Contents

Base model

To properly model the interaction of *E. Coli* Nissle in the large intestine it needs to be inserted into a model of the colon. This model is based on the model of the colon published by Rafael Muñoz Tamayo in 2010 and after the *E. coli* addition tracks 18 different state variables, an overview of which can be seen in Table 1 [1].

Table 1: State variables for the model

Microbes	Metabolites	Gasses
• Sugar utilising group	• Sugar	• Hydrogen
• Lactate utilising group	• Lactate	• Methane
• Acetogenesis group	• Acetate	• Carbon dioxide
• Methanogenesis group	• Butyrate	
• *E. coli Nissle* population	• Propionate	
	• Hydrogen	
	• Methane	
	• Carbon dioxide	
	• Water	
	• Polysaccharides	

The model consists of 6 compartments, divided into a proximal, transversal, and distal colon with each of these having a mucosal and luminal compartment, an overview of which can be seen in Figure 1. Each compartment is assumed to be perfectly mixed, and a constant volume is maintained for every compartment except for the distal luminal compartment, which functions as a semi-batch reactor to mimic the accumulation and secretion of faeces.

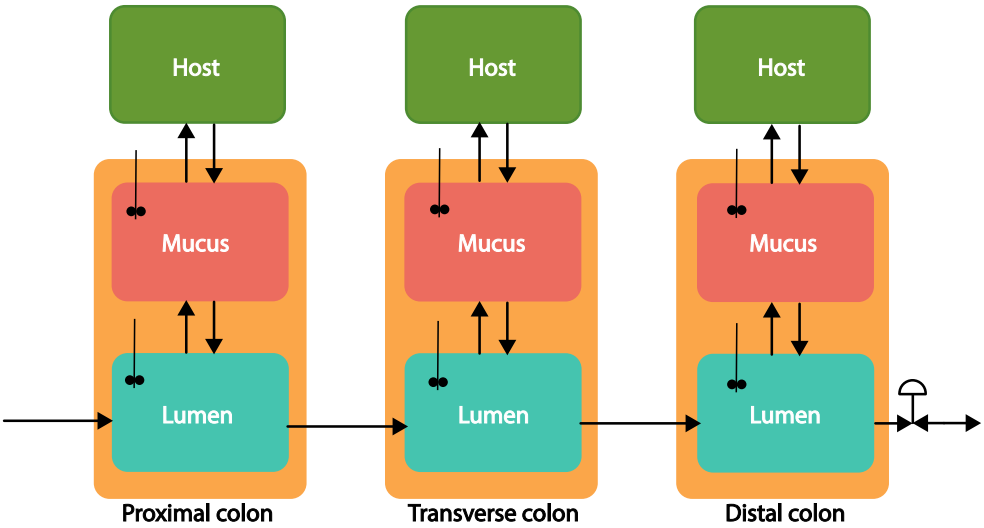

Figure 1: Overview of the model. A continuous volumes are maintained in the proximal and transverse lumen compartments creating a continuous flow, while the distal colon accumulates and dumps volume periodically. From the lumen compartments the tracked variables are exchanged with the mucus, and the mucus exchanges with the lumen and host. Each compartment is perfectly mixed.

In each of the compartments a number of metabolic processes are present, an overview of which can be seen in. Besides the metabolite input from diet, metabolites concentrations are influenced through degradation and creation by microbes following the scheme in Figure 2.

