AppGuide: Calculation of evaporation using off-gas parameters and the ideal gas law

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1 Introduction

The usage of off-gas analysis is a well-known application in fermentations, but is still considered as an add-on instead of an obligatory tool such as pO_2- or pH-determination. A continuous exhaust gas analysis can help to achieve a better understanding of a fermentation process, but can also directly improve the process itself. For example, the loss of media due to evaporation is an often-ignored factor that can be monitored using exhaust gas sensors [1]. The evaporation in submerse processes is related to the humidity of the exhaust gas which leaves the reactor. To guarantee a sufficient supply of oxygen during aerobic processes the cultures are sparged continuously with air. Since dry air is normally used for these applications, water is stripped permanently from the medium and escapes the reactor with the exhaust gas in form of vapor [2]. During processes with low aeration rates the evaporation is neglectable, but with increased aeration more evaporation takes place, resulting in losses of around 0.2 %/h [3].

The evaporation of water can be determined using a **BlueVary** or **BlueInOne** sensor. These sensors always contain additional sensors for pressure and humidity measurement, which are normally used for automatic compensation of O_2- and CO_2-vaules. Using these values enables the calculation of evaporated water. Current evaporation and corrected fermentation volume were directly calculated by soft sensors integrated in the bioprocessing software **BlueVIS**.

2 Parameters and Values

2.1 Adjustment of the ideal gas law

$$p * V = n * R * T \leftrightarrow n = \frac{p * V}{R * T}$$

$$with \quad n = \frac{m}{M} \qquad \rightarrow \qquad m_{H_2O} = \frac{p * V * M_{H_2O}}{R * T}$$

$$with \quad f = \frac{V_{H_2O}}{V_{gas}} \leftrightarrow V_{H_2O} = f * V_{gas} \qquad \rightarrow \qquad m_{H_2O} = \frac{p * f * V_{gas} * M_{H_2O}}{R * T}$$

$$with \quad \rho = \frac{m}{V} \qquad \rightarrow \qquad V_{H_2O} = \frac{p * f * V_{gas} * M_{H_2O}}{R * T * \rho_{H_2O}}$$

$$with \quad F_{gas} = \frac{V_{gas}}{t} \leftrightarrow V_{gas} = F_{gas} * t \qquad \rightarrow \qquad V_{H_2O} = \frac{p * f * F_{gas} * t * M_{H_2O}}{R * T * \rho_{H_2O}}$$

Table 1: Abbreviations, common symbols and units of the parameters used for the calculation based on the ideal gas law

Sign	Parameter	Unit
p	Pressure	N/m²
V_{gas}	Volume gas	L
V_{H2O}	Volume of water content	mL
n	Amount of substance	Mol
R	Universal gas constant	N*m/mol*K
T	Temperature	K
M	Molecular mass	g/mol
f	Absolute humidity	m³/L
ρ	Density	g/mL
F	Aeration rate	L/min
t	Time	h

2.2 Constants

The following parameters are physical constants and can be directly used for the calculation:

Table 2: Abbreviations, common symbols and values of the constants used for the calculation based on the ideal gas law

Sign	Parameter	Value
R	Universal gas constant	8.31442 N*m/mol*K
M_{H2O}	Molecular mass of water	18.02 g/mol
ρ_{H2O}	Density of water	1.0 g/mL

2.3 Exhaust-gas parameters

The following parameters can be measured using a **BlueVary** or **BlueInOne** of **BlueSens gas sensor GmbH**:

Table 3: Abbreviations, common symbols and units of the parameters measured by off-gas analysis

Sign	Parameter	Unit
p	Pressure	bar
T	Temperature	°C
f	Absolute humidity	Vol%

In order to calculate the evaporation as shown before, the values must be converted according to their units.

