STBRsim – A MATLAB Application for Stirred Tank Bioreactor Simulation

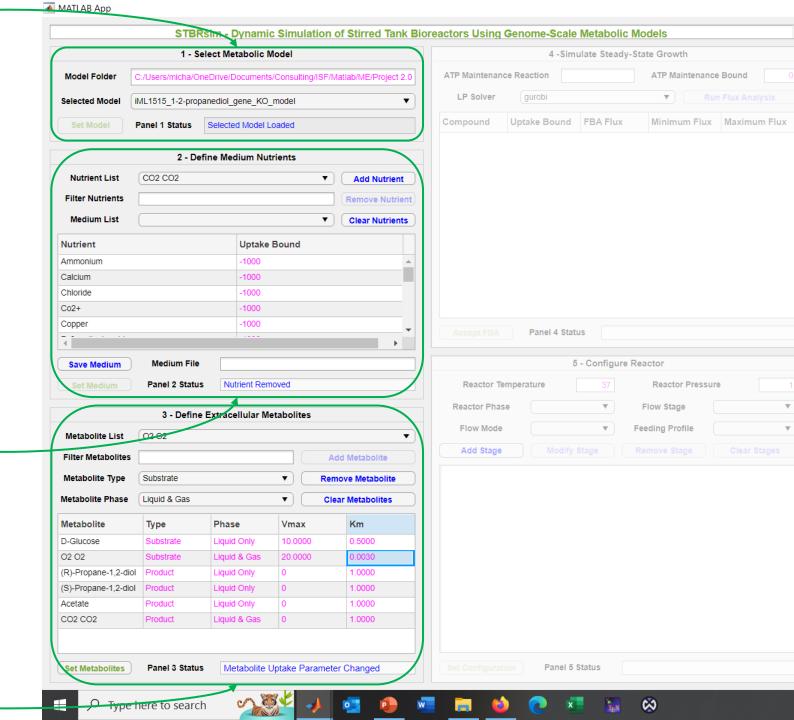
				of Stirred Tank Bi								:o Ferment		
	1 - Sel	ect Metabolic N	lodel			4 -Simulate Stea	ady-State Growt	h			6 - Specify D	Dissolved Ga	s Parameter	rs
Model Folder	C:/Users/micha/One	C:/Users/micha/OneDrive/Documents/Consulting/ISF/Matlab/ME/Project 2.0			ATP Maintenance R	ATP Maintenance Reaction ATPM ATP Maintenance Bound 6.86		und 6.86	Dissolved Gas	Henry's	s Constant	Mas	s Transfer Coefficie	
elected Model	iML1515_1-2-propa	nediol_gene_KO_	model	T	LP Solver	gurobi	•	Run Flu	x Analysis	CO2 CO2	0.6080		100.	
Set Model Panel 1 Status Selected Model Loaded			Compound Uptake Bound FBA Flux			FBA Flux	02 02	0.0260		100.	0000			
				E. coll biomass objective function (IML1515) - core - with 0				0.6787 🔺						
2 - Define Medium Nutrients				(R)-Propane-1,2-diol 0 2.4367										
Nutrient List CO2 CO2 Add Nutrient		(S)-Propane-1,2-diol		0		0		Panel 6 Status Dissolved Gas Parameters Set		iore Sot				
	002 002				Acetate		0		0.4727	Set Parameters	Panel 6 Status Dissolved Gas Parameters Set			
Iter Nutrients				Remove Nutrient	Ammonium		-10	00	-7.3301		7 - Specify Ex	tracellular in	itial Conditi	ons
Medium List			•	Clear Nutrients	CO2 CO2		0		23.8864		7 - opeony Ex		nun oonun	0115
					Calcium		-10	00	-0.0035	Variable		Туре	Phase	Initial Condition
itrient		Uptake	Bound		Chloride		-10	00	-0.0035	E. coli biomass obje	tive function	Biomass	Liquid	0.1000
imonium		-1000			C02+		-10	00	-1.6968e-0{	(R)-Propane-1.2-diol		Product	Liquid	0
lcium		-1000			Copper		-100	00	-4.8121e-04	(S)-Propane-1,2-diol		Product	Liquid	0
loride		-1000			D-Glucose		-10		-10 🗸	Acetate		Product	Liquid	0
2+		-1000			4				•	CO2 CO2		Product	Liquid	0
oper		-1000				Panel 4 Status FB	BA Results Accepte	d						
				•						D-Glucose		Substrate	Liquid	250.0000
Save Medium	Medium File					5 - Config	jure Reactor			02 02		Substrate	Liquid	0.2145
							_			Liquid Volume		Volume	Liquid	80
	Panel 2 Status	Nutrient Rem	noved		Reactor Tempe	erature 37	Reactor	Pressure	1	CO2 CO2		Product	Gas	0
	3 - Define	Extracellular Me	etabolites		Reactor Phase	Liquid	_				Panel 7 Status	Initial Con	ditions Set	
letabolite List	02 02			•	Flow Mode	Batch 🔻	Feeding Pro	file No F	low 🔻	8 - Perform Bioreactor Simulation				
Iter Metabolites	s		Ac	dd Metabolite	Add Stage	Modify Stage	Remove Sta	ge C	lear Stages	ODE Solver	ode15s		LP Solution	Fast
etabolite Type	Substrate		▼) Rem	nove Metabolite	Flow Stage		Stage 1			ODE Tolerance		.0001	Simulation	
tabolite Phase	Liquid & Gas		▼ Cle	ar Metabolites	Start		0							
abonte i nase	Elquid & Oas			ar metabolites	End		18			Run Simulation	Result Nar	ne gas_	_switch_4h	
tabolite	Туре	Phase	Vmax	Km	Flow		N/A			Discard Result	Add Resu	lt Re	emove Result	t 🔰 🔹 Clear Resu
Glucose	Substrate	Liquid Only	10.0000	0.5000	Parameter		N/A			Result List	gas switch 4h			
02	Substrate	Liquid & Gas	20.0000	0.0030	D-Glucose		N/A				<u> </u>			
-Propane-1,2-di	iol Product	Liquid Only	0	1.0000	02 02		N/A			Visualize Results	Panel 8 Stat	Resi	ult Added	
Propane-1,2-di	iol Product	Liquid Only	0	1.0000	52.02						•	- Manage Ca		
tate	Product	Liquid Only	0	1.0000							9	- Manage Ca	ise	
2 CO2	Product	Liquid & Gas	0	1.0000						File Folder C:	/Users/micha/One	Drive/Docume	nts/Consultin	g/ISF/Matlab/DFBA/Fi
										Save Case	Case File	case_ecoli_mu	utant_propane	diol
	Panel 3 Status	Extracellula	r Metabolites Set	t	Set Configuration	Panel 5 Status	Reactor Flow C	onfiguration Se	et	Load Case Pa	inel 9 Status	Case File Cre	eated	

