The Calculations in LIMS

Contents

[1. Volume Calculations 1](#_Toc505938008)

[2. Titer and Productivity Calculations 2](#_Toc505938009)

[3. Yield, Product, Byproduct, and Substrate Calculations 3](#_Toc505938010)

[3.1 Product of Interest (POI) Produced 4](#_Toc505938011)

[3.2 Carbon Source (CS) Consumed by Cells 4](#_Toc505938012)

[3.3 Carbon Source (CS) Fed to Cells 5](#_Toc505938013)

[3.4 Carbon Source (CS) Consumed in Seed 5](#_Toc505938014)

[4. Off-gas Calculations 6](#_Toc505938015)

[5. Basic Fermentation Performance Calculations 6](#_Toc505938016)

[6. Additional Rate Calculations 7](#_Toc505938017)

[7. Carbon Balance Calculation 9](#_Toc505938018)

[8. Degree of Reduction Calculation 9](#_Toc505938019)

[9. Optional Calculations 10](#_Toc505938020)

[9.1 More precise fermenter volume calculation 10](#_Toc505938021)

[9.2 Yield% Calculations 11](#_Toc505938022)

[9.3 Water Off-Gas Estimation 11](#_Toc505938023)

[9.4 Ethanol Off-Gas Estimation 12](#_Toc505938025)

[10. Appendix 13](#_Toc505938026)

[10.1 Table of Constants for calculations 13](#_Toc505938027)

The following document details the calculations to be performed in LIMS to analyze batch or fed-batch fermentations. It was developed based on calculations currently used for the melatonin project, as well as knowledge gained from previous fermentation experience. The calculations are applicable to any water-soluble product made from one or more carbon sources (including the serine project). Modifications or additions would need to be made for fermentations involving an organic (non-aqueous) phase or gaseous products.

# Volume Calculations

Fermenter volume is determined from the initial volume and the cumulative weights of substrates added to the vessel. Currently, these include substrate(s) and base addition, but could be generalized to include any number of feeds. Substrate feeds are measured online as mass flowrates, in g of liquid per hour, which can be converted to mL per hour by dividing by the density, and integrated over time to get the cumulative liquid volume added.

The current approach makes a number of assumptions and simplifications.

1. Water vapor or other evaporated liquids lost to the off-gas are not considered.
2. Any volume change due to differences between O2 input and CO2 output is not considered.
3. Volume or broth density changes resulting from products are not included.

Fermenter volume is therefore calculated using the following equation.

Where Vol0 is the initial volume (L)

Volinoc is the inoculum volume (L)

fd is the feed density (g/mL).

B is the cumulative volume of base added at the timepoint of interest (ml)

is the piecewise integral of the flowrate over all time intervals up to the timepoint of interest (g). The simplest method is to use the trapezoid rule:

Where k and k-1 subscripts refer to the kth timepoint at which flowrate is recorded, tk is the time interval between the kth and (k-1)th measurements, and n is the total number of measurements made up until time t.

 Vs@t is the volume of material lost to sampling, summed over each sample up to the previous timepoint

# Titer and Productivity Calculations

Titer and productivity are calculated based on the selected POI (product of interest) (i.e. XYZ). If the product of interest is measured by more than one method/test (i.e. HPLC and LCMS), the analytical team will select in advance which measurement is to be used.

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Where

* is the concentration of the product of interest at time t in mmol/L
* is the molecular weight of the product of interest in g/mol

Where

* is the titer of the product of interest at time t in g/L
* is elapsed time from inoculation (time 0) to the current time t in hours.

Productivity can also be calculated for any time interval t1 to t2

# Yield, Product, Byproduct, and Substrate Calculations

In this section we describe the determination of total product/byproduct produced and substrate consumed, so that yields can be determined. If more than one carbon source is available (as in the case of biomass/mixed sugars, or a substrate co-feed like tryptophan) the user can select which of the feed components to include in the yield calculation.

For the product of interest, a gram/gram yield is calculated. Where

Where POI is the defined Product of Interest (i.e. XYZ) and CS is one or more carbon sources.

An additional yield is calculated based on the total carbon source fed to the cells (not consumed). If at the end of fermentation the residual carbon source is 0, then yield based on fed will equal yields based on consumed.

Analogous equations exist for molar yield:

It is also useful to calculate yield in terms of C moles, in particular where multiple substrates with different numbers of C atoms are used (e.g., glucose and xylose)

Where C mol of CS consumed can in general be calculated by summing the contribution of n carbon sources

The total g or mmol of product and substrate are calculated according the following sections.