$$p\left[\frac{N}{m^2}\right] = p\,[bar] * 100{,}000$$

$$T\,[K] = T\,[°C] + 273.15$$

$$f\left[\frac{L}{L}\right] = f\,[Vol\%]\ \div 100$$

$$f\left[\frac{m^3}{L}\right] = f\left[\frac{L}{L}\right] \div 1000 \rightarrow f\left[\frac{m^3}{L}\right] = f\,[Vol\%]\ \div 100{,}000$$

Since the absolute humidity of the air supply can vary between different systems, it is necessary to determine the offset value of the “dry air” to get accurate values for the absolute humidity. For this the exhaust-gas sensor should be flushed with dry air until an equilibrium is reached. The value for the absolute humidity at equilibrium shows the offset value for the used air supply system (f_0).

2.4 Further parameters

The aeration speed is an important factor for the evaporation itself and is therefore necessary for this calculation. If the aeration speed is always at the same rate, it can be used as a constant. Otherwise, the current aeration speed has to be used for the calculation using the current flow rate measured by a mass flow controller (MFC).

3 Calculations

3.1 Calculation of evaporation for each time point

Since the **BlueVIS** software exports the current values every 5 s the aeration speed has to be transformed into [L/s] and the time has to be set to t = 5 s (this counts only for a calculation with exported data and not for live data). To get the evaporated volume of water in mL the formular has to be used with the following units:

$$V_{H_2O}[mL] = \frac{p\left[\frac{N}{m^2}\right] * (f - f_0)\left[\frac{m^3}{L}\right] * F_{gas}\left[\frac{L}{s}\right] * t\,[s] * M_{H_2O}\left[\frac{g}{mol}\right]}{R\left[\frac{N * m}{mol * K}\right] * T\,[K] * \rho_{H_2O}\left[\frac{g}{mL}\right]}$$

Entering all constants and known values leads to the following formular, which can further be simplified:

$$V_{H_2O}[mL] = \frac{p * 100{,}000\left[\frac{N}{m^2}\right] * (f - f_0) \div 100{,}000\left[\frac{m^3}{L}\right] * F_{gas}\left[\frac{L}{s}\right] * 5\,s * 18.02\,\frac{g}{mol}}{8.31442\,\frac{N * m}{mol * K} * (\,T + 273.15\,[K]\,) * 1.0\,\frac{g}{mL}}$$

$$V_{H_2O}[mL] = \frac{p\left[\frac{N}{m^2}\right] * (f - f_0)\left[\frac{m^3}{L}\right] * F_{gas}\left[\frac{L}{s}\right] * 5\,s * 18.02\,\frac{g}{mol}}{8.31442\,\frac{N * m}{mol * K} * (\,T + 273.15\,[K]\,) * 1.0\,\frac{g}{mL}}$$

Since the conversion factors for pressure and absolute humidity cancel each other out, the values measured by the off-gas analyzer can be directly used as an input for this formular.