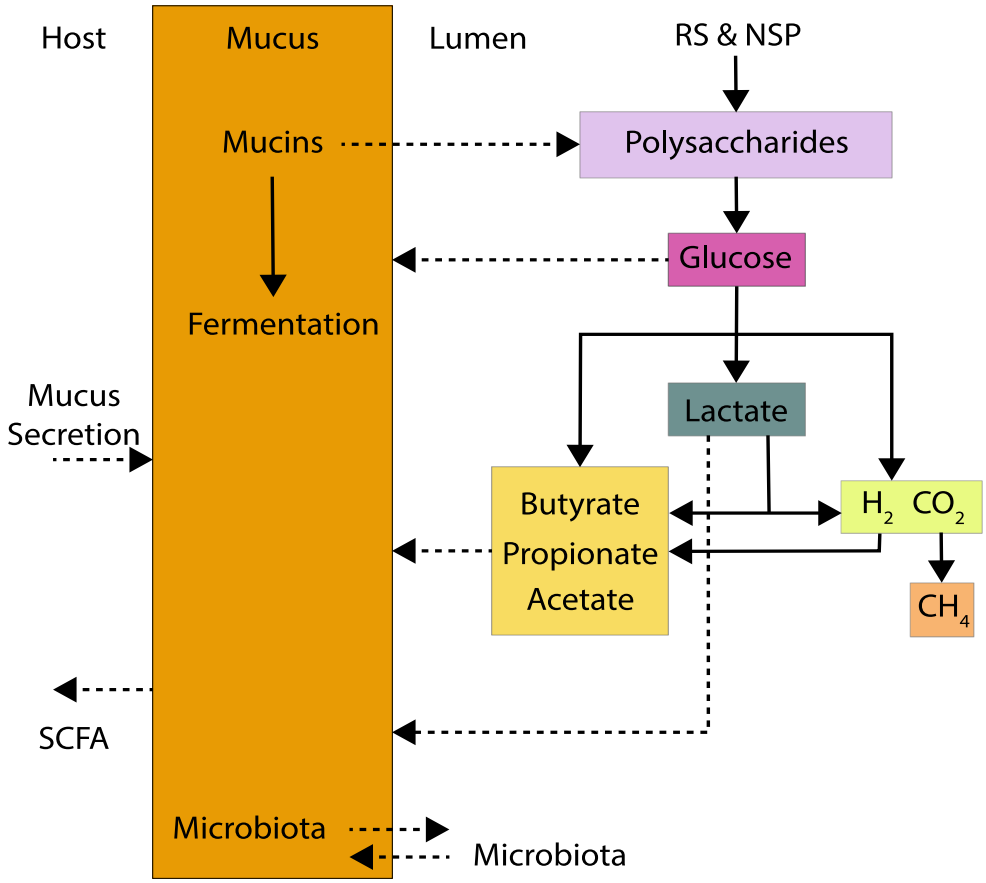

Figure 2: Overview of the metabolic processes in each compartment. Fermentation in the mucus stands for the same metabolite flow as is visible in the lumen. RS stands for resistant starch and NSP stands for non-starch polysaccharides.

Equations of the base model

Each of the state variables in the model are derived from generalised equations for each of the subgroups: soluble liquid components, soluble gas components, microbes, and polysaccharides. Furthermore, there are generalised equations for the exchange of gas and the flow. For an overview of the symbols see appendix I; an overview of constants is given in appendix II.

The small intestine

To start, while the small intestine is not the focus of this model, it is modelled in a simple series of 3 continuous stirred tank reactors to simulate the modulating role of the small intestine. According to equation 1, with dilution set to 9 d^{-1} [2].

$$z_j^{(1)} = D\left(z_{j-1} - z_j\right) \tag{1}$$

Proximal and transversal lumen liquid phase

The changes in the concentration of the liquid soluble metabolites lactate, acetate, butyrate, propionate, methane, carbon dioxide, and hydrogen are described by equation 2.

$$s^{(1)}{}_i^l = \frac{q_{in}}{V^l} s_{i,in}^l - \frac{q_{out}}{V^l} s_i^l - \gamma_i^l s_i^l + \sum_{\mathrm{j}=1}^{5} Y_{i,j}^l \rho_j^l - Q_i^l \tag{2}$$

The first term describes the inflow of the component to a specific compartment and the second term the outflow. In these terms q_{in} and q_{out} stands for the flux in an out the compartment, V stands for the volume of the compartment, s_{in} stands for the input concentration and s stands for the concentration of the soluble compound in the studied compartment. The third term describes the absorption of the compound by the host, where γ is the absorption rate. The fourth term describes the production and consumption of the compound by the different microbial groups, an elaboration of which can be seen in Muñoz-Tamayo et al., 2010. In this term Y stands for the yield of the specific reaction and ρ is the kinetic rate associated with that reaction. For example, sugar utilising bacteria consume sugar and produce lactate with a specific yield and with their relevant positive and negative reaction rates. Finally, where applicable, the fifth term describes the liquid-gas exchange of the compound, where s_g is the concentration of the compound in gas form, $k_L\, a$ is the liquid-gas transfer coefficient, K_H is the henry's law coefficient, R is the ideal gas coefficient, and T is the absolute temperature.

