange	mn2[e] <=>	-1000	1000
EX_mobd(e)	Molybdate exchange	mobd[e] <=>	-1000	1000
EX_na1(e)	Sodium exchange	na1[e] <=>	-1000	1000
EX_tungs(e)	tungstate exchange	tungs[e] <=>	-1000	1000
EX_zn2(e)	Zinc exchange	zn2[e] <=>	-1000	1000
EX_ni2(e)	Ni2+ exchange	ni2[e] <=>	-1000	1000
EX_sel(e)	Selenate exchange	sel[e] <=>	-1000	1000
EX_slnt(e)	selenite exchange	slnt[e] <=>	-1000	1000
EX_so4(e)	Sulfate exchange	so4[e] <=>	-1000	1000
EX_nh4(e)	Ammonia exchange	nh4[e] <=>	-1000	1000
EX_pi(e)	Phosphate exchange	pi[e] <=>	-1000	1000
EX_cbl1(e)	Cob(I)alamin exchange	cbl1[e] <=>	-0.01	1000

Monk, J. M., P. Charusanti, et al. (2013). "Genome-scale metabolic reconstructions of multiple Escherichia coli strains highlight strain-specific adaptations to nutritional environments." Proc Natl Acad Sci USA 110(50): 20338-20343.

iJO1366 Amino Acid Exchange Reactions

Note: Amino acids are only allowed to be secreted in the basic model (LB = 0). The model can be modified to allow amino acid uptake.

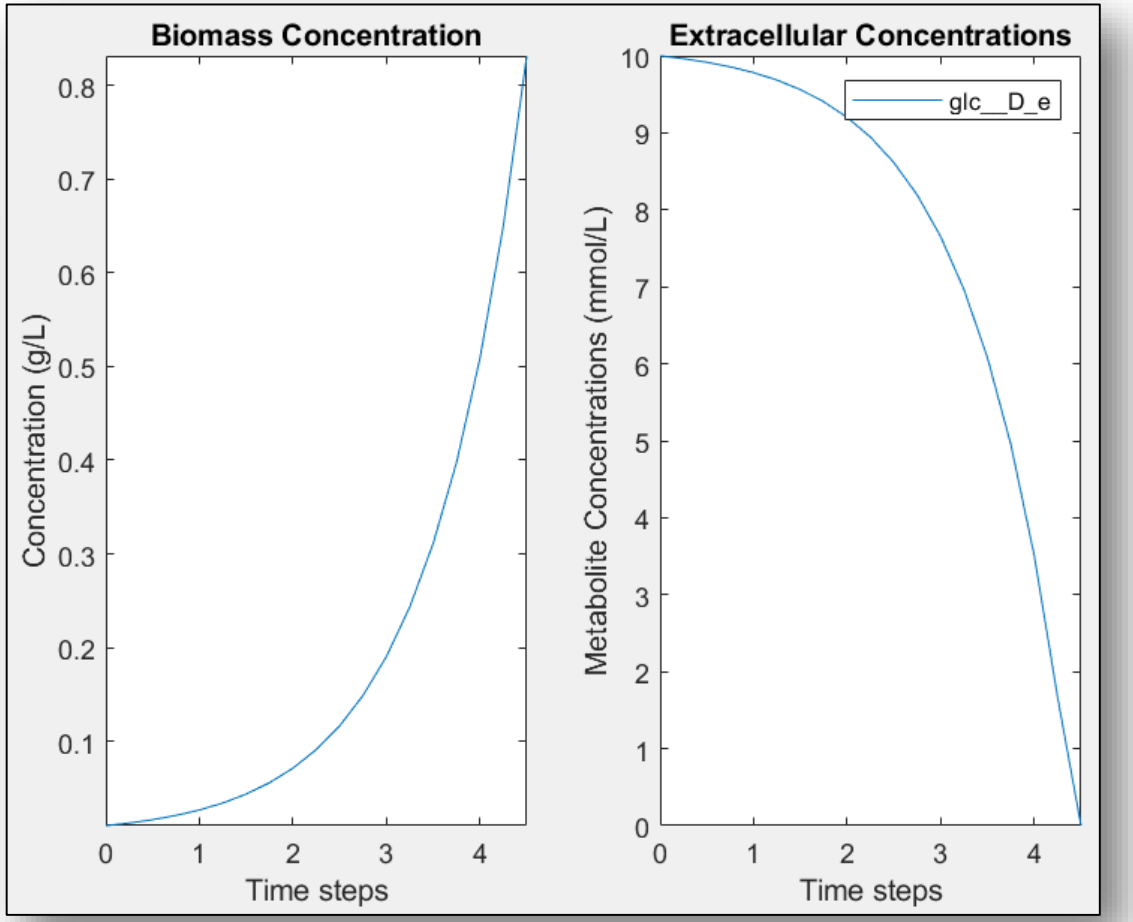
No essential amino acids for *E.coli*

Rxn name	Rxn description	Formula	LB	UB
EX_ala_L(e)	L-Alanine exchange	ala-L[e] <=>	0	1000
EX_arg_L(e)	L-Arginine exchange	arg-L[e] <=>	0	1000
EX_asn_L(e)	L-Asparagine exchange	asn-L[e] <=>	0	1000
EX_asp_L(e)	L-Aspartate exchange	asp-L[e] <=>	0	1000
EX_cys_L(e)	L-Cysteine exchange	cys-L[e] <=>	0	1000
EX_gln_L(e)	L-Glutamine exchange	gln-L[e] <=>	0	1000
EX_glu_L(e)	L-Glutamate exchange	glu-L[e] <=>	0	1000
EX_gly(e)	Glycine exchange	gly[e] <=>	0	1000
EX_his_L(e)	L-Histidine exchange	his-L[e] <=>	0	1000
EX_ile_L(e)	L-Isoleucine exchange	ile-L[e] <=>	0	1000
EX_leu_L(e)	L-Leucine exchange	leu-L[e] <=>	0	1000
EX_lys_L(e)	L-Lysine exchange	lys-L[e] <=>	0	1000
EX_met_L(e)	L-Methionine exchange	met-L[e] <=>	0	1000
EX_phe_L(e)	L-Phenylalanine exchange	phe-L[e] <=>	0	1000
EX_pro_L(e)	L-Proline exchange	pro-L[e] <=>	0	1000
EX_ser_L(e)	L-Serine exchange	ser-L[e] <=>	0	1000
EX_thr_L(e)	L-Threonine exchange	thr-L[e] <=>	0	1000
EX_trp_L(e)	L-Tryptophan exchange	trp-L[e] <=>	0	1000
EX_tyr_L(e)	L-Tyrosine exchange	tyr-L[e] <=>	0	1000
EX_val_L(e)	L-Valine exchange	val-L[e] <=>	0	1000

