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Kombucha: An Empirical Model

Sugars, Acids, Cellulose and Alcohol

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Abstract This paper attempts to fill a gap in the existing literature on Kombucha fermentation by providing an empirical model for *open* systems that are either static (study 1) or agitated (study 2). We analyzed the conservation of carbon-mass, and the data from study 1 suggests fructose could be synthesized to gluconic acid by Kombucha bacteria (fig. 2), which is not well documented in existing literature. We developed a theoretical system of ordinary differential equations (ODEs), established from the underlying biology and chemical reactions. We fit the general model to each study's data, where the parameters were optimized using the Nelder-Mead method. The resulting models (one for agitated ferments, one for static ferments) predict the Kombucha fermentation process for up to 20 days, a time-frame consistent with most commercial brewing applications. The models could therefore be adapted for further study of Kombucha fermentation, or commercial forecasting.

Keywords Kombucha · ODE · Model

Mathematics Subject Classification (2010) MSC 92D40 · MSC 92C42

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1 Introduction

1.1 History

Kombucha is an ancient traditional beverage consisting of a sweetened tea base, cultured with specific strains of bacteria in symbiosis with yeasts [16]. The result is a sour, sometimes vinegary beverage with naturally low levels of carbonation, which is a byproduct of the fermentation process. In commercial applications it is common to force carbonate the beverage to higher concentrations of CO_2. Kombucha also yields bacterial cellulose via specific strains of bacteria (typically *Komagataeibacter xylinus*), usually requiring a non-agitated fermentation methodology.

The commercial product is typically prepared by mixing a determinate quantity of black or green tea leaves (a nitrogen source) with boiled water (80-100°C), followed by stirring in a sweetener (a carbon source) while the mixture is still quite hot in order to help it dissolve. The most common sweetener is probably sucrose, however, a plethora of options are available, such as molasses, grape juice, sour cherry juice, coconut water, and more. This mixture is left to cool down (20-30°C) before adding a prepared innoculum of yeast and bacteria. The consortium can be prepared from a previously fermented culture and broth left in refrigeration. This is then placed into the sweetened tea in sufficient quantities (8-15% v/v) and left to ferment. During fermentation a cellulosic pellicle layer (otherwise referred to as the mother culture) is formed by the bacteria by metabolizing certain substrates. It is typical to add both the solid cellulosic pellicle and the sour liquid broth when inoculating the present batch.

There are two general "environments" for fermenting Kombucha, the first being an *open* system, which is when the brew is left in a vessel to ferment with only a porous cloth covering the vessel's opening. This allows the system to effectively breathe, as oxygen is required for the obligately aerobic bacteria (OAB). The second environment is a *closed* system, in which a sealed lid is affixed to the fermentation vessel, requiring oxygen to be supplied at an appropriate rate for the OAB so as to prevent oxygen starvation. An open ferment is generally *wild*, meaning the exact consortia of yeasts and bacteria is to some degree unknown, subject to varying population sizes and even possibly some species between batches. The closed fermentation process can allow for much stricter control over species, and provides grounding for spending the effort to identify and quantify the species present. This study focuses on two types of open, wild ferments.

In either open or closed systems, there are both agitated (stirred) and static (not stirred) methodologies. In the static ferment there is opportunity for a biofilm to develop (the aforementioned cellulosic pellicle layer or mother culture). This symbiotic culture of yeast and bacteria (SCOBY) grows from the top down, pushing old layers into the broth as new layers form on top. However, a critical thickness of the SCOBY can occur, effectively starving the OAB of oxygen, a consequence of at least three factors.

1.2 Considerations

First, the *maximum transport distance* of oxygen [15] is much less than for sucrose, due to oxygen being much less soluble in water (0.004 g/L at 25°C) than sucrose

(2100 g/L). This presents a limiting factor for metabolic activity as the SCOBY thickens. Vershuchren *et al.* discovered that the saturated oxygen profiles (SOP) started from 200 to 500μm depending on the initial broth of the pH, with all relevant cases (having $pH_0 \leq 5$) seeing a reduction in the SOP to under 100μm by day 3. Those mixtures then maintained approximately 50μm during the ferment. Moreover, they found new layers of cellulose form exclusively in the 50 to 100 μm depths, which also quickly become oxygen deprived.

Second, the amount of dissolved oxygen also critically depends on the interfacial area within the fermentation vessel [1], granting that fermentation occurs in a sufficiently deep vessel such that saturation is possible. Worthy of note is that in the agitated case, the cellulose pellicle cannot form in a suspended matrix over the broth as the network of cells is broken and cannot form thick layers, which hinders growth. Thus oxygen starvation is less of a concern in the open and agitated system, which is the methodology of one of the studies we examine herein.

Third, a byproduct of fermentation is CO_2 release due to cellular respiration, which can build up under the SCOBY and cause the partial pressure of oxygen to drop seriously enough to impact the bacteria and cause die-off [5]. This is only an issue in static cases where the SCOBY remains undisturbed (the second methodology we examine), such that the trapped CO_2 is incapable of escaping the critical metabolic zone (for the OAB) within 50μm of the surface. This causes an oxygen deprived environment and effectively starves the OAB of a primary metabolic supplement.