The change in the concentration of sugar has one difference. The third term is exchanged for a diffusion equation taking the difference of concentration between the mucosal and luminal compartments into account instead of an absorption equation. This is described in equation 3.

$$s^{(1)}{}_{su}^l = \frac{q_{in}}{V^l} s_{i,in}^l - \frac{q_{out}}{V^l} s_i^l - \frac{\gamma_i \left(s_i^l - s_i^m\right)}{V^l} + \sum_{\mathrm{j}=1}^{5} Y_{i,j}^l \rho_j^l \tag{3}$$

The change in concentration for polysaccharides is given by equation 4.

$$z^{(1)}{}^l = \frac{q_{in}}{V^l} z_{in}^l + \frac{q_{out}^m}{V^l} z^m - \frac{q_{out}}{V^l} z^l - \rho_1^l \tag{4}$$

Where the first term describes the inflow from the previous lumen compartment, the second term describes the flow of mucin from the mucus, and the third term describes the

flow to the next compartment. The last term is the reaction rate given by ρ_1, describing the hydrolysis of polysaccharides.

The change in microbe population is given by equation 5.

$$x^{(1)}{}_i^l = \frac{q_{in}}{V^l} x_{i,in}^l - \frac{1}{\tau_i + \frac{V^l}{q_{out}}} x_i^l + b_i^l \frac{V^m}{V_l} x_i^m - a_i x_i^l + \sum_{j=2}^{11} Y_{i,j}^l \rho_j^l \quad (5)$$

The first term represents the inflow of microbes from the previous compartment. The second term represents the outflow to the next compartment with an addition, a residence time τ is added to simulate staying power of the microbe. The third term represents the microbes that are sheared from the mucus by the stool, and the fourth term represents the adherence of microbes in the lumen to the mucus. The fourth term once again represents the reactions that can happen according to the Petersen matrix.

Finally, to maintain a constant volume in the compartment the flow is calculated according to equation 6.

$$q_{out} = q_{in} + \sum_{i=11}^{15} \frac{b_i x_i^m V^m w_i}{r_i} + q_{out}^m - \sum_{i=2,i\neq 3,7,8}^{9} \frac{\gamma_i^l s_i^l w_i}{r_i} - \sum_{i=11}^{15} \frac{a_i x_i^l V^l w_i}{r_i} \quad (6)$$

Where the first term is the inflow from the previous lumen compartment. The second term describes the volume of microbes that is sheared from the mucus. The third term is the flow of volume from the mucus. The fourth term describes the absorption of metabolites from the lumen and the fifth term describes the loss of volume due to adherence of microbes to the mucus.

Proximal, transversal, and distal mucus liquid phase

In the mucus the equations are slightly modified, and the general equations remain constant over all compartments. Equation 7 describes the changes in concentration for soluble components for the mucus.

$$s^{(1)}{}_i^m = -\gamma_i^m s_i^m + \sum_{j=1}^{5} Y_{i,j}^m \rho_j^m - Q_i^m \quad (7)$$

Where the first term is absorption of the compound by the host, the second term gives the creation and usage of the compound, and the last term, where applicable, is the gas transfer.

The mucus version of the change in sugar is given by equation 8. It has only two terms: the diffusion equation between the mucus and the lumen, and the reaction rate equations.

$$s^{(1)}{}_{su}^m = \frac{\gamma_i^l s_i^l V^l}{V^m} + \sum_{j=1}^{5} Y_{i,j}^m \rho_j^m \quad (8)$$

The change in polysaccharide concentration in the mucus is given by equation 9. The first term describes the production of mucus by the host. The second term describes the flow of volume from the mucus to the lumen and the third term describes the degradation of polysaccharides into glucose.

$$Z^{(1)^m} = \frac{\Gamma}{V^m} - \frac{q_{out}^m}{V^m} z^m - \rho_1^m \quad (9)$$

The change of microbial population in the mucus is given by equation 10.

$$x_i^{(1),m} = a_i \frac{V^l}{V^m} x_i^l - b_i x_i^m + \sum_{j=2}^{9} Y_{i,j}^m \rho_j^m \quad (10)$$

Where the first term describes the adherence of bacteria from the lumen to the mucus, and the second term describes the shear of bacteria to the lumen. The third equation describes the biomass production and the degradation of the microbes.

Finally, to keep the volume constant the flow to the lumen is given by equation 11.

$$q^m{}_{out} = \frac{\Gamma}{1000*0.05} + \sum_{i=11}^{14} \frac{a_i x_i^l V^l w_i}{r_i} - \sum_{i=2,i\neq3,7,8}^{9} \frac{\gamma_i^m s_i^m w_i}{r_i} - \sum_{i=11}^{14} \frac{b_i x_i^m V^m w_i}{r_i} \quad (11)$$

Where the first term describes the volume gained through the mucus production by the host, the second term describes the adherence of microbes to the mucus, the third term describes the adsorption of metabolites by the host, and the fourth term gives the shear volume to the lumen.