Aerobic, Glucose Substrate DynamicFBA Growth

```
% DynamicGrowth_Aerobic_JO1366_FBC.m
clear;

model = readCbModel('iJO1366.mat');
model = changeRxnBounds(model, {EX_glc__D_e','EX_o2_e'},[-10 -30], 'l');
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M');

% Set-up variables for dynamicFBA
substrateRxns = {' EX_glc__D_e '};
initConcentrations = [10];
initBiomass = .01;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ac_e','EX_acald_e','EX_etoh_e','EX_for_e', ...
            'EX_glc__D_e','EX_lac__L_e','EX_succ_e'};

[concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
    dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, ...
    timeStep, nSteps, plotRxns);

% Plot labels
subplot(1,2,1);
title('Biomass Concentration');
xlabel('Time steps');
ylabel('Concentration (g/L)');
subplot(1,2,2);
title('Substrate Concentrations');
xlabel('Time steps');
ylabel('Concentrations (mmol/L)');
```

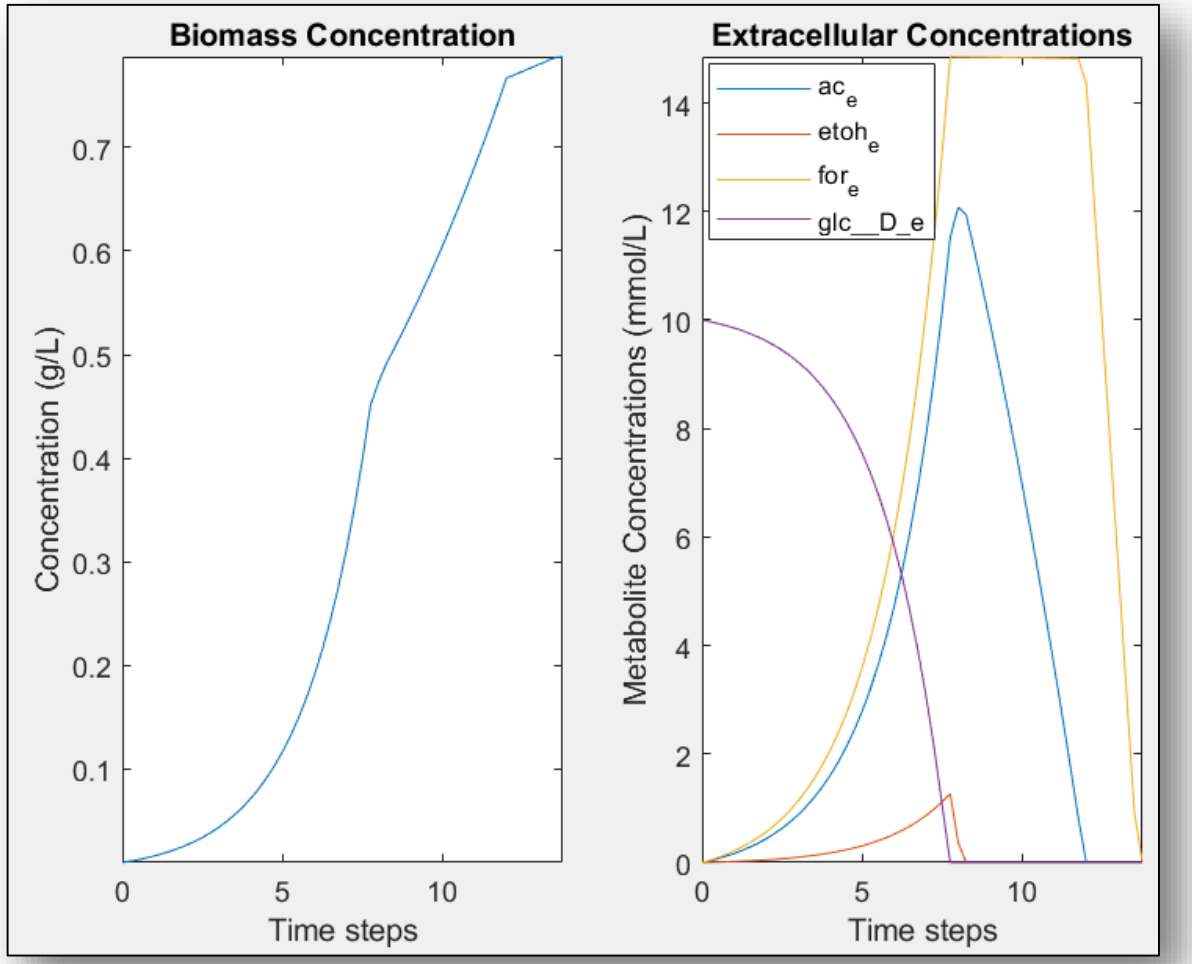
In the SBML level 3 FBC protocol, square brackets are illegal so the compartment identifier is preceded by an underscore*; e.g.
EX_GLC-D[e] -> EX_glc__D_e ;
EX_ac[e] -> EX_ac_e



*https://prince.lcsb.uni.lu/cobratoolbox/tutorials/base/IO/iframe_tutorial_IO.html

Low Aerobic, Glucose Substrate DynamicFBA Growth

```
% DynamicGrowth_Aerobic_JO1366_FBC.m
clear;

model = readCbModel('iJO1366.mat');
model = changeRxnBounds(model, {EX_glc__D_e','EX_o2_e'},[-10 -5], 'l');
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M');

% Set-up variables for dynamicFBA
substrateRxns = {' EX_glc__D_e '};
initConcentrations = [10];
initBiomass = .01;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ac_e','EX_acald_e','EX_etoh_e','EX_for_e', ...
            'EX_glc__D_e','EX_lac__L_e','EX_succ_e'};

[concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
    dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, ...
    timeStep, nSteps, plotRxns);

% Plot labels
subplot(1,2,1);
title('Biomass Concentration');
xlabel('Time steps');
ylabel('Concentration (g/L)');
subplot(1,2,2);
title('Substrate Concentrations');
xlabel('Time steps');
ylabel('Concentrations (mmol/L)');
```



Substrate Maximum Growth Rate with iJO1366 Model

The iJO1366 *E. coli* model allows 180 different organic compounds to be used as the sole carbon source under aerobic or anaerobic conditions.