Example – Determine the optimal time for aerobic-to-anaerobic switch to maximize the production of R-1,2-propanediol production in engineered *Escherichia coli*

1. Select the metabolic model for the strain to be simulated. Here we select an *E. coli* strain designed for 1,2-propanediol synthesis.

2. Define the culture medium in terms of unlimited nutrients and their maximum uptake rates. The nutrients can be extracted from the model, specified manually, or loaded from a saved file.

3. Define balanced extracellular metabolites, including growthlimiting substrates and secreted products. Each metabolite is characterized by its phases(s) and uptake kinetic parameters.



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4. Perform flux balance analysis to analyze steady-state growth. Maximum uptake rates can be changed to determine their effects on cellular growth, substrate uptake and product secretion rates.

5. Configure liquid and gas flow stages to implement time-based feeding strategies. The flow mode (e.g., fed-batch), feeding profile (e.g., ramp), start/end times, flow rate parameters, and feed substrate values can be specified for each stage.

	1 - S	elect Metabolic N	/lodel		4 -Simulate Steady-State Growth					
Model Folder	0.44	- D. i /D	· · · · · · · · · · · · · · · · · · ·		ATP Maintenance Pe		7	ound 6.9		
Model Folder	C:/Users/micha/Oi	neDrive/Documents	s/Consulting/IS	F/Matlab/ME/Project 2.0	ATP Maintenance Reaction ATPM ATP Maintenance Bound 6.8					
Selected Model	iML1515_1-2-prop	oanediol_gene_KO_	_model	▼)	LP Solver	gurobi	▼ Run F	lux Analysis		
Set Model	Panel 1 Status	Selected Model L	oaded		Compound		Uptake Bound	d FBA Flux		
					E. coli biomass objecti	ive function (iML1515) - core	- with 0	0.6787		
	2 - De	fine Medium Nut	trients		(R)-Propane-1,2-diol		0	2.4367		
Nutrient List	CO2 CO2			Add Nutrient	(S)-Propane-1,2-diol		0	0		
					Acetate		0	0.4727		
Filter Nutrients				Remove Nutrient	Ammonium		-1000	-7.3301		
Medium List				Clear Nutrients	CO2 CO2		0	23.8864		
Nutrient		Uptake	Bound		Calcium		-1000	-0.0035		
		-1000	Dound		Chloride		-1000	-0.0035		
Ammonium Calcium		-1000		^	Co2+		-1000	-1.6968e-0{		
Chloride		-1000			Copper		-1000	-4.8121e-04		
Co2+		-1000			D-Glucose		-10	-10		
Copper		-1000						•		
				•	Accept FBA	Panel 4 Status FBA R	esults Accepted	/		
•				•						
Save Medium	Medium File					5 - Configure	Reactor			
Save Medium	Medium File Panel 2 Status		noved		Reactor Temper		Reactor Reactor Pressure			
			noved			rature 37	Reactor Pressure			
	Panel 2 Status				Reactor Temper Reactor Phase		Reactor Pressure	age 2 🗸		
	Panel 2 Status 3 - Define	s Nutrient Rem				rature 37	Reactor Pressure Flow Stage	-		
Set Medium Metabolite List	Panel 2 Status	s Nutrient Rem			Reactor Phase	Gas V	Reactor Pressure Flow Stage	-		
Set Medium	Panel 2 Status 3 - Define	s Nutrient Rem		▼ Add Metabolite	Reactor Phase Flow Mode Add Stage	rature 37 Gas ▼ Constant ▼ Modify Stage	Reactor Pressure Flow Stage State Feeding Profile Coord Remove Stage Image: Coord	onstant v		
Set Medium Metabolite List	Panel 2 Status 3 - Define	s Nutrient Rem	etabolites		Reactor Phase Flow Mode	rature 37 Gas ▼ Constant ▼ Modify Stage Stage 1	Reactor Pressure Flow Stage Sta Feeding Profile Co	onstant v		
Set Medium Metabolite List Filter Metabolites Metabolite Type	Panel 2 Status 3 - Define	s Nutrient Rem	etabolites	Add Metabolite	Reactor Phase Flow Mode Add Stage	rature 37 Gas ▼ Constant ▼ Modify Stage	Reactor Pressure Flow Stage State Feeding Profile Coord Remove Stage Image: Coord	onstant v		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas	s Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolites	Reactor Phase Flow Mode Add Stage Flow Stage	rature 37 Gas ▼ Constant ▼ Modify Stage Stage 1	Reactor Pressure Flow Stage Stage Feeding Profile Co Remove Stage Stage 2	onstant v		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Metabolite	Panel 2 Status 3 - Define O2 O2 Substrate Liquid & Gas Type	s Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolite Km	Reactor Phase Flow Mode Add Stage Flow Stage Start	rature 37 Gas ▼ Constant ▼ Modify Stage Stage 1 0	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Remove Stage Stage 2 6	onstant v		
