Modeling Mouth as a Bioreactor

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1. Abstract

The human (S.K.Subramanian[1]) mouth is modeled as a bio-reactor in which essentially two reactions take place: the conversion of starch to maltose and isomaltose[2] by the enzyme salivary amylase, a component of Saliva and the growth of microbes inside the mouth. The digestion of starch is modeled in a Chemostat while the microbial growth is modeled in a Fed-batch reactor.

2. Introduction

The food that humans consume enters the body through the mouth where it is masticated. The mouth secretes saliva containing the enzyme salivary amylase which hydrolyses the 1-4 glycosidic bonds in starch[2].

Once the bolus (as the food is now called) enters the stomach, the salivary amylase is deactivated by the acidic pH in the stomach. The remaining starch is now hydrolyzed by pancreatic amylase in the small intestine. In this exercise, I have modeled the mouth as a batch reactor and a chemostat and have compared the conversions achieved in these with data available on the web[10].

Saliva also contains lysozymes- enzymes that lyse the cell wall of bacteria. These lysozymes kill the gram positive bacteria in the mouth[3] and keep the microbial flora in check. The microbes present in the oral cavity include Gram-positive bacteria such as the *a-haemolytic streptococci* and *actinomycetes*, and Gram-negative bacteria such as Veillionella, the anaerobic *Bacteroides spp.* and *Eikenella corrodens*[3]. The upper limit for the microbial population is around 10^5 microbes in the mouth to avoid any pathological condition[4].

The microbes present in the mouth feed on the nutrients sticking to the walls of oral cavity and teeth, and harm the teeth, gums and are also know to cause bad breadth when the number of microbes exceeds a threshold value[4].

I have modeled the microbial growth as a fed-batch reactor with the aim to determine 'brushing' or 'mouth washing' frequency.

3. Models

3.1 *Variables Used*

τ	Residence time of Chemostat
V	Volume of Chemostat
V_m	Maximum velocity of Salivary Amylase
C_{A0}	Initial concentration of starch
X	Percentage conversion of starch.

3.2 *Model for starch digestion*

The mouth is modeled as a chemostat and a batch bioreactor and the results are compared.
Vm of salivary amylase was determined by an enzymatic assay[2] with 50 fold diluted enzyme. The residual starch(substrate) is determined by freezing the reaction in 0.1N HCl, adding iodine solution and measuring the O.D. The L-B plot gave the following equation:

$$\frac{1}{V} = 3.1894\frac{1}{S} + 62.622 \qquad (1)$$

V_m=15.96 g/l/min

Thus, V_{max} for the enzyme in saliva is $50V_m$

V_{max}=798.4 g/l/min // highly evolved enzyme!

K_m= 50.9 g/l

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[7]

Volume of saliva secreted per second = 0.4ml/s
Quantum of food in a mouthful = 10 ml
Time taken to chew food = 0.266 min
Volume of bioreactor= 10+16*0.4 = 16.4 ml
Starch concentration in feed = 95.12g/l

The bioreactor is the oral cavity enclosed by the walls of the mouth. The tongue acts as the stirrer, maintaining uniform mixing. Both saliva and food is taken as input and the bolus is the output.

3.2.1 Chemostat

$$\tau = \frac{V}{v_0} = \frac{C_{A0} - C_A}{(-r_a)} \quad (2)$$ [13]

Rate of reaction ($-r_a$) = 103.9 g/l/min [9]

$C_{A0} - C_A = XC_{A0}$ X is the degree of conversion (3)

(2), (3) => $X = \frac{\tau(-r_a)}{C_{A0}}$

Calculating, we get X=0.29 or 29% conversion of starch in the mouth.