Substrate	Aerobic (hr^{-1})	Anaerobic (hr^{-1})
acetate	0.507518	0
acetaldehyde	0.720937	0.0669269
ethanol	0.85598	0
D-fructose	1.97781	0.517819
formate	0.0323058	0
fumarate	0.912399	0.0786957
D-glucose	1.97781	0.517819
L-glutamine	1.41043	0.199669
L-glutamate	1.40768	0.198604
glycerol	1.13903	0.192276
D-lactate	0.869278	0.0185858
L-malate	0.912399	0.0786957
pyruvate	0.732137	0.114473
succinate	0.998535	0

Mixed Fermentation Products

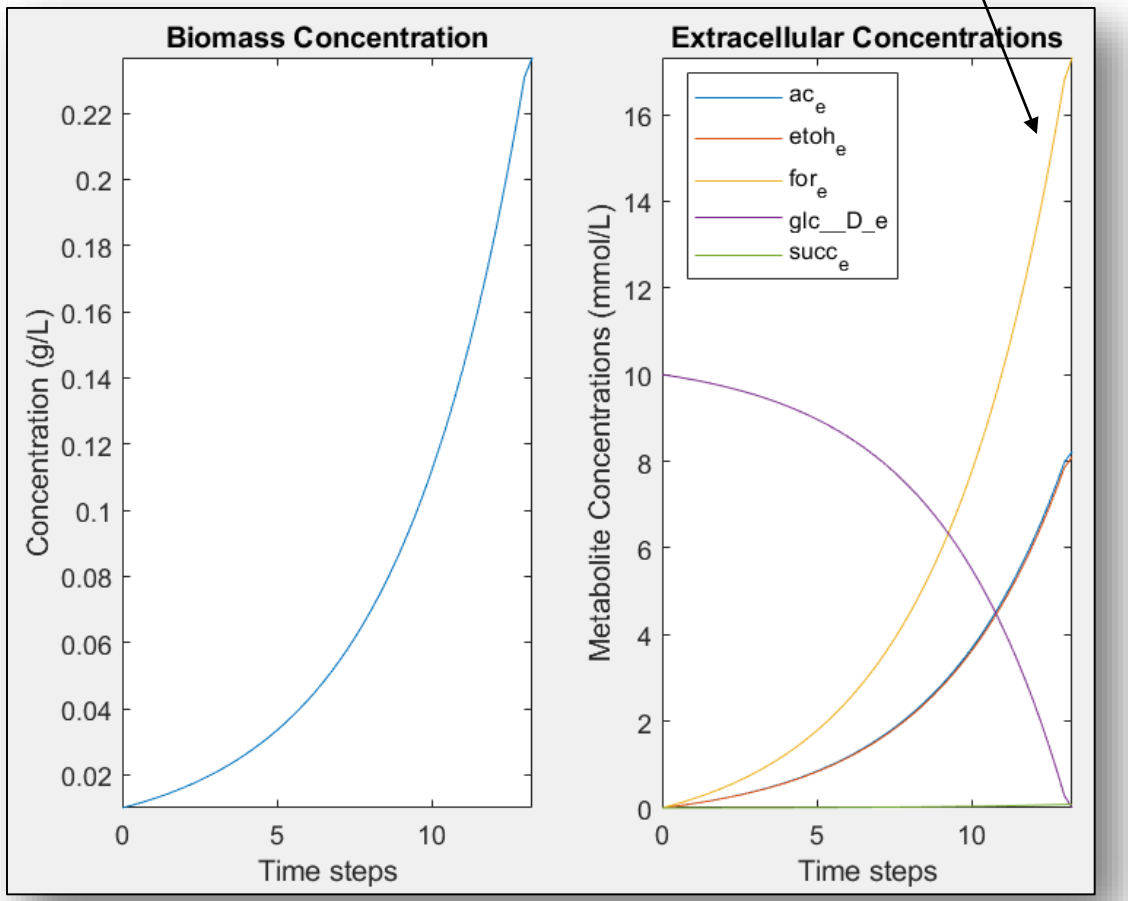
CarbonSourceGrowth_iJO1366.m

Orth, J. D. and B. O. Palsson (2012). "Gap-filling analysis of the iJO1366 Escherichia coli metabolic network reconstruction for discovery of metabolic functions." BMC systems biology 6(1): 30.

DynamicFBA: Anaerobic, Glucose Substrate

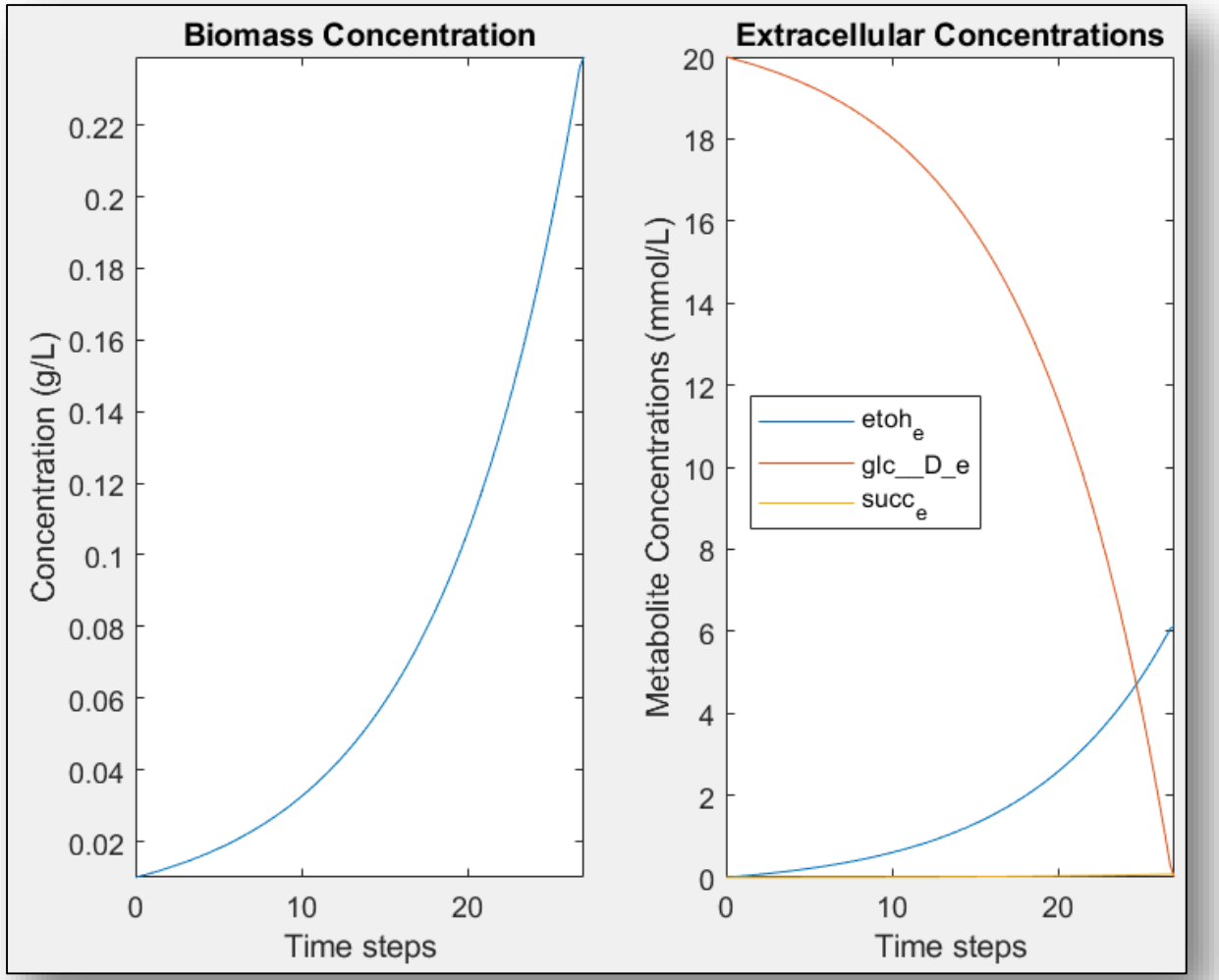
Will not grow on these substrates anaerobically

```
% DynamicGrowth_Aerobic_JO1366_FBC.m
clear;

model = readCbModel('iJO1366.mat');
model = changeRxnBounds(model, {EX_glc__D_e','EX_o2_e'},[-10 -0], 'l');
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M');

% Set-up variables for dynamicFBA
substrateRxns = {' EX_glc__D_e '};
initConcentrations = [10];
initBiomass = .01;
timeStep = .25; nSteps = 100;
plotRxns = {'EX_ac_e','EX_acald_e','EX_etoh_e','EX_for_e', ...
           'EX_glc__D_e','EX_lac__L_e','EX_succ_e};

[concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
    dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, ...
    timeStep, nSteps, plotRxns);

% Plot labels
subplot(1,2,1);
title('Biomass Concentration');
xlabel('Time steps');
ylabel('Concentration (g/L)');
subplot(1,2,2);
title('Substrate Concentrations');
xlabel('Time steps');
ylabel('Concentrations (mmol/L)');
```