Proximal and transversal lumen and all mucus gas phase

All lumen compartments have the same equations for gas phase compounds, which is given by equation 12.

$$s_{g,i}^{(1)} = \frac{q_{g,in}}{V_g} s_{g,i,in} - \frac{q_{g,out}}{V_g} s_{g,i} + \frac{Q_i V}{V_g} \quad (12)$$

Where the first term is the flow of gas from the last compartment, the second term gives the outflow of gas, and the third term describes the exchange of gas with the liquid phase.

The mucus compartments almost have the same equation, as shown in equation 13. The only difference is that there is no term for the inflow of gasses.

$$s_{g,i}^{(1)} = -\frac{q_{g,out}}{V_g} s_{g,i} + \frac{Q_i V}{V_g} \quad (13)$$

The liquid-gas exchange is described by equation 14.

$$Q_i = k_L a \left(s_i - K_{H,i} R\, T\, s_{g,i}\right) \quad (14)$$

Where $k_L a$ = the liquid-gas transfer coefficient, $K_{H,i}$ is the henry's law coefficient, R is the ideal gas coefficient, and T is the absolute temperature.

Finally, the outflow of gas is calculated described in equation 15.

$$q_{g,out} = q_{g,in} + q_{g,lgt} \tag{15}$$

Where $q_{g,lgt}$ is given by equation 16, as based on Batstone et al. (2002).

$$q_{g,lgt} = \frac{RT}{P_{atm} - p_{H_2O}} V \left(Q_{H_2} + Q_{CH_4} + Q_{CO_2}\right) \tag{16}$$

Distal lumen

The distal lumen is the most divergent compartment, it is the only compartment with a non-constant liquid volume, instead being modelled as a semi-batch reactor. The liquid volume initiates at a value of 100 mL and is reset as soon as it hits 300 mL to simulate excretion from the bowels, in accordance with (REFERENCE).

The change of volume in the distal lumen satisfies equation 17, so that the total volume of the distal lumen remains the same.

$$V_{l,d}^{(1),l} = -V_{g,d}^{(1),l} = q_n = equation\ 5 \tag{17}$$

This changes the equations for the state variables slightly. The change of soluble compounds in the distal lumen is now given by equation 18.

$$s^{(1)}{}_i^l = \frac{q_{in}}{V^l} s_{i,in}^l - \frac{q_n}{V^l} s_i^l - \gamma_i^l s_i^l + \sum_{j=1}^{5} Y_{i,j}^l \rho_j^l - Q_i^l \tag{18}$$

Where only the q_{out} is changed to q_n. This is the same for the change in sugar, polysaccharides, and microbes, given by equations 19, 20 and 21, respectively.

$$s^{(1)}{}_i^l = \frac{q_{in}}{V^l} s_{i,in}^l - \frac{q_n}{V^l} s_i^l - \frac{\gamma_i \left(s_i^l - s_i^m\right)}{V^l} + \sum_{j=1}^{5} Y_{i,j}^l \rho_j^l \tag{19}$$

$$z^{(1)}{}^l = \frac{q_{in}}{V^l} z_{in}^l + \frac{q_{out}^m}{V^l} z^m - \frac{q_n}{V^l} z^l - \rho_1^l \tag{20}$$

$$x^{(1)}{}_i^l = \frac{q_{in}}{V^l} x_{i,in}^l - \frac{q_n}{V^l} x_i^l + b_i \frac{V^m}{V_l} x_i^m - a_i x_i^l + \sum_{j=2}^{9} Y_{i,j}^l \rho_j^l \tag{21}$$

Finally, the change to gas phase concentrations in this compartment is then changed to equation 22.

$$s_{g,i}^{(1)} = \frac{q_{g,in}^{l}}{V_g^l} * \left(s_{g,i,in}^{l} - s_{g,i}^{l}\right) - \frac{q_{g,lgt}^{l} - q_n}{V_g^l} s_{g,i}^{l} + \frac{Q_i^l V_l^l}{V_g^l} \quad (22)$$

