Metrological innovation in regulatory water analysis

Feedback on the 5-days Biochemical Oxygen Demand (BOD5)
The BOD parameter – environmental context

- Closely related to the influence of organic pollution on the level of dissolved oxygen in surface water
- Mineralization of organic matter by aerobic bacteria causes dissolved oxygen depletion
- Excess of organic pollution can decrease the biodiversity and lead to non-drinkable/unusable water

Feedback on the 5-days Biochemical Oxygen Demand (BOD5)
The BOD parameter – historical context

- **Index of water quality** (freshwater and wastewater) = biodegradable organic pollution
- BOD/COD = wastewater biodegradability
- Suggested by the **Royal Commission on Sewage Disposal** in 1912 in UK
- Initially, dissolved oxygen uptake after 5 days at 18.3°C (standardized later at 20°C) = BOD5
- Early, the time and temperature of incubation were sharply criticized
- Alternative incubation periods (2 or 7 days) and higher temperatures (27°C) were suggested in 1913
- Today, **BOD is used worldwide**: surface water & treatment plant monitoring, tax amount calculation
The BOD parameter – principle

- The BOD test is based on the **mineralization of organic matter by aerobic bacteria** which requires oxygen at the **final metabolic step = respiration** (like in the natural ecosystems).
- BOD depends on the **time** and **temperature** according the well-known biochemical model:

\[
\text{BOD}(t,T) = \text{BOD}_u \cdot \left( 1 - e^{-k(T) \cdot t} \right)
\]

with:
- \( \text{BOD}(t,T) \) = BOD after the \( t \) time at the \( T \) temperature
- \( \text{DBO}_u \) = ultimate BOD ; intrinsic to the sample
- \( k(T) \) = reaction rate at the \( T \) temperature
- \( t \) = time of incubation

\[
k(T) = k(20^\circ C) \cdot \theta (T - 20^\circ C)
\]

with:
- \( k(20^\circ C) \) = reaction rate at 20°C
- \( \theta \) = temperature coefficient
- \( T \) = temperature of incubation

- Standardized conditions are **5 days** at **20°C** in darkness (cf. NF EN 1899, ISO 5815 or SM 5210 B)
- Excepted in Nordic and Baltic countries - **7 days/20°C** = BOD7 - and India - **3 days/27°C** = eq. BOD5

**Dissolved oxygen measurement**

*Previously...* titrimetric analysis
*Usually...* electrochemical probe
*And soon...* optical probe

**Organic matter + O₂**

\( \text{CO}_2 + \text{H}_2\text{O} \)

*Metabolic reactions*

\( \text{Bacterial respiration} \)

**Bacterial inoculum**

+ Buffer
+ Sample
or Quality Control

\( \text{O}_2 \) limitation
A innovative idea – 2003-2009

- Results from collaborative academic research was the first step of the story

- A new way to measure organic matter biodegradability by soil bacteria using the resazurin
- Intensity of the bacterial respiration ($O_2$ uptake) can be revealed by fluorescence measurement
- Reaction and measurement can be carried out in a 96-wells microplate
- European patent n° 1844330 in 2007 and scientific publication by Dudal et al. in 2006
A innovative idea – 2003-2009

- measuring organic matter biodegradability through bacterial respiration… it looks like BOD!
- Is this innovative analytical solution could be used for BOD5 measurement in water sample?
- The question was investigated along with a project of business creation based on it
- This project was supported at the local and national scale and benefited from a grant:

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2010 – The Envolure company is founded

- The goal was to turn the original idea into a reliable product which could be commercialized

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Exclusive license agreement on European patent EP 1844330

Business development unit
From the idea to the product – 2010-2013

• R&D program to adapt, improve and validate the invention for BOD measurement
• Numerous supports at the local, national and European scale :

- Industrial partnership with the SIAAP
  Public sewerage service of the greater Paris
  Since 2012 and in the framework of the Mocopee research project (2014-2017)
  Test, improvement and validation of the prototype by end-users

• Finally, new innovative solutions & new patent applications: EP 2597461 (2011); WO 2016184848 (2015)
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Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

From the idea to the product – 2010-2013

- Final product is a ready to use analytical kit requiring a basic fluorescence microplate reader
- Main advantages are: easy-to-use; space-saving; rapidity; low investment and operating cost