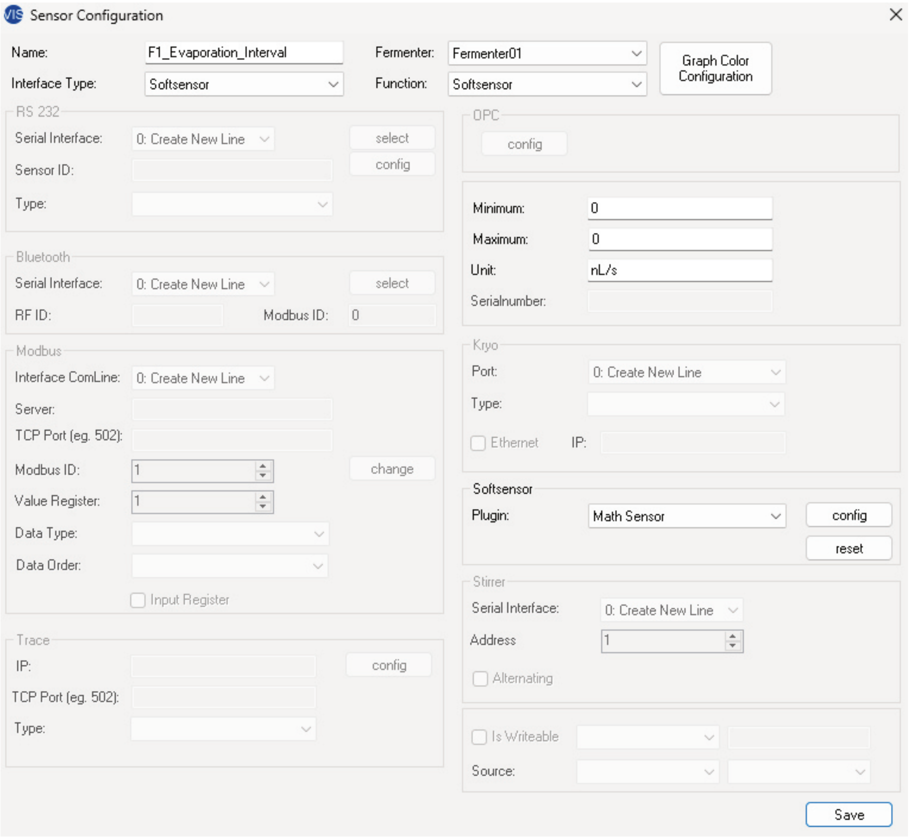
3.2 Calculation of total evaporation

To obtain the total amount of evaporated water, the values of the individual time points must be summarized as follows:

$$\sum_{k=1}^{n} V_{H_2O,1} + V_{H_2O,2} + \cdots + V_{H_2O,n}\,[mL]$$

4 Calculations in BlueVIS

Two softsensors are required to calculate the evaporation in **BlueVIS**. The first one calculates the loss of water at each time point. As shown in Figure 1 the softsensor must be defined as a **Math Sensor** to perform the mathematic operation.

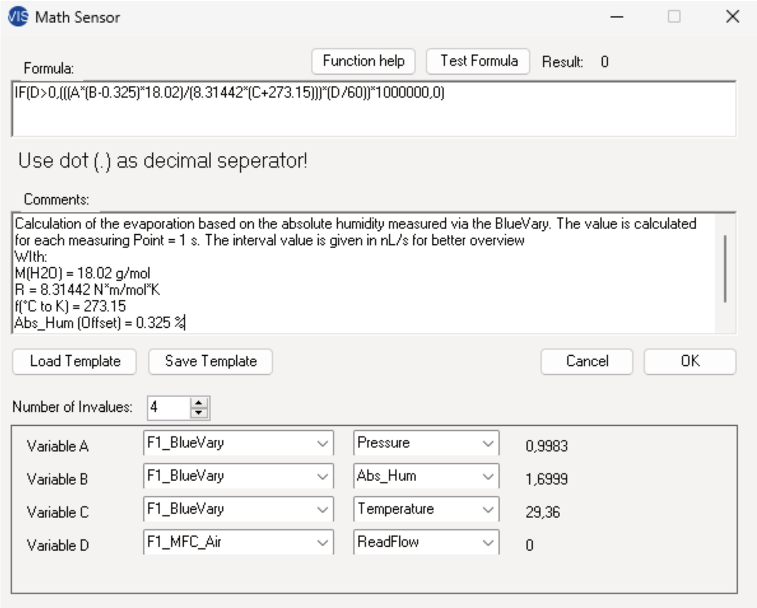


Sensor Configuration

Name: F1_Evaporation_Interval
Fermenter: Fermenter01
Interface Type: Softsensor
Function: Softsensor
Serial Interface: 0: Create New Line
Interface ComLine: 0: Create New Line
Modbus ID: 1
Value Register: 1
Modbus ID: 0
Minimum: 0
Maximum: 0
Unit: nL/s
Port: 0: Create New Line
Plugin: Math Sensor
Serial Interface: 0: Create New Line
Address: 1