Set Medium Metabolite List Filter Metabolites Metabolite Phase Metabolite D-Glucose	Panel 2 Status 3 - Define O2 O2 Substrate Liquid & Gas Type Substrate	s Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolites Km 0.5000	Reactor Phase Flow Mode Add Stage Flow Stage Start End	rature 37 Gas ▼ Constant ▼ Modify Stage Stage 1 0 6	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Remove Stage Stage 2 6 15	onstant 🔹		
Set Medium Metabolite List Filter Metabolites Metabolite Phase Metabolite D-Glucose O2 O2	Panel 2 Status 3 - Define O2 O2 Substrate Liquid & Gas Type Substrate Substrate	S Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolites Km 0.5000 0.0030	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000	Reactor Pressure Flow Stage Stage Remove Stage Stage 2 6 15 0 0	onstant 🔹		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Metabolite D-Glucose 02 02 (R)-Propane-1,2-diol	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product	s Nutrient Rem	etabolites ▼ F ▼ Vmax 10.0000 20.0000 0	Add Metabolite Remove Metabolite Clear Metabolites Km 0.5000 0.0030 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000 N/A	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	onstant		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Metabolite D-Glucose D2 O2 (R)-Propane-1,2-diol (S)-Propane-1,2-diol	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product	s Nutrient Rem	etabolites ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼	Add Metabolite Remove Metabolite Clear Metabolite Km 0.5000 0.0030 1.0000 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000 N/A	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	onstant		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Occurse D2 O2 (R)-Propane-1,2-diol (S)-Propane-1,2-diol Acetate	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product Product	s Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolite Km 0.5000 0.0030 1.0000 1.0000 1.0000 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000 N/A	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	onstant		
Set Medium Metabolite List Filter Metabolites	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product	s Nutrient Rem	etabolites ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼	Add Metabolite Remove Metabolite Clear Metabolite Km 0.5000 0.0030 1.0000 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000 N/A	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	onstant		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Metabolite D-Glucose D2 O2 (R)-Propane-1,2-diol (S)-Propane-1,2-diol	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product	s Nutrient Rem	etabolites ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼	Add Metabolite Remove Metabolite Clear Metabolite Km 0.5000 0.0030 1.0000 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage 0 Stage 1 0 0 6 1000 N/A	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	onstant		
Set Medium Metabolite List Filter Metabolites Metabolite Type Metabolite Phase Metabolite D-Glucose D2 O2 R)-Propane-1,2-diol S)-Propane-1,2-diol Acetate	Panel 2 Status 3 - Define (O2 O2 Substrate Liquid & Gas Type Substrate Substrate Substrate I Product Product	s Nutrient Rem	etabolites	Add Metabolite Remove Metabolite Clear Metabolites Km 0.5000 0.0030 1.0000	Reactor Phase Flow Mode Add Stage Flow Stage Start End Flow Parameter	rature 37 Gas ▼ Constant ▼ Modify Stage Stage 1 0 6 1000 N/A 0.2100	Reactor Pressure Flow Stage Stage Feeding Profile Colspan="2">Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2" Colspan="2"	Clear Stages		

