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A bioelectronic tongue to estimate the toxicological intensity of pollutants in wastewater treatment plant

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ABSTRACT

With 360 km³ of wastewater produced each year in Europe, the management and control of units responsible for their treatment appear as major challenges in preserving the environment. Nevertheless, these processes remain vulnerable to the presence of toxic compounds likely to compromise their performance. Although many toxicity tests exist to evaluate the impact of pollutants on the environment, these are generally not easily transferable to the monitoring of wastewater treatment processes (constraints of implementation, representativeness of the information provided).

This innovative project leverages the concept of a "bioelectronic tongue" integrated into a biosensor to evaluate the toxic impact of pollutants on wastewater treatment plant (WWTP) microbiomes. This work presents a holistic approach, covering the entire process from the selection of representative microorganisms to in situ application. The strategy hinges on the synergistic implementation of 8 bioreporters, coupled with a data processing algorithm to generate relevant toxicity assessments. In parallel, a significant focus was placed on developing a biosensor optimized for in situ deployment of this innovative measurement strategy.

The developed approach (TOXLAB) has been compared to reference methods currently used to assess the toxicological intensity of effluents. As expected, significant differences were highlighted between the standardized methods, particularly the method based on marine bioluminescent bacteria (lack of representativeness – 9 out of the 11 tested conditions could not be quantified with this method). However, the results provided by the TOXLAB approach show a certain adequacy with the toxicity data obtained on the urban WWTP microbiome. On the other hand, the results obtained on the industrial site (34 samples) are much more contrasted. Indeed, no correlation ($r^2 = 0.033$) could be established between the data from the TOXLAB approach and the effects induced on the autochthonous microbial community of the site. To explain these results, the work focused on the composition of these specific ecosystems, thus showing important differences between the microbiomes of WWTPs and the industrial site. Thus, in light of these results, the conclusion of the study shows the need to use a specific set of bioreporters, dedicated to each industrial site, in order to ensure a relative representativeness of the information provided.

1. Introduction

Wastewater treatment is a major challenge for modern societies, both in terms of public health and environmental protection. According to a 2021 study by Jones et al. (2021), the annual global production of domestic wastewater in Europe is around 360 km³. Of this total production, 52 % is treated by domestic wastewater treatment units such as activated sludge plants.

Operators of these facilities are subject to performance targets (regulations related to the quality of discharges into the environment)

focusing on certain classes of major pollutants (organic matter, nitrogen, phosphorus) (European communities, 1991). However, achieving such levels depends directly on the quality of the treatments implemented, primarily based on biological processes (Prochaska and Zouboulis, 2020; Sikosana et al., 2019). The presence of toxic molecules (accidental or intentional contamination of wastewater with toxic agents) can be detrimental to the microbial flora involved in these pollution control processes (aerobic heterotrophic, denitrifying, nitrifying, dephosphorylating flora, etc.) (Madoni et al., 1996; Nyberg et al., 2008; Papadimitriou et al., 2007; Principi et al., 2006) and potentially lead to a

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decrease in the efficiency of these processes (Grau and Da-Rin, 1997). In this context, assessing the toxicity of inputs seems necessary (Hernando et al., 2005) to protect facilities, guarantee the qualitative compliance of discharges, and protect the receiving environment.

Currently, the management of wastewater treatment plants relies on physicochemical indicators related to the pollutant load. These measurements, based on parameters such as total organic carbon, chemical/biological oxygen demand (COD/BOD) (Hernando et al., 2005; Jouanneau et al., 2019; Liwarska-Bizukojc et al., 2016), are not sufficient to predict the potential toxic effect of inputs on the activated sludge biomass involved in the pollution control action.

The most commonly used methods (Ren, 2004; Xiao et al., 2015) for measuring toxicity of inputs in wastewater treatment plants are based on four reference standards, i.e., ISO 11348 - inhibition of bioluminescence of *Aliivibrio fischeri* (Hernando et al., 2005; Libralato et al., 2010; Tobajas et al., 2016; Vasquez and Fatta-Kassinos, 2013), ISO 10712 - inhibition of growth of *Pseudomonas putida* (Tobajas et al., 2016), ISO 6341 - inhibition of mobility of *Daphnia magna* (Hernando et al., 2005; Ra et al., 2007; Vasquez and Fatta-Kassinos, 2013) and ISO 8692 - inhibition of growth of *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum* or *Pseudokirchneriella subcapitata*) (International Organization for Standardization, 2012). These tests rely on the toxicological resistance capacities of a single bioreporter to assess the toxicity of effluents. Although reproducible, these approaches are limited in terms of interpretation since the correlation between the effect highlighted by these tests and the real effect on a complex microbial community of a wastewater treatment plant is not evident (Hernando et al., 2005; Kefford et al., 2023; Liwarska-Bizukojc, 2022; Liwarska-Bizukojc et al., 2016; Spurgeon et al., 2020) (representativeness of the bioelements in relation to the "activated sludge" community).

NCIMB (National Collections of Industrial, Food and Marine Bacteria Ltd., UK) addresses this limitation by proposing two alternative toxicity assessment methods based on a controlled set of microorganisms, ensuring high reproducibility. In the case of the "Microbial Assay for Risk Assessment" (MARA) test, the approach is based on a selection of 11 microbial strains (10 bacterial strains and 1 yeast strain) and evaluates the intensity of the toxic effect through the inhibition of their cell growth. According to the authors (Wadhia et al., 2007), the strains were selected to ensure taxonomic diversity and variability in response to different toxicants (Gabrielson et al., 2003), however, the criteria used are not explicit. In the case of the LumiMARA test (Jung et al., 2015), the strategy is essentially the same as the MARA test except that it only involves bioluminescent bacterial strains. In this case, the test focuses on the inhibition of bioluminescence to estimate the toxic effect. These approaches, while promising, present two major limitations. Firstly, each bioreporter provides a specific toxicity value (i.e. 11 different toxicity measurements) that can vary significantly due to the intrinsic sensitivity of each bioreporter. The first limitation therefore lies in the interpretation of these multi-specific data. In other words, how can we interpret the potentially very different (even contradictory) data provided by the 11 bioluminescent strains in order to propose a single toxicity value? In addition, the bioreporters used were selected from very diverse environments (marine, freshwater, terrestrial...) to ensure a certain genetic diversity. Thus, the second limitation concerns the representativeness of the bioreporters. Indeed, in light of the toxicity levels induced by a given pollutant on the different bioreporters, it is risky to predict the toxic impact on the microbiome of a wastewater treatment plant.

Additionally, to increase representativeness, another measurement strategy relies on the inhibition of oxygen consumption by activated sludge (method described in ISO 8192 or OECD 209). This strategy allows to overcome the limited representativeness of monospecific tests by directly using an environmental microbial community such as indigenous biomass (Gutiérrez et al., 2002; Ricco et al., 2004). Studies show, however, a variability of the results obtained, inherent to the biomass used (Ricco et al., 2004). These methods ultimately remain reserved for

analytical laboratories and are generally not widely deployed within wastewater treatment units (Love and Bott, 2000) by site personnel, mainly due to the technical constraints associated with their implementation (lack of training, human resource deficit, time required for protocol implementation). Therefore, autonomous biosensor solutions have been developed to assess the toxicity of effluents in situ. Among commercial solutions, several devices have been identified such as the NitriTox (according to DIN EN ISO 9509:2006 based on activated sludge, LAR Process Analysers AG) or the EZ7900 Analyzer (respirometric test based on activated sludge, Hach Lange). However, to our knowledge, no articles related to the use of such systems for wastewater monitoring have been identified in the scientific literature.

Building upon the concept of "electronic tongues" as applied to other environmental sectors (Cetó et al., 2016; Jouanneau et al., 2019; Raud and Kikas, 2013), we propose a novel approach utilizing a panel of selected microorganisms representative of activated sludge (receiving pollution) but with known, acceptable numbers, physiological characteristics, and toxic sensitivity for integration into a biosensor. Electronic tongues are bio-inspired analytical tools coupling (bio)sensors and chemometrics. According to the agreed IUPAC definition (Vlasov et al., 2005), an "electronic tongue is a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on the pattern recognition and/or multivariate analysis (artificial neural networks (ANNs), principal component analysis (PCA), etc.)". This hybrid strategy combines the advantages of using pure strains (reproducibility, controlled protocols) and microbial communities (biological diversity, representativeness). This strategic choice complicates implementation and data interpretation (combined interpretation of data from individual strains), necessitating the development of a dedicated biosensor to handle this increased complexity.

In a previous study, exploiting the concept of the electronic tongue, a panel of microorganisms was proposed to achieve a different objective, i.e., the measurement of Biological Oxygen Demand (BOD₅) (Jouanneau et al., 2019). In this study, a culture collection was established, composed of several bacterial strains ($n = 23$), exclusively isolated from activated sludge from domestic wastewater treatment plants, and meeting a number of criteria related to biosensor deployment (aerobic, non-pathogenic, capable of withstanding lyophilization). These strains were also characterized in terms of sensitivity to 23 toxic stresses and respiratory kinetics. This culture collection, along with the various sources of information, served as the starting point for the present study, which aims to answer the following question: Is it possible to select a panel of bacteria isolated from activated sludge whose sum of toxic sensitivities is close to that of a wastewater treatment plant inoculum?

