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Report

for

IFREMER

Centre de Brest

Contract 93/2 436 400/C

**ENZYMATIC ACTIVITY OF ENTEROBACTERIA
IN RELATION TO RAPID DETECTION AND
SURVIVAL IN SEA WATER: Effect of lactose.**

October 1994
B-1-1994-5



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THE UNIVERSITY OF TRONDHEIM
THE NORWEGIAN INSTITUTE OF TECHNOLOGY

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**ENZYMATIC ACTIVITY OF ENTEROBACTERIA
IN RELATION TO RAPID DETECTION AND
SURVIVAL IN SEA WATER: Effect of lactose.**

by

Liv Fiksdal, Rolf André Bohne and Ingun Tryland

October 1994

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Foreword.

The present work is part of an ongoing collaboration between IFREMER- Centre de Brest and the University of Trondheim, NTH- Division of Hydraulic and Sanitary Engineering. Results from the collaborative work has so far been presented in one scientific article¹, at two international conferences^{2,3} and in a project report⁴. Knowledge obtained was incorporated in the application for support to EU-project NEWTECH which started May 1st 1994.

- 1) Fiksdal, L., Pommepuy, M., Caprais, M-P. and Midttun, I. (1994) Monitoring of fecal pollution in coastal waters by use of rapid enzymatic techniques. *Appl. Environ. Microbiol.* **60** pp 1581-1584
- 2) **Appendix 1.** Pommepuy, M., Fiksdal, L., Caprais, M-P., Melikechi, H. and Cormier, M. (1994) Evaluation of *E. coli* by a colimetric β -D-galactosidase assay. Poster. Proc. Annual meeting ASM 23-27 May 1994, Las Vegas.
- 3) **Appendix 2.** Pommepuy, M., Fiksdal, L., Caprais, M-P. and Cormier, M. (1994) Evaluation of bacterial contamination in an estuary using rapid enzymatic techniques. Poster Proc. IAWQ 17th Biennial International Conference 24-30 July 1994, Budapest.
- 4) L. Fiksdal og Ingunn Midttun (1993) Enzymatic activity of enterobacteria in relation to rapid detection and survival in sea water. Report for IFREMER. Institutt for vassbygging, NTH. B-1-1993-4.

Summary.

The effect of lactose on rapid detection of fecal coliform bacteria in enzyme assays for MUGalase and MUGluase activity has been investigated. Water samples from different locations contaminated by sewage were analyzed. Lactose did not have an effect on enzyme activity and did not seem to be important as an inductor during enzyme assays. Enzyme activity versus fecal coliform numbers in the polluted water samples were in agreement with data found previously. The correlation between MUGalase and MUGluase activity in the different water samples indicated that the relationship between fecal coliform bacteria and bacterial population with β -D-galactosidase was the same in all samples, i.e. that no major addition of non sewage bacteria had occurred.

Results from the optimization of MUGal-concentration showed that maximum rate of hydrolysis was obtained at 0.2 g MUGal/l. Because cellulose acetate/nitrate filters absorb the fluorescent hydrolysis product, MU, polycarbonate filters should be used during the rapid 25 min assay even if filtration time is increased.

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Evaluation of *E.coli* by a colimetric β -D-galactosidase assay.

Appendix 2.

Evaluation of bacterial contamination in an estuary using rapid enzymatic techniques

1. INTRODUCTION

The ongoing collaboration between IFREMER-Centre de Brest and NTH at the University of Trondheim (contract N° 91/2 430 423/C: Etude du rôle de l'activité enzymatique des entérobactéries), has resulted in increased knowledge of enzymatic activities of polluted coastal waters of France and Norway.

Measurement of enzyme activities (methylumbelliferyl- β -galactosidase (MUGalase) and methylumbelliferyl- β -glucuronidase (MUGlucose)) of fecal coliform bacteria (FC) are presently used for rapid detection of FC bacteria in water.

During the investigations the question of influence by environmental conditions has been addressed. Among these is the effect of lactose on MUGalase and MUGlucose-activity. The presence of lactose could be important for enzyme activity of cells in the environment as well as during analysis of enzyme activity.

Sarhan and Foster (6) showed that lactose prevented the activity of MUGlucose assessed as fluorescing colonies on solid media after 7.5 hours of incubation. The mechanism of this inhibition was not clear and several possibilities were suggested, e.g acid production by fermentation of sugars which may inhibit enzyme production or enzyme activity during growth.

When lactose was added to the rapid MUGalase test medium, Berg and Fiksdal (2) demonstrated increase of MUGalase activity of sewage bacteria (10^2 - 10^3 FC/100 ml). Induction of the galactosidase in the presence of lactose was suggested as an explanation. Apte and Bately (1) did, however, not observe increase of galactosidase activity when lactose was added in their one hour MU galactoside test, rather the opposite effect was observed.

The objective of this work has been to contribute to our understanding of the mechanism of MU-substrate hydrolysis by fecal coliform bacteria, by investigating the effect of lactose on rapid hydrolysis of these substrates.