## Product of Interest (POI) Produced

The product of interest produced is the current amount of product in the tank at time t plus all the product that was removed previously by sampling.

Where is the concentration in mM of the product of interest (i.e. XYZ) at time t, MWPOI is the molecular weight of the product of interest, and Vt is the volume of the fermentor at time t (as calculated in Section 1). The second term is the volume of all samples taken up until (but not including) time t multiplied times the POI concentration at the sample time and the molecular weight.

Analogous equations exist for any soluble (non-gaseous) product that is measured as a mass or molar concentration in the fermentation broth.

## Carbon Source (CS) Consumed by Cells

The carbon source consumed by the cells is calculated based on the initial amount in the tank, the amount added during the fermentation and the amount remaining as residual in the tank and in the samples.

* is the initial carbon source in the tank in g. It is calculated using , the inoculation event volume, and the carbon source concentration measurements at time 0 (pre-inoculation).
* is the sum of all carbon source added to the tank via bulk feed events up to and including time t. It is calculated using , the bulk feed volume and user entered feed concentrations. For carbon sources added only by continuous feed pump, this is normally 0.
* is the cumulative amount of carbon source added to the fermentor via continuous feed. It is calculated using the g of material fed by the pumps and the user entered feed concentration. The online data records feed pump rate as g of liquid per hour, so this must be first converted to volumetric weight and then multiplied by the substrate concentration.

Where the piecewise integral over each time step can be calculated by the method of choice (e.g., trapezoidal or parabolic), as described in Section 1 for the volume calculation.

* is the residual carbon source concentration measured in the sample at time t (g/L) multiplied by the fermenter volume at time t (as calculated in Section 1).
* is the amount of carbon source lost to sampling. It is the sum of all samples that have been taken up until (but not including) time t. It is calculated using , the volume of the sample and the measured carbon source concentration at the time of the sample. As above, this term is not currently used.

## Carbon Source (CS) Fed to Cells

The carbon source fed to the cells ignores the residual in the tank. This is relevant from a commercial perspective, since any carbon source added contributes to the cost, whether the cells actually consume it or not.

## Carbon Source (CS) Consumed in Seed

The carbon source in consumed the seed does not need to be measured, but can be calculated based on estimated parameters. Note that this value is not the amount consumed in the entire seed tank, but only that amount corresponding to the portion of seed used to inoculate the fermentor.

Where is the initial volume, OD0 is the OD measurement immediately following inoculation, the OD conversion factor is a user-defined value (default should be 0.4 g/L biomass per OD), and the biomass yield in seed is the g biomass produced per g sugar consumed under the seed culture conditions. For *E. coli* growing aerobically on glucose, this is approximately 0.45 g/g. The user should enter notes if deviating from default.

# Off-gas Calculations

The online off-gas measurements are used in conjunction with the air flowrate measurement to determine the oxygen update rate (OUR) and CO2 evolution rate (CER). These rates are calculated as follows:

Where T is the temperature in Kelvin, O2 and CO2 are the % of each gas in the off-gas, Fair@t is the inlet gas flowrate in L per minute, and R is the universal gas constant. R = 0.08314 atm-L/(mol-K). P is the vessel pressure, in atm. For most bench-scale fermentation vessels, P=1 atm. However, larger tanks such as the 30L may be run at elevated pressure. When these units are used, the calculated OUR and CER values are in mmol/hr. X is the % O2 in the inlet gas, equal to 21% for pure air. If a mixture of air and pure oxygen is used, the concentration of O2 in the mixture is given by

% O2 in inlet = (volume fraction of air fed)\*21 + (volume fraction of O2 fed)\*100

These equations assume that the gas flowrate out is equal to the flowrate in; i.e., that there is no significant gain or loss of gas material in the fermentation process. This assumption could be relaxed if an inert gas component such as argon were measured. Loss or gain of argon % would indicate production or consumption of total gas, respectively. This is currently beyond the scope of our calculations, but equations could be developed in the future.

The total amount of O2 consumed and CO2 produced is calculated by integrating OUR and CER, respectively, over all time intervals up to time t. The simplest method is to use the trapezoid rule:

Where k and k-1 subscripts refer to the kth timepoint at which offgas measurements are made, tk is the time interval between the kth and (k-1)th measurements, and n is the total number of measurements made up until time t.