1.3 Ecology

The typical yeasts present are of genus *Saccharomyces*, especially *Candida sp.*, and *Zygosaccharomyces*. [13]. The typical bacteria are *Acetobacter xylinum* (acetic acid bateria –AAB), *Gluconacetobacter* (gluconic acid producing bacterium), and *Komagataeibacter xylinus*, which is the species best known for cellulose production [16]. Modeling specific strains is considerably intractable, and so we instead model "global" behaviors per family. This guides some nomenclature for this paper, in referring to the consortia of yeasts as simply *the yeast*, and the various Kombucha bacteria as *the bacteria*.

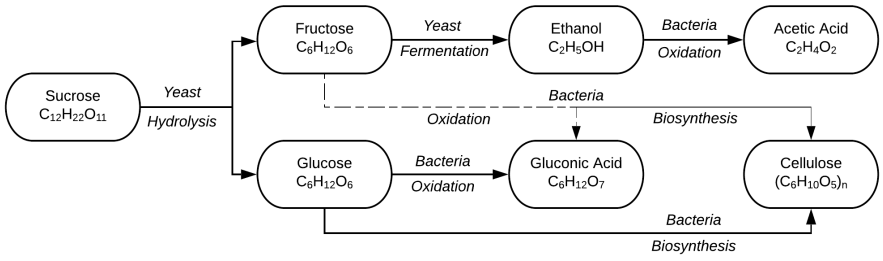

Fig. 1 Substrate Compartment Flow; (Solid) Known mechanism; (Dashed) Postulated mechanism (in this paper)

Guided by the literature, figure 1 demonstrates the substrate flow of the typical ferment. The yeast play a critical role via hydrolysis of the polysaccharide sucrose, producing two monosaccharides, fructose and glucose, by the invertase enzyme [2]. Fructose is fermented into ethanol by yeast, which in turn is oxidized into acetic acid by the *Acetobacter aceti*, this is the first chain of reactions to consider [2]. The second is the conversion of glucose into gluconic acid by *Gluconobacter Oxydans* [3]. Finally, *A. aceti* and *K. xylinus* biosynthesize cellulose from various subtrates [16], however in our research we found glucose to be the predominant substrate for this process. As aside, the theoretical compartmental flow for our ODE model is reflected in figure 1.

As per a study done by Sievers *et al.* in an open and agitated ferment, they found conservation of mass without evidence of significant anabolism [13]. The good stoichiometry they observed led them to conclude the major fermentation products (sucrose, fructose, ethanol, acetic acid, glucose, gluconic acid) have all been accounted for, with other substrates and species having had negligible impact.

1.4 Motivation

The undertaking of this paper is to establish working mathematical models of both the static open ferment and the agitated open ferment – closed ferments are not considered at this time. The research comprises the data of two studies, we obtain agitated open ferment data from Sievers *et al.* [13], and the static open ferment data comes from a paper by Chen and Liu [2]. In the latter case, the mentioned substrates do not account for all the chemistry (as opposed to Sievers *et al.* as discussed in section 1.3). Hence, here we will simply posit that the formation of cellulose sufficiently encapsulates the missing mass-balance for the models at hand, and investigate this further in section 3.4

2 Preamble to Formulation of the Models

Here we turn to the various underlying chemical reactions that determine the flow of our compartmental model of ODEs, as expressed in figure 1. We consider the reactants: sucrose, $C_{12}H_{22}O_{11}$; fructose and glucose, $C_6H_{12}O_6$; ethanol, C_2H_5OH; gluconic acid, $C_6H_{12}O_7$; and acetic acid, $C_2H_4O_2$.

2.1 Chemical Reactions

First, sucrose is converted to fructose and glucose via the yeast invertase enzyme (a process known as hydrolysis) [8]

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{Y} C_6H_{12}O_6 + C_6H_{12}O_6$$

One fructose molecule is fermented to two ethanol molecules, with carbon dioxide byproduct [7]

$$C_6H_{12}O_6 \xrightarrow{Y} 2C_5H_5OH + 2CO_2$$

and each resulting ethanol molecule is oxidized by bacteria into acetic acid [3]

(really a two step process, but herein simplified to one step)

$$C_2H_5OH + 2[O] \xrightarrow{B} C_2H_4O_2 + H_2O$$

Acetic acid dissociates into acetate ion and a hydrogen ion

$$C_2H_4O_2 \xrightarrow{\text{Dissociation}} C_2H_3O_2^- + H^+$$

while glucose is oxidized to gluconic acid by the bacteria [16]

$$C_6H_{12}O_6 + [O] \xrightarrow{B} C_6H_{12}O_7$$

which dissociates to form a gluconate ion and a hydrogen ion

$$C_6H_{12}O_7 \xrightarrow{\text{Dissociation}} C_6H_{11}O_7^- + H^+$$

and there is no consideration of cellulose here. Thus, accounting for molarity, we chart the chemical reactions in figure 2.