For the first time, this work presents a comprehensive approach, covering the entire process from the selection of representative microorganisms to in situ application, including the development of a dedicated biosensor. It highlights the importance of carefully selecting and adapting bioreporters to the target ecosystem, namely the microbiome of a wastewater treatment plant.

2. Experimental section

2.1. Bacterial strains

2.1.1. Bacterial strains

The list of the 23 preselected strains used in this project is provided in the supplementary data (Table SD-1). This preselection, resulting from a previous study (Jouanneau et al., 2019), is composed of non-pathogenic bacterial strains identified as representative of the microbiome involved in domestic wastewater treatment processes.

2.1.2. Characterisation and selection of representative bacterial strains (bioreporters)

Representative strains (bioreporters) were selected based on (i)

phenotypic criteria and (ii) a study of the respiratory capacities of bacterial strains in the studied environment (wastewater). The protocols used, as well as the raw data, are from a previous work and are detailed in the publication by [Jouanneau et al. \(2019\)](#).

The resistance capacities of the bacterial strains (i) were characterized by 23 toxic stress conditions (see Table SD-2) using the BIOLOG GEN III approach. Thus, from the collected data ([Jouanneau et al., 2019](#)), a toxicological resistance index (IdR) ([Thouand and Jouanneau, 2019](#)) was calculated for each tested strain based on the number of inhibitory conditions reported over the total number of tested conditions. This individual index varies between 0 and 1, with 0 being the value assigned when the strain resists none of the tested toxic stress conditions, and 1 being the value assigned to bioreporters that have shown the ability to maintain metabolic activity under all imposed conditions. In other words, the higher the toxicological resistance index, the more resistant the tested strain is to the conditions to which it has been exposed.

The objective of the second characterization phase (respiratory capacity) (ii) was to select bioreporters capable of providing a rapid and significant biological signal to quickly determine the toxicity level of the analyzed sample. To achieve this, the selection was based on respiratory kinetics (the toxicity assessment criterion in this study), evaluated by respirometry. The 23 preselected bacterial strains were characterized by measuring their oxygen consumption in a synthetic wastewater environment (as described in OECD standard 209 ([OECD, 1984](#)) - representative of the studied environment). The strains were then compared based on their observed respiratory kinetics. The protocol is detailed in [Jouanneau et al. \(2019\)](#).

2.2. Conservation and growth of the bioreporters

2.2.1. Growth conditions and freezing

The Luria Bertani medium (LB medium) was prepared in distilled water and supplemented with 5 g.L⁻¹ NaCl (Merck, Germany), 5 g.L⁻¹ tryptone (Biokar Diagnostics, France) and 1 g.L⁻¹ yeast extract (Biokar Diagnostics, France). 15 g.L⁻¹ of agar type E (Biokar Diagnostics, France) was added to obtain the solid medium. The pH was adjusted to 7 using a solution of HCl (Sigma Aldrich, USA) or NaOH (Merck, Germany) before autoclaving at 120 °C for 20 min.

Glycerol solution (GlyS) at 50 % (v/v) was prepared in distilled water and sterilized by autoclaving.

All strains were cultivated and stored under the same conditions as follow. The cells were stored at -80 °C in a mixture (v/v) of LB medium (50 %) and GlyS (50 %). The cells were isolated on solid LB medium at +30 °C during 12–24 h. Fresh medium was inoculated from the isolated colonies and cells were grown overnight (14 h) in LB medium at +30 °C under shaking at 250 rpm. Then, the cells were diluted to an optical density of OD_{620 nm} = 0.2 in fresh LB medium and cultivated at +30 °C under shaking at 250 rpm until an OD_{620 nm} = 0.45 (exponential growth stage) was reached.

2.2.2. Freeze-drying of bioreporters

The Freeze-drying medium was prepared in distilled water as following: 200 g.L⁻¹ tryptone (Biokar Diagnostics, France), 100 g.L⁻¹ yeast extract (Biokar Diagnostics, France) and 10g.L⁻¹ NaCl (Merck, Germany). The pH was adjusted to 7 using a solution of HCl (Sigma Aldrich, USA) or NaOH (Merck, Germany) before autoclaving at 120 °C for 20 min.

Saccharose solution (Freeze-drying protectant) was prepared at 240 g.L⁻¹ in distilled water, sterilized by filtration through 0.2 µm cellulose acetate filters (146,560, Dutscher, France) and stored at +4 °C.

The protocol is adapted from a previously validated protocol ([Jouanneau et al., 2012, 2011](#)). Briefly, cells from an exponential growth phase bacterial culture (optical density OD_{620 nm} = 0.45) are washed. For this purpose, the cultivated cells in LB medium were centrifuged (6400g, 5 min, +4 °C) to remove the growth medium and

resuspended in a cold washing solution of MgSO₄ ● 7H₂O (10⁻² M). This operational step was repeated three times. After the final centrifugation, the bacterial pellet was resuspended in a solution composed of 50 % Saccharose solution and 50 % Freeze-drying medium to reach an optical density (OD_{620 nm}) of 5. The bacterial suspension was then aliquoted into 2 mL vials (223,683/W224100-093, Wheaton, US) at 110 µL per vial and placed in the freeze-dryer (CHRIST Epsilon 2-4 LSCplus). The cells were then frozen for at least 3 h at -80 °C before the lyophilization phase. The freeze-drying procedure was carried out in several successive phases (Table SD-3). The entire freeze-drying protocol lasted approximately 26 h.

Lyophilized bacterial strains are stored at -20 °C for a maximum of 2 months prior to use.

2.3. Toxic solutions

Several toxicants were used in this study. All initial solutions are summarized in [Table 1](#).

The concentrated stock solutions were stored at +4 °C for the duration of the study. The diluted solutions used in the toxicity assays were prepared extemporaneously (Tables SD-4, SD-5 and SD-6).

2.4. Design of the TOXLAB biosensor for measurement, analysis, and data interpretation

To facilitate in situ deployment of the proposed analytical strategy, a biosensor was developed. This biosensor has been designed as a benchtop instrument, in order to be deployed in industrial contexts (indoor environments) and with the aim of meeting local operational needs. This development was divided into two complementary modules: a hardware component and a software component.

2.4.1. Hardware module

The hardware component of the TOXLAB biosensor was designed using computer-aided design (CAD) software, SolidWorks 2023 (Dassault Systèmes, France), and manufactured in-house. The control electronics (embedded board within the biosensor) were outsourced to a partner company, Tronico Alcen (France). This module was developed to meet several operational requirements, including simplified implementation of bioreporters, fluorescence-based respiration measurement, maintenance of optimal measurement conditions, and automation of measurement sequences.

The optical chain for measuring fluorescence generated by biological activity was specifically developed for 2 mL vials containing freeze-drying bioreporters ([Fig. 1A](#)). The measurement cell is based on an incident light source emitted by an LED, necessary for fluorophore (resorufin) excitation. The incident light is optically filtered to specifically select the excitation spectral band (540 nm ± 10 nm). The filtered beam then passes through the sample to excite resorufin molecules, formed under the action of bacterial enzymes, which in response re-emit a light signal between 590 and 615 nm (λ_{emission}). The emitted signal is

Table 1
Concentrated stock solution of the toxicant used in the experiments.

Solution	Concentration	Solvent	CAS number	Reference
Pentachlorophenol sodium salt	2 g.L ⁻¹	Distilled water	131-52-2	76480, Sigma - Aldrich
CuSO ₄ ●5H ₂ O	0.5 g.L ⁻¹	Distilled water	231-847-6	A4778701, Labosi
ZnCl ₂	1.5 g.L ⁻¹	Distilled water	7646-85-7	31650, Sigma - Aldrich
3,5 Dichlorophenol	1 g.L ⁻¹	Distilled water	591-35-5	D70600, Sigma - Aldrich
Norfloxacin	0.25 g.L ⁻¹	Distilled water	70458-96-7	N9890, Sigma - Aldrich