2. SAMPLING LOCATIONS

Samples were collected from:

Lade sewage treatment plant, Trondheim (effluent after sieve and sand sedimentation)
Polluted rivers in the Trondheim area (Søra and Leirelva).

3. MATERIALS AND METHODS.

3.1 Enumeration

Fecal coliform bacteria (FC) were recovered by membrane filtration (Millipore filter GA,

47 mm diameter, pore diameter 0.22 μm) with m-FC medium (survival experiments) and with MacConkey-medium (field investigations). Colony forming units (CFU) were counted after 24 hours at 44.5 °C. Results from CFU-counts on MacConkey-agar are used for determining the correlation between FC numbers and enzyme activities during field investigations..

3.2 Enzyme activity

Methylumbelliferyl- β -D-galactosidase (MUGalase) assay was done by filtering 20 ml water sample through a 0.22 μm pore size 47 mm diameter membrane filter (Poretics polycarbonate filter). The filter was then aseptically placed in a 250 ml flask containing 20 ml sterile substrate solution. The substrate solution was prepared by dissolving MUGal (0.02 g/l) in 0.05 M phosphate buffer (pH 8.0) with 0.02 % sodium lauryl sulfate (SLS) (SigmaChemical Co) by heating to 70°C. The flasks were incubated in a shaking water bath at 44.5°C and the fluorescence intensity was measured each 5 min for 25 min with a Sequel Turner spectrofluorometer model 450 (excitation at 365 nm, emission at 440 nm), after addition of 100 μl 10 M NaOH to 2.5 ml sample in the cuvette to obtain pH>10 and maximum fluorescence.

Calibration was established with solutions of MU (Sigma Chemical Co.) in buffer + SLS-solution. Enzymatic activity measured as production rate of MU ($\mu\text{M min}^{-1}$), was determined by least squares regression. Three sample parallels and two control samples (blanks) were analyzed at each concentration of lactose .

Methylumbelliferyl- β -D-glucuronidase (MUGluase) assay was done as described for the MUGal-assay. The substrate solution was prepared by adding 3 ml MUGlu-solution (50mg MUGlu/50ml sterile, triton-water; triton water was prepared by adding 1 drop of Triton X 100 added to 50 ml distilled water) to 17 ml 0.05 M phosphate buffer (pH 6.4). Three sample parallels and two control samples (blanks) were analyzed at each concentration of lactose.

Optimization of MUGluase assay. Optimization of MUGal concentration was carried out by using sewage effluent samples from Lade sewage treatment plant. The enzyme assay was performed as described in the MUGalase assay, at various concentrations of MUGal, except that 0.05% SLS was used in three of the experiments and 0.02% SLS in the last experiment (08.06.94).

Interference between methylumbelliferone (MU) and different filters was investigated by adding a filter to 20 ml 0.7M sterile methylumbelliferone (MF) solution in 250-ml flasks. Incubation of flasks and fluorescence was performed as described in the MUGalase assay. The following Millipore filter types were studied:

cellulose acetate/cellulose nitrate filters

0.45 μm pore size, 47 mm diameter, Millipore catalogue no. HAWG 047S0

0.22 μm pore size, 47 mm diameter, Millipore catalogue no. GSWP 047S0

polycarbonate filter

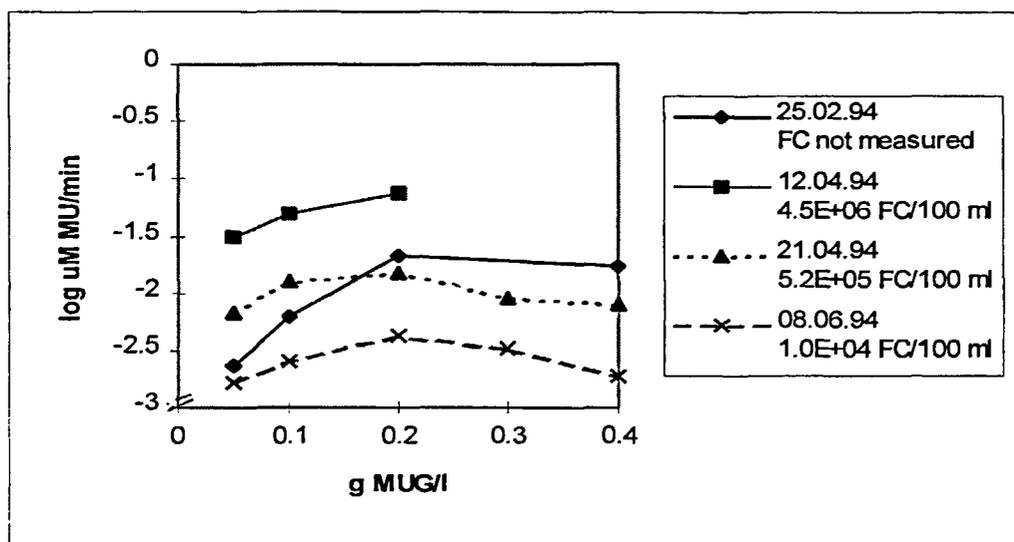
0.2 μm pore size, 47 mm diameter, Millipore catalogue no. GTTP 04700