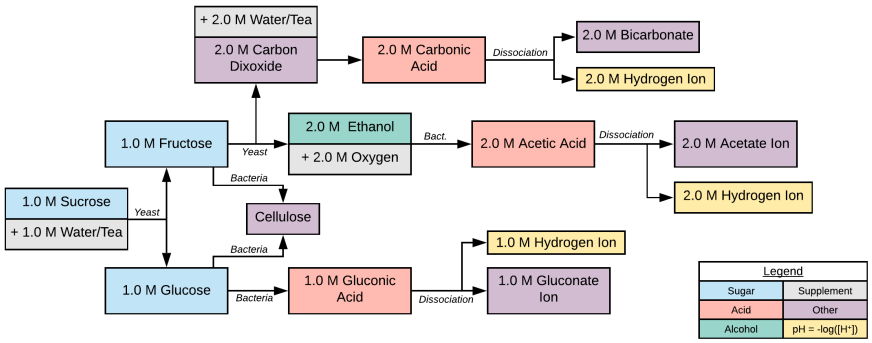

Fig. 2 Chemical reactions accounting for molarity

2.2 Acidity

The acidity (pH) is defined as the base 10 logarithm of the reciprocal of the hydrogen ion concentration, or equivalently, the negative base 10 logarithm of the hydrogen ion concentration

$$\text{pH} = \log\left(\frac{1}{H^+}\right) = -\log\left(H^+\right)$$

Here we also address that CO_2 reacts with H_2O to form carbonic acid (H_2CO_3), which dissociates to release a hydrogen ion (H^+) and bicarbonate ions (HCO_3^-) [17]. The pH of the broth serves as a buffering system for the bacteria, halting their metabolic activity and rendering them dormant at some threshold [4]. If the pH increases then the bacteria reinvigorate and continue metabolizing available substrates [4], including even metabolizing cellulose if no other substrates are available.

3 Formulation of the Models

In this section we turn to the formulation of the two models. The studies are conducted over 60 days [2][13], but most commercial Kombucha for consumable products range from 10 to 20 days, before the pH of the beverage dips below 3.0, which is considered unsafe for the human digestive tract [16]. Therefore, we will model only the first 20 days of each study, but will perform a carbon-mass analysis of their data over the whole time frame.

As mentioned in section 1.3, modeling the consortia of yeasts and bacteria *by strain* proves intractable with the current data. Switching to modeling family behaviours, however, was feasible and provided satisfactory results in optimizing model parameters to the various observations. The examination of the involved chemical reactions as in section 2.1 provided a good starting point for developing a model. As well, figures 1 and 2 may prove to be crucial cross-reference materials for the reader as we expand the model.

3.1 Background Theory

We will begin with a synopsis, then leave the rest to the reader. We first consider that the substrates for metabolic activity are finite, thus the model requires that we used proportionality and substrate concentration limiting behavior, i.e.,

$$\frac{\mathrm{d}}{\mathrm{d}t}\text{Substrate} = \frac{\text{param}_1 \cdot \text{Substrate}}{1 + \text{param}_2 \cdot \text{Substrate}} \cdot \text{Catalytic Agent} \tag{1}$$

to guide the general theory. Motivations for this formulation can be found in [6][14] which builds off Monod's work [10].

Equation 1 is communicating that the rate of change of a substrate in the broth (left-hand side) is proportional to the activity of a catalytic agent (such as the yeast) on the limiting ratio of the substrate (we'll use sucrose for illustration). The two parameters are simplifying parameters for numerical analysis, considering that many of the equations in the system (forthcoming) yield no analytical (closed-form) solutions.

Recall that the sucrose molecule is hydrolyzed into one molecule each of fructose and glucose by the yeast, and sucrose can *only* decrease (we're barring a brewer from spiking the broth with additional sucrose mid-ferment), so it will *always* appear as a non-positive rate. Let S, F, and G_s respectively denote the concentrations of sucrose, fructose, and glucose. Furthermore, denote the yeast by Y and some generic parameters a and α. Then we could represent this miniature theoretical model of the compartmental flow of sucrose into fructose and glucose by

$$\begin{aligned}
\frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{aS}{1+\alpha S}Y \\
\frac{\mathrm{d}F}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y
\end{aligned}$$

Moreover, since one fructose molecule is fermented into *two* ethanol molecules by the yeast, so we could represent this extra step by the model if we let E stand for ethanol, and include b and β parameters

$$\begin{aligned}
\frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{aS}{1+\alpha S}Y \\
\frac{\mathrm{d}F}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y - \frac{bF}{1+\beta F}Y \\
\frac{\mathrm{d}E}{\mathrm{d}t} &= 2\frac{bF}{1+\beta F}Y \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y
\end{aligned}$$

which accounts for sucrose only decreasing, and gathering up in the fructose compartment which *simultaneously* has one unit leaving to turn into two units in the ethanol compartment. In this example, glucose is a sink for the other molecule coming from sucrose hydrolysis, that is, in this example glucose is only accumulating – but this will not be reflective of the actual process. We go into the specifics of fitting the model to the data in section 4.Hence, we arrive at our model statement.

3.2 Theoretical Model

Let S, F, E, A, G_s, G_a and C be respectively the concentrations of sucrose, fructose, ethanol, acetic acid, glucose, gluconic acid and cellulose. Furthermore, let Y denote the yeast and B the bacteria, and let the parameters be

$$a, b, c, d, e, f, \alpha, \beta, \gamma, \delta, \epsilon, \lambda, \rho, \text{ and } r$$