3.2.2 Batch Bioreactor

$$-\frac{dS}{dt} = v = \frac{V_m S}{K_m + S}$$

Solving, we get

$$V_m t = (S_0 - S) + K_m \ln(\frac{S_0}{S})$$

if $S_0X = (S_0 - S)$ where X is the degree of conversion then

$$V_m t = S_0 X + K_m \ln(\frac{1}{1-X})$$

Residence time for the batch =16s = 0.266 min
// can be taken as 13s, 3 s for mixing!
V_m for the enzyme in the batch = 155.9g/l/min
Km = 50.9g/l
S_0 = 95.12g/l

Solving for X, we get
X=0.27 = 27%

3.3 Assumptions

- Amylase is assumed to follow MM kinetics model.
- In the Chemostat, it is assumed that the reaction is zero order with respect to the substrate.
- The enzyme concentration is assumed to be constant in the batch process, whereas, it is continuously changing. This constant is taken to be maximum enzyme

concentration during the reaction. Hence, the prediction of the batch reactor is slightly on the higher side than what would actually happen in a batch process.

3.4 *Microbial growth*

At night, the reactor is assumed to be operating in batch mode with a constant specific growth rate. This will give exponential growth.

$x=x_0 \exp(\tau t)$

The system is not modeled during ingestion, when there can be microbes entering the reactor in the feed, and the growth is difficult to model with varying reactor volumes.

At other times, the bioreactor consists of the oral cavity including the walls and teeth. The tongue is excluded as it is supplied by a lot of saliva, thereby having minimal microbial growth on the tongue.

Average Growth rate of microbes	$=\mu\ s^{-1}$
Death rate due to lysozymes	$=d\ s^{-1}$
(Assumed first order, acts on all bacteria)	
Flow rate	$=F$
No of microbes in the reactor	$=x$ per unit vol
Microbes sticking to teeth = 10^{-3}	$=x_w$ per unit vol
Dilution rate	$=D$
Volume of reactor	$=V$

3.4.1 Cell Balance

$I - O + G - C = Acc$

Where I = input rate $=0$;

O = output rate $=Fx$;

G = generation rate $=\mu(x+ x_w)V$;

C = Consumption rate $=dxV*V = kx$ for a given V

The concentration of enzyme depends on the volume of saliva, more saliva, better lysozyme action

Acc= accumulation $=\frac{dx}{dt}*V$

We get

$$\frac{dx}{dt}=\mu(x+ x_w)-Dx-dxV$$

$$=x(\mu-D-dV)+ \mu x_w$$

$(\mu - D - Vd)\, t = \ln\left(\frac{x(\mu - D - Vd) + \mu x_w}{x_0(\mu - D - Vd) + \mu x_w}\right)$

$= (\mu - D - k)\, t = \ln\left(\frac{x(\mu - D - k) + \mu x_w}{x_0(\mu - D - k) + \mu x_w}\right)$

$= ut = \ln\left(\frac{xU + \mu x_w}{x_0 U + \mu x_w}\right)$ where U = $(\mu - D - k)$

Typical values

Just after oral cleansing (brushing), x_0 can be assumed to be zero.

μ is taken to correspond to doubling time of 20 min; i.e. $\mu = 0.03465\ min^{-1}$

k is assumed to be one tenth of μ.

Volume of the reactor is assumed to be 1ml.

Flow rate is determined by waiting for 1 min (1ml measured with a clinical syringe) and measuring the saliva secreted. One hundredth of this is **assumed** to contribute to the reactor[12]. The remaining is swallowed, and comes into contact predominantly only with the tongue.

Thus, $D = F = \frac{1}{100} = 0.01\ min^{-1}$

To compute the time at which the threshold cell microbe concentration[3] is reached,

$x_w = 10^3$

$x = 10^5$

$U = 0.9 * 0.03465 - 0.1 = 0.021185\ min^{-1}$

Computing t, we get

t= 194.92 min

3.5 Assumptions

- Though lysozymes act only on gram positive bacteria, the model assumes that lysozymes act on all bacteria. No appreciable change in O.D. occurred when saliva was observed at different times. The small fluctuations in the O.D values were attributed to error in the instrument
- It is assumed that the person whose mouth is being modeled doesn't spit or chew gum etc. (A valid assumption in Subramanian's[1] case.)