4. Results

4.1 Optimization of MUGalase assay.

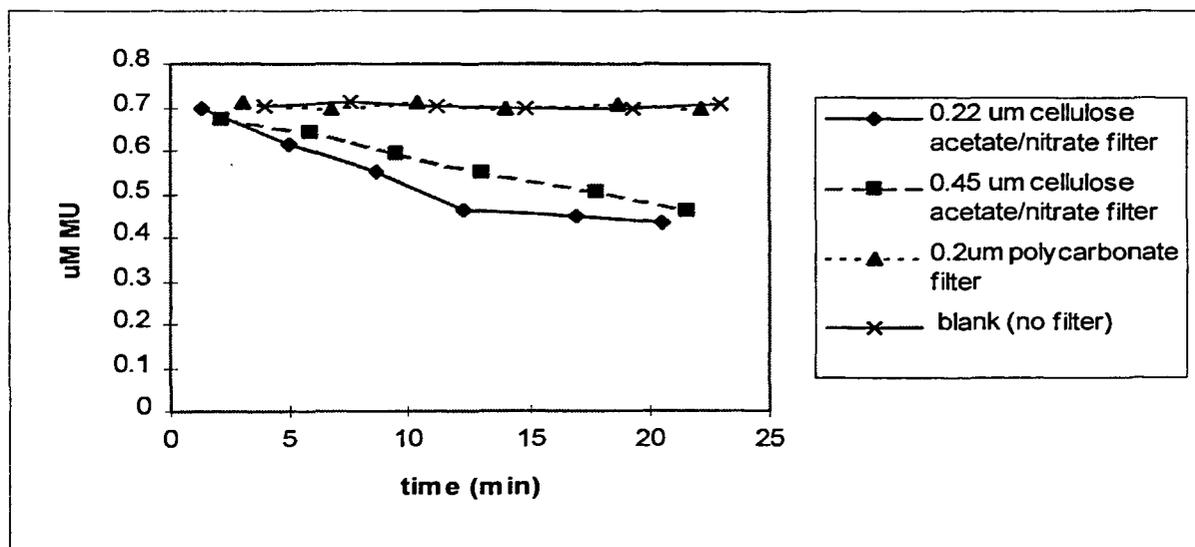
Results indicated that enzyme activities for MUGalase substrate corresponded to saturation kinetics, although a small decrease of activity was observed when substrate concentration increased from optimum value (0.2 g MUGal/l) to 0.4 g MUGal/l) (Figure 1).

Figure 1 Substrate (MUGal) optimization.



The fluorescence of MU did not change with time in flasks with no filter or with polycarbonate filter, while a decrease was observed in the presence of cellulose acetate/nitrate filters (Figure 2).

Figure 2 Effect of filters on MU fluorescence



4.2 Effect of lactose on enzyme activity

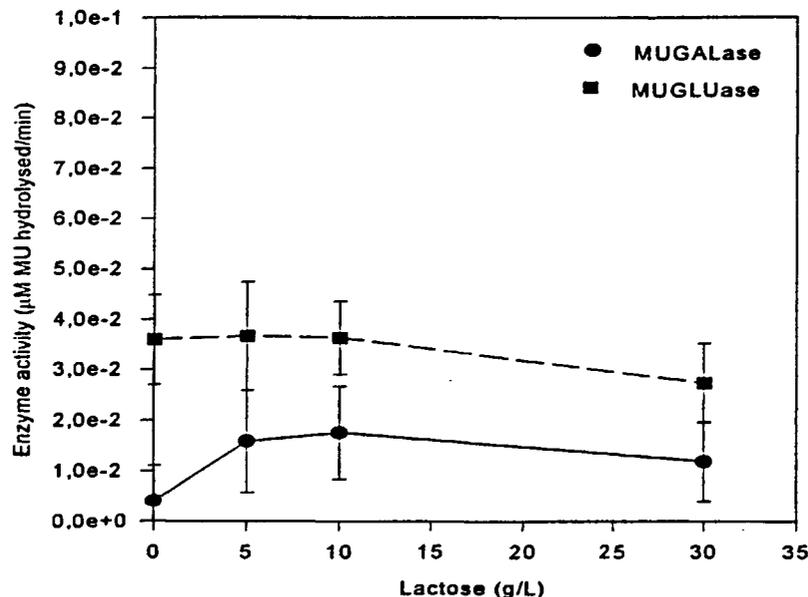
Fecal coliform numbers and enzyme activity of effluent from Lade sewage treatment plant and water samples from polluted rivers (Leirelva and Sørå), are shown in Table 1.