Taken together with the chemical formulae, we can finally represent the full theoretical model:

$$\begin{aligned}
\frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{aS}{1+\alpha S}Y \\
\frac{\mathrm{d}F}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y - \frac{bF}{1+\beta F}Y - \frac{cF}{1+\gamma F}B^{\dagger} && \text{(for † see next page)} \\
\frac{\mathrm{d}E}{\mathrm{d}t} &= 2\frac{bF}{1+\beta F}Y - \frac{dE}{1+\delta E}B \\
\frac{\mathrm{d}A}{\mathrm{d}t} &= \frac{dE}{1+\delta E}B \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y - \frac{eG_s}{1+\epsilon G_s}B - \frac{fG_s}{1+\lambda G_s}B \\
\frac{\mathrm{d}G_a}{\mathrm{d}t} &= \frac{eG_s}{1+\epsilon G_s}B \\
\frac{\mathrm{d}C}{\mathrm{d}t} &= \frac{cF}{1+\gamma F}B + \frac{fG_s}{1+\lambda G_s}B \\
\frac{\mathrm{d}Y}{\mathrm{d}t} &= \rho\left(\frac{aS}{1+\alpha S} + \frac{bF}{1+\beta F}\right)Y \\
\frac{\mathrm{d}B}{\mathrm{d}t} &= r\left(\frac{cF}{1+\gamma F} + \frac{dE}{1+\delta E} + \frac{eG_s}{1+\epsilon G_s} + \frac{fG_s}{1+\lambda G_s}B\right)B && (2)
\end{aligned}$$

Just look for matching terms between equations for compartment flows, for example, we can see the term with both parameters c and γ represents fructose biosynthesis by bacteria into cellulose (first term for C_t), but also is an *activity* term for Bacteria – moreover, this is the novel (or under-studied) biosynthesis this paper proposes. There is dual significance to notice here, first in that a substrate can be acted upon by both yeast and bacteria, and second, that these terms have some connection with the activity of the underlying populations. This is what we meant by representing the behavior of a family in section 1.3, versus actually trying to model a strain's input to the model.

3.3 Agitated Model

We fit this model (model 2) to the research data from Sievers *et al.* in the statistical computing language **R** – methodology discussed in section 4. Along with a few parameters being optimized to zero, there was a persistent lack of mass balance in the glucose parameter optimization (it was always drastically under-fitting). We were seeing that no matter the approach, there was always insufficient glucose, and thereby gluconic acid, in the fitted model. This indicated a mistaken assumption in the model that failed to correspond to what we're now positing is a flow from fructose to gluconic acid (that term with both c and γ), which is not supported in current literature. This conclusion is supported weakly by the fact that it happens to improve the fit, but stronger evidence is provided by an analysis of the raw data.

In figure 3, the sucrose data points indicate the (loss of sucrose) $S_0 - S_t$, or equivalently, the gain of fructose and glucose over time. However, since fructose and glucose are also decaying, for conservation of mass we supplemented their data values with their byproduct data values, performed in moles. As is clearly visible from the first and second graphs, glucose sits nearly to the required values until day 24, and adding the concentration of gluconic acid to it would bump the data values above the "sucrose" values, which cannot be the case. Both fructose and glucose data points ought to match the loss of sucrose (as one sucrose molecule hydrolyzes to one fructose and one glucose molecule). The problem here is that fructose is drastically low in the first graph of figure 3.

To remedy this, we added to it the ethanol and acetic acid values in moles (the known byproducts in the fructose cycle) and still saw the fructose data points fell short of the sucrose-loss. As an exploratory move, we added gluconic acid to the fructose group, producing the second chart in figure 3. After day 24 we see that the sum of the fructose, ethanol, acetic acid, and gluconic acid altogether now grow away from the sucrose-loss values, diametrically opposed to the glucose cycle, which decays away from the sucrose-loss profile at roughly the same time and rate. Taking the fructose group, and average it with the glucose values, produces the third graph in figure 3, where the data falls nearly perfectly along the sucrose loss curve. This result gestures at the good conservation of carbon-mass claimed by Sievers *et al.* [13].

This analysis then proposes fructose must be oxidized into gluconic acid quite significantly. It is possible that around day 24 the bacteria switch to metabolizing

[†]This term was not originally in the theoretical model and is explained in section 3.3

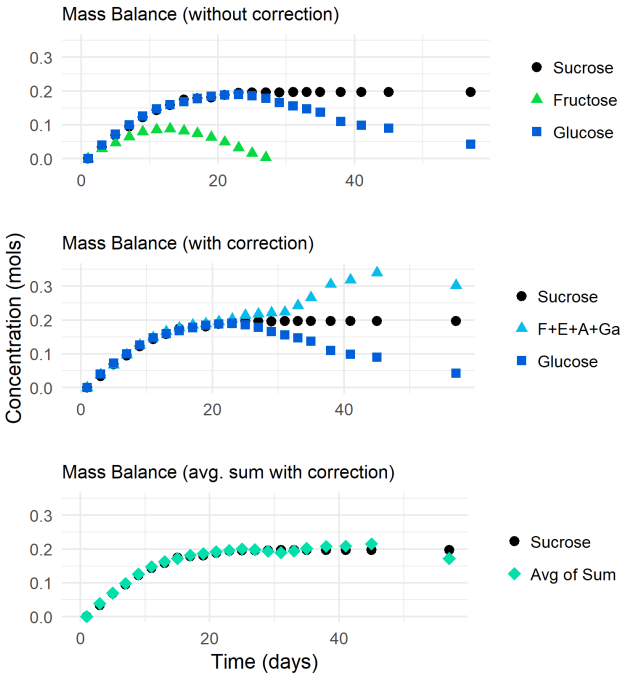

Fig. 3 Data Analysis; Sucrose here is actually $S_0 - S_t$, hence the cumulative *loss* of sucrose from its initial concentration. We plot to day 40 as the data beyond this is sparse and relevant behaviours are readily observed within this range.

glucose to gluconic acid (a well-supported process in the literature [2][3][15]). Reflecting this with the data did show significant improvements to figure 2 (figure not shown), suggesting the bacteria could be primarily utilizing fructose for gluconic acid production before switching to glucose for this purpose later in the ferment.