- Sufficient substrate is assumed to be present to maintain specific growth at maximum (constant) level.

4. Discussion

Starch hydrolysis:

The conversion attained in the mouth is 27% (when modeled as batch reactor) which compares well with 30-40% as found on the world wide web[10]

The chemostat gives comparable values for the with the batch reactor because the reaction was assumed to zero order. Moreover, the residence time for the batch reactor also included the initial mixing time where the feed is mixed with the enzyme.

Microbial Growth

The time required for the microbes to reach threshold concentration to cause harm is estimated to be just greater than 3 hours. This is the worst case scenario[12]. Since food is consumed every 4 hours or so, the reactor is literally washed out and starts from the beginning. The model predicts that if a person goes without food or water for more than 3 hours, he is bound to show symptoms of bad breadth.

5. Conclusion

Thus the mouth is modeled as a bioreactor in which starch is hydrolyzed as well as one in which microbes grow. The batch process effectively models the hydrolysis of starch. The microbe growth model suggests that one should (at least Subramanian[1] should) wash his mouth every third hour or so, to stay away from bad breadth and other periodontal infection.

6 Scope for Further Work

- The Chemostat model for starch hydrolysis can be considered with rate changing with substrate concentration, though the integrals will have to be performed using numerical techniques.
- The constants in the microbial growth model can be evaluated using a high resolution haemo-cytometer.(the one in the Microbiology lab can only look at large cells like yeast.)

7. Appendix

[7] *– Parameters for Starch digestion*

Refer to the graph and tables on the next page.

Volume of saliva secreted per second was determined by chewing a rubber band for **10s** and measuring the volume of saliva secreted, which was found to be **4ml**.

Quantum of food in a mouthful is the amount of water I could hold in my mouth which I felt was equivalent to volume of food in mouth. Value obtained was **10ml**. I think this is a reasonable volume of food intake, considering the duration of mastication.

Time taken to chew food measured with wrist watch. Food was not consciously (at least I think so) kept in the mouth for longer than necessary. Time was averaged over 3 values (15,16,17) to give **16s**.

Starch concentration in feed:

Density of food assumed to be 1.3g/cc (just heavier than milk– food sank very slowly in milk), starch content= 0.4*Carbohydrate content

=0.3*0.3=0.12% by weight. [8]

Mass of starch in a mouthful = (1.3*10)*0.12 = 1.56g

Starch Concentration $=\frac{1.56}{16.4*10^{-3}}$ = 95.12g/l

[9] *Chemostat values*

2*Km ~ S=95.12

Dilution of enzyme = 2.56 fold

Velocity = $\frac{V_{max}}{2.56}*\frac{1}{3}$=103.96g/l/min

Residence time=$\frac{V}{v_0}=\frac{16.4}{\frac{16.4}{16}}=16s$

[11] *V_m for starch hydrolysis: batch reactor*

Dilution of the enzyme= 2.56 fold.