Table 1. Bacterial numbers and enzymatic activity (MUGalase and MUGluase) of polluted water samples.

place/date	fecal coliforms /100 ml	enzyme activity $\mu\text{M MU}/\text{min}$		Standard deviation σ		Relative deviation %		Correlation coefficient R	
		MUGal	MUGlu	MUGal	MUGlu	MUGal	MUGlu	MUGal	MUGlu
Lade 28.06.94	$1.3 \cdot 10^5$	$3.9 \cdot 10^{-3}$	$4.5 \cdot 10^{-2}$	$3.6 \cdot 10^{-3}$	$4.9 \cdot 10^{-3}$	90	11	90	85
Lade 12.07.94	$1.3 \cdot 10^5$	$5.6 \cdot 10^{-3}$	$5.4 \cdot 10^{-3}$	$2.6 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	47	46	94	98
Lade 19.07.94	$1.7 \cdot 10^5$	$4.3 \cdot 10^{-3}$	$1.2 \cdot 10^{-2}$	$2.6 \cdot 10^{-3}$	$2.5 \cdot 10^{-3}$	60	21	95	94
Lade 28.07.94	$2.4 \cdot 10^6$	—	$4.6 \cdot 10^{-2}$	—	$3.6 \cdot 10^{-3}$	—	8	—	96
Leirelv19.0894	$4.4 \cdot 10^3$	$8.4 \cdot 10^{-4}$	$1.3 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$	$1.8 \cdot 10^{-3}$	214	138	98	94
Sørå 21.08.94	$5.0 \cdot 10^3$	$6.4 \cdot 10^{-4}$	$1.9 \cdot 10^{-3}$	$2.4 \cdot 10^{-3}$	$1.9 \cdot 10^{-3}$	375	100	76	78

Results from initial experiments (Fig. 3) when lactose was added to effluent from Lade sewage treatment plant, indicated a slight increase of MUGalase activity when concentration of lactose increased from 0 to 5 g/l. When the concentration was further increased, from 10 to 30 g/l, enzyme activity remained the same. MUGluase activity was not effected by presence of lactose (Fig. 3).

Figure 3. Effect of lactose (0-35 g/l) on activity of MUGalase and MUGluase activity of sewage effluent, 28.06.94 (error bars represent standard deviation).



Previous experiments (1) demonstrated a positive effect, i.e. increased MUGalase activity, after addition of 3.5 g lactose/l. Further experiments were therefore performed in the lower concentration range (0-5 g lactose/l), with sewage effluent and polluted river water (Figs. 4 and 5).

Figure 4. Effect of lactose (0-5 g/l) on activity of MUGalase (A) and MUGluase (B) activity of sewage effluent (error bars represent standard deviation).