DynamicFBA: Ethanol Production with Glucose Substrate

```
% DynamicEthanolProduction_JO1366_FBC.m
clear;

model = readCbModel('iJO1366.mat');
model = changeRxnBounds(model, {'EX_glc__D_e','EX_o2_e'},[-10 -0], 'l');
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M');

% Knockouts
model = changeRxnBounds(model, {'PFL','PPC','PPKr'},[-0 -0 -0], 'b');

% Set-up variables for dynamicFBA
substrateRxns = {'EX_glc__D_e'};
initConcentrations = [20];
initBiomass = .01;
timeStep = .25; nSteps = 125;
plotRxns = {'EX_ac_e','EX_acald_e','EX_etoh_e','EX_for_e',
            'EX_glc__D_e','EX_lac__L_e','EX_succ_e'};

[concentrationMatrix,excRxnNames,timeVec,biomassVec] = ...
    dynamicFBA(model,substrateRxns,initConcentrations, initBiomass,
    timeStep, nSteps, plotRxns);

% Plot labels
subplot(1,2,1);
title('Biomass Concentration');
xlabel('Time steps');
ylabel('Concentration (g/L)');
subplot(1,2,2);
title('Substrate Concentrations');
xlabel('Time steps');
ylabel('Concentrations (mmol/L)');
```



Lesson Outline

- Dynamic Flux Balance Analysis (dynamicFBA)
- "dynamicFBA" Basic Operation
- Minimal Media Examples
- Limiting Media Examples

K12 Media

- Growth in K12 media was simulated by adjusting lower bounds of exchange reactions to correspond to media conditions

	Chemical	Concentration
K12 Medium:	KH_2PO_4	2 g/L
	$K_2HPO_4.3H_2O$	4 g/L
	$(NH_4)_2HPO_4$	5 g/L
	Yeast Extract	5 g/L
	Glucose	25 g/L
	$MgSO_4.7H_2O$	0.5 g/L
	Thiamine	2.5 mg/L
	K12 trace metal	5 ml/L
K12 trace metal solution:	NaCl	5 g/L
	$ZnSO_4.7H_2O$	1 g/L
	$MnCl_2.4H_2O$	4 g/L
	$FeCl_3.6H_2O$	4.75 g/L
	$CuSO_4.5H_2O$	0.4 g/L
	H_3BO_3	0.575 g/L
	$NaMoO_4.2H_2O$	0.5 g/L
	6N H_2SO4	12.5 ml/L

Assigned Metabolite Uptake Rates for K-12 Media

(Metabolite lower bound determined by initial concentration of metabolite times the ratio of the initial glucose concentration/lower bound)

Metabolite	MW (g/mol)	g/L in media	mmol/L in media	Lower bound (mmol $gDW^{-1}h^{-1}$)
Glucose	180.16	25	138.7655417	-11
Ammonium	18.03851	1.365829484	75.71742258	-6.002150375
Phosphate	94.9714	6.655736264	70.08147994	-5.555386948
Potassium	39.0983	1.945099309	49.74894838	-3.943619038
Sulfate	96.07	0.203323529	2.11641021	-0.167768684
Chloride	35.453	0.062050364	1.750214773	-0.138740225
Copper	63.546	0.000509009	0.008010093	-0.000634963
Iron (III)	55.845	0.00490684	0.087865335	-0.00696512
Magnesium	24.305	0.049304203	2.028562155	-0.160804933
Manganese	54.938044	0.005551956	0.101058487	-0.008010947
Molybdate	95.95	0.001826052	0.019031286	-0.001508618
Sodium	22.98976928	0.029775544	1.295164976	-0.102668246
Thiamine	265.35	0.0025	0.009421519	-0.000746848
Zinc	65.38	0.001136847	0.017388304	-0.001378378
Alanine	89.09	0.225	2.525535975	-0.200200247
Arginine	174.2	0.145	0.832376579	-0.065982824
Asparagine	132.12	0.16	1.211020285	-0.095998062

Metabolite	MW (g/mol)	g/L in media	mmol/L in media	Lower bound (mmol $gDW^{-1}h^{-1}$)
Aspartic acid	133.1	0.16	1.202103681	-0.09529124
Cysteine	121.16	0.02	0.165070981	-0.013085243
Glutamine	146.14	0.345	2.360749966	-0.187137594
Glutamic Acid	147.13	0.345	2.344865085	-0.185878393
Glycine	75.07	0.135	1.798321567	-0.14255367
Histidine	155.15	0.06	0.386722527	-0.030655649
Isoleucine	131.17	0.145	1.105435694	-0.08762833
Leucine	131.17	0.21	1.600975833	-0.126909995
Lysine	146.19	0.22	1.504890895	-0.119293303
Methionine	149.21	0.045	0.301588365	-0.02390703
Phenylalanine	165.19	0.12	0.726436225	-0.05758489
Proline	115.13	0.115	0.998870842	-0.079180891
Serine	105.09	0.13	1.237034922	-0.098060253
Threonine	119.12	0.135	1.133310947	-0.089838012
Tyrosine	181.19	0.09	0.496716154	-0.039374888
Valine	117.15	0.165	1.408450704	-0.111648451

For more accurate results these uptake rates need to be measured

Sarah Allred, "Metabolic Modeling of Spider Silk Production in *E. coli*," MS Thesis, USU, 2014