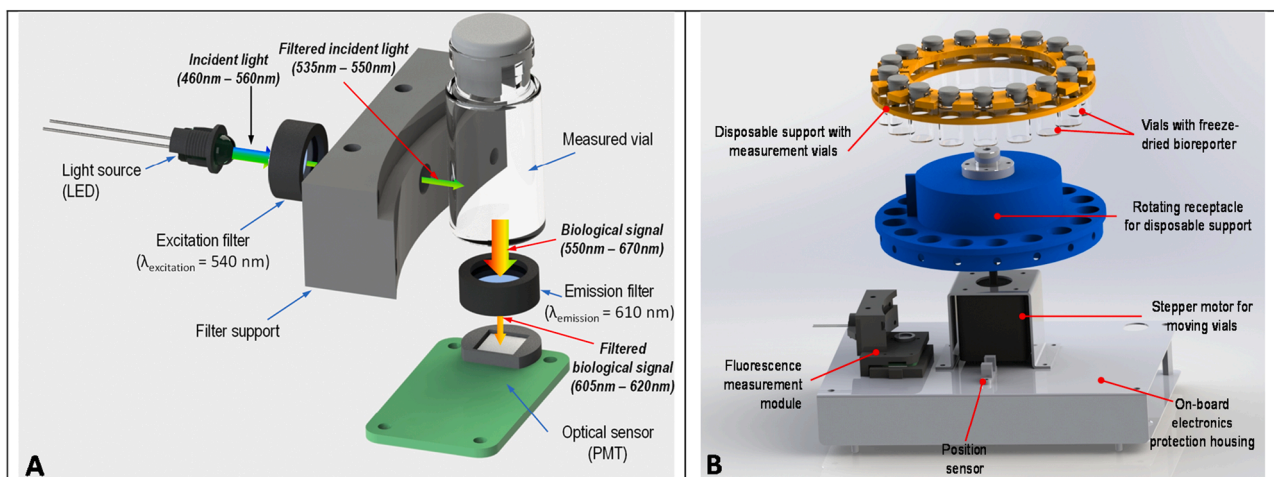


Fig. 1. TOXLAB biosensor designed for this study. (A) Schematic diagram illustrating the optical measurement setup. (B) Exploded view of the fluorescence measurement platform, providing a detailed view of its components. The 3D model was generated using Solidworks 2023.

filtered using a second optical filter at $610 \text{ nm} \pm 10 \text{ nm}$ and measured with a photosensitive sensor (photomultiplier). To limit parasitic signals induced by the incident light source on the optical sensor, the optical paths of the excitation and emission beams were oriented perpendicularly.

For ease of use, vials containing lyophilized bioreporters are pre-installed in a reusable support designed for this purpose (Fig. 1B) with 17 positions: 8 positions dedicated to toxicity measurements (1 position per bioreporter), 8 positions for biological activity controls (1 position per bioreporter), and 1 "sample" control (measuring the reducing effect of the sample on resazurin without bioreporter). During a test, this receptacle is positioned in the biosensor chamber (Fig. 2), which is temperature-controlled ($30 \pm 1 \text{ }^\circ\text{C}$) using a heating system developed for this project. The temperature is controlled using a probe. A stepper motor allows the vials to be moved, by a rotational movement, in front of the optical cell to allow measurement of the fluorescence intensity

emitted by the different vials (Fig. 1B). A position sensor has been integrated into the device to ensure reliable and continuous control of the vial positioning relative to the optical measurement cell (see all references in Table SD-7).

2.4.2. Software module

In parallel, a human-machine interface (HMI) was specifically designed to control the hardware module. This HMI (Fig. 2) was developed using the integrated development environment (IDE) Lab-Windows/CVI 2017 (National Instruments, USA) to allow simplified management of the biosensor. Once the bacteria are installed in the biosensor, the operator is guided by the HMI to perform the various tasks of the protocol (rehydration, exposure to the sample analyzed, and addition of resazurin). Throughout the operation, the temperature is regulated at $30 \text{ }^\circ\text{C}$ to ensure optimal measurement conditions. Finally, the HMI displays, in real time, the monitoring of biological activity by

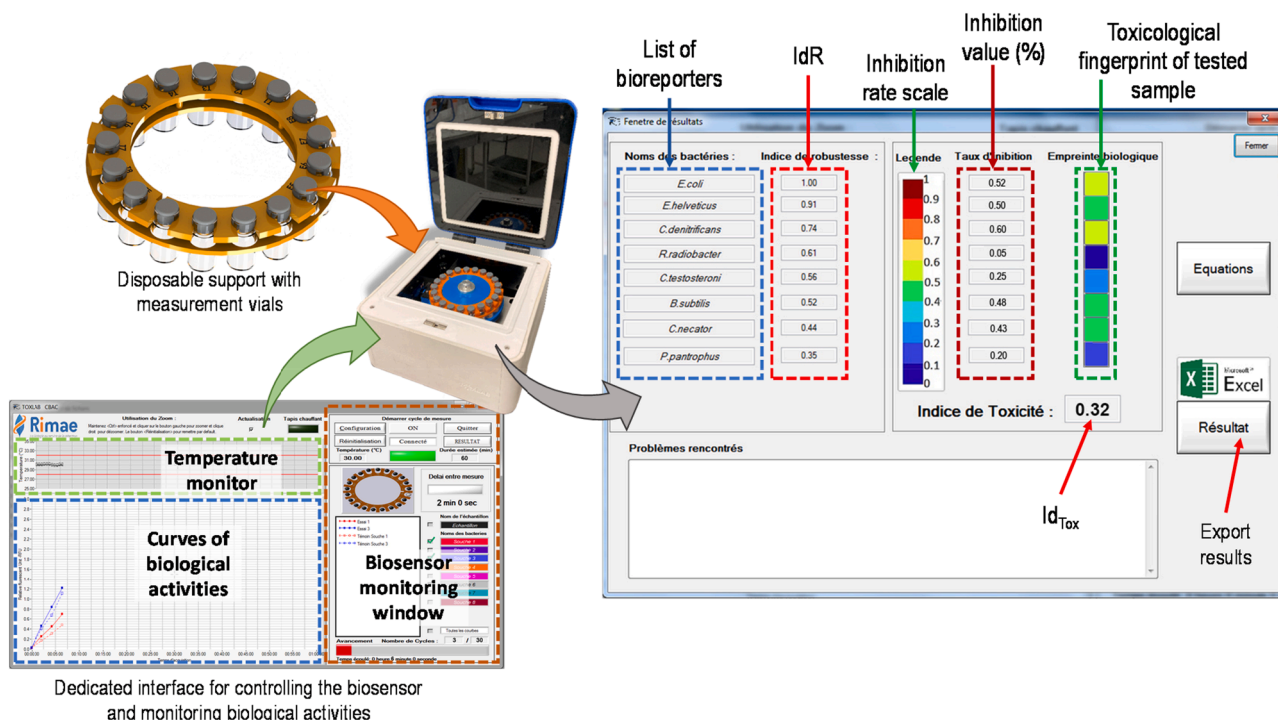


Fig. 2. Human-machine interface (HMI) for the management and analysis of data acquired from the TOXLAB biosensor.

fluorimetry induced by the different bioreporters.

Once the analysis is complete, the collected data is processed by a secondary HMI module. To do this, the data undergoes several successive treatments in order to propose an interpretation to the operator, namely: modeling of respiratory kinetics by linear regression (least squares method), subtraction of controls, determination of the specific inhibition rate, and calculation of the global toxicity index (IdTox).

The HMI provides a simple, rapid visualization of the processed data. Two interpretation levels are offered (Fig. 2): an intermediate level in the form of a fingerprint related to the intensity of the toxic effects induced on each bioreporter individually, and a second level, compiling all the data collected by the entire panel, through the global toxicity index (IdTox).

2.5. Toxicity assessment

2.5.1. Toxicity assay on selected strains (in microplate)

Freeze-fried bacterial strains are reconstituted in 110 µL sterile distilled water and incubated for 30 min at 30 °C. After the rehydration phase, 10 µL of bacterial suspension was added to each well of a 96-well microplate (Nunc, 236108). The bacteria are then exposed, for 45 min at +30 °C, to 90 µL of solutions to be analyzed (or MgSO₄ ● 7H₂O - 10⁻² M control solution) (the concentrations of the tested solutions and the preparation methods are detailed in supplementary data - Table SD-4).

After the 45-minute exposure, 10 µL of resazurin (fluorescent respiration dye - PrestoBlue™, Invitrogen, cat. no. 12083745, Fig. SD-2) was

added to each well. The respiratory kinetics are then monitored by fluorimetry for 1 hour at 30 °C using the CLARIOstar Plus multi-mode microplate reader (BMG Labtech, λ_{Excitation} = 540 nm, λ_{Emission} = 610 nm). To ensure accurate toxicity assessment, biotic controls (one per bioreporter, without toxicant) and abiotic controls are included in each measurement. Biotic controls assess baseline bioreporter activity, while abiotic controls account for the sample's inherent reducing power on the resazurin dye.

Due to the variability of microplate assays, each condition is systematically tested in triplicate in the microplates.

2.5.1.1. Inhibition rate calculation. Initially, respiratory kinetics for the different assays (toxicity tests and controls) are determined by linear regression from the raw fluorescence data (slope of the regression line - Fig. SD-1). Inhibition rates are then calculated from the calculated kinetics values, taking into account the effects observed with biotic and abiotic controls (Eq. 1).

$$Ir_i = 1 - \frac{(Tox_i - C_{Ab})}{(C_{Bi})} \tag{1}$$

Ir_i : Inhibition rate calculated for bioreporter *i*.

i : bioreporter identifier.

Tox_i: Kinetic value determined for toxicity assay with bioreporter *i*.

C_{Ab}: Kinetic value determined for abiotic control.