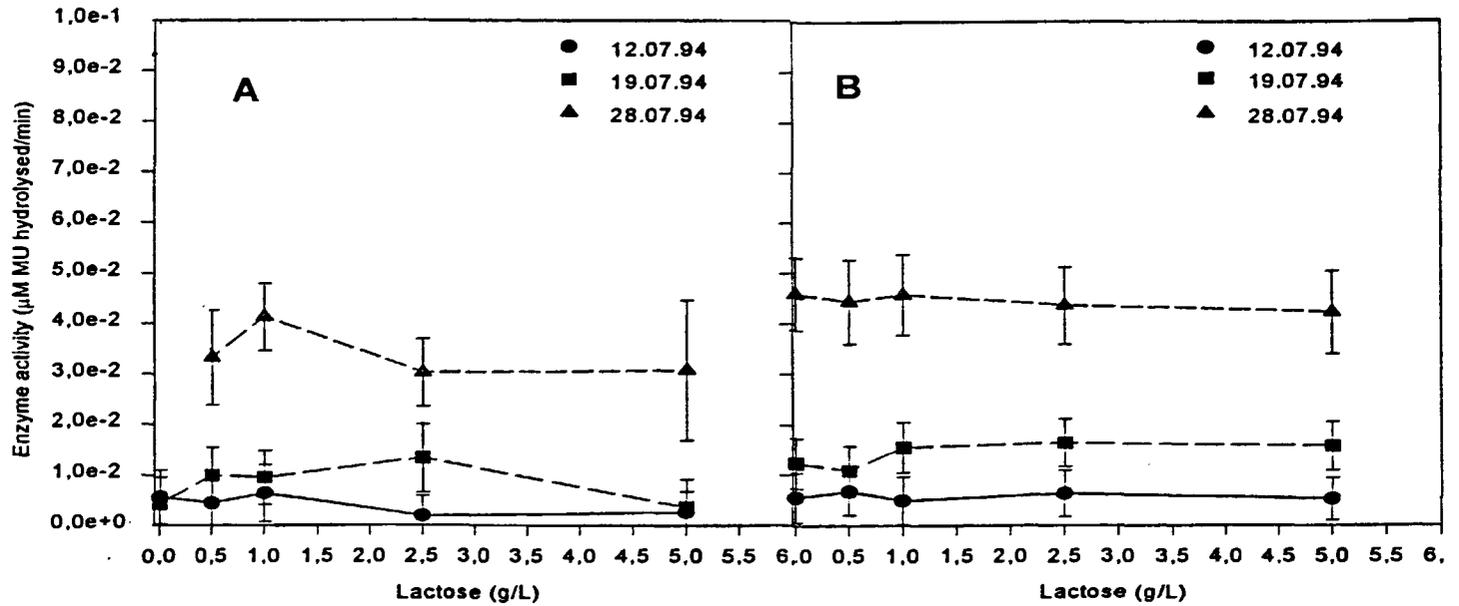
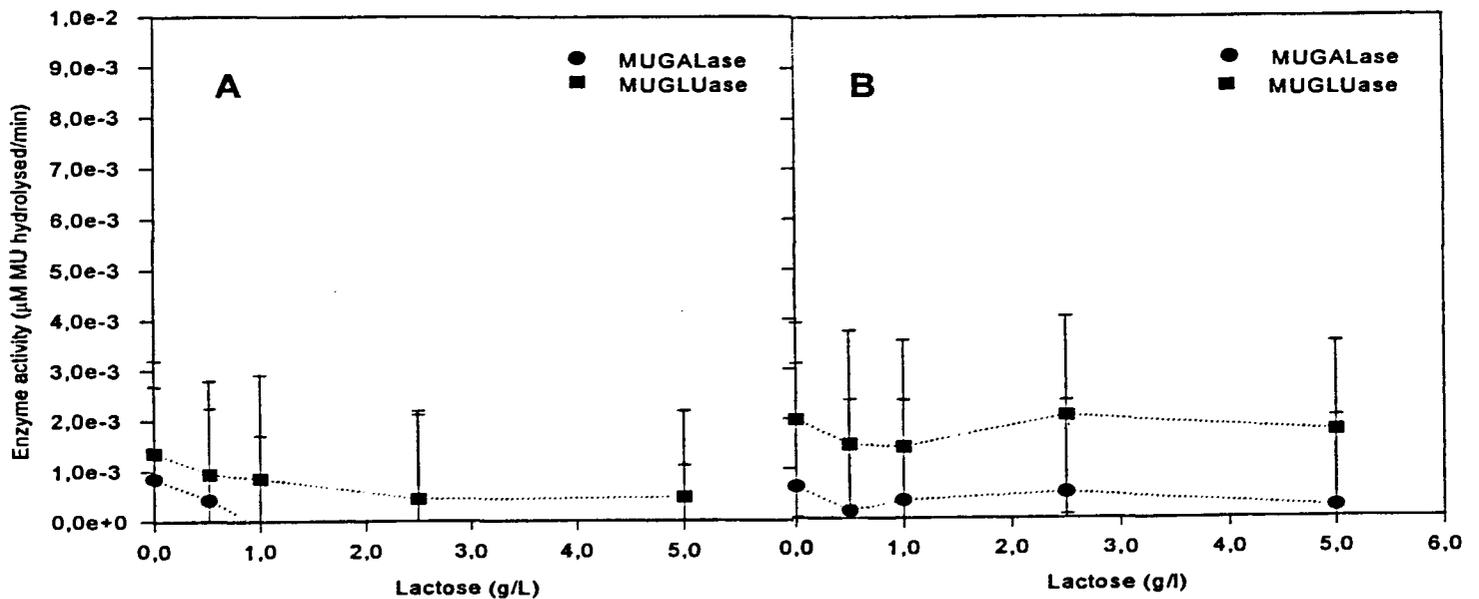