Coding the K-12 Uptake Rates

(The estimated metabolite lower bounds are determined by initial concentration of a metabolite times the ratio of the initial glucose concentration/lower bound - Experimentally measured values would be better)

```
% Set uptake values for amino acids;
model = changeRxnBounds(model,'EX_ala__L_e',-0.200200247,'l');
model = changeRxnBounds(model,'EX_arg__L_e',-0.065982824,'l');
model = changeRxnBounds(model,'EX_asn__L_e',-0.095998062,'l');
model = changeRxnBounds(model,'EX_asp__L_e',-0.09529124,'l');
model = changeRxnBounds(model,'EX_cys__L_e',-0.013085243,'l');
model = changeRxnBounds(model,'EX_gln__L_e',-0.187137594,'l');
model = changeRxnBounds(model,'EX_glu__L_e',-0.185878393,'l');
model = changeRxnBounds(model,'EX_gly_e',-0.14255367,'l');
model = changeRxnBounds(model,'EX_his__L_e',-0.030655649,'l');
model = changeRxnBounds(model,'EX_ile__L_e',-0.08762833,'l');
model = changeRxnBounds(model,'EX_leu__L_e',-0.126909995,'l');
model = changeRxnBounds(model,'EX_lys__L_e',-0.119293303,'l');
model = changeRxnBounds(model,'EX_met__L_e',-0.02390703,'l');
model = changeRxnBounds(model,'EX_phe__L_e',-0.05758489,'l');
model = changeRxnBounds(model,'EX_pro__L_e',-0.079180891,'l');
model = changeRxnBounds(model,'EX_ser__L_e',-0.098060253,'l');
model = changeRxnBounds(model,'EX_thr__L_e',-0.089838012,'l');
model = changeRxnBounds(model,'EX_tyr__L_e',-0.039374888,'l');
model = changeRxnBounds(model,'EX_val__L_e',-0.111648451,'l');
```

```
% Set uptake values for minerals;
model = changeRxnBounds(model,'EX_ca2_e',-0.00237348,'l');
model = changeRxnBounds(model,'EX_cobalt2_e',-1.14e-05,'l');
model = changeRxnBounds(model,'EX_ni2_e',-0.000147288,'l');
model = changeRxnBounds(model,'EX_nh4_e',-6.002150375,'l');
model = changeRxnBounds(model,'EX_pi_e',-5.555386948,'l');
model = changeRxnBounds(model,'EX_k_e',-3.943619038,'l');
model = changeRxnBounds(model,'EX_so4_e',-0.167768684,'l');
model = changeRxnBounds(model,'EX_cl_e',-0.138740225,'l');
model = changeRxnBounds(model,'EX_cu2_e',-0.000634963,'l');
model = changeRxnBounds(model,'EX_fe3_e',-0.00696512,'l');
model = changeRxnBounds(model,'EX_mg2_e',-0.160804933,'l');
model = changeRxnBounds(model,'EX_mn2_e',-0.008010947,'l');
model = changeRxnBounds(model,'EX_mobd_e',-0.001508618,'l');
model = changeRxnBounds(model,'EX_na1_e',-0.102668246,'l');
model = changeRxnBounds(model,'EX_thm_e',-0.000746848,'l');
model = changeRxnBounds(model,'EX_zn2_e',-0.001378378,'l');
```

Sarah Allred, "Metabolic Modeling of Spider Silk Production in *E. coli*," MS Thesis, USU, 2014

DynamicFBA: Growth on Glucose with limiting K12 Media

```
% Dynamic_Growth_K12media_iJO1366_FBC.m
clear;

% Load iJO1366 model
model = readCbModel('iJO1366.mat');
model = changeObjective(model,'BIOMASS_Ec_iJO1366_core_53p95M');

%Setting carbon source and oxygen
model = changeRxnBounds(model,'EX_glc__D_e',-10,'l');
model = changeRxnBounds(model,'EX_o2_e',-0,'l');

% Set uptake values for amino acids & Minerals;
...
% Set-up variables for dynamicFBA: % NOTE- substrate rxns and plot rxns need to be in the order that they appear in the model
initBiomass = .01;
timeStep = 0.5; nSteps = 100;

substrateRxns =
{'EX_ala__L_e','EX_arg__L_e','EX_asn__L_e','EX_asp__L_e','EX_cl_e','EX_cu2_e','EX_cys__L_e','EX_fe3_e','EX_glc__D_e','EX_gln__L_e','EX_glu__L_e','EX_gly_e','EX_his__L_e',
'EX_ile__L_e','EX_k_e','EX_leu__L_e','EX_lys__L_e','EX_met__L_e','EX_mg2_e','EX_mn2_e','EX_mobd_e','EX_na1_e','EX_nh4_e','EX_phe__L_e','EX_pi_e','EX_pro__L_e','EX_ser__L_e',
'EX_so4_e','EX_thm_e','EX_thr__L_e','EX_tyr__L_e','EX_val__L_e','EX_zn2_e'};

initConcentrations =
[2.525535975,0.832376579,1.211020285,1.202103681,1.750214773,0.008010093,0.165070981,0.087865335,138.7655417,2.360749966,2.344865085,1.798321567,0.386722527,1.105435694,
49.74894838,1.600975833,1.504890895,0.301588365,2.028562155,0.101058487,0.019031286,1.295164976,75.71742258,0.726436225,70.08147994,0.998870842,1.237034922,2.11641021,
0.009421519,1.133310947,0.496716154,1.408450704,0.017388304];

plotRxns = {'EX_ac_e','EX_acald_e','EX_ala__L_e','EX_arg__L_e','EX_asn__L_e','EX_asp__L_e','EX_cl_e','EX_cu2_e','EX_cys__L_e','EX_etoh_e','EX_fe3_e','EX_for_e','EX_glc_e',
'EX_gln__L_e','EX_glu__L_e','EX_gly_e','EX_his__L_e','EX_ile__L_e','EX_k_e','EX_lac__L_e','EX_leu__L_e','EX_lys__L_e','EX_met__L_e','EX_mg2_e','EX_mn2_e','EX_mobd_e',
'EX_na1_e','EX_nh4_e','EX_phe__L_e','EX_pi_e','EX_pro__L_e','EX_ser__L_e','EX_so4_e','EX_succ_e','EX_thm_e','EX_thr__L_e','EX_tyr__L_e','EX_val__L_e','EX_zn2_e'};

dynamicFBA(model,substrateRxns,initConcentrations, initBiomass, timeStep, nSteps, plotRxns);

%labeling
subplot(1,2,1); title('Biomass Concentration'); xlabel('Time steps'); ylabel('Concentration (g/L)');
subplot(1,2,2); title('Substrate Concentrations'); xlabel('Time steps'); ylabel('Concentrations (mmol/L)');
```

Active Exchange Reactions

(Media Comparison.xlsx)

Glucose Growth on Minimal Media

(409 Active Reactions)