C_{Bi}: Kinetic value determined for biotic control (bioreporter *i*).

Calculated inhibition rates for the different bioreporters are used to

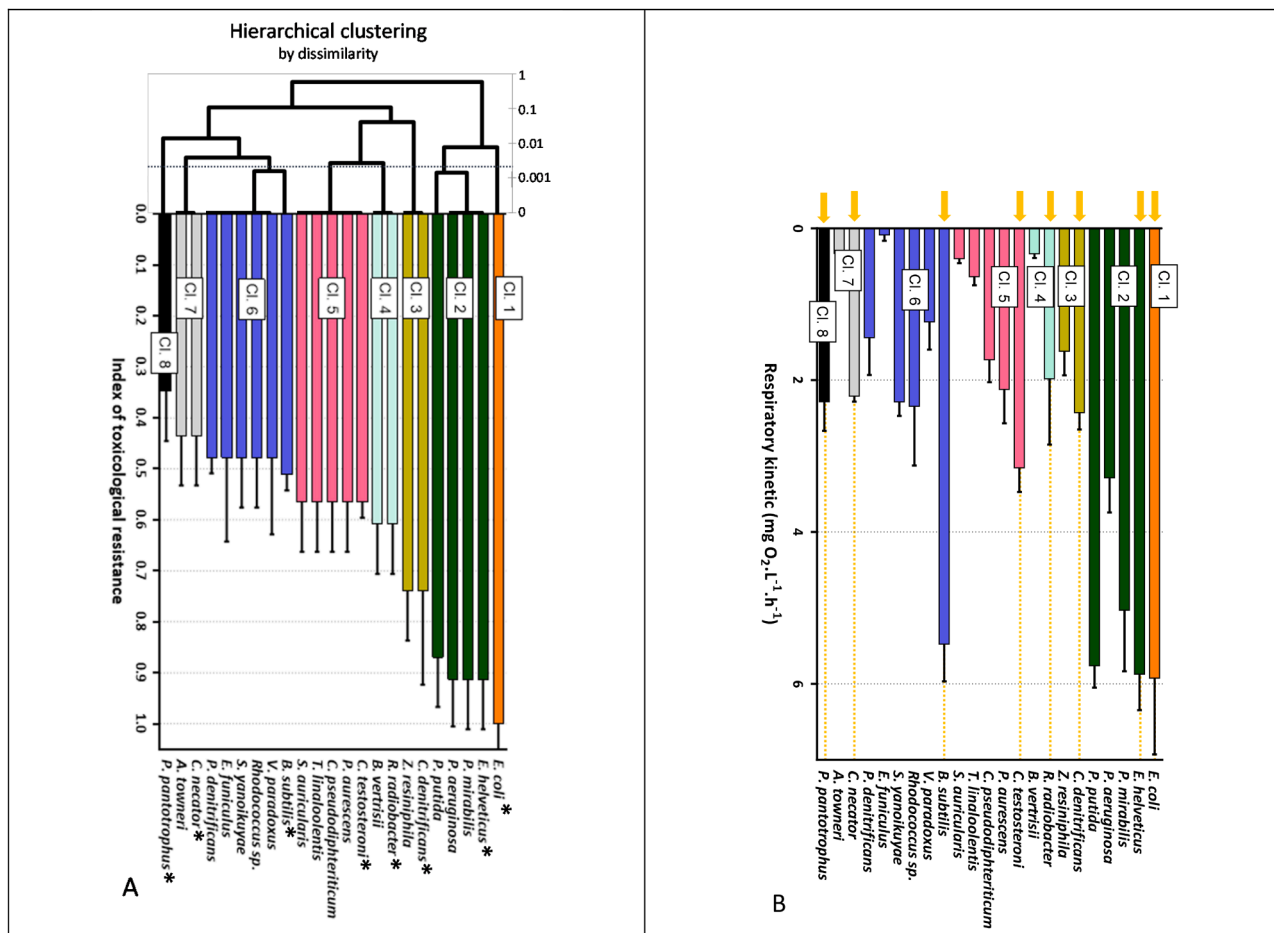


Fig. 3. (A) Hierarchical clustering of the 23 strains according to their toxicological resistance to 23 toxic conditions. Clusters defined by hierarchical ascending classification (Euclidean distance, classification level = 0.002). Each cluster is symbolized by a different color and noted as Cl. *n*. / (*) Strain selected based on respiratory kinetics criteria. IdRi: Index of toxicological resistance of bioreporter *i*. (B) Respiratory activities of the 23 bacterial strains, obtained from standardized wastewater without toxicants (Jouanneau et al., 2019; OECD, 1984). Yellow arrows indicate the strains selected for each cluster.

establish specific heatmaps of the samples in the HMI.

2.5.1.2. Overall toxicity index calculation. The overall toxicity index (see Eq. 2) is determined from the individual inhibition rates obtained after exposing the sample to the panel of bioreporters (selected bioreporters), weighted by the individual toxicological resistance indices (IdR - see § 2.1.1).

$$Id_{tox} = \frac{\sum_{i=1}^8 IdR_i \times Ir_i}{\sum_{i=1}^8 IdR_i} \quad (2)$$

Id_{tox}: Global toxicity index.

i : bioreporter identifier.

IdR_i: Index of toxicological resistance of bioreporter i.

Ir_i : Inhibition rate calculated for bioreporter i.

The bioreporter-based measurement strategy is patent-protected (WO2019162619) (Thouand and Jouanneau, 2019).

2.5.2. TOXLAB biosensor toxicity assay

For toxicity tests performed with the TOXLAB biosensor, vials containing lyophilized isolated strains are pre-arranged in a dedicated disposable rack (see Fig. 3B). After introducing 110 µL of sterile distilled water into each vial, the support is installed in the measuring device to respect the rehydration conditions (30 min, +30 °C). At the end of this step, 990 µL of toxic solutions to be analyzed (or MgSO₄ ● 7H₂O - 10⁻² M control solution) are introduced into the vials (the concentrations of the tested solutions and the preparation methods are detailed in supplementary data - Table SD-4). After 45 min of exposure at 30 °C in the device, 110 µL/vial of resazurin (fluorescent respiration dye - Presto-Blue™, 12083745, Invitrogen) are added. Fluorescence intensity is then monitored for 1 hour at 30 °C, with a measurement taken every 90 s.

The collected biological data (fluorescence intensity) is automatically processed by the HMI to determine the inhibition rates induced by the analyzed sample on the different bioreporters and the overall toxicity index (see §2.5.1).

2.5.3. Toxicity test performed according to OECD 209 method (Urban WWTP)

In this test, toxicity is assessed based on the inhibition of respiratory activity in a microbial community exposed to a sample, in accordance with the procedure described in the OECD guideline. To ensure test conditions in accordance with OECD standard 209 (OECD, 1984), a synthetic wastewater (SWW) solution was prepared as follows: 1 L of distilled water was supplemented with 16 g of tryptone (Biokar Diagnostics, France), 11 g of meat extract (Biokar Diagnostics, France), 3 g of urea (Sigma-Aldrich, USA), 0.7 g of NaCl (Merck, Germany), 0.4 g of CaCl₂ ● 2H₂O (Merck, Germany), 0.2 g of MgCl₂ ● 7H₂O (Sigma-Aldrich, USA), and 2.8 g of K₂HPO₄ (Merck, Germany).

Microbial communities were obtained from aeration basins of two urban wastewater treatment plants (WWTP of La Roche sur Yon (France); 8816 m³.d⁻¹ / 46.638237°, -1.429149° and WWTP of Bellevigny (France); 501 m³.d⁻¹ / 46.787613°, -1.441787°). For the test, environmental inocula were washed to remove traces of any dissolved toxicants present within the community. For this, 500 mL of inoculum was centrifuged at 5000 G for 5 min. The supernatant was removed, the pellet was resuspended in 200 mL of sterile MgSO₄ ● 7H₂O - 10⁻² M solution and homogenized. This operation was performed 3 times. Finally, the bacterial suspension was adjusted to 3 g.L⁻¹ (dry matter).

Each test was prepared according to the following mixture: 6.4 ml of SWW, 20 mL of concentrated test solution (10X) (Table SD-5), 73.6 mL of distilled water and 100 mL of washed bacterial suspension.

The test protocol involves exposing the test sample to the bacterial suspension under agitation and aeration for 3 h at 20 °C. Following this exposure, aeration is stopped and agitation is reduced, and respiratory activity is measured using an oximeter (Hach Lange). The intensity of

the toxic effect is calculated based on the respiratory kinetics obtained with the analyzed sample (ratio between the activity measured on the sample and the reference activity without toxicant).

2.5.4. Toxicity test performed according to ISO 11348 method

This assay measures the inhibition of bioluminescence emitted by the marine bacterium *Aliivibrio fischeri* NRRL-B 11177 (International Organization for Standardization, 2007). The test was conducted according to a standard procedure using a LUMISTox® device (Hach Lange, USA).