Analogous equations to those for CER and CERT could be used for other gaseous products and byproducts that are analyzed in the off-gas (e.g., hydrogen).

# Basic Fermentation Performance Calculations

The calculations in this section require just online data and measurements recorded by the fermentation team, and not any analytical measurements. Thus they provide a first look at fermentation performance.

* Biomass in grams

Where is the OD measured at time t, is a constant as defined in Section 3.4 (default of 0.4), and is the volume of the fermentor at time t as calculated in section 1.

* sOUR is the specific oxygen uptake rate, or OUR per biomass.
* Growth Rate,

Where are the previous time point’s OD, fermentor volume, and elapsed time.

* SUR, substrate uptake rate – calculated for each carbon source (CS) defined in the experiment. SUR is the rate at which the cell is using the carbon source.

Where CSt and CSt,prev are the current and previous time point’s cumulative carbon source consumed, as calculated in Section 3.2. ETt and ETt,prev are the elapsed time at the current and previous time point. The units for SUR can be g/hr or mmol/hr, depending on whether mass or molar substrate consumption is used.

* POI/Biomass Yield – yield of product of interest (in mmol/L) per gDCW

# Additional Rate Calculations

The following calculations are done when analytical data is available. Formulas below are given for the product of interest (POI) but could be calculated for any byproduct as well. Grams of POI (or byproduct) produced are calculated according to Section 3.1.

* Mass and Molar concentration conversions - All the measured analytes are converted between their measured units to their corresponding non-measured unit (i.e. if measured in mass concentration, system will calculate molar concentration and vice versa).

Mass concentration = Molecular weight \* Molar Concentration

Molar concentration = Mass Concentration/Molecular weight

* Interval Time – the average elapsed time between two time points at which instantaneous values are calculated
* Overall POI Mass Rate
* Instantaneous POI Mass Rate
* Instantaneous specific POI Productivity Mass
* Instantaneous specific POI Productivity Molar
* Instantaneous specific CS SUR Mass
* Instantaneous specific CS SUR Molar
* Instantaneous specific OUR Molar

Where OURT is the cumulative OUR as recorded in the online data.

* Instantaneous POI Yield g/g
* Instantaneous POI Yield mol/mol

The instantaneous rate calculations above are using linear interpolation between the timepoints. An alternate approach, which may be considered more accurate, is to curve fit the data and then differentiate at each timepoint.

# Carbon Balance Calculation

The carbon balance calculation attempts to account for all carbon molecules consumed by the cell as carbon source and produced by the cell as byproducts and biomass.

Where the mmoles of each product/byproduct is calculated as described in Section 3.1 and CERT as described in Section 4.

For *E. coli*, the #C in OD has been determined by modeling to be 17.04.

# Degree of Reduction Calculation

The degree of reduction is similar to carbon balance in that is gives information about the ins and outs of the system and is a measure of the how ‘closed’ the loop is between inputs and outputs. It is based on the elements in each input/output and a calculated degree of reduction for each reagent/compound.

Each compound has a calculated degree of reduction (DOR) that is entered by the user. (See appendix table)

For reference, the degree of reduction of a compound is calculated as:

Degree of reduction for a compound = 4*a* + *b* –2*c* – 3*d* + 6*e* + 5*f*

Where a, b, c, d, e, and f are the coefficients in the compound formula (C*a*H*b*O*c*N*d* S*e*P*f*)

Where CERT and OURT are calculated as described in Section 4, and total mmoles of each product/byproduct determined as in Section 3.4.

# Optional Calculations

This section covers calculations that are not currenly done at the CfB, but could be added in the future to improve accuracy or provide additional insight into fermentation performance.

## More precise fermenter volume calculation

In the future, if we want to relax the assumptions given in Section 1, the volume could be calculated as follows:

The first 4 terms are identical to the equation in Section 1, except that the base density (bd, g/L) is used instead of being assumed to be 1000. The additional terms provide corrections to the volume based on relaxing the assumptions. Any number of these can be added if available.