Figure 1: Softsensor configuration for the calculation of evaporation at every time point

Further the formular as described in 3.1 has to be implemented in the softsensor as shown in Figure 2. Since BlueVIS generates new values every second, the time parameter is 1 and can therefore be ignored. The output values of the gas analyzer und the mass flow controller (MFC) are used as inputs for this calculation. To avoid errors during the calculation, the operation is controlled by an IF-function to start the calculation only if aeration is turned on (If the aeration is turned on perform the calculation, else value is 0). To get a better overview of the interval values the results of the calculation are given in nL/s. A calculation in mL is possible but will be displayed as 0 in **BlueVIS** due to the high number of decimals places.



Math Sensor

Formula:

```
IF(D>0,(((A*(B-0.325)*18.02)/(8.31442*(C+273.15)))*(D/60))*1000000,0)
```

Result: 0

Use dot (.) as decimal seperator!

Comments:

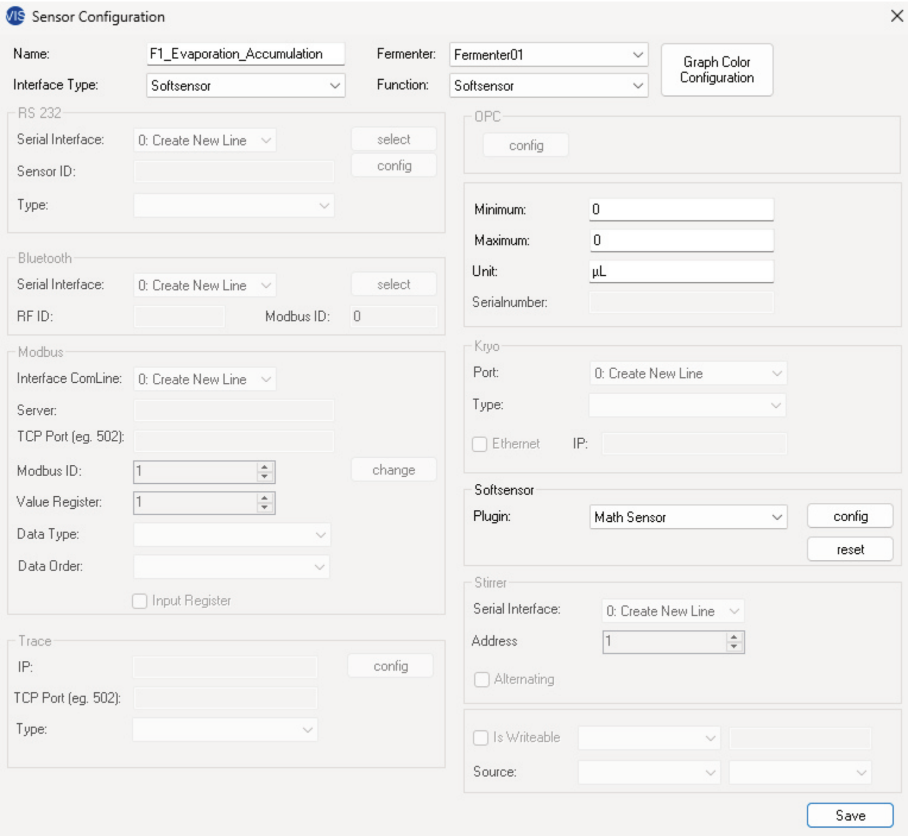
Calculation of the evaporation based on the absolute humidity measured via the BlueVary. The value is calculated for each measuring Point = 1 s. The interval value is given in nL/s for better overview
WIth:
M(H2O) = 18.02 g/mol
R = 8.31442 N*m/mol*K
f(°C to K) = 273.15
Abs_Hum (Offset) = 0.325 %

Number of Invalues: 4

Variable A	F1_BlueVary	Pressure	0,9983
Variable B	F1_BlueVary	Abs_Hum	1,6999
Variable C	F1_BlueVary	Temperature	29,36
Variable D	F1_MFC_Air	ReadFlow	0

Figure 2: Calculation of the evaporation for each time point in nL/s

The second softsensor is used to accumulate the single values of each time point. With this we generate a value which can be used as an output value to e.g., correct the volume calculation of the fermenter or to control a pump to replace the lost volume. For this, a **Math Sensor** has to be added as shown in Figure 3.



