**######**

We measured the concentration of metabolites in the affluent and gas composition in the headspace. Then, we can evaluate how much of each metabolite and gas are generated per day by converting the unit to “mole/day”

We only consider Acetate-,Propionate-, Butyrate-, CO2, CH4, H2, and HCO3-

For Acetate-,Propionate-, Butyrate-

We convert the value to the unit “mole/day” by concentration (M/L) × flow rate (L/day)

For CO2, CH4, and H2

We convert the value to the unit “mole/day” by partial pressure (%) × gas production rate (L/day) × 1/24.5 (mol/L)

We assume that the degradation of the source is described as

Sucrose(aq) +y1iH2O(l) →x1iAcetate-(aq) +x2iPropionate-(aq) +x3iButyrate-(aq) +x4iCO2(g) +x5iCH4(g) +x6iH2(g) +x7iHCO3-(aq) +x8iH+(aq)

where i suggested day

once we get the a, b, c, d, e, f mole/day of Acetate-,Propionate-, Butyrate-, CO2, CH4, and H2

the ratio of the x1:x2:x3:x4:x5:x6:x7 will equal to a:b:c:d:e:f:g

and we can assume that

x1 = a×λi; x2 =b×λi; x3 =c×λi; x4 =d×λi; x5 =e×λi; x6 =f×λi

where λi is the daily constant

and we fix the coefficient of sucrose as 1

then, we can start to balance the equations by the elements carbon, hydrogen, oxygen, and electron

C: 2x1 + 3x2 +4x3 + 1x4 + 1x5 + x7 = 12

H: 3x1 + 5x2 +7x3 + 4x5 + 2x6 + x7 + x8 = 22 + 2y1

O: 2x1 + 2x2 +2x3 + 2x4 + 3x7 = 11+ y1

e-:-(x1 + x2 +x3) - x7 +x8 =0

we can rewrite the equations as follows

(2a+3b+4c+d+e) λ+ x7 =12 ------Eq1

(3a+5b+7c+4e+2f) λ + x7 + x8 = 22 + 2y1 ------Eq2

(2a+2b+2c+2d) λ + 3x7 = 11+ y1 ------ Eq3

-(a+b+c) λ - x7 +x8 =0 ------ Eq4

Eq2- Eq4

(4a+6b+8c+4e+2f) λ + 2x7 = 22 + 2y1 ------Eq5

Eq5- Eq3×2

(2b+4c-4d+4e+2f)λ= 4

λ= ------Eq6

Plug Eq6 into Eq1 and we can get value of x7 and λ

x7 = and λ =

once we get the λ we can calculate the value of x1 to x7

Plug values of x7 and λ into Eq3 and Eq4 then we can get y1 and x8

y1 = (2a+2b+2c+2d) λ + 3x7 -11

x8 = (a+b+c) λ + x7

The detail of calculating the equation equilibrium is shown on the sheet “Overall equation equilibrium” in the file thermodynamics calculation\_v9\_Figure2\_20231019\_upload

**######**

Once we have the overall equation:

Sucrose(aq) +y1iH2O(l) →x1iAcetate-(aq) +x2iPropionate-(aq) +x3iButyrate-(aq) +x4iCO2(g) +x5iCH4(g) +x6iH2(g) +x7iHCO3-(aq) +x8iH+(aq)

We can calculate the overall Gibbs free energy change (ΔG33′) was by the equation

ΔG33′ = ΔG330′+RT×ln

Where

ΔG33′ is the Gibbs free energy corresponding to the metabolite composition at 33 °C and pH= 7

ΔG330′ is equal to summary product’s ΔGf0(298K) minus summary reactant’s ΔGf0(298K)

The detail of calculating the equation equilibrium is shown on sheet “Overall Gibbs free energy change” in the file thermodynamics calculation\_v9\_Figure2\_20231019\_upload

**######**

In this study we correct the ΔGf0 (at 298K) to ΔGf330 (at 306K) by Van ‘t Hoff equation

ln = -

=

ΔG0 = RTlnK → K = exp()

exp() =

Where T1=298K, T2=306K

ΔGf0 is standard Gibbs energy change of formation

ΔHf0 is standard enthalpy of formation

R is gas constant

T is absolute temperature

The detail of calculating the correction is shown on sheet “Correction” in the file thermodynamics calculation\_v9\_Figure2\_20231019\_upload

**######**

The ΔG33′ of equation 1-9 in Figure2 E-G was calculated by the following equation

ΔG33′ = ΔG330′+RT×lnQ

Where Q is reaction quotient

The detail of calculating the correction is shown on sheet “Syntroph and Methanogenesis” and “Fermentation” in the file thermodynamics calculation\_v9\_Figure2\_20231019\_upload