BM RESPIROMETRY APPLICATIONS GUIDE

for wastewater treatment control, research and education

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Chapters Index

Link of video: https://youtu.be/UeMvk7U5ZMo

This manual is made on the approach to get the necessary guidelines for the main respirometry tests and applications. It was not intended to describe closed and strict procedures; on the contrary, we have to take into account that BM respirometers are not closed systems and normally the user could carry out his own applications by making use of these guidelines and taking advantage of the wide field of possibilities that BM software is offering.

1. Measurements & Automatic tests in BM Respirometers

BM lab respirometers

Automatic measurements

BM Model	Automatic measurements	Description	Programming capability
T+ EVO, EVO2 Advance, Advance2 Advance Pro	**DO** (mg O_2/L)	Dissolved oxygen	Programmable threshold in Cyclic operation mode
EVO, EVO2 Advance, Advance2, Advance Pro	**Temperature** (ºC)	Temperature	Programmable
Advance, Advance2, Advance Pro	**pH**	pH	Programmable
Advance Pro	**ORP** (mV)	Redox potential	Non-Programmable

All programmable measurements can be set before and during the respirometry test

Operation modes and Automatic parameters

OUR & Cyclic OUR modes

OUR: Oxygen Uptake Rate (mg O_2/l.h)
It measures the oxygen uptake rate for only one measurement or serial o measurements.

SOUR: Specific OUR (mg O_2/g VSS.h)
Specific OUR related to MLVSS. SOUR = OUR / MLVSS

R mode

Rs: Dynamic Respiration Rate (mg O_2/l.h)
It measures the oxygen uptake rate from the mixture of the activated sludge and certain amount of wastewater sample or compound within a continuous chain of measurements.

Rsp: Dynamic specific respiration Rate (mg O_2/g VSS.h)
Specific Rs referred to MLVSS. Rsp = Rs / MLVSS

bCOD: Biodegradable COD (mg O_2/l)
Biodegradable or soluble readily biodegradable COD fraction, based on Rs measurements integration from a mixture of activated sludge and biodegradable sample.

U: COD removal rate (mg COD/l,h)
Speed at which the COD is being removed.

q: Specific COD removal rate (mg COD/ mg VSS.d)
Specific U referred to MLVSS concentration.

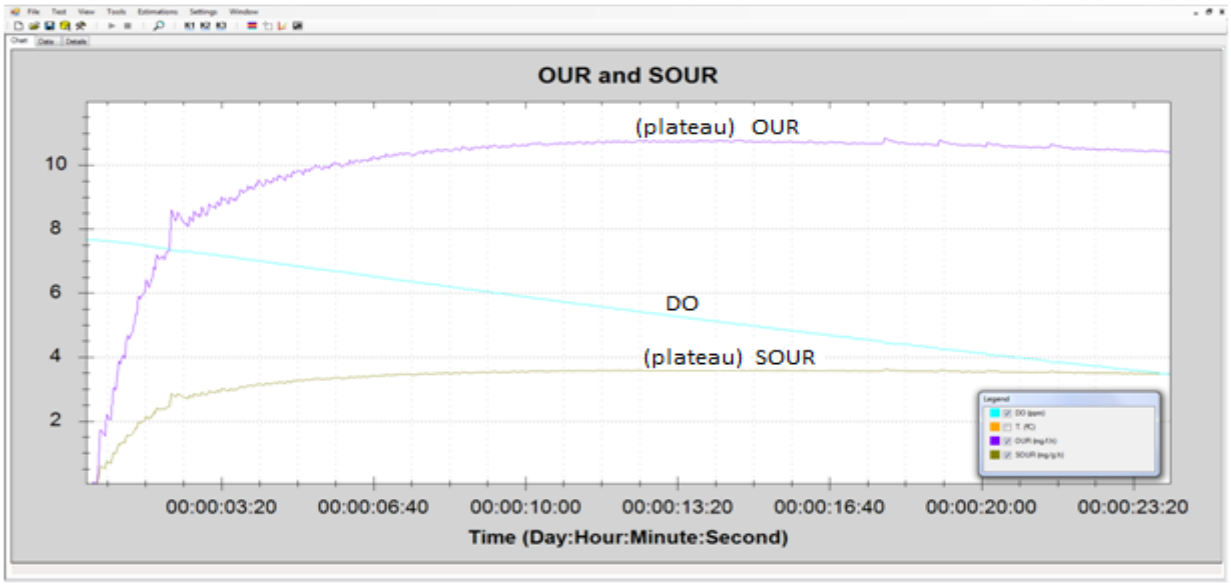
Operation modes description

(See Operation Manual)

OUR mode

Normally the reference temperature for all these tests is 20ºC, and should be carried out within the same pH, temperature conditions and MLVSS concentration as the actual process.

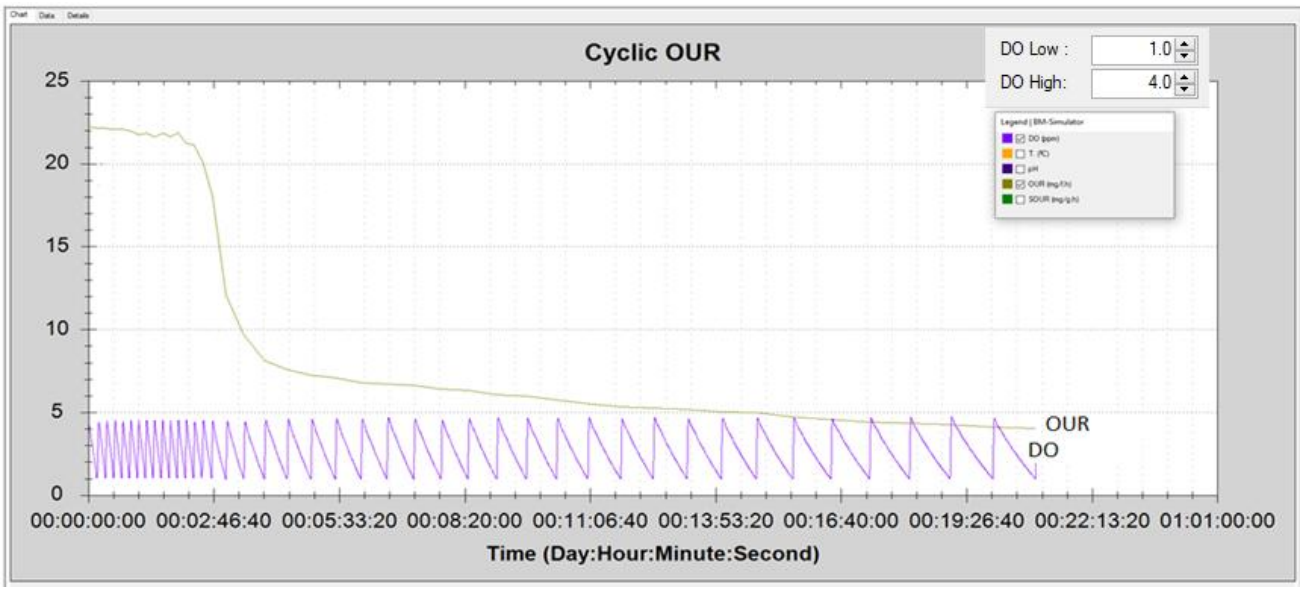
In a OUR test, it is assumed that the representative OUR & SOUR values correspond to their maximum values, which are normally reached within the plateau in the respirogram.



Overlapping respirograms of OUR, DO and SOUR

Cyclic OUR mode

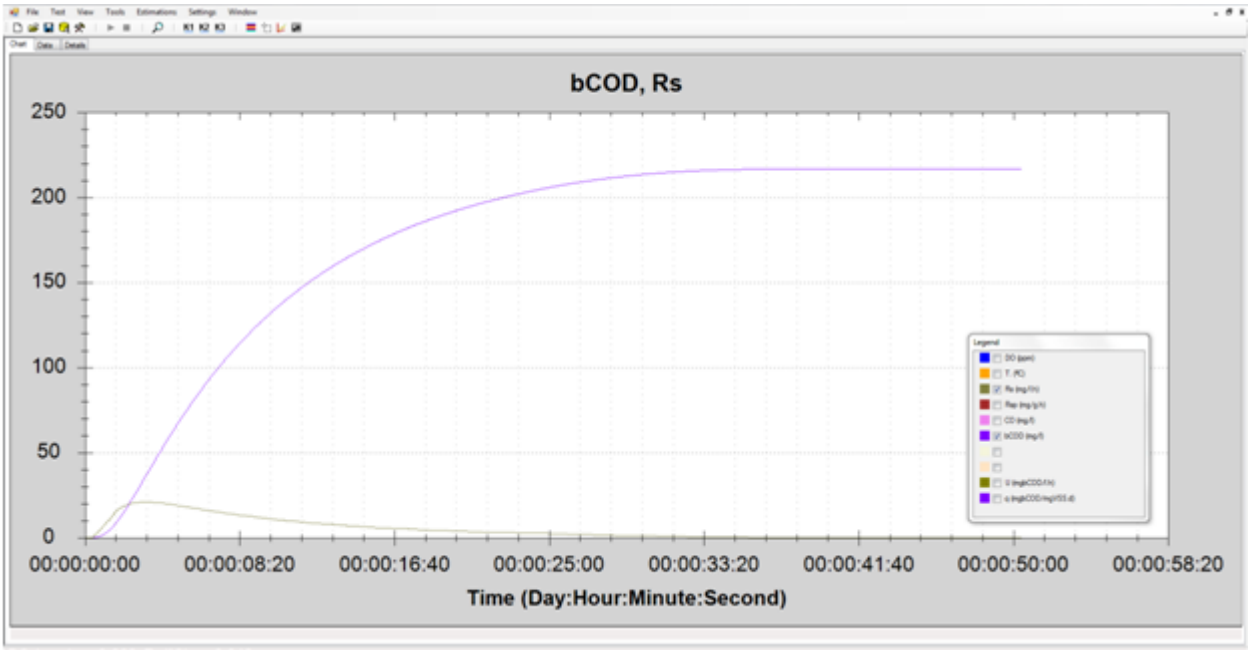
This mode works by programming two DO setpoints (high DO and low DO) in the tests board settings, between which the oxygen rises and falls, generating the corresponding continuous series of OUR and SOUR values.



Overlapping respirograms of cyclic OUR and DO

R mode

Normally R test must be carry out with activated sludge of 2 – 5 g/l of MLVSS concentration; and, in case that this concentration cannot be achieved, we can make use of the returned activated sludge (RAS) by diluting it if necessary.



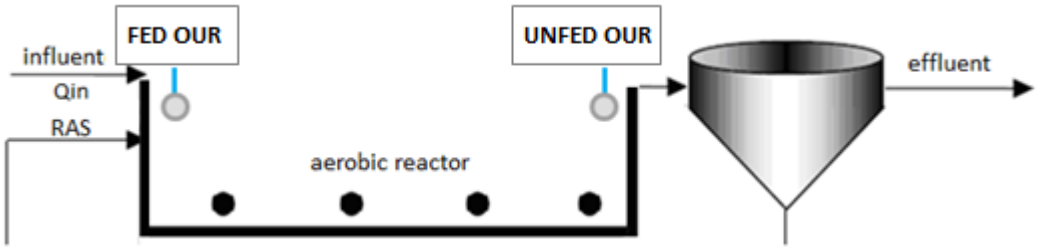
Overlapping respirograms of bCOD and Rs

2.
Pulse of the process

Early evaluation of the treatment process

Taking the pulse by means the Loading Factor (LF)

The one-day pulse of the process can be taken by means the assessment of the ratio of two OUR tests corresponding to the influent sludge (FED OUR) and effluent sludge (UNFED OUR)



Loading Factor: **LF = FED OUR / UNFED OUR)**

LF	Assessment
LF < 1	Inhibition / Toxicity already present in reactor
1 < LF < 2	Low efficiency or low BOD loading
2 < LF < 5	Good process performance
LF < 5	Overloading

Taking the pulse by means the UNFED SOUR

This is a representative SOUR test of the effluent activated sludge in the biological reactor.

Throughout this test we can obtain a fast qualitative assessment of how the process is performing and sludge health.

In a plug-flow process, the sludge can be directly collected from the end of the reactor within a representative loading period.

Reference guide-table

Process type	F/M (BOD/SS.d)	SRT (d)	UNFED SOUR ref ($mgO_2/gVSS.h$)
High loading	> 0.4	4	10 - 15
Medium loading	0.2 – 0.4	4 - 10	7 - 10
Low loading	0.07 – 0.2	10 - 30	3 - 7
Very low loading (extended aeration)	< 0.7		

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Actual UNFED SOUR Vs. UNFED SOUR ref. (Table)	Assessment
>> ref	Overloading
= or +/- ref	Good performance
< ref	Low loading
<< ref	Very low loading or Toxicity symptoms

To assess the results, we compare the UNFED SOUR from the test with the UNFED SOUR ref. in the guide-table.

The values of the guide-table should not be understood on the criterion that the result can vary within the showed range, but on the way that a determined process has its specific range which normally would be included within the range in the guide-table.

3.
Endogenous oxygen uptake rate

The endogenous phase in activated sludge is a state in which there is no biodegradable substrate and oxygen consumption is due solely to the microorganisms for their survival.

In the BM Respirometry, obtaining a reliable endogenous sludge is extremely important, not only to evaluate the real state of the biomass but also because its oxygen level (measured in the BM Respirometer) represents the baseline for tests carried out in R mode.

Total endogenous oxygen uptake rate

This endogenous level can be achieved when the effluent sludge is aerated with fine bubble diffusers for long enough to remove any remaining substrate type.

Normally the endogenous respiration state can be recognized when the oxygen readings are stable within its oxygen saturation level.

The necessary time to reach the endogenous respiration can be very much variable (from a couple of hours until > 24 hours) and depends of the process type, actual loading rate state, physic-chemical conditions, biodegradable character of the material under treatment.