Forging ahead with this presupposition, plus factoring in that glucose *should* be converted to gluconic acid at some point, and removing terms optimized to zero, we obtained the following model which provides a significant fit to the data. Additionally, we relabel some parameters to maintain a stylistic convention.

$$
\begin{aligned}
\frac{\mathrm{d}S}{\mathrm{d}t} &= -aSY \\
\frac{\mathrm{d}F}{\mathrm{d}t} &= aSY - \frac{bF}{1+\beta F}Y - \frac{cF}{1+\gamma F}B \\
\frac{\mathrm{d}E}{\mathrm{d}t} &= 2\frac{bF}{1+\beta F}Y - \frac{dE}{1+\delta E}B \\
\frac{\mathrm{d}A}{\mathrm{d}t} &= \frac{dE}{1+\delta E}B \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= aSY - \frac{eG_s}{1+\alpha G_s}B \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= \frac{cF}{1+\gamma F}B + \frac{eG_s}{1+\alpha G_s}B \\
\frac{\mathrm{d}C}{\mathrm{d}t} &= 0 \qquad \text{(left for explicitness)}
\end{aligned}
$$

$$\begin{aligned}\frac{\mathrm{d}Y}{\mathrm{d}t} &= \rho\left(aSY + \frac{bF}{1+\beta F}\right)Y \\ \frac{\mathrm{d}B}{\mathrm{d}t} &= r\left(\frac{cF}{1+\gamma F} + \frac{eG_s}{1+\alpha G_s} + \frac{dE}{1+\delta E}\right)B\end{aligned} \tag{3}$$

Cellulose was left for explicitness as all parameters tended to zero during optimization. This reflects the nature of the agitated ferment, any actual growth would be insignificant, reflecting the poor chance for a biofilm to grow between agitations, since once stirred it would merely disintegrate or float in very insignificant clumps along the surface. When the structural integrity of the biofilm is regularly compromised its growth is seriously hindered [5].

We found that model 3 is a good selection for at least the first 20 days of Kombucha fermentation in an agitated and open ferment. The results from fitting the theoretical model follow in figure 4 and table 1. This was done by fitting model 2 in **R**, using log-likelihood estimation with a least squares objective function optimizing with the Nelder-Mead method.

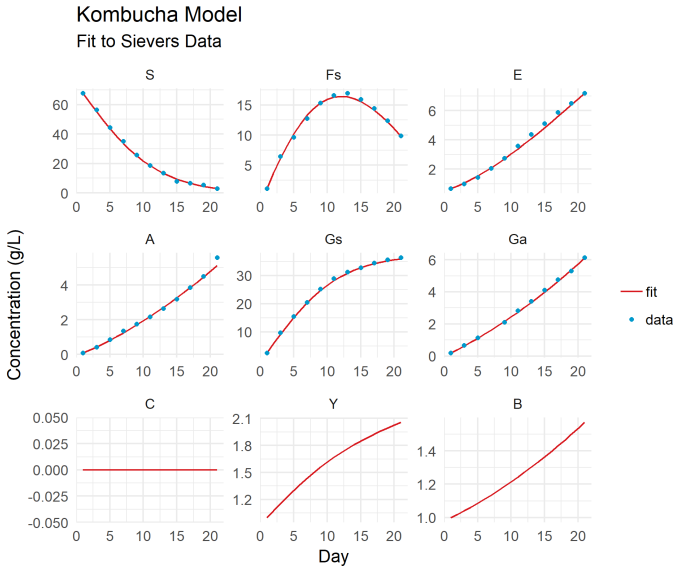

Fig. 4 Agitated Kombucha model fit to Sievers *et al.* data over 20 days

Table 1 Parameter Estimates for Sievers *et al.* data

Parameter	Value	Parameter	Value	Parameter	Value
a	9.41e-02	e	7.14e-04	δ	3.40e+01
b	3.53e-01	α	7.65e-04	ρ	3.71e+00
c	1.15e+00	β	8.81e+01	r	2.13e+00
d	1.85e-01	γ	8.81e+02		

It was considered briefly whether the original authors, Sievers *et al.*, switched their sugar labels in their study or even just on their graphs. However, switching fructose and glucose in the analysis for figure 3 and rerunning did not conserve carbon-mass. Thus, truly against expectation, the data itself suggests gluconic acid is at least partly a product of bacterial activity on the fructose substrate for some period of time in the early stages. This encourages an interesting potential line of research regarding which strains of relevant bacteria may produce gluconic acid from fructose, or else to disprove this hypothesis.

Finally, a comment on the yeast and bacteria activity in figure 4. It appears the model interprets growing yeast and bacterial activity throughout the 20 days analyzed. The bacterial activity closely resembles the general curves for ethanol, acetic acid, and gluconic acid, receiving an approximately linear rate increase of 0.08 per day. Yeast had activity growth closer to .105 per day, but tapering off with the decreases in fructose and glucose substrates. However, these are proxies, and despite adhering to assumptions, caution is advised against reading too much into the charts for yeast and bacteria.