$V_m = \frac{V_{max}}{2.56}$=311.875g/l/min

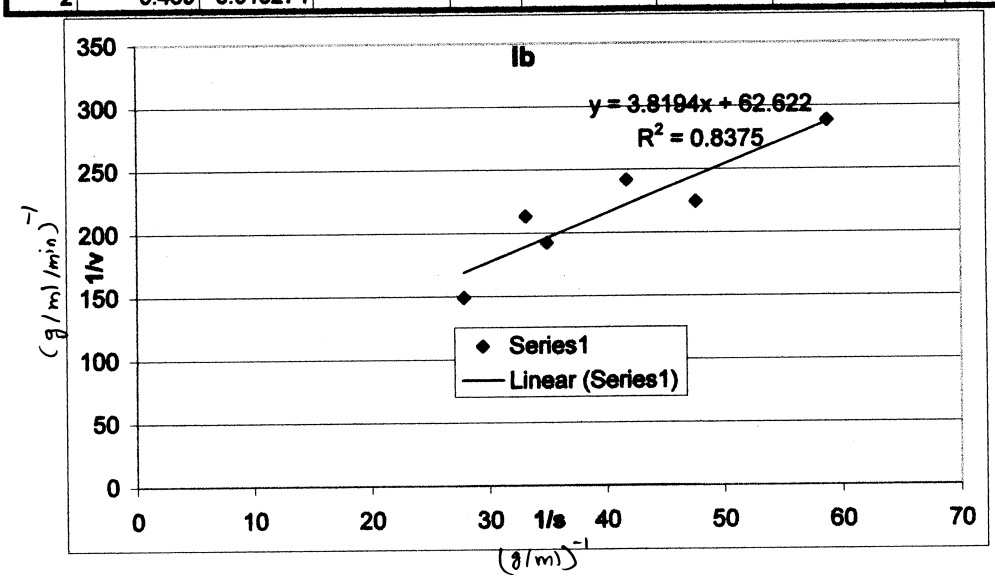
<V_m> (arithmetic mean of initial and final)= 155.9g/l/min

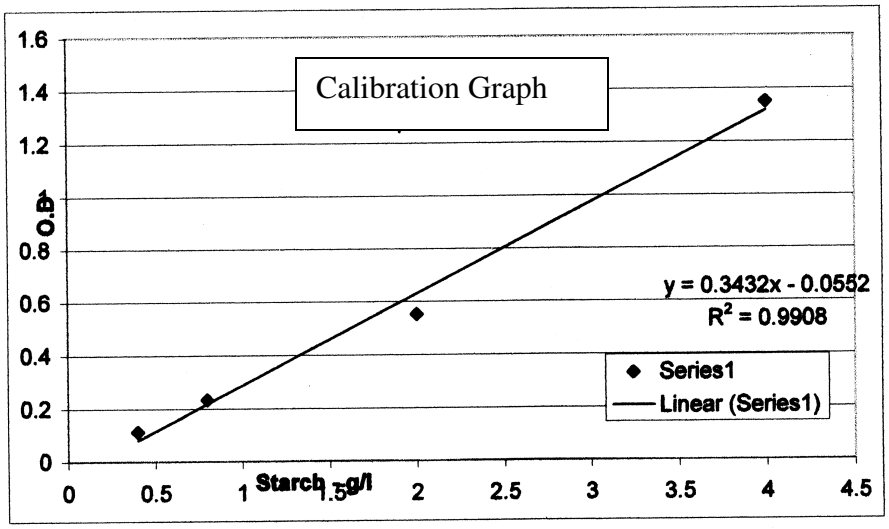
This is computed to account for the variable enzyme concentration in the batch.

[12]

When one hundredth of flow rate is considered, the worst case scenario is considered, hence, the threshold is reached only after the time given with this assumption.

t min	S(t) O.D	S(t) g/ml	S(t+dt) g/ml	dt min	V g/ml/min	Sm g/ml	1/V (g/ml/min)$^{-1}$	1/Sm (g/ml)$^{-1}$
0	-	0.0392	0.03249417	1	0.00670583	0.035847	149.1240267	27.89627009
1	1.06	0.032494	0.02780303	1	0.00469114	0.030149	213.1677019	33.1690345
2	0.899	0.027803	-	-	-	-	-	-
0	-	0.0312	0.0259965	1	0.0052035	0.028598	192.1784706	34.96717243
1	0.837	0.025997	0.02185897	1	0.00413753	0.023928	241.6901408	41.79249878
2	0.695	0.021859	-	-	-	-	-	-
0	-	0.0232	0.01874126	1	0.00445874	0.020971	224.2785445	47.68574096
1	0.588	0.018741	0.01527389	1	0.00346737	0.017008	288.4033613	58.79732739
2	0.469	0.015274	-	-	-	-	-	-





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8. References

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