To check if the sludge is already under endogenous respiration, place it in the reactor vessel of the respirometer, set to ON stirrer, aeration (55) and pump. Then, check for a stable DO reading. If DO is still going up is because it is not yet under endogenous and will need more time to be aerated.

Once we get the sludge under endogenous state, we can perform a normal OUR test to get the OURend.

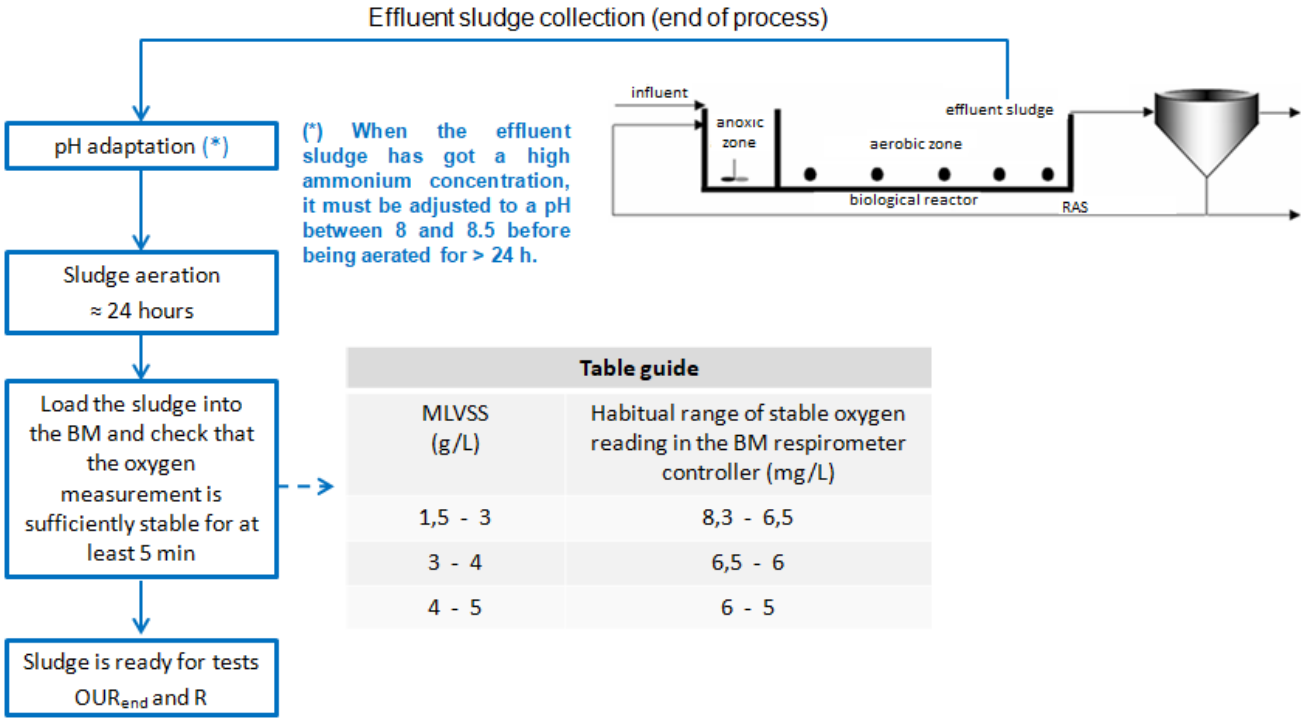
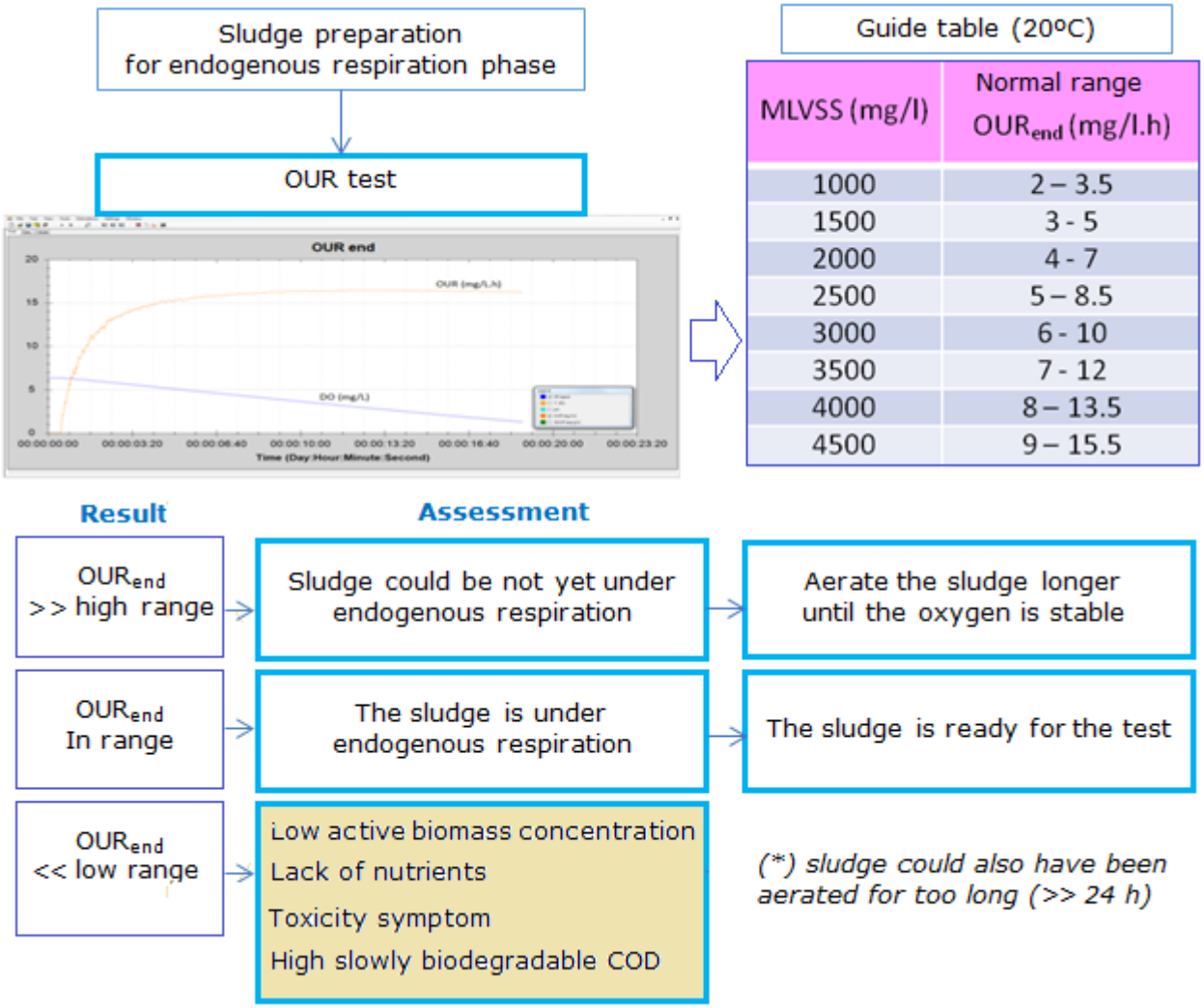


Table guide	
MLVSS (g/L)	Habitual range of stable oxygen reading in the BM respirometer controller (mg/L)
1,5 - 3	8,3 - 6,5
3 - 4	6,5 - 6
4 - 5	6 - 5

Total OUR_{end} assessment



Endogenous oxygen uptake rate of the heterotrophic biomass

OUR_{endH}: OUR of the sludge for the heterotrophic biomass

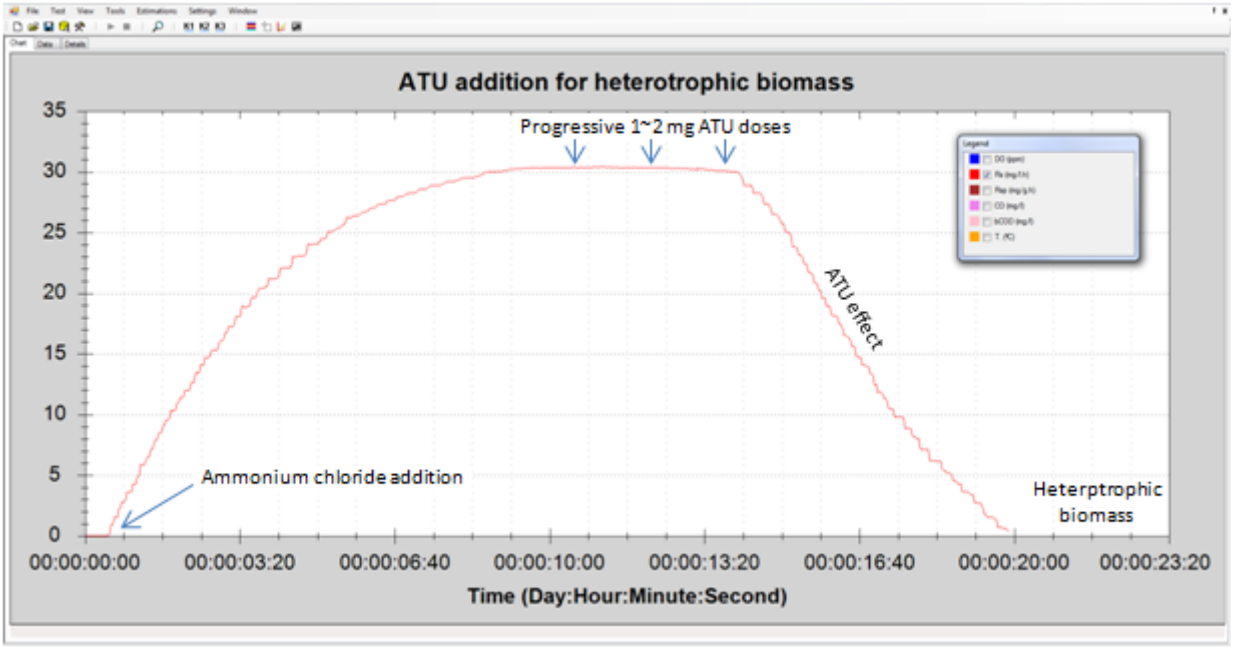
In a process with nitrification

In case there are active heterotrophic and autotrophic bacteria, the first thing to do is to eliminate the autotrophic in order to get a type of sludge where there are only heterotrophic microorganisms.

On that purpose, we will follow this procedure:

1. Get 1 litre of endogenous sludge for a R test.
2. Prepare a solution of 100 mg of ATU (Allyl Thiourea) in 100 ml of distilled water. In this way for 1 ml of solution would correspond 1 mg AEU.
3. Prepare an amount of ammonium chloride on 60 mg per g of MLVSS basis.
4. Prepare the dose of the ammonium chloride amount diluted in a small volume of distilled water (5~10 ml)
5. Program the R test, taking into account the temperature and pH, but not taking into account the Vf, Vm and Solids settings (you can leave the default values)

6. In the respirometer, be sure about the DO stability of the endogenous sludge.
7. Start the R test and, when "insert sample in the reactor" displays, add the ammonium chloride dose to the reactor.
8. Observe the respirogram and wait until it reaches a plateau of maximum Rs measurements.
9. As the plateau is formed, start adding the solution of ATU by doses of 1 ml every 20 seconds (approx.) until the Rs value in the plateau starts to decrease clearly. – The amount of ml you have added will give you the amount of mg of ATU necessary to inhibit the nitrifiers activity -
10. Wait until Rs value fall completely until reaching the base-line (horizontal axis)
11. Stop the test.



Now we have an endogenous sludge without nitrifiers and you can carry out an OUR test with this sludge that you get in the reactor.

NOTE1: Once you know the amount of ATU needed for a specific sludge, it would be not necessary to repeat the R test with ammonium chloride.

NOTE2: In the R test with ammonium chloride, the maximum value of Rs (in the plateau) would correspond to the representative maximum respiration rate due to nitrification on the conditions in which the test was done.

NOTE3: It could be possible that we were not sure if the activated sludge has or not active nitrifier biomass. In this case this R test with ammonium chloride we will confirm if there is nitrification or not. So that, the absence of very poor Rs generation when we were adding the ammonium chloride would indicate the absence of nitrifier biomass and it would be not necessary to add any ATU dose to get the heterotrophic biomass.