ATP Maintenance Re	4 -	Simulate Stea	ady-State G	rowth				
	action	АТРМ	ATI	P Maintenar	nce Bou	nd <u>6.</u> 8	36	Dissolved Gas
LP Solver	gurobi			T	Run Flux	Analysis		CO2 CO2
	J							02 02
Compound				Uptake B	ound	FBA Flux		
E. coli biomass objecti	ve functio	on (iML1515) - c	ore - with	0		0.6787	^	
(R)-Propane-1,2-diol				0		2.4367		
(S)-Propane-1,2-diol				0		0		Set Parameters
Acetate				0		0.4727		
Ammonium				-1000		-7.3301		
CO2 CO2				0		23.8864		
Calcium				-1000		-0.0035		Variable
Chloride				-1000		-0.0035		E. coli biomass obje
Co2+				-1000		-1.6968e-0{		(R)-Propane-1,2-diol
Copper				-1000		-4.8121e-04		(S)-Propane-1,2-diol
D-Glucose				-10		-10	-	Acetate
<								CO2 CO2
		5 - Config	ure Reactor	•				O2 O2 Liquid Volume
Reactor Temper	ature	37	Re	actor Press	ure		1	CO2 CO2
Reactor Phase	Liquid	•	Flow	Stage	Stage	1 1	•	Set Initial
Flow Mode	Batch	▼)	Feeding	g Profile	No Fl	ow v	•	
Add Stage	Mo	dify Stage	Remov	e Stage		ear Stages		ODE Solver
Flow Stage			Stage 1					
Start			0					ODE Tolerance
End			18					Run Simulation
Flow			N/A					Discard Result
			N/A					Result List
Parameter			N/A					Visualize Results
Parameter D-Glucose								