Implementation of *E. coli* Nissle

The model for *E. coli* Nissle is based on the general microbe equation for each respective compartment, see equations 5, 10, and 21. For *E. coli* Nissle the standard yield and shear constants, while the degradation rate, $k_{d,ecn}$, the specific consumption rate, $k_{m,ecn}$, and the adherence, a_{ecn}, were changed to reflect changes that were engineered or required to match literature. First, to match literature, a standard adherence of a regular *E. coli* Nissle to mucus was calculated from literature at 0.038 [3]. Then, with a standard adherence, a degradation rate was found through parameter optimisation and latin hypercube sampling to reach the same residence time, as found in literature, of around 4 days [4].
With a standard healthy *E. coli* Nissle model completed, a modification of the substrate uptake rate was needed to represent the toll that engineering a bacterium takes on its regular functions. For this purpose, the data from one of its main purposes was analysed, the production of chromoproteins. From literature the growth rate was estimated at 75% of full growth rate using the chromoproteins spisPink and asPink [5]. This growth rate could then be related to a relative substrate uptake rate of 83% [6].
The *E. coli* Nissle was injected into the model as a single burst of an input concentration at 10 days with a duration of 0.2 days to simulate the taking of a pill.

Implementation of strategies to enhance *E. coli* Nissle residence time

To better predict the usage of an engineered *E. coli* Nissle in the colon as a diagnostic vector several strategies were developed to research the opportunities to increase the residence sstime allowing it to perform its diagnostic function in the colon, while influencing the microbiome as little as possible. For this purpose the input concentration was kept at a standard probiotic dose of around 4 times the normal daily intake of microbes from the environment [4,7].

Adherence

The adherence was evaluated by sampling a parameter space from 0 to 0.248 [d^{-1}] using latin hypercube sampling with 100 samples. Since in literature no data on the planned modification could be found, this range was assessed for the purpose of sensitivity, advising experiments, and relating to other adhesion improvement strategies from literature.

Dosing strategies

Another way of improving the residence time of the engineered microbe is by using a technique called frequency dosing, where instead of modifying the microbe, it is dosed a couple of times to bolster the population that exists in the colon [8]. This was implemented in this model as two doses, where the time between doses was variable which was then subject to sensitivity analysis and comparison to a single dose variant.

Prebiotic strategy

Prebiotics as a microbe supporting vector has been used in clinical settings for a long time [9]. The basic idea is to support the microbes you wish to flourish with nutrients and environments that are conducive to their health. In this model this was implemented similar to the input of the modified *E. coli* Nissle, as a burst of sugar input at the same time as the *E. coli* input at different concentrations.

Modelled kill switch

The proposed engineered *E. coli* Nissle includes a kill switch as a safety measure, due to the usage of engineered microbes in the human microbiome. This kill switch is based on disabling the reproduction of the cell instead of outright killing it. To analyse the effectiveness of this strategy for *E. coli* Nissle, the kill switch was implemented by introducing a function that changes the yield of the microbe to 0 at a designated time point. This represents the taking of the kill switch triggering pill.

Model performance

The base model and the additions resulted in a model that contains 108 different differential equations over 6 compartments. The total model's performance was 2.5 hours on an AMD opteron 6272 with 2 terabytes of RAM, or 30 minutes on a MacBook air m1 with 16 gigabytes of RAM when simulating 100 days.

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Appendices

Appendix I

State variables

- s_i = concentration of soluble component i in liquid phase [M] or [mol/l]
- $s_{g,i}$= concentration of soluble component i in gas phase [M] or [mol/l]
- x_i= concentration of bacterial group i [M] or [mol/l] or optical density (OD)
- z = concentration of polysaccharides [g/l]
- ζ= the concatenated vector of (s, z, x, s_g) (see Petersen matrix)

Parameters

- a_i= adherence coefficient of bacteria in lumen [d^{-1}]
- b_i= shear loss coefficient of bacteria in mucus [d^{-1}]
- f_i= fraction of the component i used for catabolic reactions
- k_d= decay constant of bacteria [d^{-1}]
- $K_{H,i}$ = henry's law coefficient of component i [M/bar]
- $k_{hyd,i}$= maximum specific hydrolysis rate [d^{-1}]
- k_i= inactivation constant of bacteria [d^{-1}]
- $k_L a$= liquid-gas transfer coefficient multiplied by the specific transfer area [d^{-1}]
- $k_{m,i}$= maximum specific rate of substrate consumption [mol s_j d^{-1} / mol x_j]
- $K_{s,j}$= half-saturation constant for microbial growth (constant of Monod) [M]
- $K_{x,i}$= half-saturation constant for ratio $\frac{s}{x}$ [Ms_j/Mx_j]
- r_i= density of component i [g/l]
- w_i= molecular weight of component i [g/mol]
- $Y_{i,j}$= yield component of i in process j [mol s_i/ mol s_j]
- Y_j= biomass yield factor for bacteria using substrate j [mol x_j/mol s_j]
- γ_i= transport coefficient of component i [d^{-1}]
- η_i= reaction yield
- λ_i= reaction coordinate
- $\mu_{max,i}$= maximum specific growth rate [d^{-1}]
- τ_i= additional residence time of bacteria in lumen [d]