Toxicant solutions were prepared as described in supplementary data (Table SD-6). Samples were incubated with the bacterial suspension (50/50 ratio) for 30 min at 15 °C. The intensity of the toxic effect is estimated by measuring the bioluminescence (ratio between the bioluminescence observed with the sample and the bioluminescence emitted without toxicant).

2.6. Toxicity test on the industrial site (SYENSQO)

2.6.1. Sample collection

A wastewater sampling campaign was conducted at the SYENSQO industrial site (Tavaux, 47.052254, 5.408974) from October 17th to November 6th, 2019. This industrial site specializes in the synthesis of polymers (polyvinylidene chloride, polyvinyl chloride). The study aimed to assess the toxicity of untreated effluents to evaluate their potential impact on the wastewater treatment plant's microbial community. 34 samples from various production lines were collected by the operator in 50 mL sterile tubes (reference 352098, Corning Falcon) (2 × 40 mL per sample). Stored at -20 °C, these samples were kept until analysis using the OECD 209 reference method or with the TOXLAB biosensor.

2.6.2. Toxicity analysis (SYENSQO)

OECD 209 analyses were carried out directly at the SYENSQO industrial site in order to benefit from fresh biomass sampled extemporaneously from the aeration basin of the site's wastewater treatment plant. The protocol was nevertheless adapted for these samples. The site's activated sludge was prepared following the procedure outlined in Section 2.5.3. The tests were prepared by respecting the following volumes: 2.6 mL of SWW, 40 mL of sample to be tested (industrial wastewater) and 37.4 mL of washed bacterial suspension (final dry matter concentration of 3.1 g.L⁻¹ ± 0.4 g.L⁻¹). Toxicity measurements followed the OECD 209 protocol (Section 2.5.3). TOXLAB biosensor analyses were conducted off-site using the same protocol as described in Section 2.5.2.

2.7. Microbial community diversity analysis using 16S rRNA gene

To study the composition of the microbial communities of the environmental inoculum (urban WWTP of La Roche sur Yon (France) - 46.638237°, -1.429149° and industrial WWTP of SYENSQO - Tavaux), 16S rRNA-encoding genes were sequenced by NGS (Illumina MiSeq). Thus, 45 mL of activated sludge was sent for analysis to the service laboratory ADNid (Groupe Qualtech). The ADNid laboratory performed DNA extraction, and amplification of 16S rDNA by PCR via the V3-V4 region with primers 341F/805R according to the primers of Klindworth et al. (2013) (Klindworth et al., 2013). Here are the sequences of these primers: 341F: 5'-CCTACGGGNGGCWGCAG-3' and 805R: 5'-GACTACHVGGGTATCTAATCC-3'. Raw data were analyzed using the Galaxy Migale open access platform developed by Université Paris-Saclay and INRAE (Bioinformatics, MIGALE bioinformatics facility). The FROGS and FROGSSTAT packages were used for this analysis (Bernard et al., 2021; Escudé et al., 2018). Operational taxonomic units (OTUs) were compared with the SILVA database (v.138, Bremen, Germany) using the BLAST tool for species or genus assignment (Murali et al., 2018). Unclassified sequences at domain level were classified using VSEARCH global alignment (90 % identity) and those for which

Table 2

Table of the final set of bioreporters used in the measurement strategy.

Bacterial strain	Collection Id	IdR _i	Phylum
<i>E. coli</i>	ATCC 700926	1.000	<i>Pseudomonadota</i>
<i>Enterobacter helveticus</i>	DSM 18396	0.913	<i>Pseudomonadota</i>
<i>Comamonas denitrificans</i>	DSM 17887	0.739	<i>Pseudomonadota</i>
<i>Rhizobium radiobacter</i>	DSM 7215	0.609	<i>Pseudomonadota</i>
<i>Comamonas testosteroni</i>	DSM 38	0.565	<i>Pseudomonadota</i>
<i>Bacillus subtilis</i>	DSM 10	0.522	<i>Bacillota</i>
<i>Cupriavidus necator</i>	DSM 428	0.435	<i>Pseudomonadota</i>
<i>Paracoccus pantotrophus</i>	DSM 65	0.348	<i>Pseudomonadota</i>

this was inconclusive were deleted (Rognes et al., 2016).

Experiments were four times independently replicated.

2.8. Data processing

Several data processing tools were employed for this study. The presented graphs were generated using Excel (Microsoft Office Professional Plus 2021) and Prism 6 (GraphPad). Hierarchical classification trees were constructed using XLSTAT (Addinsoft 2023) based on toxicological resistance data from the 23 preselected strains.

3. Results and discussion

3.1. Selection of bioreporters

The primary objective of this step was to determine a sufficient number of bioreporters to be used in the biosensor while ensuring representativeness in terms of "toxicological sensitivity" relative to the activated sludge microbiome. To this end, two criteria were selected to rank and choose the most suitable bioreporters: (i) diversity of sensitivities and (ii) respiratory kinetics (indicative of the ability to rapidly reveal inhibition).

Phenotypic data related to bioreporter resistance to environmental stress conditions (Table SD-2) allowed for hierarchical classification of the 23 strains based on their specific profiles (Fig. 3A). This initial analytical treatment highlighted a redundancy of sensitivity among certain bioreporters. Indeed, several strains exhibit similar capabilities in terms of toxicological resistance to the 23 stress conditions tested. By grouping strains with similar profiles, 8 clusters were obtained. According to these data, with a resistance index (IdR, see §2.1.1.) of 1.00, cluster #1 (1 unique bioreporter: *Escherichia coli*) appears as the most resistant, in contrast to cluster #8 (1 unique bioreporter: *Paracoccus pantotrophus*), the most sensitive (IdR = 0.35). Among the obtained clusters, some group up to 6 bioreporters with a substantially similar profile, as is the case for cluster #6 (*Bacillus subtilis*, *Variovorax paradoxus*, *Rhodococcus* sp., *Sphingobium yanoikuyae*, *Ectobacillus funiculus* and *Paracoccus denitrificans*). Based on these results, the number of bioreporters to be used in the biosensor was set to 8 representatives (1 per cluster).

The second selection step, based on the study of respiratory kinetics (Fig. 3B), was conducted to select, within each cluster, the bioreporters with the most intense respiratory activity. For example, in the case of cluster #2, 4 strains exhibit a similar toxicological profile (*Enterobacter helveticus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Pseudomonas putida*); however, the *E. helveticus* strain shows more significant respiratory kinetics. This strain was therefore selected as the representative of cluster #2 (Fig. 3B).

This initial phase of the study identified the 8 bacterial bioreporters of the final panel used in the measurement strategy, the list of which is presented in Table 2.

To validate the approach, toxicity assessment tests were conducted to evaluate the impact of a model pollutant (ZnCl₂) on the different selected bacterial strains. The obtained results are presented in Fig. 4.

A dose-response effect was observed: as the toxicant concentration (here ZnCl₂) increased, the respiratory kinetics decreased, showing an

increase in the intensity of the induced toxic effect (Fig. 4A). When extended to the entire selected set, the observed toxic effects were specific to each bioreporter. In other words, the induced effects did not impact all bioreporters homogeneously. According to the results, *R. radiobacter* did not appear to be significantly affected by ZnCl₂ at the tested concentrations; the observed inhibition rates did not exceed 5.7 %. Conversely, the *C. denitrificans* strain seemed to be particularly impacted with inhibition rates of 40.5 % and 59.7 % for 50 mg.L⁻¹ and 150 mg.L⁻¹, respectively. These results are entirely in agreement with the literature, which mentions differences in sensitivity between bioreporters in toxicity tests (Jung et al., 2015; Kowalik et al., 2024; Zubaidi et al., 2021).

By combining all data provided by the bacterial panel, weighted by the specific sensitivities (IdR) of each bioreporter, a global toxicity index is calculated ($0 < Id_{Tox} < 1$), which, in the case of ZnCl₂, increases with increasing concentration. This index was developed to facilitate the interpretation of multiparametric toxicity data (inhibition rates) by non-specialist operators by proposing a simple global index. The proposed weighting emphasizes the contribution of bioreporters identified as "resistant" compared to "sensitive" bioreporters in the calculation of this index. Another toxicity index has been proposed in MARA and LumiMARA approaches, with a different calculation algorithm (Gabrielsson et al., 2003; Wadhia et al., 2007). For each bioreporter used, a Microbial Toxic Concentration (MTC) index is calculated based on the response of the different bioreporters. Based on these individual indices, the authors propose a global toxicity index based on the average of the MTCs, but at the same time masking the richness of the information carried by the biological fingerprint.

3.2. Comparison of the biosensor with other reference methods

The objective was to assess the biosensor's ability to anticipate the toxic effects of pollutants on activated sludge, impacting the purification performance of the pollution treatment process. To this end, the TOX-LAB approach was compared, based on 11 toxic conditions, with 2 other standardized approaches: OECD 209 and ISO 11348. All data obtained from the different approaches are presented in Fig. 5.