**Bacterial inoculum**

+ Buffer
+ Sample

**or calibration standard**

or **Quality Control**

Unlimited O₂

**Incubation at 30°C for 48h in darkness**

**Organic matter + O₂**

**Metabolic reactions**

**Bacterial respiration**

- **CO₂ + H₂O**

**BOD5 (mgO₂/L)**

Fluorescence converted in mgO₂/L thanks to standard solutions of known BOD5

Feedback on the 5-days Biochemical Oxygen Demand (BOD5)
Scientific and technical validation – 2014-2016

- Enverdi® BOD values are statistically equivalent to BOD5 values obtained from standard methods.
- According to criteria of the ISO/TS 16489 standard; comparison versus NF EN 1899-1 & SM 5210 B.
- Nearly 400 samples collected and analysed by 6 laboratories (using 6 different inocula) in Fr & USA.

For 98% of the samples, results are strictly similar.

Communications:
Magnin et al. 2015 “Validation of a BOD5 alternative method” 17th International Congress of Metrology, Paris (Fr).
Scientific and technical validation – 2014-2016

- Analytical specifications of the Enverdi® BOD method are in compliance with regulatory requirements
- According to criteria of standards in force for water quality: NF T90-210 and NF ISO 11352
- Specifications were ascertained by 2 laboratories (using 2 different inocula) in Fr

Analytical specifications of the Enverdi® method are comparable to the specifications of the European standard NF EN 1899-1

<table>
<thead>
<tr>
<th>Limit of quantification</th>
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<td>in mgO₂/L (according to NF T90-210)</td>
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<tr>
<th>Confidence interval (95%)</th>
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<th>Confidence interval (95%)</th>
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<table>
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<tr>
<th>Accuracy verified at</th>
<th>± 30</th>
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<tr>
<td>in % (according to NF ISO 11352)</td>
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Scientific and technical validation – 2014-2016

- Enverdi® BOD results match the average values obtained by tens of labs on the same samples
- According to criteria of the NF EN ISO 16140-2 standard
- 3 ILCs (inter-laboratory comparisons) based on real wastewater samples without additional spiking

In all case, z-score < ±1

(-2 > compliant results < 2)
Scientific and technical validation – 2014-2016

- No chemical interference detected by any cation, anion or organic surfactant at concentrations expected in municipal wastewater samples (according to NF T90-210)

- Under Enverdi® conditions, influence of nitrifying bacteria is negligible, even without ATU addition

- Enverdi® is responsive to the particulate BOD as shown by results from the Mocopee project or results obtained by Nehmtow et al. in 2016 regarding pig manure samples with high particle content

**Feedback on the 5-days Biochemical Oxygen Demand (BOD5)**

![Graph showing particulate BOD and soluble and colloidal BOD](image-url)
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Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

Scientific and technical validation – 2014-2016

Field of application and main limits

- **like the standard method**, Enverdi® BOD result is **sensitive to bacterial inhibitors and pH**
  ⇒ If necessary, sample must be diluted and/or pH adjusted between 6 and 8

- **The Enverdi® BOD method is suitable for analysis of municipal wastewater samples**
  ⇒ Analysis of industrial wastewater samples should be considered with caution and should
  require a preliminary study ;
  However, up to now, satisfactory results were obtained with agri-food, farming and paper mill effluents
  ⇒ The current version of the Enverdi® BOD kit is **not sensitive enough for analysis of surface water** ; particularly, the LOQ at 0.5 mgO₂/L is not guaranteed

Adaptation to surface water analysis is under development ; a new version of the kit is expected for 2018

- **Commercially available bacterial seeds are not compatible** with the Enverdi® BOD kit
  ⇒ Currently, a natural source of bacteria must be used as inoculum

Development of a compatible standardised inoculum is under consideration
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Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

Regulatory and normative obstacles

• Result equivalence, high performances and widely recognized advantages
  ⇒ Not sufficient for a routine use by laboratories

• Regulatory framework is highly restrictive regarding the analytical method used... whatever the performances and the valuable advantages of the surrogate method

Directive 91/271/EEC
BOD5 i.e. 5 days/20°C is pointed out
No mention of particular standard

BOD7 is used in Finland, Sweden and Lithuania
Standard method 5210 D is allowed in Italy

Decree 21/07/2015
The NF EN 1899 standard method is pointed out

Other methods are not allowed

• No procedure in France (in EU ?) to apply for the regulatory acceptance of an innovative method

Lack of support from public authorities at the crucial step of the technological transfer and innovation adoption by end-users
Regulatory and normative obstacles

- Regulatory and normative framework guarantees the quality of analytical results but...