<u>OUURend In a process without nitrification</u>

In this case the total OUR_{end} will coincide with the $OUR_{end.H}$

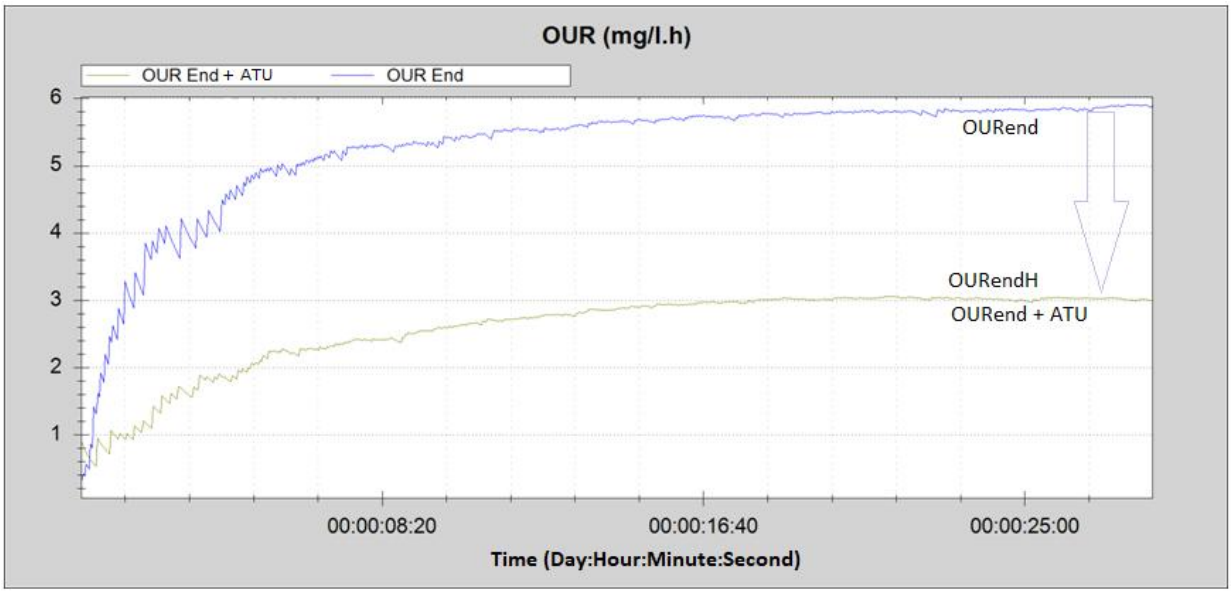
$OUR_{end.H} = OUR_{end}$

Endogenous oxygen uptake rate of the autotrophic biomass

The endogenous respiration rate for nitrifiers can be obtained by the difference between the total OUR_{end} and the $OUR_{end.H}$

$OUR_{endA} = OUR_{end} - OUR_{endH}$

OURendA: Endogenous oxugen uptake rate (mg O_2/L.h)



4.
Stoichiometric coefficients of the heterotrophic biomass

Yield coefficient of heterotrophic biomass referred to oxygen demand

$Y_{H,COD}$: Yield coefficient of heterotrophic biomass (mg CO{bact.}/mg COD{soluble.})

Note: Y value corresponds to Y that is showed in the assay Configuration as 0,67. In case that we determine a different value, it should be modified in each R assay.

The $Y_{H,COD}$ value is specific for each ASP (activated sludge process)

$Y_{H,O2}$ is determined from an R assay where the activated sludge should prepared in optimal conditions:

- Free of big / hard solids in activated sludge. For that is convenient to filter the sludge by means a narrow bandwidth cook strainer.
- Activated sludge in basic or endogenous phase.
- The process should be without any symptom of inhibition or toxicity.

Most effective method for YH determination is by a R test with sodium acetate as standard sample.

In any case, there are some observations we have to take into account:

$Y_{H,COD}$ determination from sodium acetate

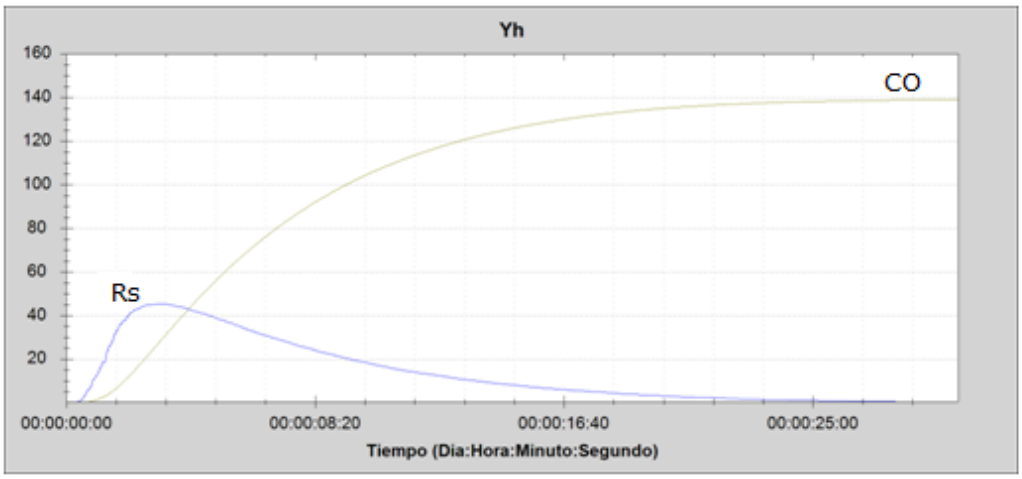
First thing is to make a solution of 400 mg of sodium acetate in 1 litre of distilled water. For this solution, we must obtain (from the lab.) the actual COD value (COD_{ac} ≈ 300 mg/l

The sample volume should be in between 30 and 50 ml and peristaltic pump flow at 2.

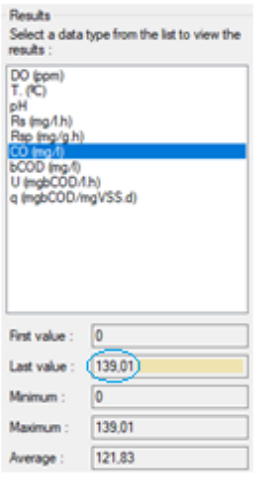
We carry out an R test in order to determine CO.

CO: Consumed oxygen = Δ O_2 (mg/l)

Sample volume shoul be set in between 30 to 50 ml. and pump speed to 2



Overlaid Rs and CO Respirograms



CO result

$\mathbf{Y_{H,COD}} = 1 - CO / COD_{ac}$

$Y_{H,COD}$: Heterotrophic yield coefficient (mg O_2/mg COD)

Yield coefficient of heterotrophic biomass referred to the biomass concentration

$\mathbf{Y_{H,VSS}} = Y_{H,COD} / f_{cv}$

$Y_{H,VSS}$: Heterotrophic yield coefficient referred to the biomass concentration (mg VSS / mg CODs)
f_{cv} = rbCOD {bact.} / VSS = 1.42 (mg COD/mg VSS) - (normal value commonly accepted)

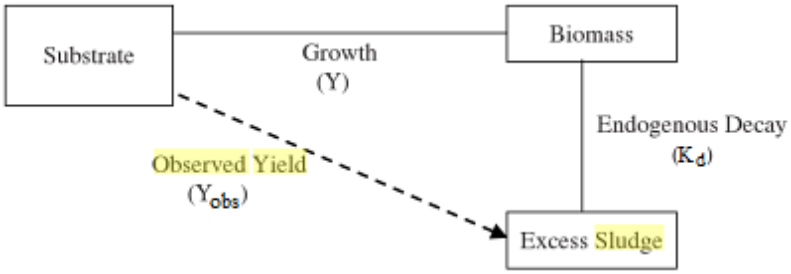
Source: The Activated Sludge Resource Book - K.C. Landrea - La Trobe University, Bendigo (Australia)

Typical values

Coefficient	Unit	Range	Typical	Remark
$\mathbf{Y_H}$	g MLSS/g COD	0.4-0.6	0.5	Convertible each other assuming COD/BOD=2 and MLVSS/MLSS=0.8 for municipal wastewater
	g MLVSS/g COD	0.3-0.5	0.4	
	g MLSS/g BOD	0.8-1.2	1.0	
	g MLVSS/g BOD	0.6-1.0	0.8	

Observed yield coefficient

Observed yield (Y_{obs}) is the observed amount of total biomass generated per unit amount of substrate utilized. As opposed to the true growth yield (Y), this coefficient is not a constant and it is inversely proportional with the sludge age (SRT). The main application of the observed yield is the calculation of the excess sludge production.



$\mathbf{Y_{obs}} = Y / (1 + K_d * SRT)$

Y_{obs}: Observed Yield (MLVSS/DQO)
$Y \approx Y_{H.VSS}$ (0.42 VSS/COD – Typical value)
K_d (d^{-1}): Biomass fraction per day, oxidized during endogenous respiration ($K_d \approx 0.06$ – Typical value)
SRT (d^{-1}): Sludge residence time (Sludge age)

Typical values

SRT	Y_{obs}		MLVSS/ MLSS	F/M
days	g VSS/ g COD	g MLSS/ g COD		g COD/ g VSS/d
2	0.477	0.558	0.855	1.048
5	0.384	0.461	0.833	0.521
10	0.329	0.396	0.831	0.304
20	0.298	0.358	0.832	0.168
30	0.268	0.328	0.817	0.124

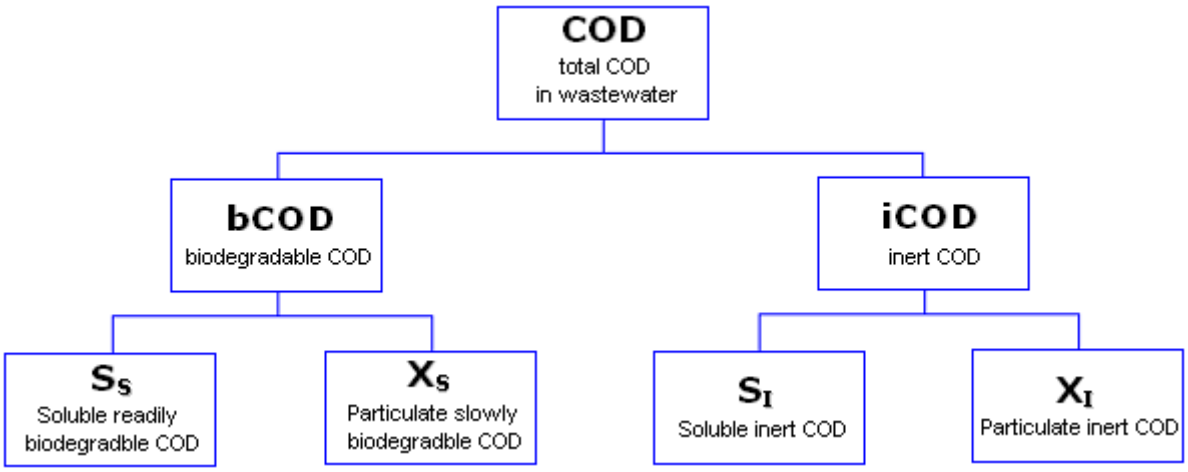
Source: Cicek, 2001; Macomber, 2005

5.
COD fractions
&
Biodegradability

Main COD fractions

We can determine and estimate some fractions through the R mode. For that, we are making use of the Allil Thiourea for nitrification inhibition)

For COD fractioning we are making use of R mode assays. Here the BM analyser is carrying out the continuous Rs integration corresponding to the substrate oxidation in order to calculate the corresponding CO (mg/l) on timely basis.



For the assays related with COD fractioning we refer to the fractions that a determined activated sludge process is able to degrade.

Total biodegradable COD (bCOD) from R mode

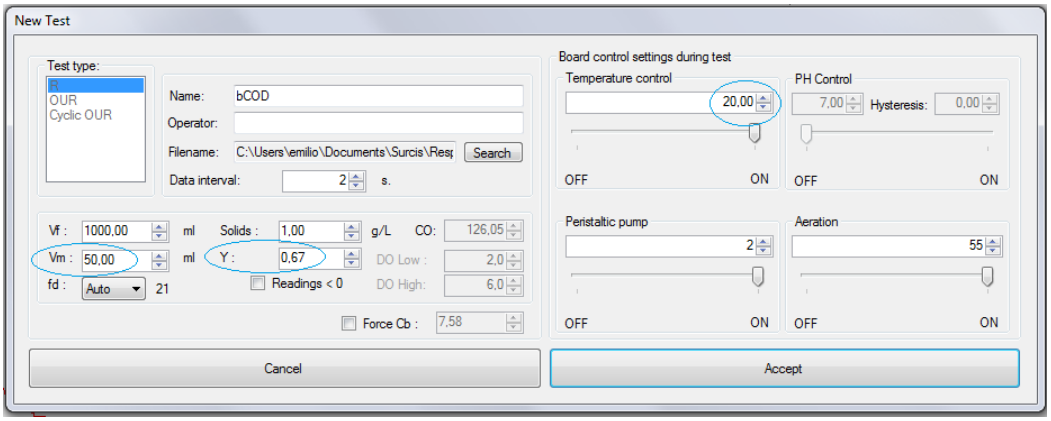
We can determine the total biodegradable COD (bCOD) by means one R test with influent wastewater to the biological reactor and endogenous sludge.

Just in case the process has nitrification, you must add a dose o Allyl Thiourea (ATU) to the endogenous sludge (stirring and aerating it) ½ hour before the test (2 to 3 mg ATU / g VSS)

If you do not use the Y default value (0.67), set the actual value of $Y_{H.COD}$ in the settings board of the bCOD test in the box for Y value.

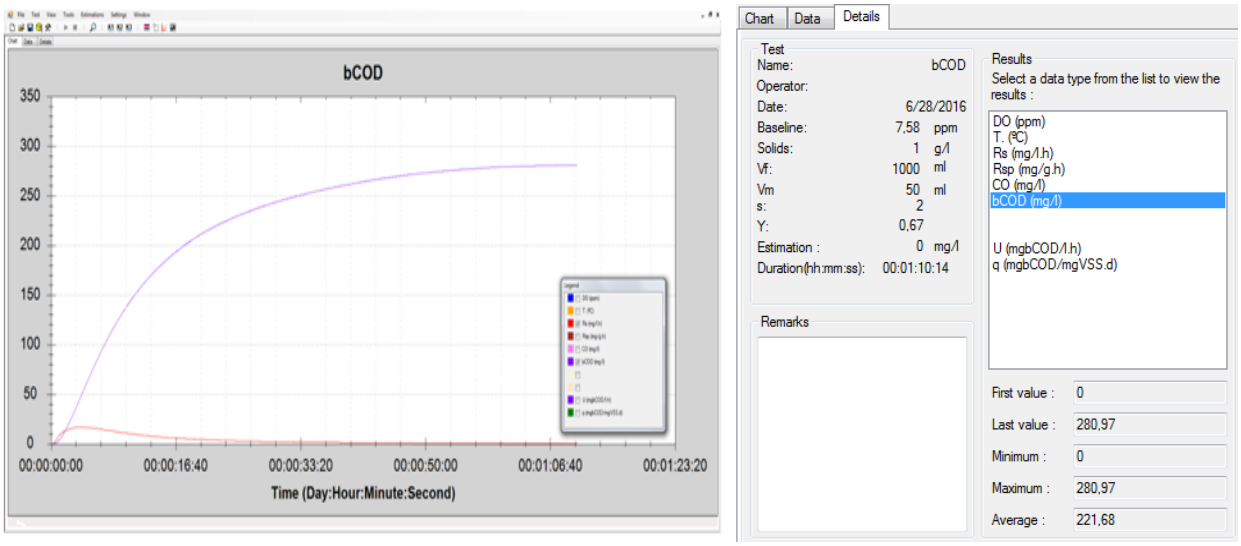
Normally we will place the peristaltic pump at 2 and the default value of aeration (55)
For the volume of sample, we should follow more or less the following guide table:

Total COD (mg/l)	Sample volume (ml)
< 500	50
500 – 5000	50 - 30
5000 - 10000	30 - 20
10000 – 25000	20 - 10
> 25000	10



IMPORTANT
R test must carry out with activated sludge of 2 – 5 g/l of MLVSS concentration. In case that this concentration cannot be achieved, we can make use of the returned activated sludge (RAS) by diluting it to get a concentration in between 2 – 5 g/l (if necessary)

BM-respirometer software will automatically calculate the on-going bCOD value by making use of the Y and the accumulated consumed oxygen (CO) It means that at any moment during the test performance we can see the bCOD that is being utilized.



