RAPID ENZYMATIC METHOD FOR THE ENUMERATION OF FAECAL BACTERIA (*ENTEROCOCCI)* IN BATHING SEAWATER

Marion Peirache [1]*, Stephane Mounier [1], Nathalie Patel [1] and Yvan Martin [2]
[1] Laboratoire PROTEE, 83957 La Garde, France - marion-peirache@etud.univ-tln.fr
[2] Institut Oceanographique Paul Ricard, Île des Embiez - Le Brusc

Abstract
The bathing water monitoring has a great interest for both bathers and authorities. The European community has actually lowered the suitable tolerance levels of the two major fecal indicators: *Escherichia coli* and *Enterococci*. The enzymatic assay enables the microbiological quality evaluation in less than an hour. Now available for *Escherichia coli*, this method doesn't work for *Enterococci* yet. Our present work first of all aims at adapting this method for *Enterococci* and in a second time at explaining biotic and abiotic discrepancies met during on site application.
Keywords: Beach, Bacteria, Pollution, Monitoring

Since the new requirements of the European Community (Directive 2006/CE 07), rules have changed and the bathing water quality monitoring has to be improved. The challenge is to combine rapidity and sensitivity with faster techniques than those currently available, allowing real-time results. Such kinds of methods are relevant for a good beaches management, since public health and economic issues are concerned.
The rapid enzymatic detection method is able to produce a fast result, but may be disturbed by interference. The principle of this technique, developed for *Escherichia coli* [1], is to filter a defined volume of sea water through a membrane (0.22 µm) which retains bacteria. Then, the membrane (*i.e.* bacteria) is submitted to a specific substrate enriched with a fluorescent compound, Methylumbelliferone (MUF). The more bacteria are present in the sample, the faster is the lysis of the substrate. The kinetic of the fluorescence apparition during 30 minutes gives a straight line which slope is proportional to enzymatic activity. This slope is correlated to the enumeration of bacteria by standard methods (membrane filtration and/or microplate) giving the number of bacteria in function of enzymatic activity.

To explain discrepancies (figure 1), this work first establish the original response for *Enterococci* by working on pure strain of bacteria, then test different parameters, biotic or abiotic ones, on the enzymatic response.

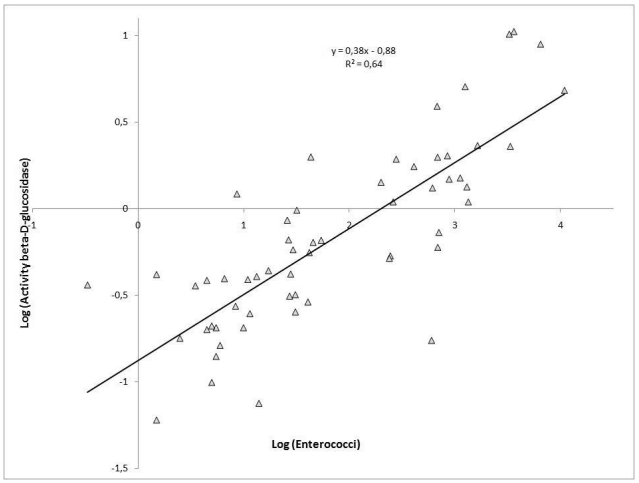

Fig. 1. Log-log linear regression between β-D-glucosidase activity (ng MUF/ mL) and *Enterococci* enumerated by standard method (CFU/100mL) *N=58*

The presence of micro-organisms, of plankton or suspended matter in natural samples can actually induce both biotic and physical interferences. Biotic interferences are confirmed, as previously found in literature for *Escherichia coli* [2] : some phytoplankton organisms are able to degrade the substrate. In the same way, autochthonous bacteria (*Vibrio* spp.) enhance the enzymatic activity. Suspended matter can moreover lead to an underestimation of bacteria numeration : a phenomenon of adsorption may occur, depriving contact of bacteria with substrate. Futhermore organic matter could have influence as its natural fluorescence wavelengths are located around the MUF ones (figure 2).

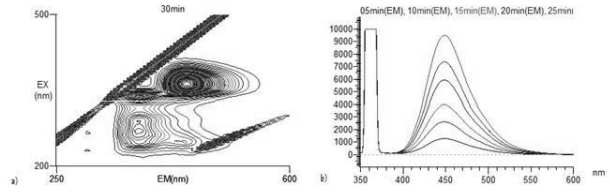

Fig. 2. A) The 3D spectral representation of the fluorescence of the MUF (λ_{ex}= 362 nm; λ_{em}= 445 nm) and natural fluorescence of bacteria at the end of measure (T=30 minutes).The biggest peak (MUF) is over the characteristic peak C (visible humic-like). b) The degradation of the substrate is showed towards the 2D curves of fluorescence of released MUF every 5 minutes along the kinetic (λ_{em}= 445 nm)

The first results of this work is the optimal conditions (pH, temperature, MUF concentration, ...) for enzymatic response of Enterococci (6 strains) found by simplex optimization design.

The second result is obtain by chemometric experimentation on both Enterococci and *Escherichia coli* (3 strains). Coefficient of factorial analysis for 7 parameters (suspended matter, hydrophobic and hydrophilic organic matter, phytoplankton, autochtonous bacteria, heavy metals). are extracted giving the impact of each parameter on the method efficiency.

References
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