3.4 Static Model

We begin the determination of the static model for Kombucha fermentation from the Sievers *et al.* model *mutatis mutandis* for Chen and Liu's research [2]. In the static case we expect cellulose production, and so we cannot expect a similar carbon-mass conservation without the cellulose mass data, which is unfortunately not included in their research. That is, we expect cellulose to accumulate and thus remove carbon from the system. Being charitable, we even added gluconic acid to glucose this time in the right hand chart of figure 5, and averaging the fructose-group and glucose-group profiles by eye shows the carbon balance still tends away from the conservation equilibrium profile (loss of sucrose), as expected in this case.

From the theoretical model (model 2), optimization sent c and γ to zero, indicating this biosynthesis is not significant to at least this study. In the Chen & Liu data, it is apparent that acetic acid decays after some time. In their research paper they also noted that accumulated CO_2 (or equivalently, the decreasing partial pressure of oxygen – pO_2), in the critical interfacial volume (50-500μm) [15] caused the bacteria to die [2].

This presented no issue in the Sievers *et al.* study as there was a constant supply of oxygen at the surface due to the agitation which, i) prevented biofilm build-up which incidentally prevented decreasing pO_2, and ii) oxygenated the broth which effectively increased pO_2. Due to these facts, we've introduce a die-off term for yeast (μY) as well as for bacteria (νB) (we did in fact include these in the agitated model, but they were optimized to zero). Modeling the CO_2 is intractable at present, without a comprehensive data set of sucrose, fructose, ethanol, acetic acid, glucose, gluconic acid, cellulose mass, yeast counts, bacterial counts, CO_2 concentrations, et cetera. Instead, the *impact* of increasing CO_2 levels are modeled via population die off.

Now that bacterial cellulose (BC) production is a significant element of the system we'll spend a brief moment expanding on the biology. The bacteria biosynthesize cellulose from glucose and fructose [4][5][15][16]. Sucrose *is* another known

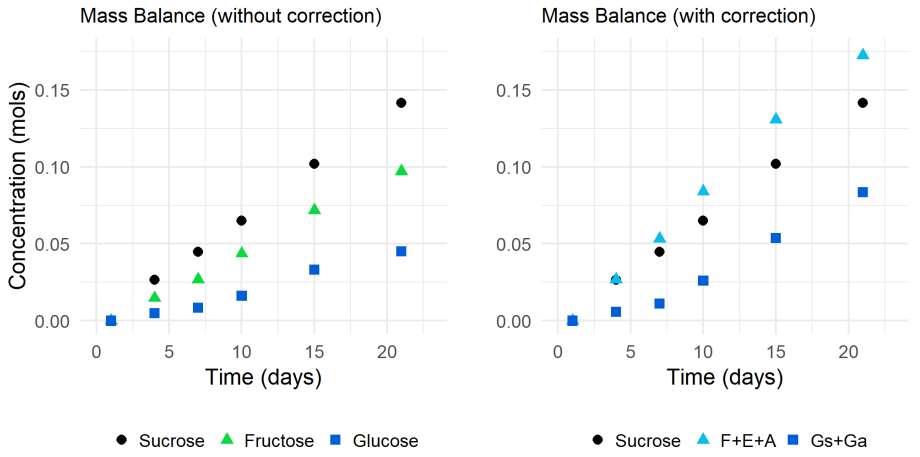

Fig. 5 Data Analysis; Sucrose is actually $S_0 - S_t$, hence the *loss* of sucrose. We plot 30 days only as the data beyond this is sparse and relevant behaviours are readily observed within this range.

carbon source for bacterial sucrose synthesis [11], however, we neglect that here as there was no obvious benefit to including it in the optimization (no closer fit), also it doesn't seem to be a preferred carbon source for the bacteria. Favorable conditions are high levels of carbon sources and low levels of nitrogen sources (from steeping the tea) [11]. Sufficient oxygen pO_2, along with having ethanol present in the system, both supplement cellulose production [12], while carbon dioxide restricts it [5]. Finally, acetic acid promotes, but gluconic acid hinders, BC production [9].

Similarly for this model, several parameters tend to zero and upon removing them and relabelling the remaining parameters, we obtain the static model

$$\begin{aligned}
\frac{\mathrm{d}S}{\mathrm{d}t} &= -\frac{aS}{1+\alpha S}Y \\
\frac{\mathrm{d}F}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y - \frac{bF}{1+\beta F}Y \\
\frac{\mathrm{d}E}{\mathrm{d}t} &= 2\frac{bF}{1+\beta F}Y - dEB \\
\frac{\mathrm{d}A}{\mathrm{d}t} &= dEB \\
\frac{\mathrm{d}G_s}{\mathrm{d}t} &= \frac{aS}{1+\alpha S}Y - cG_sB - \frac{eG_s}{1+\gamma G_s}B \\
\frac{\mathrm{d}G_a}{\mathrm{d}t} &= cG_sB \\
\frac{\mathrm{d}C}{\mathrm{d}t} &= \frac{eG_s}{1+\gamma G_s}B \\
\frac{\mathrm{d}Y}{\mathrm{d}t} &= \rho\left(\frac{aS}{1+\alpha S} + \frac{bF}{1+\beta F}\right)Y - \mu y \\
\frac{\mathrm{d}B}{\mathrm{d}t} &= r\left(cG_s + dE + \frac{eG_s}{1+\gamma G_s}\right)B - \nu B
\end{aligned} \tag{4}$$