* (Broth Density)t is the current density in g/L of the fermentation broth. If this is not measured, it can be estimated based on the initial browth density, the density of the feed, and the amount of feed added relative to the initial volume.
* bd is the base density (g/L). In section 1 this is assumed to be 1000.
* is the mass gained from O2 input. It is calculated using the cumulative moles O2 taken up (see below) and the molecular weight (MW) of O2 of 32 g/mol
* is the mass lost from CO2output. It is calculated using the cumulative moles CO2 produced (see below) and the molecular weight (MW) of CO2 of 44 g/mol.
* is the mass lost from Water vapor in the offgas. See separate Water loss section below
* is the mass lost from Ethanol vapor in the offgas. See separate Ethanol loss section below
* is the cumulative mass lost to sampling. It is the sum of all samples that have been taken up until (but not including) time t. It is calculated using , the volume of the sample and the calculated broth density at the time of the sample.

## Yield% Calculations

The Yield % calculation shows what fraction of the carbon is going to each byproduct, so can be helpful in comparing multiple fermentations to see how performance improved or differed.

Where

Where the production constant is based on the pathways used (different for each POI) and tells how many moles of carbon source are needed to produce a mole of product. For example, it takes 1 glucose to make 1 XYZ. However, 1 glucose can make 2 of the C2 and C3 compounds like acetate, ethanol, etc. In this case the production constant is 0.5 for acetate and ethanol. See appendix for full table of values.

Note for Ethanol there are 2 sources, fermentation broth and off gas, that need to be summed to get total.

An unknown compound yield can be calculated as 100% - elemental carbon balance.

## Water Off-Gas Estimation

# This calculation will provide the term MH2O@t in the volume equation in Section 9.1. It is difficult to measure the water lost to the off gas; however it can be estimated based on an empirical formula developed via experimentation based on the formulas below:

First you need to calculate the vapor pressure in atm of H2O via Antione’s equation where

Where T is the temperature of the fermentation broth measured as an online analyte, A = 8.07131, B=1730.63, C = 233.426, and 0.00131578947 converts mmHg to atm.

Next, calculate the mole fraction of H2O in the exhaust,

Where Ptop­ is the absolute pressure of the fermentor. 1atm for 0.5, 1, and 5L. 1.0986 for 30L. Note this comes from the online Pressure data where available. If not available, assume 1.

H2O is the condenser efficiency for water.

Next calculate the exhaust water flow rate which is the total exhaust gas flow rate times the mole fraction of water.

is in mmol/hr. Where AIRF (sparge), OUR, and CER come from online data.

## Ethanol Off-Gas Estimation

This calculation will provide the term MEtOH@t in the volume equation in Section 9.1 Again, this is difficult to measure but an estimate can be calculated. To calculate MEtOH@t , first you need to calculate the vapor pressure in atm of H2O via Antione’s equation where

Where 190 mol/L/atm is the Henry Law constant for EtOH at 25C

Calculate the mole fraction of EtOH in the exhaust using Henry’s law,

Where Ptop­ is the absolute pressure of the fermentor. 1atm for 0.5, 1, and 5L. 1.0986 for 30L. Note this comes from the online Pressure data where available. If not available, assume 1.

etoh is the condenser efficiency for EtOH.

Next calculate the exhaust EtOH flow rate which is the total exhaust gas flow rate times the mole fraction of EtOH.

is in mmol/hr. Where AIRF (sparge), OUR, and CER come from online data.

# Appendix

## Table of Constants for calculations

|  |  |  |  |
| --- | --- | --- | --- |
| **Compound** | **Degree of Reduction** | **Carbon Count** | **Production Constant for Yield %** |
| Glucose | 24 | 6 | 1 |
| Melatonin | 58 | 13 | 2.5 |
| Serotonin | 44 | 10 | 2 |
| Tryptophan | 46 | 11 | 2 |
| N-acetyl-serotonin | 52 | 12 | 2.5 |
| N-acetyl-tryptamin | 54 | 12 | 2.5 |
| Serine | 10 | 3 | 1/2 |
| Glycine | 6 | 2 | 1/2 |
| Glutamate | 18 | 5 | 1 |
| Alanine | 12 | 3 | 1/2 |
| Pyruvate | 10 | 3 | 1/2 |
| Lactate | 12 | 3 | 1/2 |
| Acetate | 8 | 2 | 1/2 |
| Ethanol | 12 | 2 | 1/2 |
| CO2 | 0 | 1 | 1/6 |
| O2 | -4 | 0 | 0 |
| Biomass | 20.35 | 17.04 | 2.84 |
| C5 sugar | 20 | 5 | 5/6 |
| C12 sugar | 48 | 12 | 2 |
| Fructose | 24 | 6 | 1 |
| Butyrate | 20 | 4 | 1 |