Tests were conducted using microbial communities freshly collected from two local urban wastewater treatment plants. The results obtained show that all tested conditions induced an inhibition of respiratory activity in the environmental inocula. The intensity of the observed effects varied between 1.1 % ± 2 % and 91 % ± 0.9 % for the conditions CuSO₄•5H₂O at 5 mg.L⁻¹ and 3,5-Dichlorophenol at 100 mg.L⁻¹, respectively. These results demonstrate that these different conditions are likely to induce more or less significant disturbances in the microbial communities of urban wastewater treatment plants and, by extrapolation, a decrease in the purification capacities of processes designed to treat domestic wastewater before its discharge into the environment. Although this approach is particularly relevant in the context of assessing the toxicity of pollutant(s) on a community and on the associated process, it remains nevertheless underutilized due to its very time-consuming nature, induced in particular by the use of fresh communities (approximately 7 h per sample).

However, the results obtained for the ISO 11348 test were significantly different from those obtained using the OECD 209 approach. Indeed, of the 11 tested conditions, 4 conditions did not induce any observable toxic effect (CuSO₄•5H₂O at 5 and 50 mg.L⁻¹ and Norfloxacin at 2.5 and 25 mg.L⁻¹) and 5 conditions induced toxic effects too intense to be quantified (> 100 % inhibition of bioluminescence – Pentachlorophenol at 50, 100 and 200 mg.L⁻¹). Only 2 conditions, i.e. ZnCl₂ at 50 and 150 mg.L⁻¹ induced a measurable effect by this method, inducing, respectively, 16.6 % ± 8 % and 79.8 % ± 18.4 % inhibition of bioluminescence. These results, which contrast strongly with those obtained by the OECD 209 method, have already been observed by several authors (Liwarska-Bizukojc et al., 2016) showing differences in sensitivity between bioreporters: indigenous biomass of WWTP versus pure

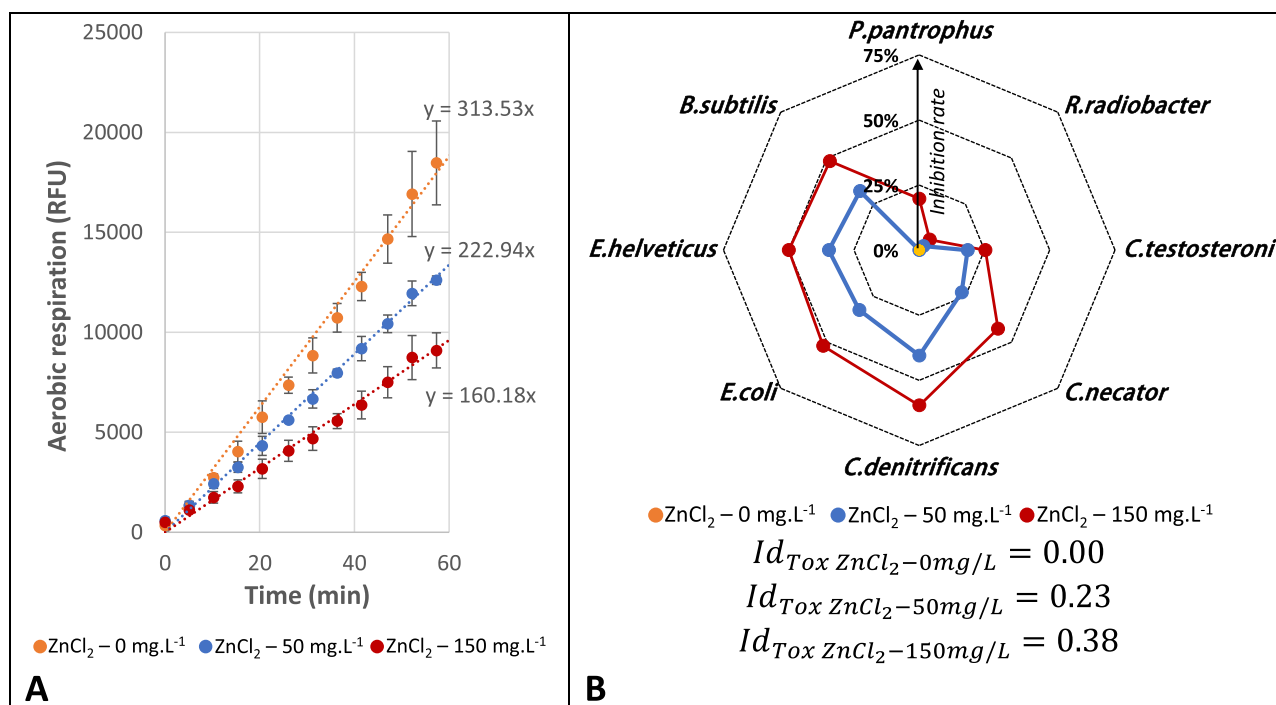


Fig. 4. Effect of ZnCl_2 on bioreporter respiratory activity. (A) Effect of different ZnCl_2 concentrations on the respiratory kinetics of the *E. coli* strain, (B) Toxicological fingerprints of ZnCl_2 on the bacterial set strains and overall toxicity index (Id_{tox}) for the 3 tested ZnCl_2 concentrations.

strain (*E. coli*). These results initially question the relevance of mono-specific approaches in the context of assessing the toxic effect on a community (WWTP) in all its diversity and complexity. In addition, the work of Yotova *et al.* in 2019²⁶ demonstrates, once again, that the response of a monospecific ecotoxicity test depends mainly on the bioreporter chosen. Consequently, one can also question the relevance of an approach based on a marine species, from a very distant environment, to assess the impact of a pollutant on a microbial community such as that of activated sludge in WWTPs.

The TOXLAB biosensor was used with the same toxicants at the same concentrations as for the previous two tests. The intensity of the toxic effect is expressed through the global toxicity index (calculated from all the responses provided by the 8 selected bioreporters). The higher the measured toxic intensity, the higher the value of this index. These results show that the approach allows for the evaluation and quantification of the toxic effect induced by the 11 tested conditions. The lowest intensity obtained with this approach concerns the condition with norfloxacin at 2.5 mg.L^{-1} , with a toxic effect evaluated at 0.0756 ± 0.002 (i.e., approximately 3 % variability; $n = 2$). Conversely, the condition with PCP at 200 mg.L^{-1} obtains a global toxicity index of 0.96 ± 0.013 (i.e., approximately 1.3 % variability; $n = 2$).

In 55 % of cases, the results obtained were not significantly different between data from microbial community tests (OECD 209) and the TOXLAB approach. For ZnCl_2 and 3,5-dichlorophenol, the TOXLAB approach appeared to be slightly less sensitive than the OECD 209 approach, and vice versa for $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ at 5 mg.L^{-1} . Although differences exist, the results obtained with the TOXLAB approach remain consistent and of the same order as those provided by the microbial communities of domestic wastewater treatment plants. Consequently, the results obtained via this new approach tend to validate the interest of a multi-species approach in the context of assessing the toxic impact of pollutants on more complex communities and, more broadly, in the context of process monitoring (WWTP).

4. Implementation of the TOXLAB strategy within an industrial environment

Industrial effluents can have a very different composition from domestic wastewater. These differences in inputs can lead to significant differences in biological diversity, compared to a domestic wastewater treatment plant community. These differences have been highlighted in particular by the work of Ibarbalz *et al.* (2013). The authors highlight a significant restructuring of the phylogenetic composition of microbial communities between the different wastewater treatment plants studied (domestic and industrial). However, the main phyla involved in the purification process seem to be conserved, although their relative abundances vary, suggesting a common bacterial core. It is in this context that the deployment of the TOXLAB strategy makes sense. Indeed, under the hypothesis that a common microbial core exists between these different communities, it is likely that the TOXLAB approach will appear relevant, subject to some adjustments (notably regarding the weight of the bioreporters (IdR) in the calculation of the overall toxicity index (Id_{TOX})). In other words, to what extent can the TOXLAB approach, based on a bacterial set from an urban wastewater treatment plant, be used to assess the toxicity of an effluent with a certain representativeness?

To answer this question, the biosensor was deployed at the SYENSQO industrial site. This site, located in Tavaux (France) on nearly 200 hectares, is dedicated to the synthesis of various molecules of interest such as polymers (polyvinylidene chloride, polyvinyl chloride). Each year, nearly a million tonnes of products leave this production plant. The waters produced, through the various processes, are collected and treated in a dedicated wastewater treatment unit on the site before being discharged into the environment.