Functions

- ρ_j= kinetic rate of process j
- Q_i= liquid-gas transfer rate of component i

Subscripts and superscripts

- g = gas phase
- in = influent
- l = lumen
- lgt = liquid-gas transfer
- m = mucus
- out = effluent
- n = net

for metabolites

- ac = acetate
- bu = butyrate
- la = lactate
- pro = propionate
- su = glucose

for bacteria

- H_{2a} = hydrogen utilising bacteria (acetogenic)
- H_{2m} = hydrogen utilising bacteria (methanogenic)
- la = lactate utilising bacteria
- su = glucose utilising bacteria
- ecn = *E. coli* Nissle

Physiological parameters

- L = length of colon section [cm]
- P_{atm}= atmospheric pressure [bar]
- p_{H_2O}= water pressure [bar]
- R = ideal gas constant [bar/M*K]
- q = flow rate [l/d]
- q_n= net flow rate in the distal lumen [l/d]
- T= temperature [K]
- V= volume in liquid phase [l]
- V_g= volume in gas phase [l]
- Γ= endogenous production of mucins [g/d]
- ϕ= diameter of the colon [cm]

Appendix II

Physiological parameters

Parameter	Value	Source	Comments
P_{atm}	1.013 bar		
p_{H_2O}	0.08274 bar	[1]	
R	0.08314 bar M^{-1} K^{-1}	[1]	
T	310.15 K	[1,10]	
V_g	10% of total volume		Assumed that the gas volume is 10% of the total volume in each compartment
q_{in}	1.5 l d^{-1}	[1,11]	Input flow into the proximal lumen
Γ	5 g d^{-1}	[1,12,13]	Total production of mucus, thus it is assumed that each mucus compartment produces 5/3 g d^{-1}

Microbe based parameters

The adherence (a_i), shear (b_i), and residence time (τ_i) parameters for the original microbes were set to 0, 0.08, and 1.0 d^{-1} respectively, in accordance with the published work of Rafael Muñoz-Tamayo in 2010. For *E. coli* Nissle the shear value is set to the same 0.08 d^{-1}, while the residence time is assumed to be 0 for *E. coli* Nissle due to there being no established colonies of it in the colon. Finally, the standard adherence of *E. coli* Nissle to the mucus is calculated at 0.038 d^{-1} in accordance with literature [3], where they quantified *E. coli* Nissle adherence. The adherence of *E. coli* Nissle was varied from the standard in simulation experiments.

Mass transfer related parameters

Parameter	Value	Source
$k_L a$	200 d^{-1}	Muñoz-Tamayo et al. 2010 Batstone et al. 2002
K_{H,H_2}	7.29*10^{-4} M bar^{-1}	Muñoz-Tamayo et al. 2010 Batstone et al. 2002
K_{H,CO_2}	0.0255 M bar^{-1}	Muñoz-Tamayo et al. 2010 Batstone et al. 2002
K_{H,CH_4}	0.0011 M bar^{-1}	Muñoz-Tamayo et al. 2010 Batstone et al. 2002

Diffusion and absorption rates [1,11,14]			
Parameter	**Value**		
	Proximal	**Transverse**	**Distal**
$\gamma_{\mathrm{su}}^{\mathrm{l}}$	1.60 l d^{-1}	3.80 l d^{-1}	6.30 l d^{-1}
$\gamma_{\mathrm{la}}^{\mathrm{l}}$	0.88 d^{-1}	0.43 d^{-1}	2.03 d^{-1}
$\gamma_{\mathrm{ac}}^{\mathrm{l}}$	1.32 d^{-1}	0.64 d^{-1}	3.05 d^{-1}
$\gamma_{\mathrm{pro}}^{\mathrm{l}}$	1.07 d^{-1}	0.62 d^{-1}	2.47 d^{-1}
$\gamma_{\mathrm{bu}}^{\mathrm{l}}$	0.90 d^{-1}	0.57 d^{-1}	2.49 d^{-1}
$\gamma_{\mathrm{H_2O}}^{\mathrm{l}}$	1.60 d^{-1}	0.77 d^{-1}	3.66 d^{-1}
γ_{la}^{m}	12.60 d^{-1}	12.60 d^{-1}	12.60 d^{-1}
γ_{ac}^{m}	18.90 d^{-1}	18.90 d^{-1}	18.90 d^{-1}
$\gamma_{\mathrm{pro}}^{m}$	15.32 d^{-1}	15.32 d^{-1}	15.32 d^{-1}
γ_{bu}^{m}	12.88 d^{-1}	12.88 d^{-1}	12.88 d^{-1}
$\gamma_{\mathrm{H_2O}}^{m}$	0.01 d^{-1}	0.01 d^{-1}	0.01 d^{-1}