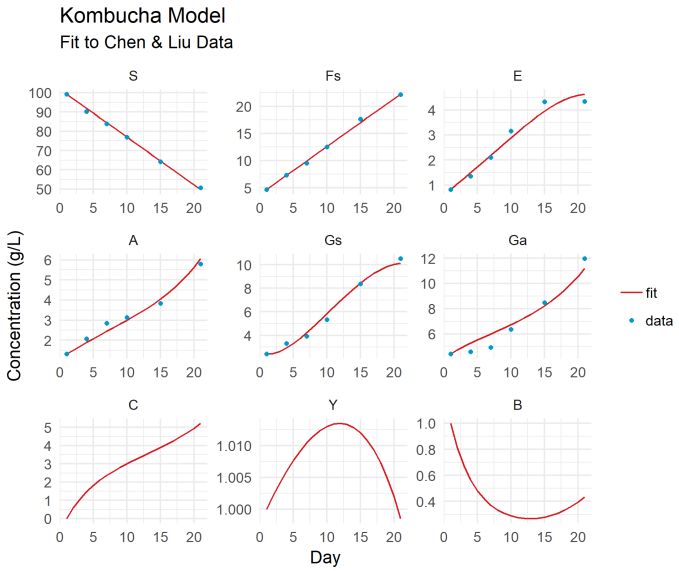

Fig. 6 Static Kombucha model fit to Chen & Liu's data over 20 days.

Table 2 Parameter Estimates for Chen & Liu *et al.* data

Parameter	Value	Parameter	Value	Parameter	Value
a	1.01e+06	e	2.71e+05	ρ	1.32e+06
b	9.96e+05	α	1.34e+08	r	1.35e+01
c	1.08e-01	β	3.56e+08	μ	1.37e+04
d	2.18e-01	γ	4.16e+07	ν	2.75e-01

Considering that the Chen & Liu study was conducted on nine independent batches (different mother cultures) with 8 trials each, their data points represent the mean of all their samples. Therefore, an absolute fit is not as fundamentally necessary as with the research from Siever's *et al.*. Moreover, our fit is fairly narrowly within the given averages of Chen and Liu's observations (see figure 6), so any error is less meaningful for a narrow variance since it still falls well within the range of their data.

Finally, to speak to the yeast and bacteria activity charts in figure 6 as we did at the end of section 3.3, the yeast seems to peak at day 10 and then rapidly decline, this might be an artifact of only fitting to the 20th day of the data. The bacteria activity declines rapidly from the first day, but starts increasing after the tenth day, perhaps this could signify a lack of resources to utilize if the yeast was dominating substrate usage, then as yeast declined in activity for whatever reason, the bacteria began to reinvigorate. The uptick in gluconic acid production between days 6 and 10, and thereafter could support this. To conclude, these charts are not necessarily interpretable, caution is advised.

4 Computations

The model was coded in **R** along with a list of parameters and initial conditions. We wrote a log-likelihood function to compute a least squares regression model. Using the **R** package `deSolve`, we got a time series for each i) substrate, ii) byproduct, and iii) the yeast and bacteria. This required converting the gathered data points (provided in gL^{-1}) to moles, then running the model (with a good first approximate parameterization) through an ODE solver from the `deSolve` package, which called the Runge-Kutta(4,5) ODE solving method. The solution matrix then had to be converted back to gL^{-1} in order to perform the least squares regression with the data. For sucrose, fructose, ethanol, acetic acid, glucose and gluconic acid, we programmed an objective function $F_{obj.}$

$$F_{obj.} = \sum \frac{(\theta_{\text{predicted}} - \theta_{\text{observed}})^2}{|\theta_{\text{observed}}|} \tag{5}$$

which got passed to the base **R** optimization function `optim()`. This function was passed the initial list of parameters and the objective function $F_{obj.}$ (equation 5). The objective function value was then minimized, corresponding to the closest fit to the data. In this case, $F_{obj.}$ was an n-dimensional surface upon which the Nelder-Mead method searches for decreasing local minima by utilizing simplicial complices (generalization of a triangle or tetrahedron to n dimensions). The minima found by the method represents the parameter selection that minimizes $F_{obj.}$.

The values of the objective functions found for Sievers *et al.* and Chen & Liu are 1.381 and 0.956 respectively.

5 Assessing Acidity

pH serves as a major quality control measure in brewing Kombucha as a commercial beverage. We worked on extending our model to predict the pH of the beverage, which had mixed results. To establish a connection to pH from our model, we had to find a way to use our predictions of acetic acid and gluconic acid to calculate pH from the associated hydrogen ion concentrations. A quick look at the reactions in section 2.1 and the chart in Figure 2 might help guide the reader through the following discussion on acid dissociation.