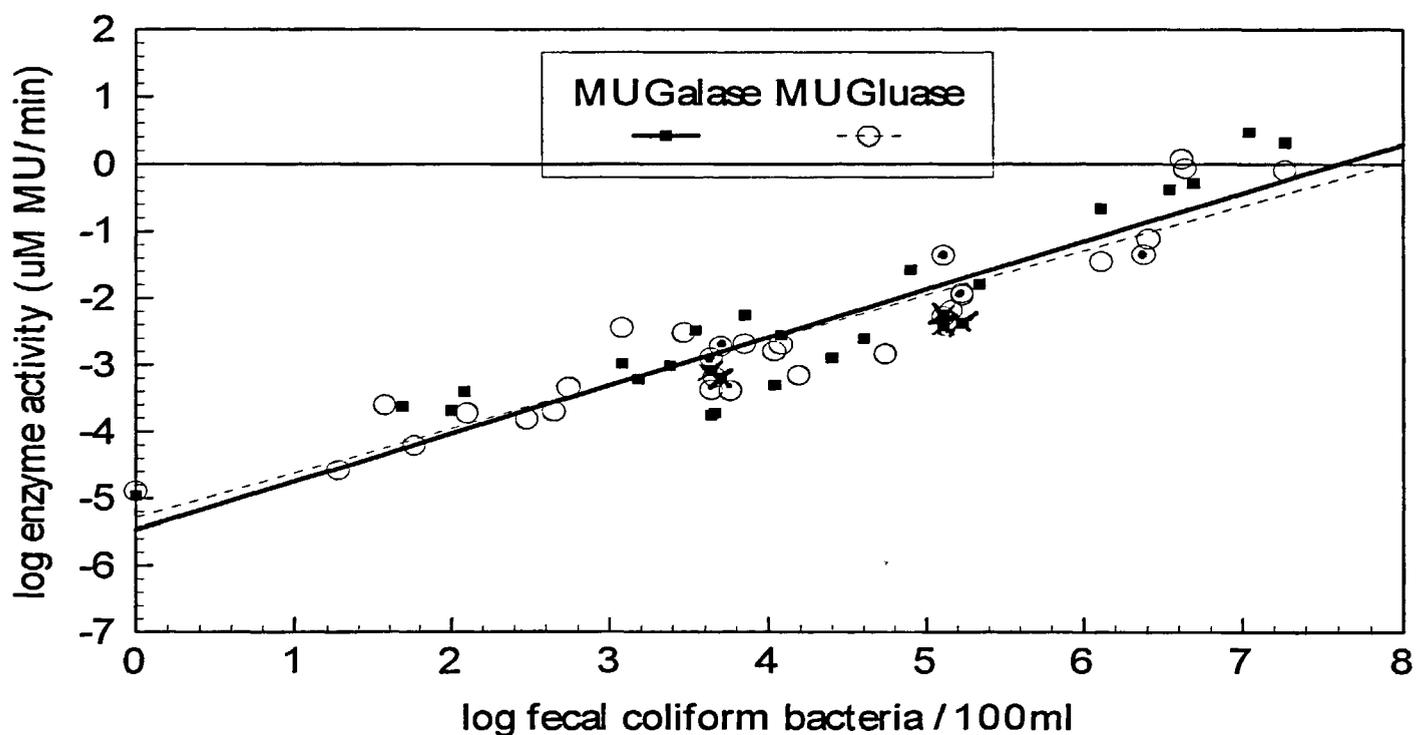
Figure 5. Effect of lactose (0-5 g/l) on activity of MUGalase and MUGluase activity of polluted river water, Leirelva (A) and Sørå (B) (error bars represent standard deviation).



These experiments demonstrated, however, that MUGalase and MUGluase activities were not influenced by varying lactose concentrations. Enzyme activities of polluted river waters (Fig.5) were low and approached the detection limit of the method; relative standard deviations of these samples were high (Table 1).

Enzyme activity versus fecal coliform number of polluted water samples without addition of lactose is presented in Figure 6.

Figure 6 Enzyme activities versus fecal coliform numbers of polluted water. Results from previous (3) (■ MUGalase, ○ MUGluase) and present (Table 1) (✕ MUGalase, ⊙ MUGluase) investigations.



Correlation curves changed from:

MUGalase: $\lg y = 0.74 \lg x - 5.44$ (3)

MUGluase: $\lg y = 0.68 \lg x - 5.31$ (3)

to:

MUGalase: $\lg y = 0.72 \lg x - 5.46$ (Fig. 4)

MUGluase: $\lg y = 0.67 \lg x - 5.28$ (Fig. 4)

after combination of present data (Table 1) with data from previous investigations (3).

5. Discussion

Presence of lactose did not cause decreased MUGlucase activity in the present work, as reported by Sarhan and Foster in their study (6). This was not unexpected because the authors suggested that the decrease of enzyme activity could have been caused by metabolic compounds produced during cell growth, e.g. acid production by fermentation of sugars. In the present investigation, neither the environment (substrate solution) nor time (25 min) could support substantial growth and production of fermentation products.

Previous investigations of sewage bacteria in our laboratory have demonstrated both increase (2) and decrease (not published) of MUGalase activity of water contaminated by sewage when lactose was added in the 25 min assay. Lack of increase of MUGalase activity was shown recently by Apte and Batley (1) during investigation of sea water contaminated by sewage in their one hour assay; rather the opposite effect was observed. A competitive inhibition of the hydrolysis of o-nitrophenyl- β -galactosides by *E. coli* K 12 has been observed in the presence of lactose (5). A similar inhibition of the hydrolysis of MUGal could have occurred in the presence of lactose in the work of Apte and Batley.

However, in the present investigation no influence of lactose on MUGalase activity was observed. Water samples from different localities were investigated (effluent from a sewage treatment plant and polluted rivers). The environment of the bacteria were different and it was expected that their physiological condition were not the same. The enzyme activity per culturable fecal coliform bacteria corresponded to enzyme activity levels found previously (Fig. 6) which were similar to levels of induced *E. coli* (7). This may indicate that the investigated water samples contained induced fecal coliform bacteria, and that no further induction was obtained after addition of lactose.

The relationship between lactose and MUGal -concentrations is important if competitive inhibition can take place. In the present work the proportion of lactose to MUGal varied in the range 2.5/1 - 25/1, and no inhibition was demonstrated. Lactose did therefore have neither an inductive nor inhibitory effect on the MUGalase activity during the assays.