Thus, 34 samples were collected over 2 months (October/November 2019) from various production processes upstream of the treatment plant. These samples were tested, on the one hand, with the 8 bioreporters of the TOXLAB approach and, on the other hand, according to the OECD 209 reference method. However, for representativeness reasons, the OECD 209 test was carried out using the microbial consortium

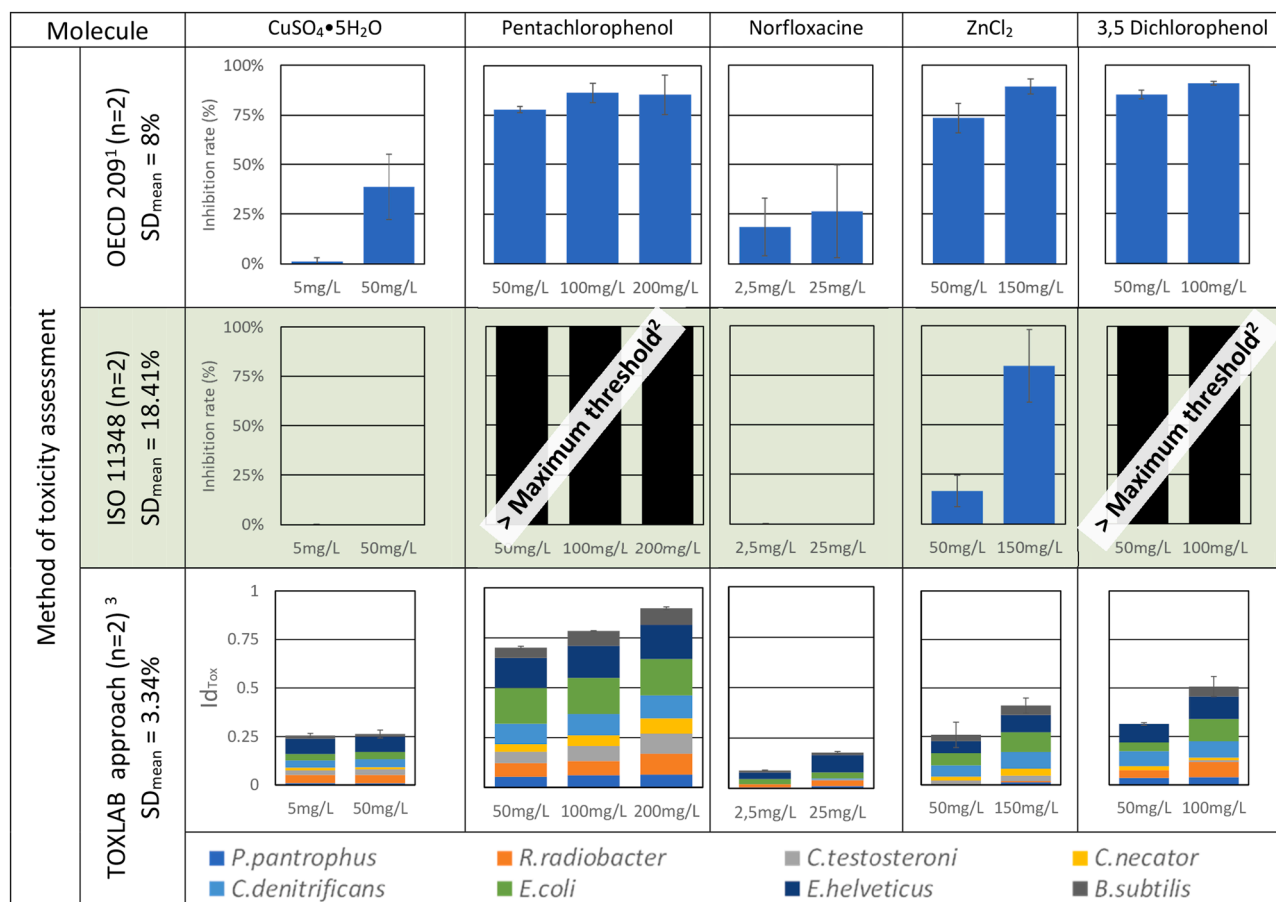


Fig. 5. Toxic effects induced by different reference molecules using TOXLAB, OECD 209 and ISO 11,348 methodologies.

¹: OECD test performed in duplicate using two distinct inoculum sources: activated sludge from La Roche-sur-Yon WWTP (8816 m³.d⁻¹) and from the Bellevigny WWTP (501 m³.d⁻¹).

²: The toxic intensity is too high for the *A. fischeri* strain. Therefore, it is not possible to quantitatively evaluate the level of toxicity caused because the tolerable threshold for this strain is exceeded.

³: Each color represents the individual contribution of each bioreporter to the calculated IdTox value.

adapted to SYENSQO (activated sludge from the SYENSQO site's wastewater treatment plant) instead of a consortium of activated sludge from a domestic wastewater treatment plant.

The obtained results are presented in Fig. 6. The results obtained with the TOXLAB approach are not correlated with the toxicity data evaluated with the reference method ($r^2 = 0.033$, $p > 0.05$). For example, sample S1 was identified as the most toxic according to the OECD 209 test based on the site's consortium with an estimated toxicity intensity of $93.82\% \pm 6.74\%$. At the same time, the TOXLAB approach estimates toxicity at a relatively low level, with a toxicological index (Id_{Tox}) of 0.122 ± 0.04 .

The most likely hypothesis to explain these discrepancies lies in the choice of bioreporters used in the TOXLAB approach. Indeed, these were exclusively selected from urban wastewater treatment plant communities, and not from activated sludge responsible for the treatment of industrial wastewater. To verify this hypothesis, the microbiome of the SYENSQO wastewater treatment unit was characterized by metagenomics (16S rRNA-encoding genes) and compared with the microbiome of one of the wastewater treatment plants used for previous tests (La Roche sur Yon, see § 3.2).

Diversity analysis (Fig. SD-7_A) shows a very significant difference in the number of Operational Taxonomic Units (OTUs) between the two microbiomes, with >5500 OTUs identified for the urban wastewater treatment plant community versus 2800 OTUs in the SYENSQO industrial treatment plant. The community structure is also very different in terms of diversity (Fig. SD-7_B). These results highlight the strict

difference between these 2 communities in terms of ecological diversity. These 2 microbiomes do not seem to possess common OTUs, showing at the same time a specific adaptation to their respective environment.

As in many environments (Shin et al., 2015), the two main phyla present are *Pseudomonadota* and *Bacteroidota*, which together represent >50% of the total microbiome abundance (Fig. 7A). However, some phyla are almost absent from the SYENSQO community, such as *Acidobacteriota*, *Planctomycetota*, *Armatimonadota*, *Myxococcota*, and *Firmicutes*, representing <0.05% of the total abundance. Focusing more specifically on the orders present within the two major phyla (Fig. 7B and 7C), we note a great structural disparity between these two communities. In the case of *Pseudomonadota*, the orders of *Rhodobacteriales*, *Sphingomonadales*, and *Pseudomonadales* have almost disappeared from the SYENSQO community, contrary to the order of *Kiloniellales* present in the SYENSQO community but not found in the WWTP microbiomes. For the phylum *Bacteroidota*, the observation is substantially the same despite a larger share occupied by the order of *Ignavabacteriales* in the SYENSQO community.

In light of these elements, the results obtained with the TOXLAB approach on water samples from the SYENSQO industrial site seem coherent. Indeed, the TOXLAB strategy relies on bioreporters exclusively from domestic wastewater treatment plants. However, the microbial communities involved in the pollution control processes are very different. The choice of bioreporters used in the TOXLAB approach is therefore, *a priori*, not appropriate to predict the impact of toxics on an industrial microbiome such as that of SYENSQO. Bioreporters were

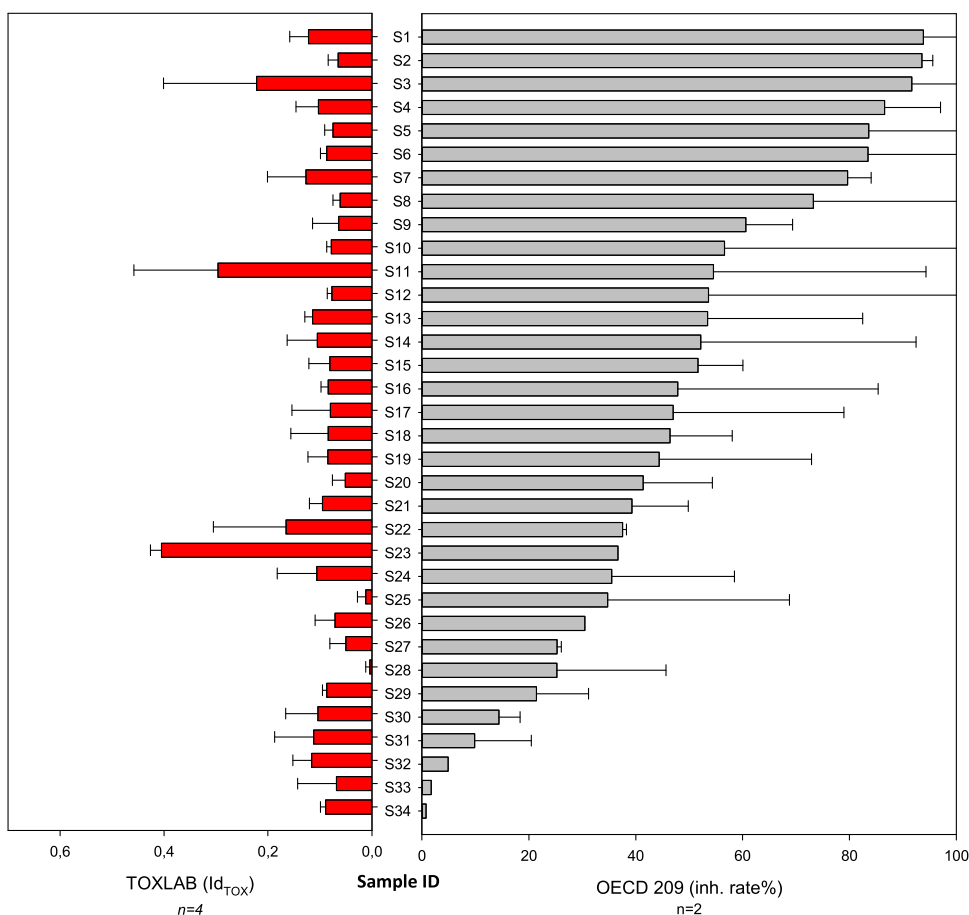


Fig. 6. Comparison between the toxicity estimated by the TOXLAB and OECD 209 approaches (carried out using activated sludge from the SYENSQO site’s wastewater treatment plant) (n = 4). Sx: with x = sample number (1 to 34).