Readily biodegradable COD determined by respirometry

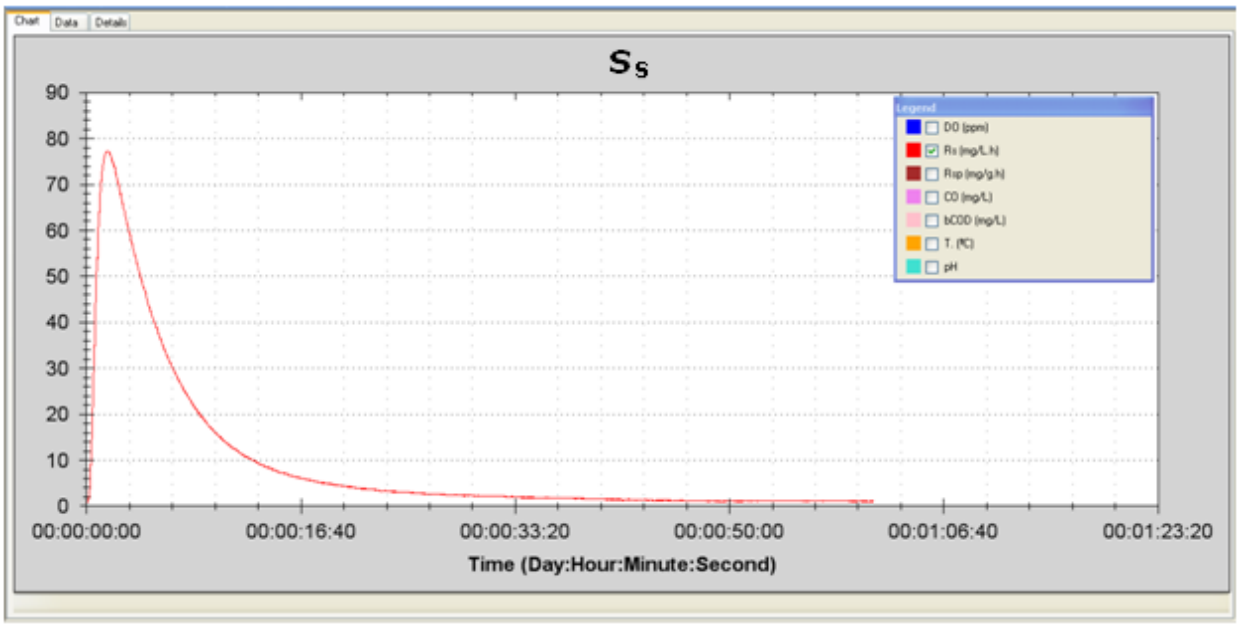
The readily biodegradable COD fraction is obtained from a truly soluble wastewater sample. Normally we can get this sample by making use of a coagulant compound (e.g. zinc sulphate: $ZnSO_4$) and then filtering the supernatant at 0.45 micron.

For test settings, we follow the same criteria as those used in the R test for bCOD (page 21)

$\mathbf{S_S} = CO / (1\text{-}Y_{H,O2})$

SS = rbCOD: Readily biodegradable COD (mg/l)
CO: Consumed oxygen in the current test (mg/L)

Note: In the following chapters, the soluble readily biodegradable COD can also be named **rbCOD**



Particulate slowly biodegradable COD

X_S = bCOD - S_S

X_S = sbCOD: Particulate slowly biodegradable COD (mg/l)

sbCOD is normally associated to particulate biodegradable COD. It corresponds to the slowly hydrolysable organic matter by the heterotrophic biomass.

Soluble inert COD

S_I = iCOD * Ss / bCOD

S_I: Soluble inert COD (mg/l)
COD_S : Soluble COD

Inert COD (non-biodegradable COD)

X_I = iCOD - S_I

X_I: Particulate inert COD

Biodegradability for a specific activated sludge process

This biodegradability as seen from the activated sludge respirometry view, under equivalent conditions to the actual ASP, should be considered not only from the biodegradable character of the wastewater sample to be analyzed but also from the sludge activity health and sample adaptation to the biomass. For that reason, this type of biodegradability should be specific for the activated sludge responsible of the organic matter oxidation of the influent wastewater.

Here we compare the biodegradable fractions with the total COD.

bCOD / COD	Character
> 0.8	Very biodegradable
0.7 ~ 0.8	Biodegradable
0.3 ~ 0.7	Very little biodegradable
< 0.3	Unbiodegradable

Biodegradability (%) = 100 * bCOD/COD

6.
Sludge production

Procedure for calculating sludge production

1) K_d: Endogenous decay coefficient (d^{-1})

This coefficient takes into account the loss of cell mass due to the oxidation of internal energy storage products for the maintenance of the cell in the endogenous respiration phase.

$\mathbf{K_d} = (24 / 1000) * SOUR_{end} / 1.42$

2) Y_{obs}: Stoichiometric observed yield coefficient (VSS/ DQO)

This coefficient represents the ratio of net biomass accumulation to the amount of excess sludge. It is related to the K_d and age of the sludge age (SRT), thus accounting for the lysis (death) of bacterial cells and the predation of bacteria by other microorganisms.

$\mathbf{Y_{obs}} = Y_{H.VSS} / (1 + K_d * SRT)$

P_X: Sludge production (kg SSV/d)

This parameter represents the net growth of biomass expressed in suspended volatile suspended solids.

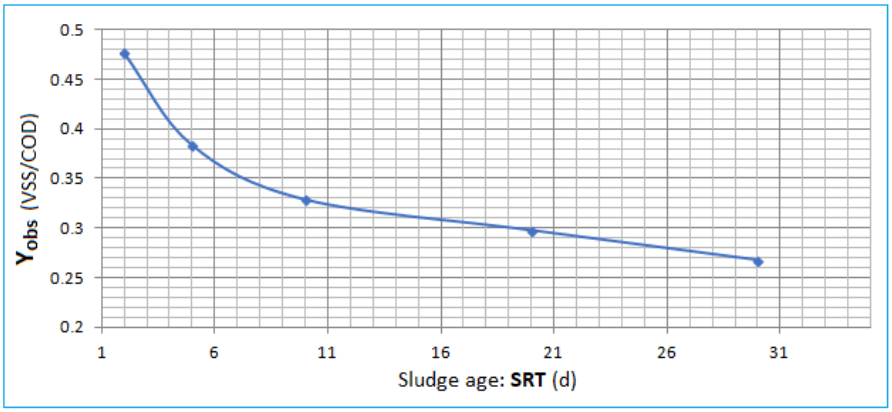
$\mathbf{P_X} = Y_{obs} * Q * bCOD_e / 1000$

P_X: Sludge production (kg VSS/d)
Y_{obs}: Observed yield coefficient (VSS/COD)
Q: Influent flow (m^3/d)
$bCOD_e$: Biodegradable COD eliminated (mg bCOD/L) = bCOD influent – bCOD effluent à bCOD effluent ≈ 1,6 * BOD effluent

Estimated calculation of the sludge production

Y_{obs} (VSS/COD)	SRT (d)	F/M (COD/VSS.d)
0,477	2	1,048
0,384	5	0,521
0,329	10	0,304
0,298	20	0,168
0,268	30	0,124

Cicek, 2001; Macomber, 2005



7.
Kinetic parameters for heterotrophic biomass

Endogenous decay coefficient for heterotrophic biomass

Fraction of heterotrophic biomass oxidized per unit time during endogenous respiration.

$\mathbf{K_d} = 0.024 * SOUR_{endH} / f_{cv}$

K_d: biomass fraction per day oxidized in endogenous respiration (d^{-1})
$SOUR_{endH}$: Specific endogenous oxygen uptake rate (mg O_2/gVSS.h)
f_{CV}: Oxygen uptake per unit of biomass = 1.42 (O_2/X_V)

Source: "Tratamiento de Aguas Residuales" R.S. Romalho 1991

For K_d estimation or assessment, the following table guide can be used:

F/M	0,03	0,05	0,1	0,15	0,20	0,25	0,30	0,40	0,50	0,60	0,70	0,80	1,00
Kd	0,024	0,041	0,067	0,080	0,092	0,1	0,109	0,118	0,123	0,128	1,131	0,133	0,136

Source: Curso de Tratamiento Biológico Aguas Residuales (CSIC) – Dr. Fco. Colmenarejo Morcillo

Oxygen uptake per day per unit of heterotrophic biomass.

Kilograms of oxygen used per day per kilogram of heterotrophic biomass during endogenous respiration

$\mathbf{b_H} = K_d / [1 - Y_{H,COD} (1 - fp)]$

b_H : Oxidation uptake per day per unit of heterotrophic biomass (d^{-1})
fp: Particulate biomass fraction = 0,08

Source: Ekama et al. (1986)

Active heterotrophic biomass concentration

Active heterotrophic biomass concentration from $OURe_{nd.H}$

$\mathbf{X_H} = 24 * OUR_{endH} / (f_{cv} * b_H)$

X_H: Active heterotrophic biomass (mg/l)
OUR_{end}: Endogenous respiration (mg/l.h) - (see page 6)
f_{CV}: Oxygen uptake per unit of biomass = 1.42 (O_2/X_v)

The active heterotrophic biomass concentration, determined from the respirometric tests, presents a variable percentage in the range of 30%–37% of the total amount of MLVSS in the activated sludge sample.

Specific substrate utilization rate in process

The specific COD utilization rate for a determined treatment process is calculated acoording to the well known equation:

$\mathbf{q} = [(1/SRT) + Kd] / Y_{H.VSS}$

q: specific COD utilization rate (mgCOD/mgSSV.d)
SRT: Current sludge retention time (d)

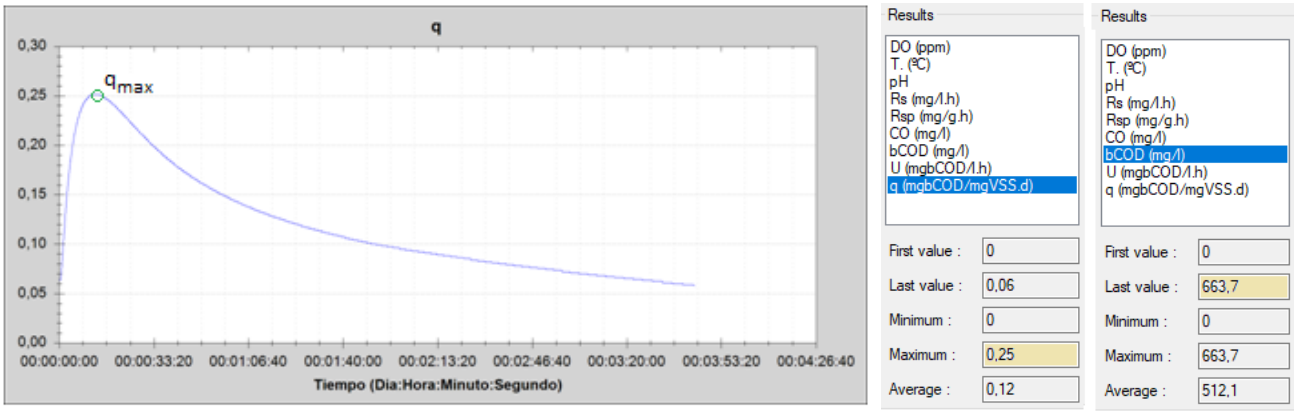
In he case that the wastewater treatment includes a nitrification process, the SRT should be calculated from the growing rate parameter of the autotrophic biomass.

Just in case some of the parameters of the equation were unknown, they can be estimated from the following table:

$1/SRT = q * Y_H - Kd$						
F/M BOD/SS.d	MLSS mg/l	SRT d	Y_H VSS/COD	Kd d^{-1}	μ_H d^{-1}	q_H COD/SSV.d
High Loading Process						
0.5	1200	2	0.6	0,123	0,62	1.03
0.47	1250	3	0,58	0.120	0.44	0.77
0.45	1300	4	0.57	0.119	0.36	0.64
Medium Loading Process						
0.4	1400	5	0.55	0.118	0,32	0,58
0.3	1800	7	0.5	0.109	0.25	0.50
0.2	2000	10	0.47	0.092	0.19	0.40
Low Loading process						
0.18	2200	12	0.45	0.085	0.16	0.37
0.1	2400	15	0.43	0.067	0.13	0.31
0.09	3200	17	0.42	0.060	0.12	0.28
Very Low Loading Process						
0.08	3400	20	0.41	0.056	0.10	0.26
0.06	3800	24	0.40	0.050	0.09	0.23
0.04	4200	27	0.39	0.032	0.07	0.18
0.03	4500	30	0.38	0.024	0.05	0.15

Maxium specific substrate utilization rate

From a R test for bCOD, the maxium specific organic substrate utilization will correspond to the maximum q value reached during the test.