Kinetic parameters

Parameter	Value	Source	Unit
$k_{hyd,z}$	$1.20 * 10^3$	a)	[d^{-1}]
$K_{X,Z}$	29.99	a)	[gram poly-saccharides/mol biomass]
$Y_{su,z}$	0.005	a)	[mol sugar/ gram biomass]
$k_{m,su}$	7.92	b)	[mol sugar/ mol biomass *d]
$k_{m,ecn}$	6.57	c)	See km, su
$K_{s,su}$	0.0026	c)	[mol sugar/ l]
Y_{su}	0.120		[mol biomass/ mol sugar]
Y_{ecn}	0.120		See Y_{su}
$Y_{la,su}$	0.499		[mol lactate/ mol sugar]
$Y_{H_2,su}$	1.440		[mol H_2/ mol sugar]
$Y_{ac,su}$	0.567		[mol acetate/ mol sugar]
$Y_{pro,su}$	0.240		[mol propionate/ mol sugar]
$Y_{bu,su}$	0.270		[mol butyrate/ mol sugar]
$Y_{CO_2,su}$	1.100		[mol CO_2/ mol sugar]
$Y_{H_2O,su}$	1.440		[mol water/ mol sugar]
$k_{m,la}$	103		[mol lactate/ mol biomass *d]
$K_{s,la}$	$6.626 * 10^3$		[mol lactate/ l]
Y_{la}	0.120		[mol biomass/ mol lactate]
$Y_{H_2,la}$	0.400		[mol H_2/ mol lactate]
$Y_{ac,la}$	0.133		[mol acetate/ mol lactate]
$Y_{pro,la}$	0.267		[mol propionate/ mol lactate]
$Y_{bu,la}$	0.200		[mol butyrate/ mol lactate]

$Y_{CO_2,la}$	0.533		[mol CO_2/ mol lactate]
$Y_{H_2O,la}$	0.493		[mol water/ mol lactate]
k_{m,H_2a}	108.84		See km, su
K_{s,H_2a}	0.0017		[mol H_2 / l]
Y_{H_2a}	0.043		[mol biomass/ mol H_2]
Y_{ac,H_2a}	0.143		[mol acetate/ mol H_2]
Y_{CO_2,H_2a}	-0.5		[mol CO_2/ mol H_2]
Y_{H_2O,H_2a}	0.629		[mol water/ mol H_2]
k_{m,H_2m}	22.581	c)	[mol H_2/ mol biomass *d]
K_{s,H_2m}	$1.563 * 10^{-6}$	c)	[mol H_2 / l]
Y_{H_2m}	0.062		[mol biomass/ mol H_2]
Y_{CH_4,H_2m}	0.095		[mol CH_4 / mol H_2]
Y_{CO_2,H_2m}	-0.450		[mol CO_2 / mol H_2]
Y_{H_2O,H_2m}	0.686		[mol water / mol H_2]
k_d	0.01	c)	[d^{-1}]
$k_{d,ecn}$	1.4		[d^{-1}]

Explanation: all values except $k_{d,ecn}$ and $k_{m,ecn}$ come originated from the base model [1], source indicates the original source referenced in the model paper.
No source given means that it was a result from kinetic experiments done by Rafael Muñoz-Tamayo in his thesis chapter 5 2010 [15].
The parameter $k_{dec,ecn}$ is was calculated by parameter optimisation to match the median residence time with full substrate uptake and yield [4].
$k_{m,ecn}$ was derived by analysing growth rate data from chromoprotein production and correlating that to substrate uptake [5,6].

a) [16] b) [17] c) [18]

Appendix III

Petersen matrix describes the reactions for a certain component. For a certain component look at the column and see what additions and subtractions happen, e.g., for sugar the reactions are defined as $Y_{su,z} * \rho_1 - \rho_2 - \rho_6$.