Dissociation is a chemical reaction in the context of acid-base pairs, specifically for us this is acetic acid and gluconic acid reacting with H_20. The acids dissolve in water, meaning the acid molecules separate into cations and anions (positively and negatively charged atoms). This occurs when the covalent bond between an electro-negative atom and a hydrogen atom undergoes heterolysis. The result is a hydrogen ion H^+, and either an negative acetate ion ($C_2H_3O_2^-$) or a negative gluconate ion ($C_6H_{11}O_7^-$), depending. We can express this dissociation (ignoring H_2O) as

$$\underset{acetic}{C_2H_4O_2 = C_2H_3O_2^- + H^+} \qquad \underset{gluconic}{C_6H_{12}O_7 = C_6H_{11}O_7^- + H^+} \tag{6}$$

One measure of the strength of an acid in solution is the acid dissociation constant K_a. It is the equilibrium constant of

$$HA \rightleftharpoons A^- + H^+$$

where equilibrium indicates the steady state of each concentration due to the forward and backward reactions occuring at the same rate. When the concentrations are at equilibrium, then the constant can be defined as

$$pK_a = \log_{10} \frac{[HA]}{[H^+][A^-]}$$

So one can find K_a* through

$$K_a = 10^{-pKa} = \frac{[H^+][A^-]}{[HA]}$$

Acetic acid has $pKa = 4.756$ and gluconic acid has $pKa = 3.86$, and so the equilibrium constants k_A for acetic acid, and k_{G_a} for gluconic acid, can be expressed as

$$k_A = \frac{[H^+][C_6H_3O_2^-]}{[C_2H_4O_2]} = 10^{-4.756} \qquad k_{G_a} = \frac{[H^+][C_6H_{11}O_7^-]}{[C_6H_{12}O_7]} = 10^{-3.86}$$

The active hydrogen ion concentrations can be expressed as

$$[H+] = [C_6H_3O_2^-] + [C_6H_{11}O_7^-]$$

We can then express the concentrations more succinctly, first we see for acetate ion

$$\begin{aligned}
[C_6H_3O_2^-] &= A(t)\frac{[C_6H_3O_2^-]}{[C_6H_3O_2^-] + [C_2H_4O_2]} \\
&= A(t)\frac{1}{1 + \frac{[C_2H_4O_2]}{[C_6H_3O_2^-]}} \\
&= A(t)\frac{1}{\frac{[H^+]}{k_A} + 1} \\
&= A(t)\frac{k_A}{[H^+] + k_A}
\end{aligned}$$

and similarly for gluconate ion

$$[C_6H_{11}O_7^-] = G_a(t)\frac{k_{G_a}}{[H^+] + k_{G_a}}$$

Wherefore, the concentration of hydrogen ions (which determine pH) are calculated by

$$\begin{aligned}
[H^+] &= [C_6H_{11}O_7^-] + [C_6H_3O_2^-] \\
&= \frac{G_a(t)k_{G_a}}{[H^+] + k_{G_a}} + \frac{A(t)k_{G_a}}{[H^+] + k_A} \qquad (7)
\end{aligned}$$

*Recall section 2.2 for the pH formula.

Since $[H^+]$ is essentially strictly increasing while the right hand side of equation 7 is decreasing, we expect one real positive root to indicate the hydrogen ion concentration at time t. However, our model was slightly inaccurate in predicting Sievers *et al.* pH data by several orders of magnitude in the logarithmic scale (equation 8). It is too large an error in prediction to chalk up to instrument calibration, so must have been introduced in the process of analysis.

$$\begin{aligned} \mathrm{pH}_{0_{exp.}} &= 3.75 & \mathrm{pH}_{f_{exp.}} &= 2.42 \\ \mathrm{pH}_{0_{obs.}} &= 3.52 & \mathrm{pH}_{f_{obs.}} &= 2.34 \end{aligned} \tag{8}$$

The error could have accrued since the initial digitization of the study data used herein, or possibly we are missing a key component for pH in the model. There are amino acids not considered and other organic acids can be present [16]. The build up of carbon dioxide (dissociating to form carbonic acid) may play a crucial role in affecting the pH. In considering the agitated model, there is no cellulose build-up over the interfacial area, and so CO_2 is not buffered in the system as it can off-gas.

As yeast can be both aerobic or anaerobic, it is possible for them to metabolize even in the lowest layers of the fluid where undissolved sucrose (a preferred substrate) may have sunk, but where pO_2 is negligible. Then any CO_2 particles rising through the fluid have ample opportunity to dissociate into carbonic acid and a hydrogen ion, effecting the pH while off gassing. This might indicate there is a non-negligible quantity of substrate acting as a base, raising the pH.

One possible way to approach a solution could be to place as proxy whatever else *should* be adding to the pH via a term

$$\frac{Tk_T}{[H^+] + kT}$$

where T is a constant body and k_T is it's associated proxy dissociation constant. Solving

$$[H^+] = \frac{G_a(t)k_{G_a}}{[H^+] + k_{G_a}} + \frac{A(t)k_A}{[H^+] + k_A} + \frac{Tk_T}{[H^+] + k_T}$$

would yield the indeterminate acidity if these elements remain unknown.

6 Discussion

Overall, our general theoretical model was able to fit well to two fairly different Kombucha fermentation processes, with some coefficients optimizing out of the equations. The fit to Sievers *et al.* study data was quite adherent to the data points, with realistic yeast and bacteria growth curves, and no cellulose growth due to frequent agitation. Moreover, the analysis of the Sievers study suggests an interesting line of research into bacteria producing gluconic-acid from fructose, which does not seem to be reflected in literature. Considering that the available data from Chen & Liu data was averaged data over multiple studies, the fit being less adherent to the data points is not as surprising, or at least not further determinable without access to the trial data underlying the averaged data. Lastly, the unimpressive pH predictions as discussed in the previous section could be accounted for with a proxy if in depth analysis of the contributors is intractable.

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