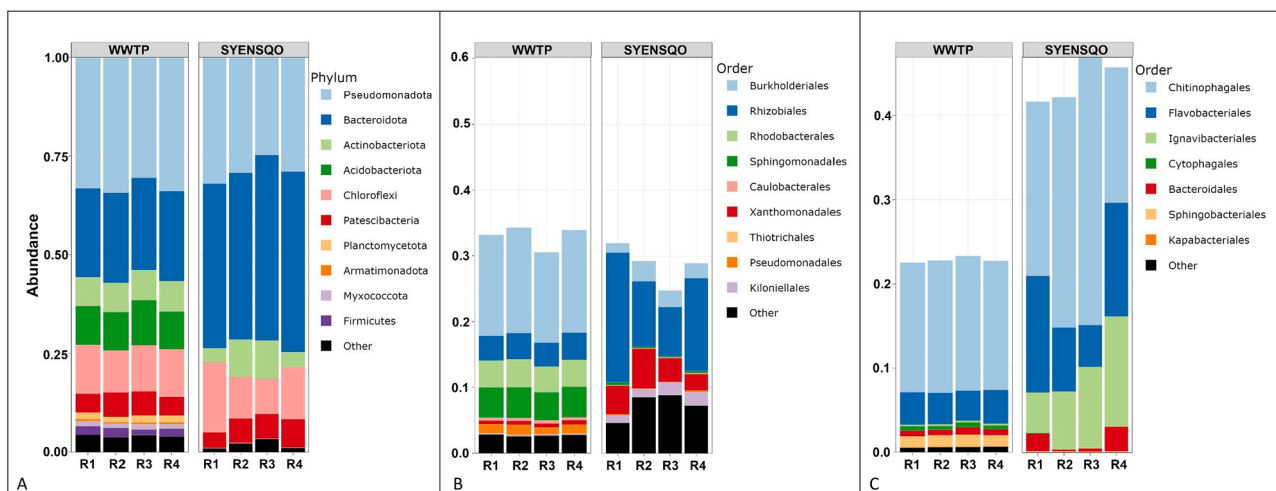


Fig. 7. Comparative analysis of microbial community composition in WWTP (La Roche sur Yon) and SYENSQO. (A) Phylum-level taxonomic profiling, (B) In-depth analysis of « *Pseudomonadota* » and (C) In-depth analysis of « *Bacteroidota* ». Each data point (R_x) represents a biological replicate (n = 4 per site).

selected, first and foremost, because of their representativeness in urban WWTP microbiomes, but these bioreporters are totally absent in the SYENSQO site communities. As such, it is unlikely that the selected bioreporters can in any way represent this specific microbiome. The lack of correlation therefore points to the need to adapt the bioreporters of the bioassay/biosensor to the context envisaged, in other words, to the microbial population of the targeted process. Consequently, proposing

such a method based on selected bioreporters will have to be the subject of an in-depth study beforehand to better understand the industrial community in order to select (or isolate) representative microorganisms.

5. Conclusion and perspectives

Within an environmental inoculum, each strain exhibits a unique

sensitivity to pollutants. As a result, evaluating the overall impact of pollutants on a microbial community is a complex and uncertain undertaking.

This study departs from traditional wastewater toxicity assessment methods, based on monospecific or environmental inoculum approaches, by employing a "bioelectronic tongue" concept. The challenge of this strategy lies in translating the multisensorial data from the biosensor into meaningful toxicity assessments for WWTP microbiomes. To address this, the study focused on the choice of bioreporters used and on their deployment in industrial sites using a dedicated biosensor (TOXLAB).

A validation phase using diverse toxicants (metals, pesticides, antibiotics) demonstrated promising coherence between the TOXLAB approach and the OECD 209 test. Indeed, the results showed a relative coherence between the toxic effects observed on the microbial community of domestic wastewater treatment plants (OECD 209) and those predicted by the TOXLAB approach. In light of these initial results, the approach developed in this study appears promising for monitoring influent to urban wastewater treatment plants and for predicting the impact of pollutants on activated sludge. In contrast, the monospecific approach (ISO 11348) exhibited less consistent results, suggesting limited relevance for monitoring toxicity impacts within on the WWTP community. This raises concerns about the suitability of the ISO 11348 standard for process monitoring.

The TOXLAB strategy was subsequently deployed in a wastewater treatment unit of an industrial site (SYENSQO, France). The results obtained show the inability of the approach to assess the toxicity of inputs and their impact on the autochthonous microbiome. 16S rRNA gene analysis revealed significant differences between the urban wastewater microbiome, upon which TOXLAB is based, and the microbiome at SYENSQO. In light of these results, the choice of bioreporters implemented in TOXLAB is not suitable and a step of selection of a specific panel will have to be carried out in order to consider using the multi-specific approach in this industrial context (Ibarbalz et al., 2013). Indeed, the strains identified within the site's microbial community are seldom, if at all, documented in existing literature. Consequently, procurement from commercial repositories such as ATCC, DMSZ, or CIP is improbable. Thus, an isolation stage conducted directly from the activated sludge will be necessary to establish a repository of potential candidates. Then, similar to the presented study, a careful selection process based on sensitivity and kinetic criteria will be essential to identify relevant bioreporters for the biosensor panel.

However, further improvements could be considered to enhance the predictive capabilities of the biosensor. Among the potential avenues for improvement, expanding the selection criteria beyond initial considerations (origin, stress resistance, and respiratory kinetics) appears to be a relevant lever in this context. Thus, by increasing the number of tested toxicity conditions, the characterization of bioreporters will be more robust, leading to a potentially more informed choice regarding the strains used in the biosensor. The study could also be opened up to a functional understanding of the ecosystem through phenotypic characterization tools (such as Phenotype microarrays BIOLOG, PICRUST2, etc.) (Acin-Albiac et al., 2020; Bochner et al., 2001; Douglas et al., 2020; Zhao et al., 2022), thus allowing the integration, during the bioreporter selection process, of criteria linked to their role within the community (carbon metabolism, nitrogen assimilation pathway, etc.).

Then a second work can be carried out on the correlation model to improve the interpretation of multisensorial data provided by the 8 bioreporters (toxicity values). In the proposed approach, the data are processed using a simple linear regression algorithm (eq. 2) to estimate toxicity (Id_{TOX}) from the inhibition data specific to each bioreporter, while taking into account the inherent sensitivity of each bioreporter through individual weighting (I_r). These models are not necessarily the most appropriate for modeling a biological phenomenon. Supervised learning algorithms such as neural networks or nonlinear models, already proven for similar approaches (Jouanneau et al., 2019), would

be a more powerful alternative for data exploration. Indeed, unlike simple linear regression, these algorithms can highlight subtle interactions between variables. Consequently, their application to the analysis of toxicity data from bioreporters could provide more accurate predictions. However, the performance of these algorithms is directly linked to the quantity and quality of the training data. A limited or unrepresentative dataset could lead to an irrelevant model with very limited predictive capabilities. Therefore, a preliminary additional step will be necessary in order to have a more substantial dataset necessary for the development of such models. The richer the database (number of trials, number of tested conditions, etc.), the more performant the model should be in terms of toxicological prediction.

The ultimate objective is to equip wastewater treatment plant managers (domestic or urban) with a predictive toxicity index (Id_{TOX} or equivalent) for assessing influent toxicity. By benchmarking this index against an internal reference threshold, operators can implement necessary process adjustments to mitigate performance impacts.

CRedit authorship contribution statement

Jouanneau Sullivan: Writing – original draft, Validation, Supervision, Project administration, Methodology, Conceptualization. **Louanneau Thomas:** Validation, Resources, Methodology, Investigation, Data curation. **Thouand Gérald:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sullivan Jouanneau reports financial support was provided by French National Research Agency. Sullivan JOUANNEAU has patent #WO2019162619 pending to No. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.123470.

Data availability

Data will be made available on request.

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