An amperometric method for the rapid detection of extended-spectrum β-lactamase producing Escherichia coli in wastewater treatment plant effluents

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Context: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL *E. coli*) are resistant to most β-lactams and have become a major concern in human and veterinary medicine. As *E. coli* and antibiotic resistant strains are part of the intestinal flora of humans, large amounts of these bacteria are present in wastewaters. Though treatments are performed in wastewater treatment plants (WWTP), large quantities of bacteria are still present in the treated effluents rejected into the environment. These releases can cause contaminations of recreational waters and thus present a health risk to exposed populations. Therefore, rapid and convenient assays are highly desired for the quantification of ESBL *E. coli* in the wastewater network and in natural environments.

Objective of the study: Development of a nitrocefin-based amperometric method for the rapid quantification of ESBL *E. coli* in WWTP effluents

Methods: Raw and treated wastewaters were filtered in duplicate through 0.45 µm filters (HAWP, 47 mm, Millipore). The amperometric assay involved two main steps: (1) the subculturing of the filtered samples in the presence of cefotaxime supplemented or not with the potassium clavulanate (ESBL inhibitor) for a few hours (4-5h) followed by, (2) the incubation of each subculture filtrate (v = 10 mL; HVLP filter, 0.45 µm, 13 mm, Millipore) with the nitrocefin substrate which hydrolysis was monitored by amperometry. i_{Cef} and i_{Clav} correspond to the intensity of the anodic current measured (~ + 0.2 V *vs*. Ag/AgCl) for the sample incubated with the cefotaxime without and with potassium clavulanate, respectively. The value $i = i_{Cef} - i_{Clav}$ was calculated and selected as the analytical response to assess the amount of EBSL *E. coli* producers.

Results: The mean calibration plots for the raw and treated wastewaters (Figure 1) were obtained by analyzing CTX-M type ESBL *E. coli* strains found in wastewaters ($bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}15}$ genes) and were used for the determination of ESBL *E. coli* in 20 raw wastewater and 20 treated wastewater samples. To check the reliability of the amperometric assay, the results were compared to a conventional counting on TBX agar plates supplemented with cefotaxime (Figure 2).

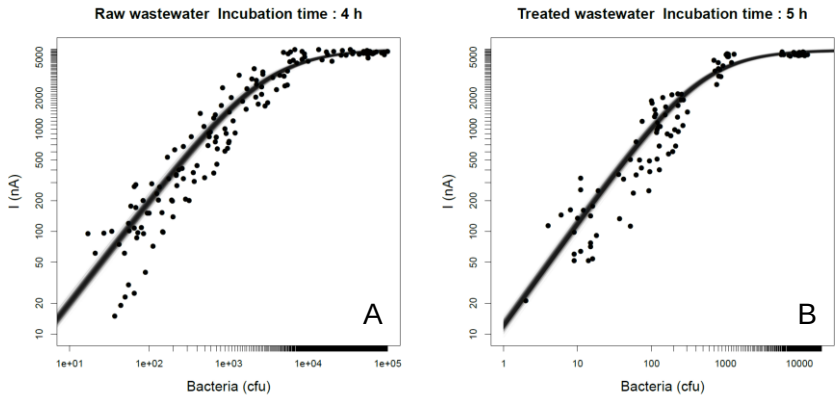

Figure 1. ESBL *E.coli* amperometric calibration curves in (A) raw and (B) treated wasterwater samples

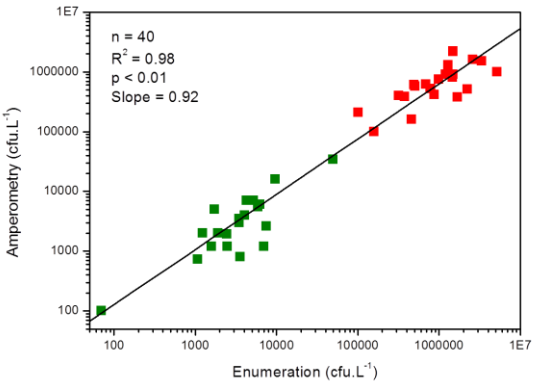

Figure 2. Correlation plot between the amperometric estimation of ESBL *E. coli* and the enumeration for **raw** and **treated** wastewater samples

Conclusion: An excellent correlation was obtained between the amperometric assay and the enumeration. This amperometric assay (5-6h) which is considerably less time-consuming than the culture-based method (24h) holds great promise for the rapid quantification of ESBL *E. coli* in the wastewater networks but also in other types of water samples (rivers, marine waters, etc.).