Half saturation coefficient

$q = q_{max} * S / (Ks + S)$ (Monod principle)

Ks $= S (q_{max} - q) / q$

Ks: Half saturation coefficient (mg/L)
S: Eliminated bCOD (mg/L)
q_{max}: Maximum specific COD utilization rate (mgCOD/mgSSV.d)

Growing rate of the heterotrophic biomass

Maximum net growing rate

$\mu_{H.max} = Y_{H.VSS} * q_{max}$

$\mu_{H.max}$: Maximum growing rate of the heterotrophic biomass (d^{-1})
$Y_{H.VSS}$: Heterotrophic yield coefficient (VSS/CODs)

Net growing rate for a determined S (COD)

$\mu_{H} = \mu_{H.max} \ S / (Ks + S)$

μ_{Hx}: Growing rate of the heterotrophic biomass (d^{-1})

8.
Operational parameters for organics removal

When wastewater treatment includes the nitrification, this process takes priority and the operating parameters must be calculated from the kinetic parameters of the autotrophic biomass and not from those of the organics.

Minimum sludge retention time for organic substrate removal in a process without nitrification

$\mathbf{SRT_{min}} = 1 / (\mu_{H.max} - K_d)$

SRT: Minimum sludge retention time (d)
K_d can be neglected when $q_{H.max}$ value is far below 0.1

SRT could be considered as MCRT (Mean cell retention time)

The actual SRT in the process should be > SRT_{min}

IMPORTANT:
In case of a process with nitrification, the SRT should always be determined in base of the nitrification rate (see chapter 10 and 11)

Specific loading rate

Actual specific BOD loading rate

$\mathbf{F/M}_{(BOD)} = BOD / (MLSS * HRT)$

$F/M_{(BOD)}$: Loading rate related to BOD (kg BOD/kg MLSS. d)
HRT: Hydraulic Residence Time (d) in the aeration tank

Actual specific rbCOD loading rate

To assess the loading to the biological reactor, it is very useful the value of F/M (rbCOD)

$\mathbf{F/M}_{(rbCOD)} = rbCOD / (MLSS * HRT)$

$F/M_{(rbCOD)}$: Loading rate related to rbCOD (kg rbCOD/kg MLSS. d)

Normally the F/M (rbCOD) is usually in the range of 60-70 % of the F/M (BOD)
Just in case the F/M (rbCOD) is >> 70% of the F/M (BOD) or << 60%, the process could run into problems (although the F/M (BOD) remains within its normal range)

Maximum specific loading rate for control and design purposes

$\mathbf{F/M_{max\ (BOD)}} = q_{H.max} * (BOD/rbCOD) * (MLVSS/MLSS)$

F/M_{max}: Maximum loading rate (BOD/MLSS.d)

Hydraulic retention time for BOD removal

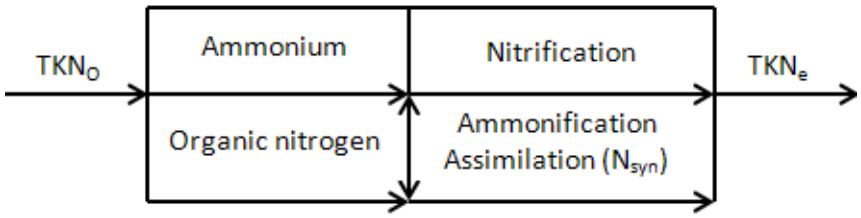
$\mathbf{HRT} = 24 * BOD / (MLSS * F/M_{max\ (BOD)})$

HRT: Hydraulic retention time (h)

9.
Kinetic parameters for autotrophic biomass

Ammonium nitrogen to nitrify in the biological process

Because of the ammonification process, where part of the organic nitrogen is going into ammonium form, the effective ammonium to nitrify must be calculated from the eliminated TKN from which we have to subtract the corresponding nitrogen directed to cell synthesis.



Actual ammonium that the process is currently nitrifying

$$\mathbf{S_N} = TKN_O - TKN_e - S_{sy}$$

S_N: Actual ammonium concentration to nitrify (mg/l NH_4-N)
TKN_O: Influent TKN (mg/l N)
TKN_e: Effluent soluble TKN (mg/l N)
S_{sy}: Nitrogen utilized in the cell synthesis = 0.05 * BOD

Ammonium that the process should nitrify

$$\mathbf{S'_N} = TKN_O - N_O - S_{sy} - S'_{Ne}$$

N_O: Organic nitrogen in effluent ≈ 2 mg N/L
S'_N: Ammonium concentration that the process should nitrify (mg/l NH_4-N)
S'_{Ne}: Maximum ammonium concentration permitted in the effluent (mg N-NH_4/l.h)

Determination of susbtrate half saturation constant for ammonium nitrogen removal in the Nitrification

The procedure for determining the substrate half-saturation in nitrification is based on the respirometry method of Dr. Adrianus C. Van Haadel – 2012.

The half-saturation constant can be calculated from an R-test by adding a sample dose (normally 50 mL) from a solution of ammonium chloride with a concentration equivalent to the actual ammonium nitrogen being treated in the process. For this, the ammonium chloride solution must be calculated on the basis of 1 mg NH4-N = 0.26 mg NH4Cl.

The R-test must be set to the same mean temperature and pH as the actual process.

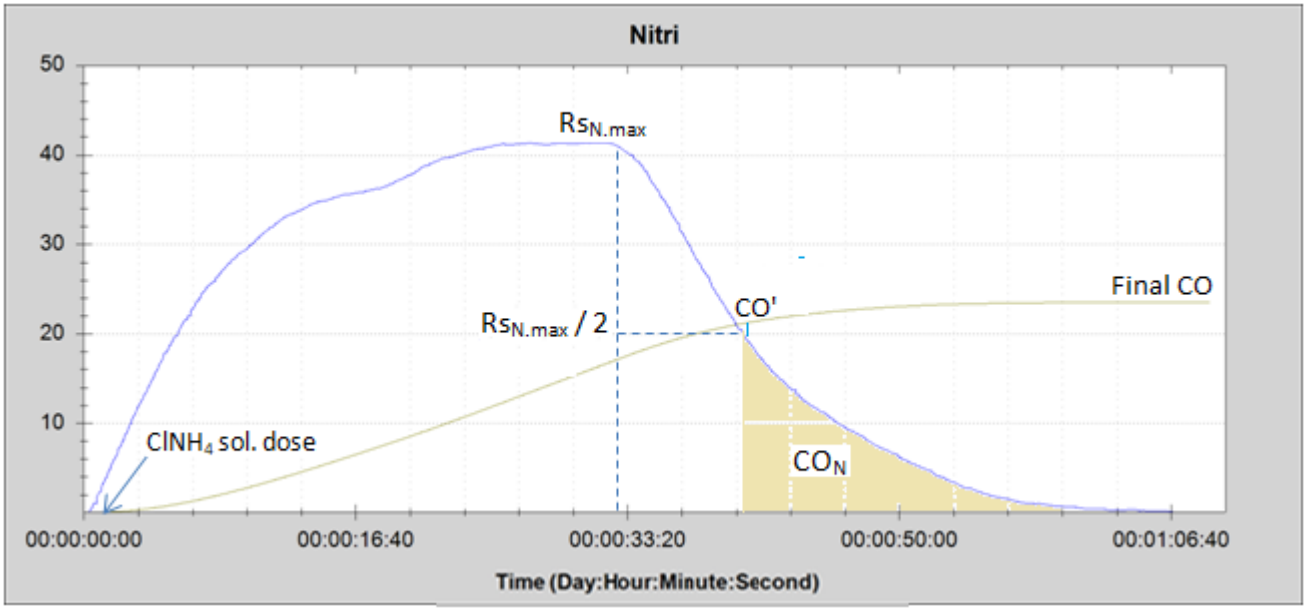
Procedure:

1. Once the Rs respirogram is completed, the maximum Rs is located ($Rs_{N.max}$) and from here the half of its value ($Rs_{Nmax}/2$)

2. By clicking with the PC mouse on the $Rs_{Nmax}/2$ value, the corresponding CO value will appear in the lower bar, which we will call CO'.
   It is also possible to obtain the CO' value by using the "Data" tab of the test, where the result string is presented here as a function of time, to locate the Rsmax/2 value and the corresponding CO for the same time interval.

3. The effective CO (CO_N) is then calculated from the difference of the final CO (maxium CO) minus the CO'.

   CO_N = Final CO – CO'



4. The ammonia nitrogen concentration corresponding to CO_N will correspond to the K_N value. Therefore, tthe K_N will be obtained by dividing the CO_N (mg O_2/L) value by 4,57.

K_N = CO_N / 4.57

K_N: Half saturation coefficient (mg NH_4-N/L)
CO_N = NOD: Consumed oxygen corresponding to $Rs_{N,max}$/2 (mg O_2/L)
4.57: mg of oxygen per mg of NH_4-N

Source: Van Haandel, Adrianus Cornelius, 2015

Maximum nitrification rate

AUR_{max} = ($Rs_{N.max}$ / 4,57)

AUR_{max}: Maximum nitrification rate (mg N/L.h)

Half saturation coefficient for oxygen

Actual nitrification eficiency

$E_N = 100\ (S_N - S_{N,ef}) / S_N$

E_N: Actual nitrification efficiency (%)
$S_{N,ef}$: Ammonium nitrogen in effluent (mgN/L)

Theoretical nitrificable nitrogen in effluent for maximum nitrification

$S_N'ef = S_N - AUR_{max} * [S_N / (S_N + K_N)] * HRT_N$

$S_{N'',ef}$: Ammonium nitrogen in effluent for maxium efficiency (mgN/L)
HRT_N: Hydraulic retention time for nitrification (h)

Maximum nitrification efficiency

$E_{Nmax} = 100 * (S_N - S_N'_{ef}) / S_N$

$E_{N,max}$: Maxium nitrification efficiency (%)

If $S_{N'ef}$ gives a negative value, a default value of zero must be taken and therefore the $E_{N,max}$ should be 100 (%)

Half saturation coefficient

$E_N = E_{Nmax} * DO / (K_{OA} + DO)$

$\mathbf{K_{OA}} = DO * (E_{N,max} - E_N) / E_N$

K_{OA}: Half saturation coefficient for oxygen (mg/L)

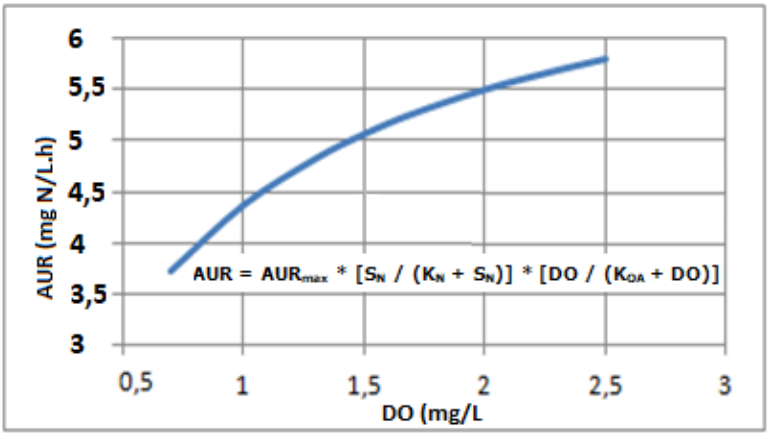
Actual nitrification rate

$\mathbf{AUR} = AUR_{max} * [S_N / (K_N + S_N)] * [DO / (K_{OA} + DO)]$

AUR: Actual nitrification rate (mg N/L.h)
DO: Bulk dissolved oxygen in the nitrification process (mg/L)

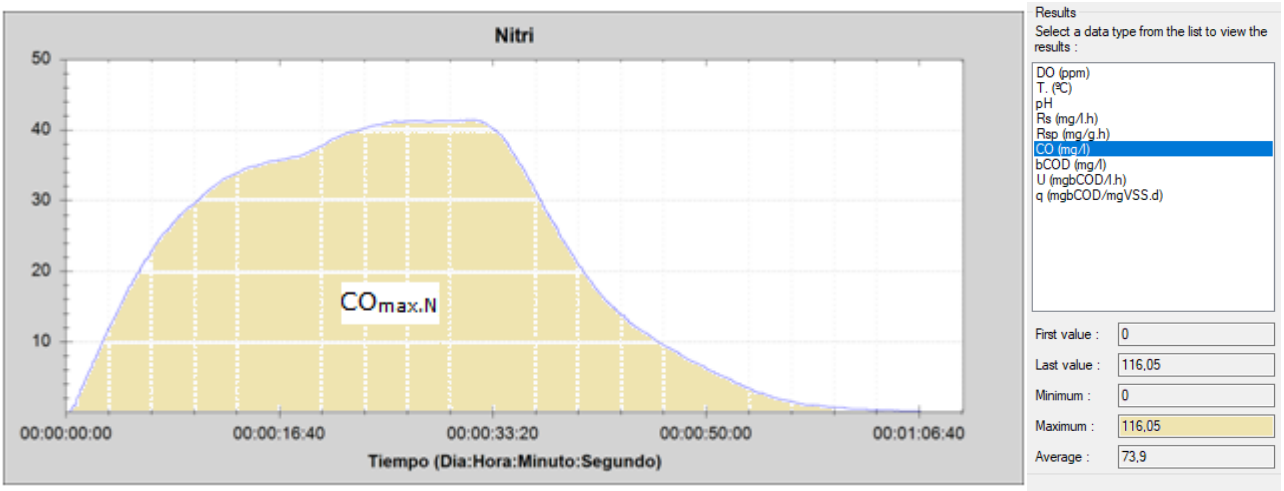
Influence of DO in the nitrification rate

Using the AUR equation, its value can be plotted for different DO values at which the nitrification process could operate. In that way, the nitrification capacity could also be analyzed for different DO values.