The slope of the linear correlation curve between logarithms of MUGalase and MUGlucase activity in the water samples was approximately 1 (1.075). This indicates that the relationship between fecal coliform bacteria and bacterial population with β -D-galactosidase was similar in all samples, i.e. that no major addition of non sewage bacteria had occurred.

Results from the optimization of MUGal-concentration indicated that 0.2 g MUGal/l was optimal when water samples with $1 \cdot 10^4$ - $4.5 \cdot 10^6$ FC/100 ml were analyzed. Apte and Batley (1) found in their one hour assay with bacterial sea water suspensions, that the assay response did not change when MUGal-concentration exceeded 0.1 g/l. Salt effects (8) could be an explanation of the observed difference of optimal substrate concentration. Enzymatic activities seemed to correspond to saturation kinetics in the present work, although a small reduction of hydrolysis rate occurred when substrate concentration was higher than 0.2 g MUGal/l. Enzyme conformation and activity can be affected by high ionic concentrations in the environment, whether this occurred during the present substrate optimization need to be further investigated. In previous studies (4, 7) 0.4 g MUGal/l was

used to avoid substrate limitation in heavily contaminated samples (sewage effluents).

Because cellulose acetate/nitrate filters absorbed 30 % of MU in the sample, polycarbonate filters should be used during the rapid 25 min assay even if filtration time is increased.

6. Conclusion

Presence of lactose had no influence on MUGalase and MUGluase activity in the present work. Lactose could therefore not be used to increase the sensitivity of the rapid method.

The enzyme activity per culturable fecal coliform bacteria corresponded to enzyme activity levels found previously, which were similar to levels of induced *E.coli*.

This may indicate that the investigated water samples contained induced fecal coliform bacteria, and therefore no further induction was obtained after addition of lactose.

The slope of the linear correlation curve between logarithms of MUGalase and MUGluase activity in the water samples was approximately 1. This indicates that the relationship between fecal coliform bacteria and bacterial population with β -D-galactosidase was similar in all samples, i.e. that no major addition of non sewage bacteria had occurred.

Results from the optimization of MUGal-concentration showed that maximum rate of hydrolysis was obtained with 0.2 g MUGal/l when water samples with $1 \cdot 10^4$ - $4.5 \cdot 10^6$ FC/100 ml were analyzed.

Because cellulose acetate/nitrate filters absorbed the fluorescent hydrolysis product, MU, polycarbonate filters should be used during the rapid 25 min assay even if filtration time is increased.

References.

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- (2) Berg, J. D. and Fiksdal, L. (1988). Rapid detection of total and fecal coliforms in water by enzymic hydrolysis of 4-methyl-umbelliferone-B-D-galactoside. *Appl. Environ. Microbiol.* **54** 2118-2122.
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- (7) Pommepuy, M., Fiksdal, L., Caprais, M-P., Melikechi, H. and Cormier, M. (1994) Evaluation of *E. coli* by a colimetric β -D-galactosidase assay. Poster. Proc. Annual meeting ASM 23-27 May 1994, Las Vegas.
- (8) Wallenfels, K. and Malhotra, M. (1961) Galactosides. *In: Advances in carbohydrate chemistry.* **16** 239

Appendix 1 Evaluation of *E.coli* by a colimetric β -D-galactosidase assay.

I-76 Evaluation of activity of E. coli by a colimetric β -D-galactosidase assay. M. POMMEPUY, L. FIKSDAL, CAPRAIS M.P., H. MELIKECHI and M. CORMIER. Ifremer Plouzané, Univ. Rennes (France) and Univ. Trondheim (Norway).

Use of 4-methylumbelliferyl β -D-galactoside (MuGal), provide a very sensitive and rapid technique for detecting and quantifying enzyme activity of Fecal coliforms (Fc). This technique was used to evaluate the viability of E. coli (H10407) exposed several days in seawater (with and without illumination). Isopropyl β -D-thiogalactosidase (IPTG) was used for induction. Initial β gal - activity of E. coli was about $5 \cdot 10^{-11}$ U (U : $\mu\text{molMU}/\text{cell} \cdot \text{min}$) and less with no induction (without IPTG) and about 2 to $5 \cdot 10^{-9}$ U (with IPTG) i.e. close to Fc activities in wastewater and rivers ($5 \cdot 10^{-8}$ to 10^{-9} U). A significant relationship was found between enzyme activity and bacterial counts just at the beginning of experiments (To : $\log \beta \text{ gal} = 0.98 \log \text{ CFU} - 5.34$ with $r = 0.95$).

Viability of E. coli was then studied in seawater for several days. Results showed bacterial counts decreased by one log/unit/day in the dark while enzymatic activity stayed more or less the same during the experimental period (7-10 days). Similar results were found in very short time period (1 day) in light (decrease in counts \approx 1 log every two hours, constant enzyme activity). Controls (E. coli in fresh water in the dark) stayed at constant levels of counts and activity all along the experimental period. If related to culturable bacteria, β gal activity/cell significantly increased by 2 log or more with time, only in stressed condition (seawater, light). Therefore, these results suggest that non culturable bacteria are able to keep β gal activity during starvation, which is according to results previously obtained in field investigations. The MuGal technique seemed to be more sensitive than the conventional techniques for recovering stressed bacteria in marine waters.