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			—	U X					
In Silico Fermentation									
6 - Specify Dissolved Gas Parameters									
Dissolved Gas Henry's Constant Mass Transfer Coefficient									
CO2 CO2	0.6080		100.0000						
02 02	0.0260		100.0000						
Set Parameters Panel 6 Status Dissolved Gas Parameters Set									
7 - Spe	cify Extracel	lular Initial Co	onditions						
Variable	Туре	Phas	e Initial Cond	dition					
E. coli biomass objective fun	ction Biom	ass Liquid	i 0.1000	^					
(R)-Propane-1,2-diol	Produ	uct Liquid	i 0						
(S)-Propane-1,2-diol	Produ	uct Liquic	i 0						
Acetate	Produ	uct Liquid	i 0						
CO2 CO2	Produ	uct Liquio	i 0						
D-Glucose	Subs	trate Liquid	250.0000						
02 02	Subs	trate Liquio	0.2145						
Liquid Volume	Volur	ne Liquio	80						
CO2 CO2 Product Gas 0									
Set Initial Panel 7	Status In	itial conditions	Set						
8 -	Perform Bior	reactor Simula	ation						
ODE Solver ode15	s 🔻	LP Solu	tion Fast	•					
ODE Tolerance	0.0001	Simul	ation Duration	18					
		J							
Result Name gas_switch_4h									
Discard Result Add Result Remove Result Clear Results									
Result List gas_s	witch_4h			•					
Visualize Results Par	nel 8 Status	Result Adde	d						
	9 - Mar	age Case							
File Folder C:/Users/m	icha/OneDrive/	Documents/Cor	sulting/ISF/Matlab/DF	BA/Files					
Save Case Case File case_ecoli_mutant_propanediol									
Save Case Case F	ile case_	ecoli_mutant_pr	opanedioi						

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6. Specify dissolution and mass transfer parameters for the dissolved gases.

7. Specify initial conditions for all extracellular variables in the liquid and gas phases.

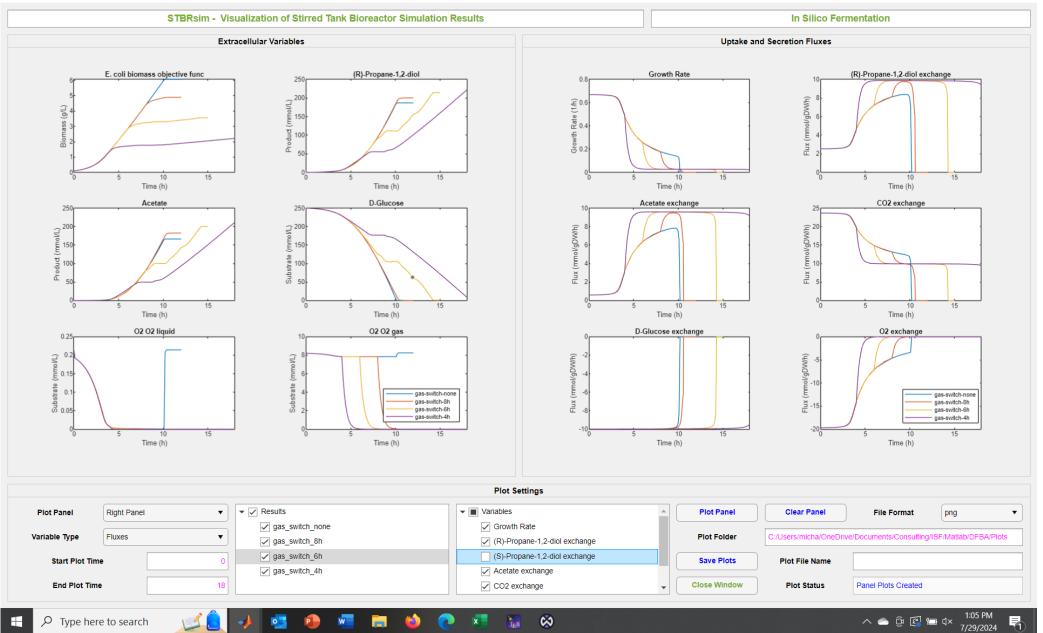
8. Perform dynamic simulation for the current parameters. Different simulations can be stored to allow comparison through a companion visualization App.

9. Save current case tocompletely capture the currentApp state for subsequent loading.

STBRsim – Companion App for Result Visualization and Comparison

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Batch Production of R-1,2-propanediol in Engineered Escherichia coli

Gas Switch Time (h)	Total Batch Time (h)	Final R-1,2-BD Titer (mmol/L)	Volumetric Productivity (mmol BD/L/h)	Product Yield (g BD/g glucose)
None	10.3	187	18.2	0.32
8	10.8	200	18.5	0.34
6	14.4	214	14.9	0.36
4	17.0	230	13.5	0.39