Yield coefficient for autotrophic biomass

To determine the autotrophic yield coefficient in relation to oxygen demand, the same test to determine K_N can be used. However, in this case, the total oxygen consumption required by the ammonium dose for its total oxidation ($CO_{max.N}$) is the value to include in the $Y_{A.COD}$ equation.



$$\mathbf{Y_{A.COD}} = (S_N * 4{,}57 - CO_{max.N}) / (S_N * 4{,}57 + i_{NOD/COD} * CO_{max.N})$$

$Y_{A.COD}$: Autotrophic yield coefficient related to COD (mgO_2/mgCOD)
$CO_{max.N}$ = NOD: Total consumed oxygen (mg/L)
$i_{NOD/COD}$: nitrogen content of bimass = 0.4 (mgNOD/mgCOD)

Sources**:** Chandran and Smets, 2000 - Kartik Chandran and Barth F. Smets, 2001

$$\mathbf{Y_{A.VSS}} = Y_{A.DQO} / 1.42$$

$Y_{A.VSS}$: Autotrophic yield coefficient related to MLVSS (mg VSS/mg N)

Nitrifier biomass concentration

$$\mathbf{F_N} = Y_{A.VSS} * S_N / (Y_{H.VSS} * S_S + Y_{A.vss} * S_N)$$

F_N: Portion of autitrophic biomass in the total MLVSS
S_S: Actual eliminated BOD in the biological process (mg/L)
$Y_{A.vss}$: Autotrophic yield coefficient ≈ 0,1
$Y_{H.VSS}$: Heterotrophic yield coefficient ≈ 0.45 (referred to VSS)

$$\mathbf{X_N} = F_N * MLVSS$$

X_A: Concentration of the nitrufier biomass (mg VSS/L)

Source: Eckenfelder, 1995

Maximum and actual specific nitrification rate referred to active biomass

$$\mathbf{q_{Nmax}} = AUR_{max} * 24 / X_N$$

q_{Nmax}: Maximum specific nitrification rate [NH_4-N/(VSS_A.d)]

$$\mathbf{q_N} = AUR * 24 / X_N$$

q_N: Actual specific nitrification rate [NH_4-N/(VSS_A.d)]

Maximum and actual growing rate in the autotrophic biomass

$\mu_{Amax} = Y_{A,VSS} * q_{N.max}$

$\mu_A = Y_{A,VSS} * q_N$

μ_A: Actual autotrophic growing rate coefficient (d^{-1})
$\mu_{.max}$: Net maximum autotrophic growing rate coefficient (d^{-1})

Actual endogenous decay coefficient for autotrophic biomass

$K_{dA} = \mu_A - 1/SRT$

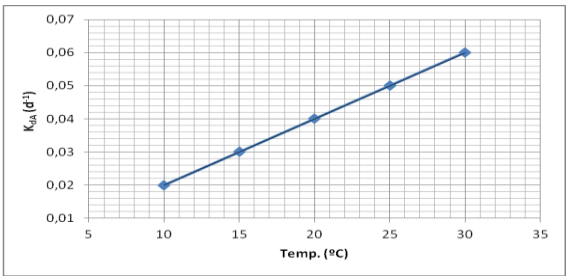
K_{dA}: Endogenous decay coefficient for autotrophic biomass (d^{-1})

Source: Metcalfe & Heddy

K_{dA} assessment

To assess the actual K_{dA}, the following guide table can be used:

Temp	Death & Decay Rate k_d ($days^{-1}$)
10°C	0.02
15°C	0.03
20°C	0.04
25°C	0.05



For practical purposes and design one can adopt the following equation:

K_{dA} (default value) = $0.04 * 1.03^{(T-20)}$ (d^{-1})

Source: Marais and Ekama, 1976

10.
Operational parameters for Nitrification

In a process with nitrification, the operating parameter SRT for ammonia nitrogen removal should prevail over the parameters related to organic substrate removal.

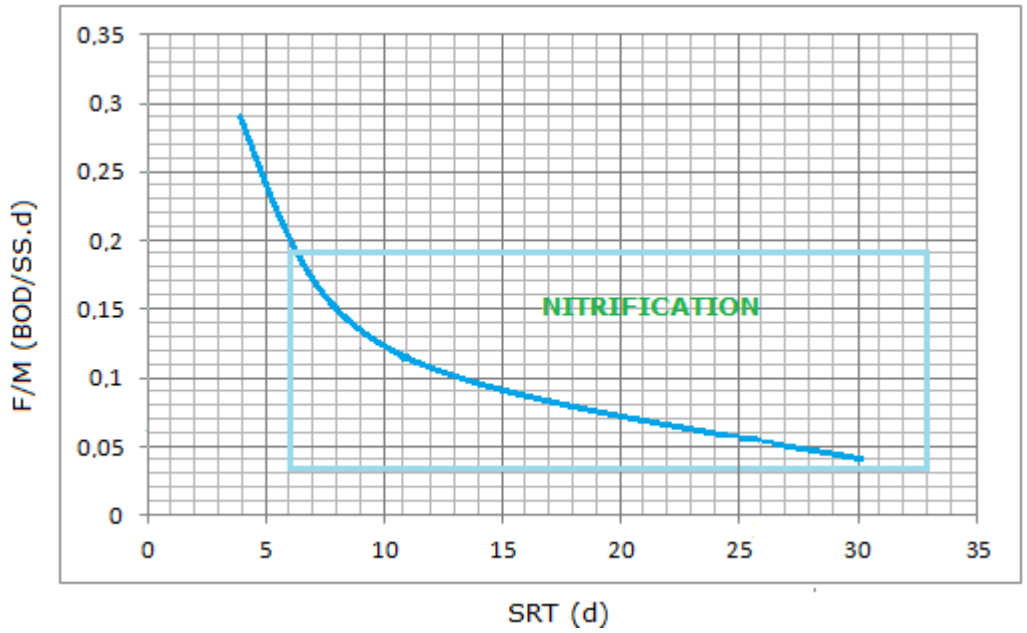
Typical design parameters for activated-sludge process modifications

Process modification	SRT, days[a]	Food-to-microorganism	MLSS, mg/L	Aeration time, hours	Return flow-to-plant flow ratio
Conventional	5–15	0.2–0.4	1 500–3 000	4–8	0.25–0.75
Complete mix	5–15	0.2–0.6	2 500–4 000	3–5	0.25–1.0
Step feed	5–15	0.2–0.4	2 000–3 500	3–5	0.25–0.75
Modified aeration	0.2–0.5	1.5–5.0	200–1 000	1.5–3	0.05–0.25
Contact stabilization	5–15	0.2–0.6	1 000–3 000[c] 4 000–10 000[d]	0.5–1.0[c] 3–6[d]	0.5–1.50
Extended aeration	20–30	0.05–0.15	3 000–6 000	18–36	0.5–1.50
High-rate aeration	5–10	0.4–1.5	4 000–10 000	2–4	1.0–5.0
High-purity oxygen	3–10	0.25–1.0	2 000–5 000	1–3	0.25–0.5
Oxidation ditch	10–30	0.05–0.30	3 000–6 000	8–36	0.75–1.50
Single-stage nitrification	8–20	0.10–0.25 (0.02–0.15)[e]	2 000–3 500	6–15	0.50–1.50
Separate-stage nitrification	15–100	0.05–0.20 (0.04–0.15)[e]	2 000–3 500	3–6	0.50–2.00

Minimum sludge retention time for nitrification

$$\mathbf{SRT_{min}} = 1 / (\mu_{Amax} - K_{dA})$$

SRT_{min}: Minimum sludge retention time (d)



11.
Denitrification

Denitrification rate (NUR)

Maximum denitrification rate by R test with sodium acetate

Based on bibliography, adapted to the R mode in the BM Respirometry

In principle, the advantage of this procedure is the relative shot time to get a reliable result

Procedure

Get 1 litre of sludge from the anoxic zone and pass it into endogenous phase (sludge should have a concentration in between 2 and 4 g/l MLVSS. If higher, the sludge should be diluted with distilled water.

Carry out the R test to obtain the yield coefficient (see procedure on page 13) and record the value (Y_H) – Optionally default value of 0,67 can be used -.

Make a solution of sodium acetate of 400 mg acetate in 1 litre of distilled water.

Take 50 mL of sample in a probe from the acetate solution.

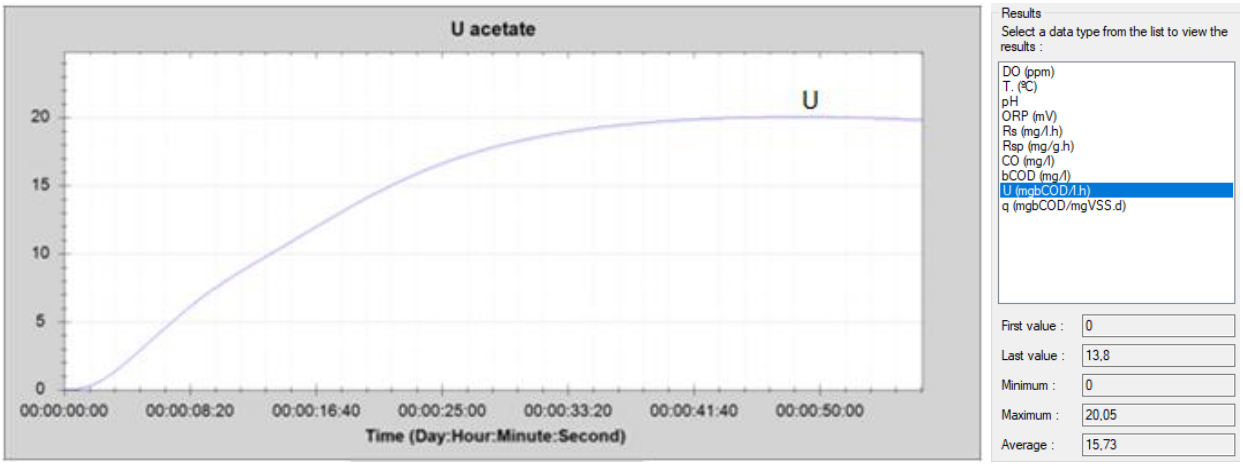
Prepare the R test by setting similar conditions of pH and Temperature as the real process.

Set sludge volume to 1000 (Vf = 1000) and sample volume to 50 (Vm = 50)

Start the test, taking special care of a stable initial baseline and adding (slowly) the 50 mL just at the time the test is asking for it.

Select the U value in the Legend option in order to obtin the respirogram of this parameter over time.

Stop the test when U (U) reaches its maximum value and record the result (it can be seen in the details window)



Calculate the NUR value, by applying the following formula:

$$\mathbf{NUR_{max}} = U_{max}\,(1 - Y_{HD}) / 2{,}86$$

$\mathbf{Y_{HD}}$ = 0,83 * Y_H: Yield coefficient for denitrification - **Habitual value $\mathbf{Y_{HD}}$** = 0,55 (mg O_2/mg COD)

Actual denitrification rate

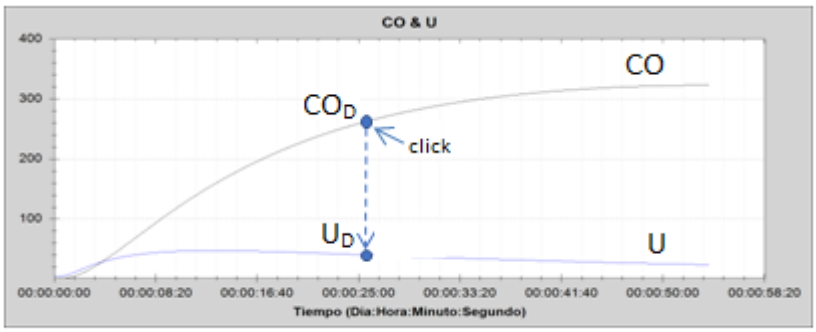
Same R respirometry test as for NUR_{max} is used for actual denitrificatio rate calculation.

In this case, the value of the cosumed oxygen should be first calculated:

$CO_D = 2.86 * S_{NO3}$

CO_D: Consumed oxygen for the actual denitrification (mg/L)
S_{NO3}: Nitrate to denitrify (mg NO_3/L)

From the bCOD test the CO and U respirogramas are overlied:



CO and U respirograms

Using the PC mouse, in the CO curve we look for the previously calculated COD value, and then the corresponding U (UD) value for the same period of time. Both values will appear in the bottom bar (attached to the Respirograms screen) – Those results can also be seen in the values table in the "Data" option –

The actual NUR is calculted as follows:

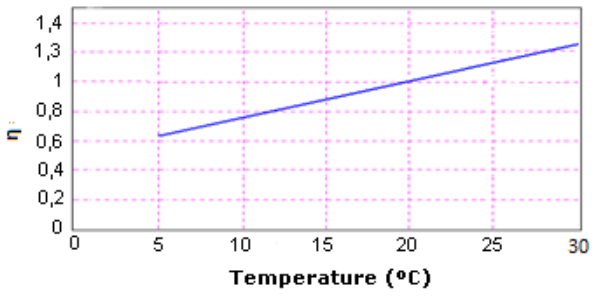
NUR = U_D (1 – Y_{HD}) * KO_D / (KO_D + OD_D)

Inhibition factor: KO_D / (KO_D + OD_D)
KO_D: Inhibition coefficient due to oxygen in the anoxic zone = 0,2 (mg/l) - Default value –
DO_D: Actual dissolved oxygen in the denitrification zone (mg O_2/L)

Sources: US-EPA, Henze et al 1987, Others

NUR correction by temperature factor

In the event that the NUR has previously been determined at 20ºC to transfer it to other temperatures, we can apply the temperature correction factor (η) by applying the following graph:



$NUR_{(Temp)}$ = η * $NUR_{(20ºC)}$

Sources: E.CHOI and R.DAEHWAN. 2000. Korea University - W.W. Eckenfekder & J.L. Musterman – 1995

However it is recommended to determine the NUR by setting the BM respirometry test at similar temperature to that of the actual process; and, in this way, no temperature correction should be necessary.