For soluble components

Component *i* ⟶	**1**	**2**	**3**	**4**	**5**	**Kinetic rate**
Process *j* ↓	S_{su}	S_{la}	S_{H_2}	S_{ac}	S_{pro}	
1 Hydrolysis	$Y_{su,z}$					ρ_1
2 Glucose utilisation	-1	$Y_{la,su}$	$Y_{H_2,su}$	$Y_{ac,su}$	$Y_{pro,su}$	$\rho_2 \& p_6$
3 Lactate utilisation		-1	$Y_{H_2,la}$	$Y_{ac,la}$	$Y_{pro,la}$	ρ_3
4 Hydrogen utilisation: homoacetogenesis			-1	Y_{ac,H_2a}		ρ_4
5 Hydrogen utilisation: methanogenesis			-1			ρ_5

Component *i* ⟶	**6**	**7**	**8**	**9**	**Kinetic rate**
Process *j* ↓	S_{bu}	S_{CH_4}	S_{CO_2}	S_{H_2O}	
1 Hydrolysis					ρ_1
2 Glucose utilisation	$Y_{bu,su}$		$Y_{CO_2,su}$	$Y_{H_2O,su}$	$\rho_2 \& \rho_6$
3 Lactate utilisation	$Y_{bu,la}$		$Y_{CO_2,la}$	$Y_{H_2O,la}$	ρ_3
4 Hydrogen utilisation: homoacetogenesis			Y_{CO_2,H_2a}	Y_{H_2O,H_2a}	ρ_4
5 Hydrogen utilisation: methanogenesis		Y_{CH_4,H_2m}	Y_{CO_2,H_2m}	Y_{H_2O,H_2m}	ρ_5

For polysaccharides and bacteria

Component *i* $\longrightarrow$		**10**	**11**	**12**	**13**	**14**	**15**	**Kinetic rate**
Process *j* $\downarrow$		Z	x_{su}	x_{la}	x_{H_2a}	x_{H_2m}	x_{ecn}	
1	Hydrolysis	-1						ρ_1
2	Glucose utilisation		Y_{su}					ρ_2
3	Lactate utilisation			Y_{la}				ρ_3
4	Hydrogen utilisation: homoacetogenesis				Y_{H_2a}			ρ_4
5	Hydrogen utilisation: methanogenesis					Y_{H_2m}		ρ_5
6	Glucose utilisation for *E. coli* Nissle						Y_{ecn}	ρ_6
7	Decay of sugar-utilising bacteria		-1					ρ_7
8	Decay of lactate-utilising bacteria			-1				ρ_8
9	Decay of homoacetogenic bacteria				-1			ρ_9
10	Decay of methanogenic bacteria					-1		ρ_{10}
11	Decay of *E.* coli Nissle						-1	ρ_{11}

Kinetic rates

ρ_1	$k_{hyd,z} * \frac{z * (x_{su} + x_{ecn})}{K_{x,z} * x_{su} + z}$
ρ_2	$k_{m,su} * \frac{s_{su} * x_{su}}{K_{s,su} + s_{su}}$
ρ_3	$k_{m,la} * \frac{s_{la} * x_{la}}{K_{s,la} + s_{la}}$
ρ_4	$k_{m,H_2a} * \frac{s_{H_2a} * x_{H_2a}}{K_{s,H_2a} + s_{H_2a}}$
ρ_5	$k_{m,H_2m} * \frac{s_{H_2m} * x_{H_2m}}{K_{s,H_2m} + s_{H_2m}} * I_{pH}$
I_{pH}	If pH >= pHU, $I_{pH} = 1$ If pH <pHU, $I_{pH} = \mathrm{Exp}\left(-3\left(\frac{pH - pH_U}{pH_U - pH_L}\right)^2\right)$
ρ_6	$k_{m,ecn} * \frac{s_{su} * x_{ecn}}{K_{s,ecn} + s_{su}} * I_{pH}$
ρ_7	$k_d x_{su}$
ρ_8	$k_d x_{la}$
ρ_9	$k_d x_{H_2a}$
ρ_{10}	$k_d x_{H_2m}$
ρ_{11}	$k_{d,ecn} x_{ecn}$

I_{pH} is a model for the inhibition of methanogenesis that is triggered when the pH of the compartment gets under the upper bound for inhibition.