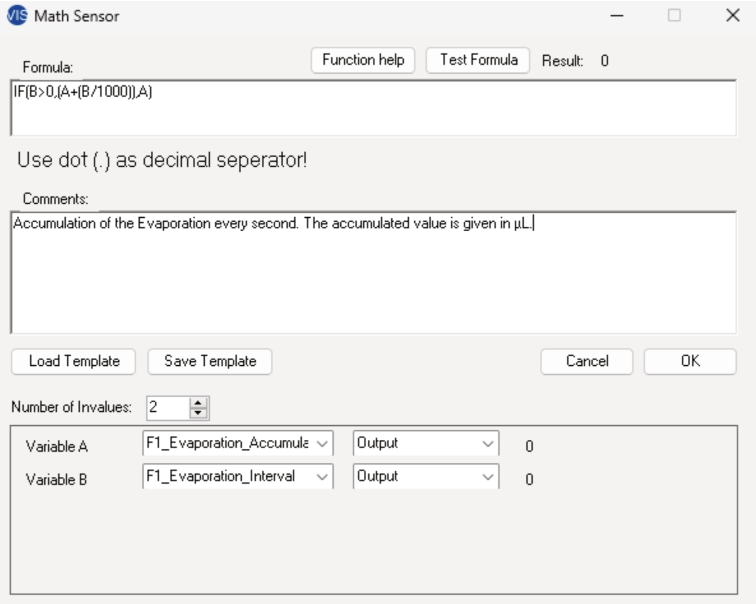
Sensor Configuration

Name: F1_Evaporation_Accumulation
Interface Type: Softsensor
Fermenter: Fermenter01
Function: Softsensor
Minimum: 0
Maximum: 0
Unit: µL
Softsensor Plugin: Math Sensor

Figure 3: Softsensor configuration for the calculation of accumulated evaporation values

To calculate the total volume of evaporated water the interval is added to the accumulated value every second. To realize this in BlueVIS the softsensor has to add the interval value to its own value at every time point. In a first step the softsensor (Evaporation_Accumulation) has to be configured without any inputs. For this just click on config and then on OK and Save, leaving the Math Sensor empty. This step is necessary, because without this step it will not be possible for the softsensor to refer to its own value.

After the softsensor is saved we can click again on edit and config to program the desired operation as shown in Figure 4. This softsensor is also controlled by an IF-function, making sure that the interval value is only added if it's greater than 0. The results of the total evaporation are displayed in µL. It is also possible to calculate the values in mL, therefore the factor inside the operation has to be changed from 1,000 to 1,000,000.



Result: 0

Formula: IF(B>0,(A+(B/1000)),A)

Comments: Accumulation of the Evaporation every second. The accumulated value is given in µL.

Number of Invalues: 2

Variable A F1_Evaporation_Accumula Output 0
Variable B F1_Evaporation_Interval Output 0

Figure 4: Calculation of the accumulated evaporation in µL

5 Literature

[1] Duboc, P. and U. von Stockar, *Systematic errors in data evaluation due to ethanol stripping and water vaporization.* Biotechnology and Bioengineering, 1998. **58**(4): p. 428-439.

[2] Doran, P.M., *Chapter 14 - Reactor Engineering*, in *Bioprocess Engineering Principles (Second Edition)*, P.M. Doran, Editor. 2013, Academic Press: London. p. 761-852.

[3] Ask, M. and S.M. Stocks, Aerobic bioreactors: condensers, evaporation rates, scale-up and scale-down. Biotechnol Lett, 2022. 44(7): p. 813-822.