Specific denitrification rate

SNUR = 24 * NUR / VSS

SNUR: Specific denitrification rate [mg NO_3 –N / (mg VSS.d)] – SNUR is also called **SDNR** –
VSS: MLVSS (mg/L)

The SNUR or SDNR assessment can be done though the following reference table:

Estimated Specific Denitrification Rates

Temp ° C	Estimated SDNR	Temp ° C	Estimated SDNR
10	0.035	18	0.076
12	0.042	20	0.091
14	0.052	22	0.110
16	0.063	24	0.132

Source: Long Island Sound Training – Nitrogen Removal - 2003 (EPA)

Procedure to calculate the necessary rbCOD, BOD and COD for denitrification

!) Consumed oxygen for denitrification: CO_D

CO_D = 2.85 * S_{NO3}

S_{NO3}: Nitratate to denitrify (mg NO_3/L)

2) rbCOD for denitrification: $rbCOD_D$

$rbCOD_D$ = CO_D / (0.83 . Y_H)

3) BOD for denitrication: BOD_D

BOD_D =(BOD/rbCOD) * $rbCOD_D$

rbCOD: Readily biodegradable COD of the wastewater sampla (mg/L)
BOD: BOD_5 of the wastewater sample (mg/L)

4) COD for denitrification: COD_D

COD_D = (COD/rbCOD) * $rbCOD_D$

COD: Chemical Oxygen Demand of the wastewater sample (mg/L)

Theoretical Calculation of the methanol Load for Maximum Denitrification

Because organic carbon is consumed mostly in the extended aeration process it is often necessary to add a carbon source such as methanol, especially when the discharge requirements for total nitrogen are low.

The procedure to calculate the methanol load is as follows:

1. Calculation of the nitrate concentration to be denitrified (S_{NO3})

2. Determination of the actual rbCOD in the influent the denitrfication process by means a BM respirometry test.

3. Theoretical calculation of methanol loading for maximum denitrification

   (Methanol Load + current rbCOD Load) / Nitrate Load = 2.86

   Methanol Load = 2.86 x Nitrate Load – rbCOD Load

   Nitrate Load (kg NO_3-N/ d) = Q * S_{NO3} / 1000
   S_{NO3}: Nitratate to denitrify (mg NO_3/L)
   rbCOD Load (kg COD/ d)= Q * rbCOD / 1000
   Q: Influent flow to the denitrification process (m^3/d)

4. Liters of Methanol per day

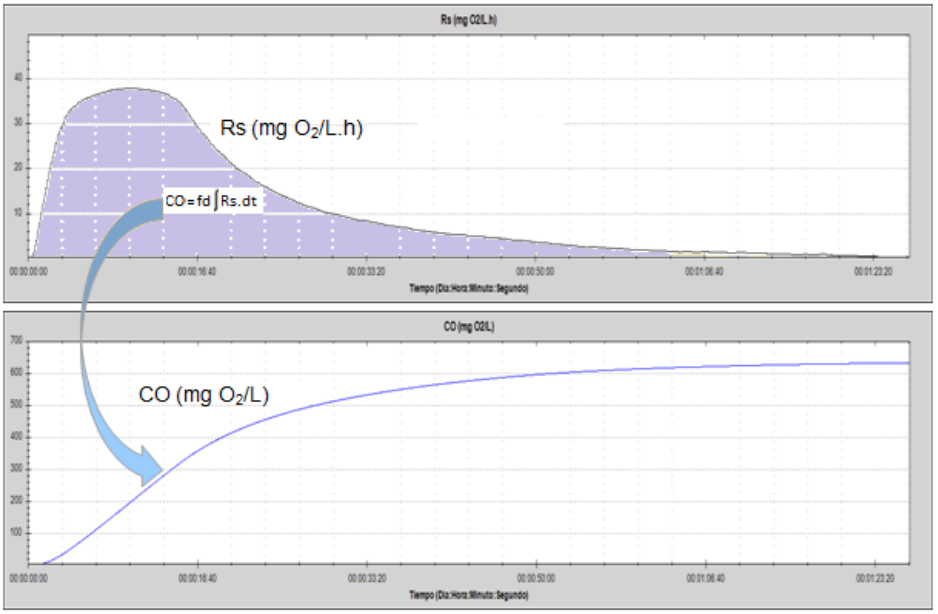
   1 liter Methanol = 1.2 kg rbCOD

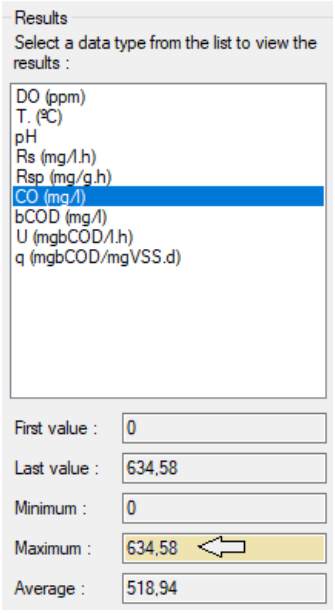
   Liters Methanol / day = Methanol Load / 1.2

12.
Aeration - Oxygen needs

Actual Oxygen Requirement (AOR) calculation in a process with nitrification-denitrification

Requirement for the carbonaceous organic matter (kg O_2/d): **AOR_C** = Q * CO / 1000





R test for bCOD

Requirement for endogenus respiration (kg O_2/d): **AOR_{end}** = 24 * V * OUR_{end} / 1000

Requirement for nitrification (kg O_2/d): **AOR_N** = 4.57 * Q * N_n / 1000

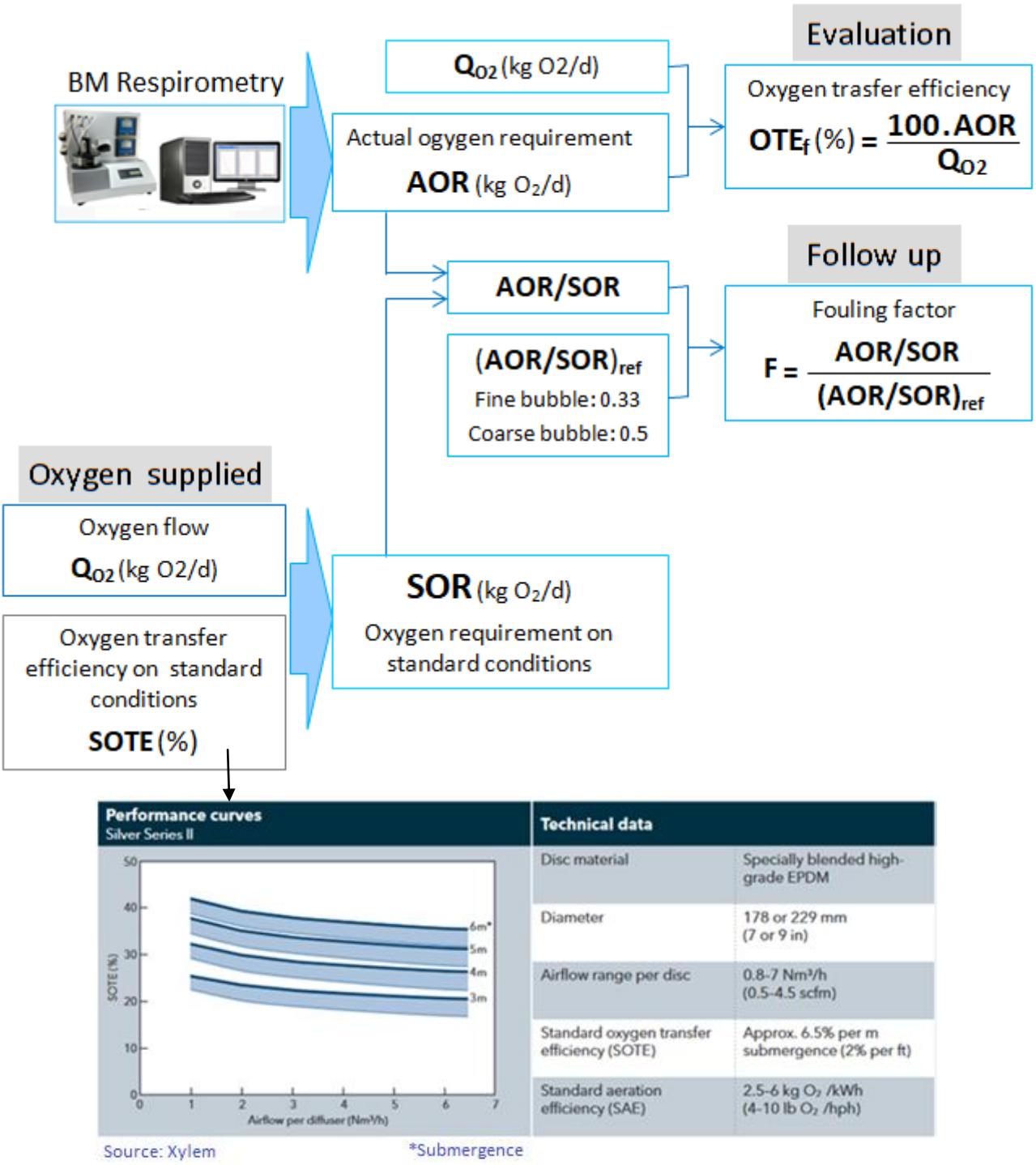
Requirement for denitrification (kg O_2/d): **AOR_{DN}** (kg O_2/d) = 2.28 * Q * N-NO_3 / 1000

Q: Influent flow (m^3/d)
CO: Consumed oxygen for the eliminated organic matter (m^3/d) - from R test for bCOD -
V: Aerobic reactor (m^3)
OUR_{end}: Oxygen uptake rate of the sludge under endogenous phase (mg O_2(L.h)
N_n: Ninitrogen for nitrification (mg N/L) ≈ NTK eliminated (mg N/L)
N-NO_3: Nitrate for denitrification (mg N-NO_3/L) ≈ N_N – N-NO_3 efluent

The oxygen requirement by denitrification (AOR_{DN}), performed under anoxic conditions, is presented as a credit against the total oxygen requirement.

AOR = AOR_C + AOR_{end} + AOR_N - AOR_N

Practical procedure to evaluate a diffused aeration system



When there is a progressive reduction in OTEf and the F is less than 0.7, it is most likely that the diffuser aeration system requires maintenance for membrane cleaning or replacement of diffusers (when > 4 years old)

13.
Toxicity

Toxicity in activated sludge

The only one toxicity of our interest is the one that could damage or activated sludge process, and never any other one where the activated sludge is not present. For that reason, this toxicity can be only assessed from the own activated sludge in one specific process.

From this evidence we can approach the toxicity in two main ways:

- Possible toxicity already in the activated sludge process (aeration tank)
- Possible toxic reaction of a waste water or compound with the activated sludge; but toxicity is not in the activated sludge process (aeration tank)

Symptoms for inhibition or toxicity that is already in the activated sludge process

Besides the external physic symptoms, by respirometry we can guess that the ASP could be under inhibition or toxicity state when we detect the following:

FED OUR / UNFED OUR < 1,3

UNFED OUR < range; and however COD in the final effluent is high.

$\mathbf{q_H}$ << F/M (rbCOD)

Toxicity detection by progressive substrate concentration increasing

To the activated sludge we add a substrate (S) in order to create a maximum respiration rate as high reference level.

IMPORTANT
The reactor sludge must be free o f any kind of Toxicity.
In case the sludge has got any toxicity, the application should be carried out by means another healthy sludge (from another plant) of similar features.

For process with nitrification
S = [½ g acetate] / g VSS + 100 mg ClNH4, in 5 mL distilled water.

For a process without nitrification
S = [½ g acetate] / g VSS, in 5 mL distilled water.

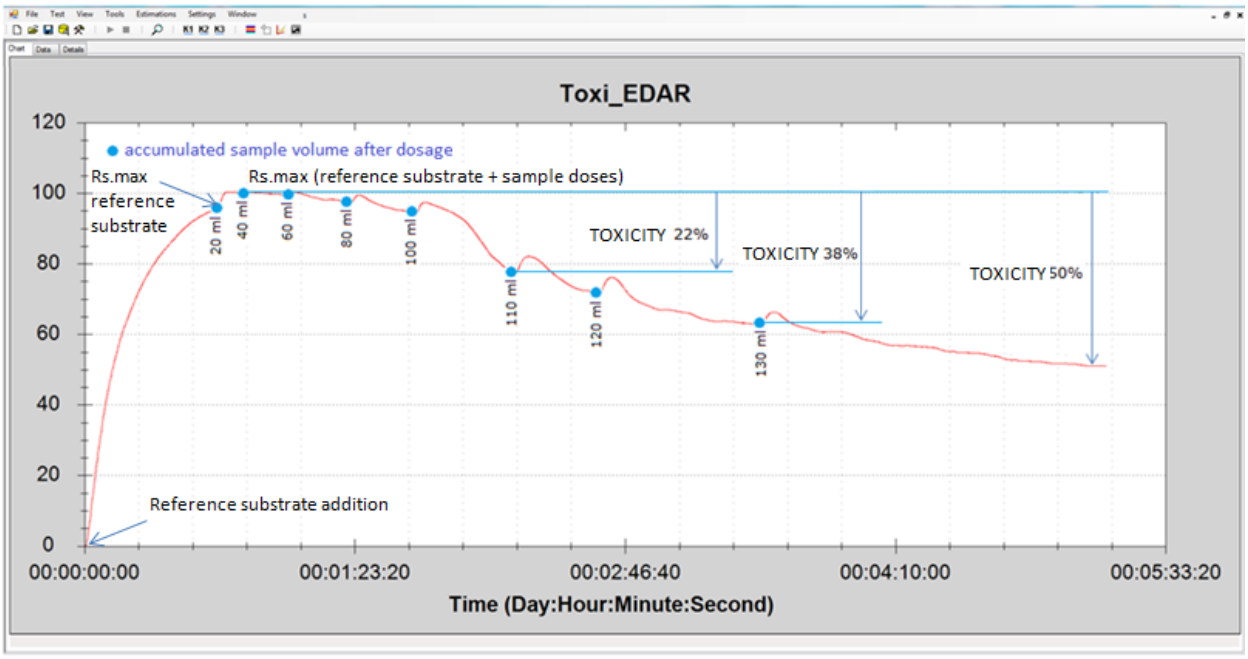
Once we have reached the maximum respiration rate (Rs.max from reference substrate) we add progressive doses of aliquot (*) in order to progressively increase the substrate concentration in the activated sludge.