EX_ac(e)	8.61685
EX_ca2(e)	-0.000891243
EX_cl(e)	-0.000891243
EX_co2(e)	-0.0911672
EX_cobalt2(e)	-0.000594162
EX_cu2(e)	-0.000594162
EX_etoh(e)	8.49536
EX_fe2(e)	-0.00142087
EX_fe3(e)	-0.00133696
EX_for(e)	17.9104
EX_glc(e)	-10
EX_glyclt(e)	0.000125869
EX_h2o(e)	-3.5678
EX_h(e)	28.3809
EX_k(e)	-0.0334146
EX_mg2(e)	-0.00148541
EX_mn2(e)	-0.000594162
EX_mobd(e)	-0.000594162
EX_nh4(e)	-2.02896
EX_pi(e)	-0.180867
EX_pi(e)	-0.180867
EX_so4(e)	-0.0470833
EX_succ(e)	0.062791
EX_zn2(e)	-0.000594162
Biomass	0.188145

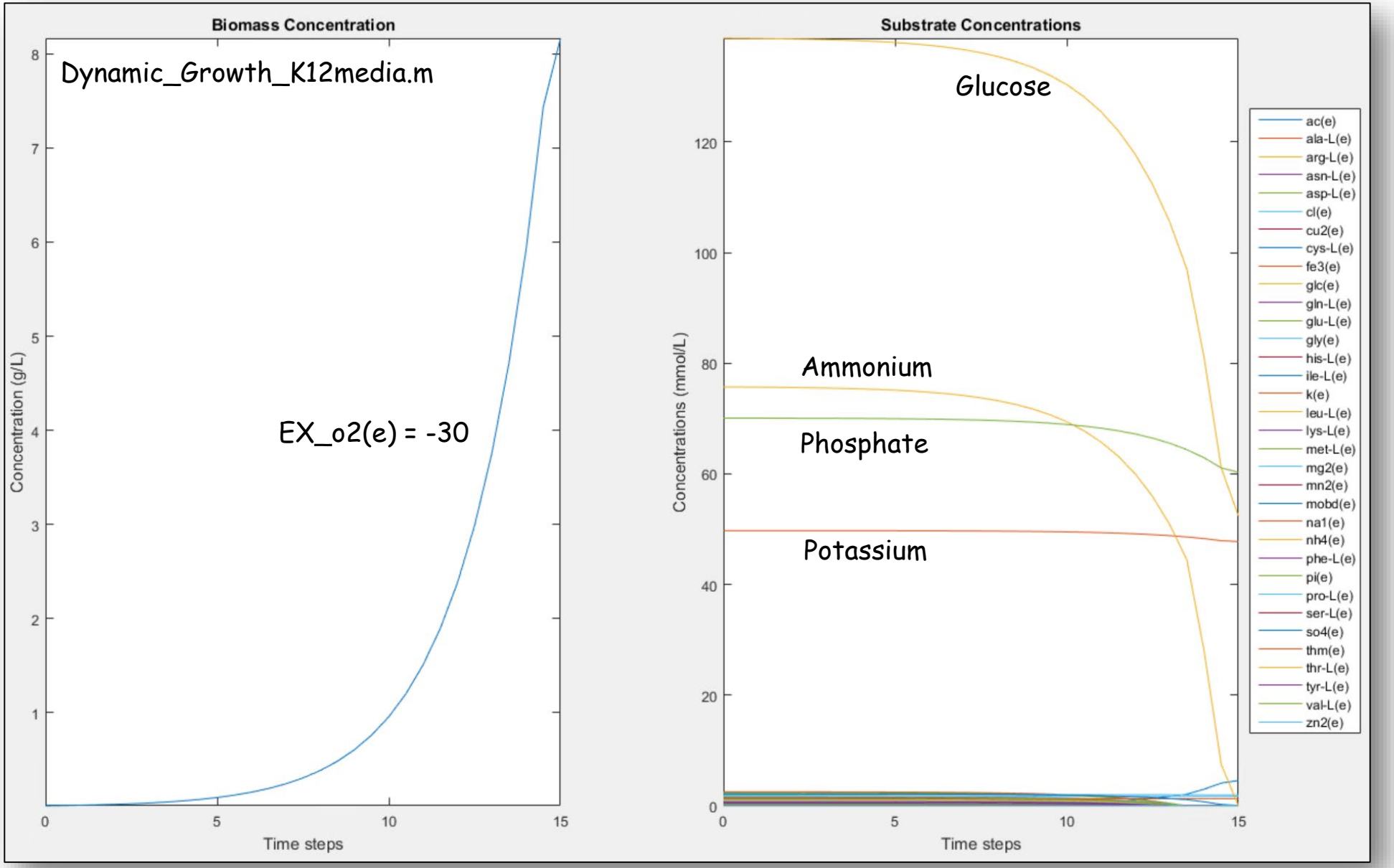
Glucose_Minimal_Map.pdf

Glucose Growth on K12 Media

(435 Active Reactions)