  ... excessive rigidity of regulations and standards constitutes a real barrier for innovation and business development in the field of water analysis

- Main Risks: slowdown of analytical progress and bankruptcy of innovative SME along with job losses
  
  ⇒ At the end of 2015, the Envolure company is bankrupt and taken over by the international AMS Alliance group leading to the creation of the AMS Envolure company
Needs and solutions

- Metrological innovation in regulatory water analysis needs a **regulated flexibility**.
- The goal is to guarantee **reliable control of water quality** together with **innovation support, analytical progress and business development**.
- This regulated flexibility requires a **clear procedure for validation and regulatory acceptance** of innovative methods... both, at the **national and European scales**.

Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

Tools and rules to validate and verify that surrogate methods match the regulatory needs, already exist = **national, EN and ISO standards / national accreditation & certification procedures**
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Needs and solutions

- Different approaches are conceivable…
  ⇒ at the national scale

  Validation by an independent 3rd party according to specifications defined by public authorities
  Regulatory acceptance on a general basis
e.g. ETV program

  Validation by end-users under the control of an accredited body (like COFRAC in France) according to specifications defined by public authorities
  Regulatory acceptance on a case-by-case basis
e.g. accreditation/certification program

⇒ at the European scale

Assessment & validation by EU authorities
European directive update
Harmonization at the EU scale

National initiative
National initiative
National initiative
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Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

Needs and solutions

• A new tool available in France since 2016 - France Experimentation

• French authorities became aware of the shift between innovation process and regulatory adjustment as well as the resulting difficulties met by innovative SME

• Because innovation highly contributes to the economic growth, the goal of this program is to dissipate the regulatory obstacles for promoting the development & transfer of innovation in France

• Based on the article n° 37-1 of the French constitution, the program proposes the adoption of experimental and temporary derogatory decrees in order to assess the effect of innovative methods, processes or services at the national or regional scale.

• If the experiment is conclusive, the innovation can be fully and durably included in French regulations

• Only the French regulations are affected (decrees need to be in agreement with EU directives)

• Similar programs exist in UK, Japan and Australia

We could also imagine an “EU Experimentation” program
Feedback on the 5-days Biochemical Oxygen Demand (BOD5)

**Needs and solutions**

- **French experimentation to come** with the Enverdi® BOD method
- Publication of the derogatory decree in **May-June 2017**; duration = **2 years**
- Samples = inlet/outlet of municipal wastewater treatment plants
- In agreement with the **91/271/EEC** directive

<table>
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<tr>
<th>STP category according to EU (PE)</th>
<th>2 000-9 999</th>
<th>10 000-49 999</th>
<th>&gt; 50 000</th>
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<tr>
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<td>&lt; 199 999</td>
<td>&lt; 299 999</td>
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**Current regulations**

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<td>12</td>
<td>52</td>
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<td></td>
<td>24</td>
<td></td>
<td>156</td>
<td>365</td>
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</table>

**Derogatory regulation about the analytical method**

<table>
<thead>
<tr>
<th></th>
<th>Standard method only (nb/year)</th>
<th>Standard or Enverdi method (nb/year)</th>
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<tbody>
<tr>
<td>Control frequency</td>
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<td>0 1\textsuperscript{st} year/6</td>
</tr>
<tr>
<td>set by Fr (nb/year)</td>
<td>12</td>
<td>0</td>
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About **50 %** of the samples could be analysed according to the Enverdi® method in 2/3 of the French hydrographic districts

- The decree precisely details:
  - the **prerequisites needed for involved laboratories** (accreditation on the standard method)
  - the **analytical validations & quality controls required to substitute** the standard method with Enverdi®
  - the **terms and conditions of the experimentation monitoring and assessment** by public authorities
To keep in mind

Support from regional, national and European authorities are efficient at the beginning of an innovative project (research and development stage).

Collaboration between SME and public research institutes based on an exclusive patent license agreement is a fruitful strategy.

Collaboration with industrial partners for end-user assessment and validation is essential for technical success of the project.

The major criticism is about the regulatory acceptance of inventions by public authorities which is vital to ensure a successful end-user adoption and business development.

The shortcoming at this particular stage should be fixed by including a reasonable flexibility in the national and European regulations in order to promote innovation in the field of regulatory water analysis.
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