(*) aliquot: sample --> starting with 1 mL sample + 5 mg acetate.

In case of toxicity, as we are sequentially adding a serial of doses: increasing progresivelly from 1 mL to 2 → 10 → 20 mL sample + 5 mg acetate.

It may happen that the Rs increase at first. However, as soon as the toxic effect is detected, the respiration rates on the respiragram will begin to progressively decrease.
We can then stop the test as soon as we have reached the target % inhibition or at any time we want.

Normally this is a screening analysis. However, by calculating the equivalence volumes corresponding to the plant's biological reactor, this procedure will give an assessment of how much toxic substrate the activated sludge can support.



I = 100 (Rs.max – Rs.tox) / Rs.max

I: Toxicity (%)
Rs.max: Maximum Rs after adding the reference substrate and aliquot doses.
Rs.tox: Rs below Rs.max causing toxicity

Global toxicity in wastewater analysis by comparison of two or more mixed liquors with a reference by means R tests with standard.

The analysis is carried out by means of two mixed liquor preparation by returned sludge (running normal)

Equivalent mixed liquors should maintain the same ratio of the actual process.

Qo/Qr = V (sample) / V (RAS)
Qo: Influent flow to reactor (m^3/h)
Qr: Recirculation sludge flow to reactor (m^3/h)
RAS: Returned Activated Sludge

Preparations:

1 litre ML ref: V returned sludge (RAS) + V (ref)

1 litre ML sample: V returned sludge (RAS) + V (influent)

ML ref: Reference mixed liquor
ML sample: Mixed liquor to analyze
V returned sludge WWTP: Volume of returned sludge from WWTP
V (ref): Volume of distilled water + reference substrate (sodium acetate)
V: Volume of influent to be analyzed

IMPORTANT

The reactor and RAS sludge must be free of any kind of Toxicity.
In case the sludge has got any toxicity, the application should be carried out by means another healthy sludge (from another plant) of similar features.

V (ref) and V should have similar COD. If not, we add sodium acetate in order to readjust the value to the other. For that, we base our corresponding dose by taking into account that approximately 100 mg acetate / litre make 75 mg COD/L.

In order to get the endogenous respiration in ML (ref) and ML sample, we leave them aerating and mixing during at least 24 hours (it could be by means of a simple aquarium aeration system)

Procedure

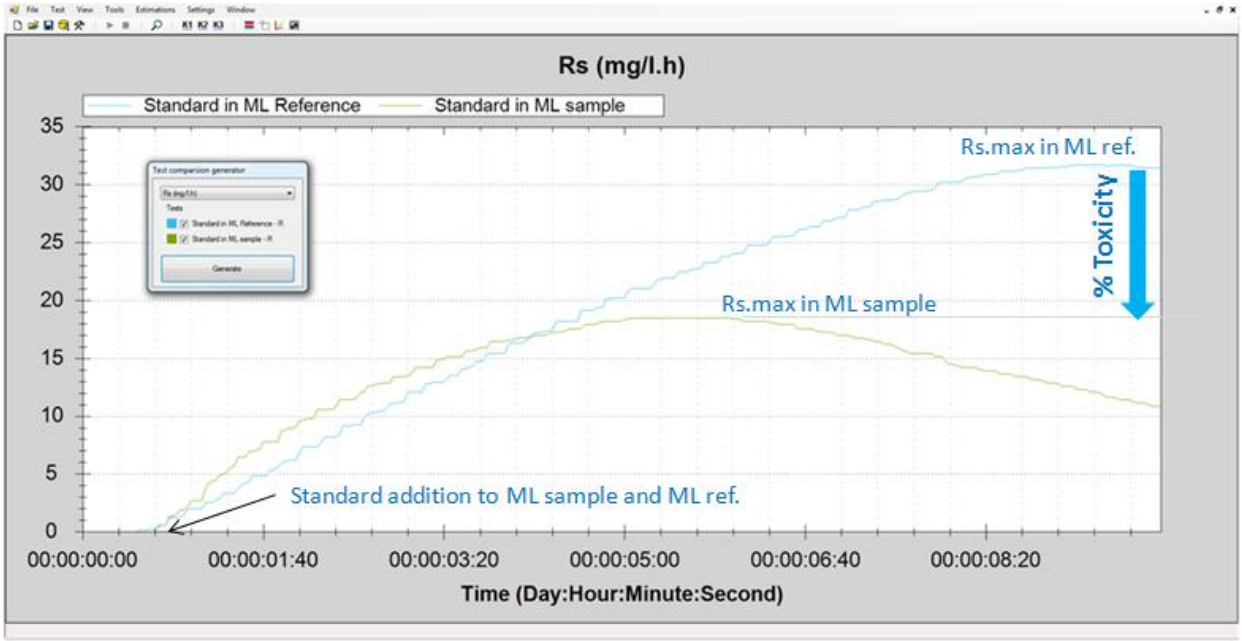
After the 24 hours of conditioning time, from ML (ref) and ML we carry out two R tests, by adding the same amount of a standard substrate (S)

S = [½ g acetate] / g VSS in 10 mL distilled water.

First, we do the reference R test [V returned sludge WWTP + V (ref) + S] and we stop the respirogram as it reaches its maximum respiration rate (Rs.max in ML ref)

Then, we do the sample respirogram [V returned sludge WWTP + V + S] and stop the respirogram as it gets the corresponding maximum respiration rate (Rs.max in ML sample)

Once obtained the corresponding respirograms, by making use of the BM software, we overlay them and analyze the Rs.max from reference on respect to the current Rs from sample test within the same time.



We calculate the % of inhibition / toxicity from the difference between RsMax in the reference test and the corresponding value of Rs in the sample test, at the same time.

I = 100 (Rs.max ML ref. – Rs.max ML sample) / Rs.max ML ref.

Specific toxicity for nitrification

The procedure is practically the same as before, but instead of sodium acetate we have to make only use of ammonium chloride in a concentration similar to the one in actual process. For that, we take into account that to calculate the actual ammonium concentration we have to multiply the mg of ammonium chloride by 0,26.

Once we have left aerating both mixed-liquors during at least 12 hours, we carry out the R tests by adding a substrate (S) composed by ammonium chloride:

S = [100 mg ClNH4] / g VSS, in 10 mL distilled water.

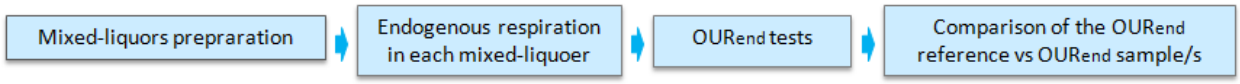
We calculate the % of inhibition / toxicity (I_N) specific for nitrification from the difference between Rs_{max} in the reference test and the corresponding value of Rs in the sample test, at the same time.

$$\mathbf{I_N} = 100 * (1 - Rs/Rs_{max})$$

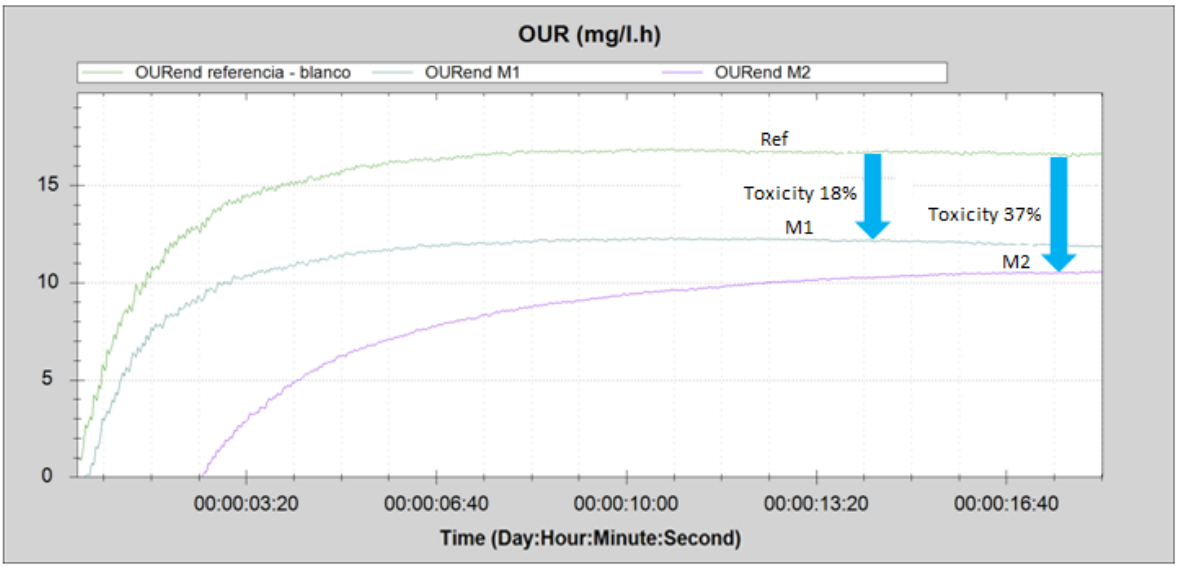
I_N: Nitrification toxicity (%)

Toxicity to the global biomass by OUR_{end} tests

With this procedure the global toxicity is assessed by comparison of the OURend of one o several mixed-liquors samples with the one for reference, all under endogenous respiration phase.



The preparations and procedure is similar to the global toxicity before described but doing endogenous OUR instead of R test and not adding any standard.

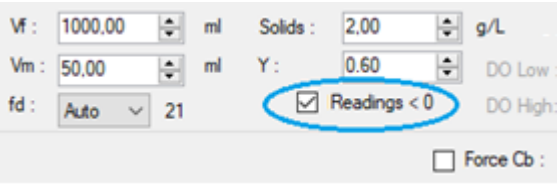


I (%) = 100 * (OURend.ref. – OURend sample M) / OURend.ref

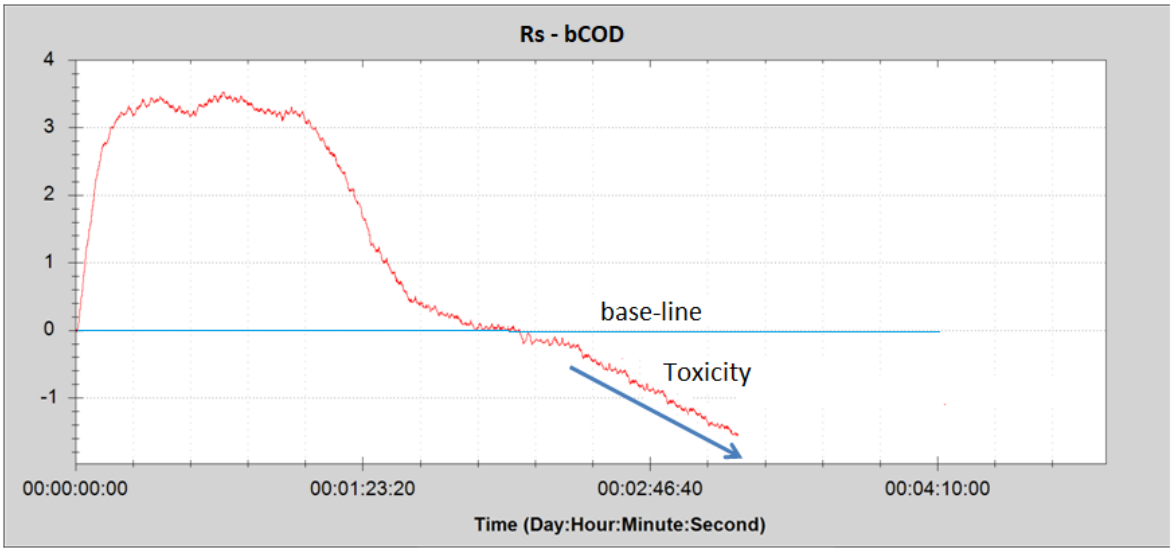
Toxicity detecion during a bCOD test

What theres is an abnormal low bCOD we can suspect that it could be a symptom of toxicity.

To check ths possibility we can modify the test by clicking od Test → set the Rs Readings < 0 → Accept and see ths pssible Rs vaiues below base line.



When the Rs exceeds the baseline and falls with a clear progressive negative slope, the test is indicating the possibility of the presence of a toxicity caused by the sample for the Vm/Vf ratio in with which the test has been performed.



14.
Conclusion

Conclusion

On this manual there are not all the applications that can be carried out with a BM-Respiromer.

BM-Respirometers are open systems and for the complexity of the different ASP types there are a huge number of applications that can be done and much more that you can discover or invent.

Each ASP is specific and many times we have to study the best daily and periodical protocol of tests in order to control and protect the process.

By means of the BM respirometer, we can also perform important studies, to develop specific control programs, D & R, support simulation software …

In case of interest of any application not related in this manual or any other consult related with BM-respirometry you can be in contact with SURCIS

By other side, SURCIS, besides manufacturing the BM analyzers, as Respirometry Engineering very much specialized in activated sludge processes, is in the best condition to act as Consulting to give a full technological customer support and perform Respirometry Services directly or associated with other or consulting, university or research centre.

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''Be open minded, but not so open minded that your brains fall out".
Groucho Marx