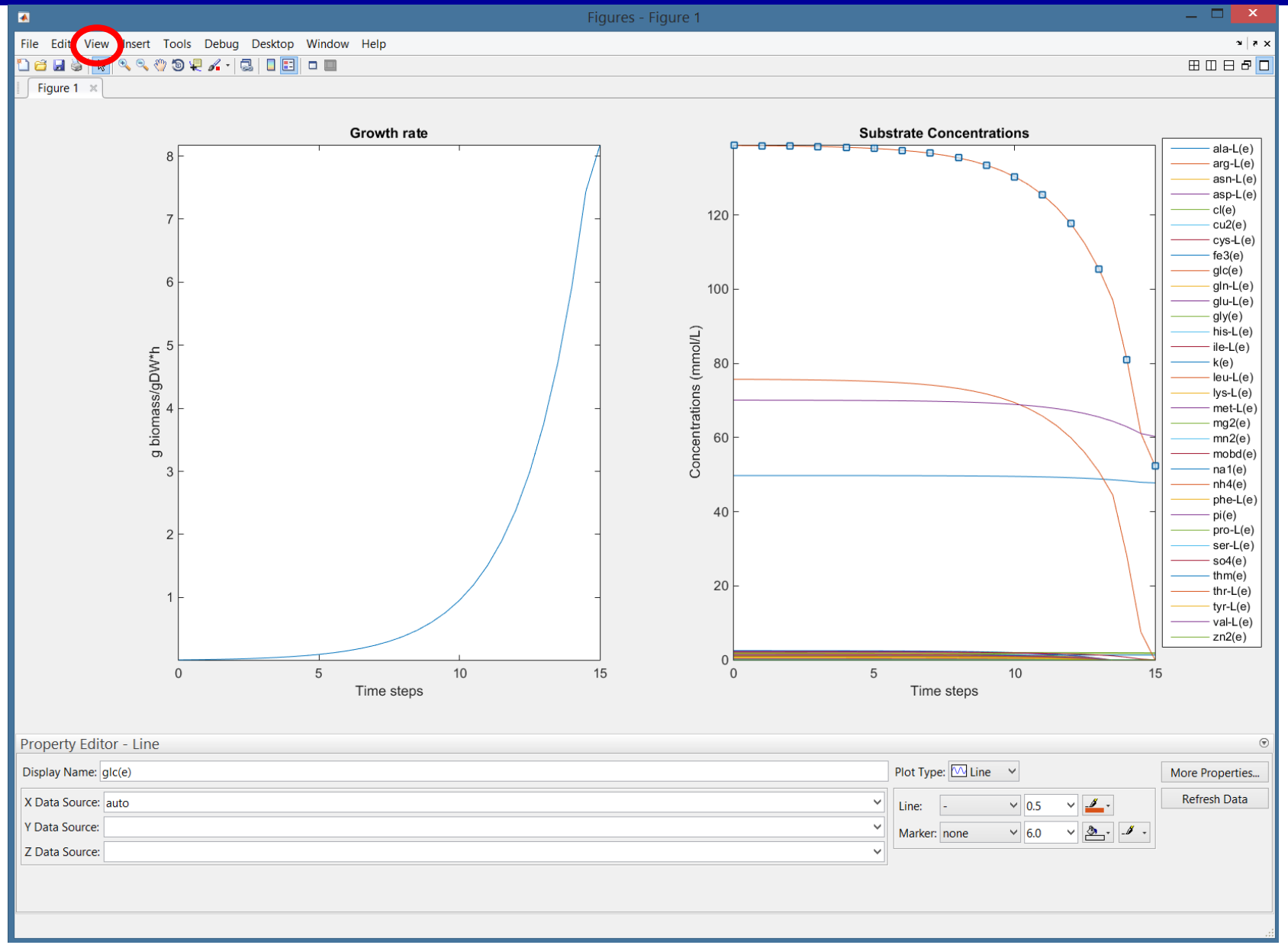
EX_ac(e)	0.00145949
EX_acald(e)	0.0404187
EX_akg(e)	0.00743512
EX_ala_D(e)	5.01E-05
EX_arg_L(e)	-0.0010678
EX_asp_L(e)	-0.0952912
EX_ca2(e)	-1.71E-05
EX_cl(e)	-1.71E-05
EX_co2(e)	0.0749214
EX_cobalt2(e)	-1.14E-05
EX_cu2(e)	-1.14E-05
EX_cys_L(e)	-0.000333477
EX_cytd(e)	0.00187757
EX_dha(e)	9.95477
EX_fe2(e)	-2.73E-05
EX_fe3(e)	-2.57E-05
EX_glc(e)	-10
EX_glu_L(e)	-0.0102491
EX_glyclt(e)	2.42E-06
EX_h2o(e)	0.415072
EX_h(e)	9.84445
EX_k(e)	-0.000641115
EX_lac_D(e)	9.7652
EX_lipa_cold(e)	0.000659534
EX_lys_L(e)	-0.00123891
EX_met_L(e)	-0.000555644
EX_mg2(e)	-2.85E-05
EX_mn2(e)	-1.14E-05
EX_mobd(e)	-1.14E-05
EX_pi(e)	-0.0047893
EX_pyr(e)	0.148429
EX_so4(e)	-1.43E-05
EX_succ(e)	0.010202
EX_trp_L(e)	0.033873
EX_tyr_L(e)	-0.000498607
EX_zn2(e)	-1.14E-05
Biomass	0.00360988

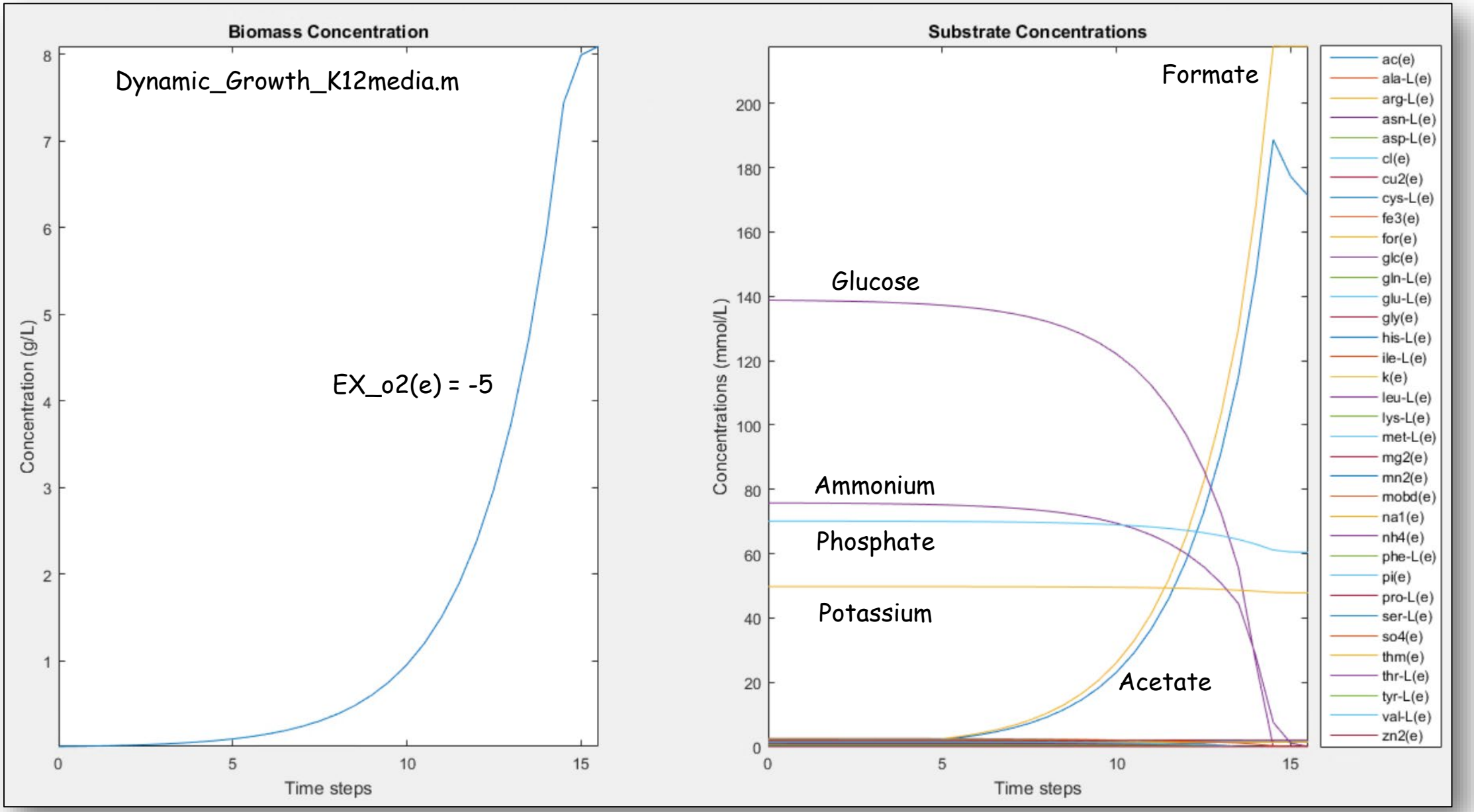
Glucose_K12media_Map.pdf


Biomass Concentration
Dynamic_Growth_K12media.m
EX_o2(e) = -30
Concentration (g/L)
Time steps
Substrate Concentrations
Glucose
Ammonium
Phosphate
Potassium
Concentrations (mmol/L)
Time steps
ac(e)
ala-L(e)
arg-L(e)
asn-L(e)
asp-L(e)
cl(e)
cu2(e)
cys-L(e)
fe3(e)
glc(e)
gln-L(e)
glu-L(e)
gly(e)
his-L(e)
ile-L(e)
k(e)
leu-L(e)
lys-L(e)
met-L(e)
mg2(e)
mn2(e)
mobd(e)
na1(e)
nh4(e)
phe-L(e)
pi(e)
pro-L(e)
ser-L(e)
so4(e)
thm(e)
thr-L(e)
tyr-L(e)
val-L(e)
zn2(e)

Matlab Property Editor

Under View Menu




Biomass Concentration
Dynamic_Growth_K12media.m
EX_o2(e) = -5
Concentration (g/L)
Time steps
Substrate Concentrations
Formate
Glucose
Ammonium
Phosphate
Potassium
Acetate
Concentrations (mmol/L)
Time steps
ac(e)
ala-L(e)
arg-L(e)
asn-L(e)
asp-L(e)
cl(e)
cu2(e)
cys-L(e)
fe3(e)
for(e)
glc(e)
gln-L(e)
glu-L(e)
gly(e)
his-L(e)
ile-L(e)
k(e)
leu-L(e)
lys-L(e)
met-L(e)
mg2(e)
mn2(e)
mobd(e)
na1(e)
nh4(e)
phe-L(e)
pi(e)
pro-L(e)
ser-L(e)
so4(e)
thm(e)
thr-L(e)
tyr-L(e)
val-L(e)
zn2(e)

dynamicFBA Limitations

- The dynamicFBA tool cannot simulate the fed batch mode. There is no way to account for substrates that enter the medium via fed batch mode, so it can only show what becomes of the initial concentrations.
- The dynamicFBA was created to optimize the biomass reaction, so there is currently no way to maximize reactions for protein production, or to maximize both the growth rate and protein production at the same time.
- The predicted growth rate can reach values higher than possible because the calculated growth rate is constantly in the exponential phase.
- These aspects make the dynamicFBA tool more useful for qualitative rather than quantitative study.

Dynamic FBA Review Questions

1. Explain the basic operation of dynamic flux balance analysis.
2. What are the key inputs required for dynamicFBA operation?
3. Why aren't the fermentation products used as carbon sources after all the glucose has been used in an anaerobic environment?
4. In an environment with a large number of plotted metabolites how can the Matlab Property Editor be useful?
5. How is minimal media modeled in the Cobra Toolbox?
6. What is the difference between minimal and K-12 media?
7. What is the purpose of the concentration matrix in dynamic FBA?
8. What is the role of each of the dynamicFBA variables: substrateRxns, initConcentrations, initBiomass, tStep, nSteps, and plotRxns?
9. What are the strengths of dynamic FBA?
10. What are the weaknesses of dynamic FBA?

Lesson Outline

- Dynamic Flux Balance Analysis (dynamicFBA)
- "dynamicFBA" Basic Operation
- Minimal Media Examples
- Limiting Media Examples

Coding the K-12 Uptake Rates

(The estimated metabolite lower bounds are determined by initial concentration of a metabolite times the ratio of the initial glucose concentration/lower bound - Experimentally measured values would be better)

```
% Set uptake values for amino acids;
model = changeRxnBounds(model,'EX_ala-L(e)',-0.200200247,'l');
model = changeRxnBounds(model,'EX_arg-L(e)',-0.065982824,'l');
model = changeRxnBounds(model,'EX_asn-L(e)',-0.095998062,'l');
model = changeRxnBounds(model,'EX_asp-L(e)',-0.09529124,'l');
model = changeRxnBounds(model,'EX_cys-L(e)',-0.013085243,'l');
model = changeRxnBounds(model,'EX_gln-L(e)',-0.187137594,'l');
model = changeRxnBounds(model,'EX_glu-L(e)',-0.185878393,'l');
model = changeRxnBounds(model,'EX_gly(e)',-0.14255367,'l');
model = changeRxnBounds(model,'EX_his-L(e)',-0.030655649,'l');
model = changeRxnBounds(model,'EX_ile-L(e)',-0.08762833,'l');
model = changeRxnBounds(model,'EX_leu-L(e)',-0.126909995,'l');
model = changeRxnBounds(model,'EX_lys-L(e)',-0.119293303,'l');
model = changeRxnBounds(model,'EX_met-L(e)',-0.02390703,'l');
model = changeRxnBounds(model,'EX_phe-L(e)',-0.05758489,'l');
model = changeRxnBounds(model,'EX_pro-L(e)',-0.079180891,'l');
model = changeRxnBounds(model,'EX_ser-L(e)',-0.098060253,'l');
model = changeRxnBounds(model,'EX_thr-L(e)',-0.089838012,'l');
model = changeRxnBounds(model,'EX_tyr-L(e)',-0.039374888,'l');
model = changeRxnBounds(model,'EX_val-L(e)',-0.111648451,'l');
```

```
% Set uptake values for minerals;
model = changeRxnBounds(model,'EX_ca2(e)',-0.00237348,'l');
model = changeRxnBounds(model,'EX_cobalt2(e)',-1.14e-05,'l');
model = changeRxnBounds(model,'EX_ni2(e)',-0.000147288,'l');
model = changeRxnBounds(model,'EX_nh4(e)',-6.002150375,'l');
model = changeRxnBounds(model,'EX_pi(e)',-5.555386948,'l');
model = changeRxnBounds(model,'EX_k(e)',-3.943619038,'l');
model = changeRxnBounds(model,'EX_so4(e)',-0.167768684,'l');
model = changeRxnBounds(model,'EX_cl(e)',-0.138740225,'l');
model = changeRxnBounds(model,'EX_cu2(e)',-0.000634963,'l');
model = changeRxnBounds(model,'EX_fe3(e)',-0.00696512,'l');
model = changeRxnBounds(model,'EX_mg2(e)',-0.160804933,'l');
model = changeRxnBounds(model,'EX_mn2(e)',-0.008010947,'l');
model = changeRxnBounds(model,'EX_mobd(e)',-0.001508618,'l');
model = changeRxnBounds(model,'EX_na1(e)',-0.102668246,'l');
model = changeRxnBounds(model,'EX_thm(e)',-0.000746848,'l');
model = changeRxnBounds(model,'EX_zn2(e)',-0.001378378,'l');
```

Sarah Allred, "Metabolic Modeling of Spider Silk Production in *E. coli*," MS Thesis, USU, 2014

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Dynamic Analysis

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Dynamic FBA Growth Simulations

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- Becker, S. A., A. M. Feist, et al. (2007). "Quantitative prediction of cellular metabolism with constraint-based models: the COBRA Toolbox." Nature protocols 2(3): 727-738. - See section