Appendix 2 Evaluation of bacterial contamination in an estuary using rapid enzymatic techniques

EVALUATION OF BACTERIAL CONTAMINATION IN AN ESTUARY USING RAPID ENZYMATIC TECHNIQUES

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ABSTRACT

Rapid enzymatic techniques were used to evaluate bacterial contamination in an estuary. Results show high contamination in the upper estuary where raw waters and sewage from a treatment plant are discharged. Strong relationships between β glucuronidase activities - β galactosidase activities and bacterial counts suggest that faecal bacteria from sewage are diluted in the estuary without bacterial flora modification. These relationships indicate moreover that rapid techniques (25 mn) can provide a rapid and precise evaluation of bacterial pollution of coastal areas.

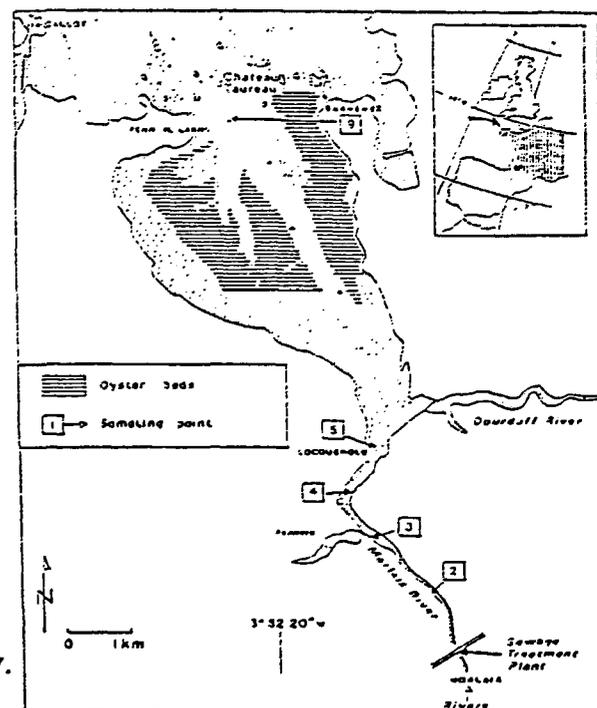
KEYWORDS

Faecal coliform, enzymatic techniques, β galactosidase, β glucuronidase, estuary.

INTRODUCTION

Coastal areas are devoted to numerous activities such as bathing and shellfishing which rely on good water quality. Bacterial water quality is routinely assessed by bacterial indicator counts, the faecal coliform tests being the most frequently used. Novel techniques based on the utilisation of fluorogenic activity have been proposed recently to detect faecal coliforms and *E. coli* ; several authors used β glucuronidase and β galactosidase as fluorogenic substrates to detect *E. coli* and faecal coliforms in fresh and sea water (Berg and Fiksdal, 1988 ; Mates and Schaffer, 1988 ; Palmer *et al.*, 1993 ; Gauthier *et al.*, 1991) ; Fluorogenic glucuronidase assay is recognized as most efficient for recovery of *E. coli* and recommended by Angoustinas *et al.* (1993) to improve the detection of faecal coliforms and *E. coli* in environmental waters.

Fig. 1. Map of Morlaix estuary.



In this study β galactosidase and β glucuronidase activities was measured to evaluate the bacterial contamination of a coastal area, using 4-methylumbelliferyl- β -D-galactoside (MUGal) and 4-methylumbelliferyl- β -D-glucuronide (MUGlu) substrates.

MATERIAL AND METHODS

Bacterial samples were carried out in the bay of Morlaix, located on the northern coast of Brittany in France (fig. 1) ; the features of the bay have been described by Salomon and Pommepey (1990).

The bacterial input in this aquatic system is mainly due to the sewage treatment plant (STP) of the city of Morlaix. This plant is situated at the upstream extremity of the estuary near the sluice and the two rivers. Estimated fluxes are 0.16×10^9 Enterobacteria/s from the rivers and 7×10^9 from the STP. These high values may be explained by the fact that only 60 % of the sewage is depurated. There are other minor tributaries around the bay which we considered as negligible.

Sampling trips were carried out in spring 1992 and winter 1993 during neap and spring tides. Waters samples were collected in sterile bottles and immediately refrigerated ; bacteria assays begun 2-4 hours after collection. Salinity was measured using a Beckman induction salinometer.

Faecal coliforms were enumerated by standard membrane filter techniques (Millipore HAWG 0.22 μm) with incubation on m-FC medium (DIFCO) and Mac Conkey agar (oxid). Colony-forming units (CFU) were counted after 24 hours of incubation at 44.5 °C.

β -D-galactosidase (MUGalase) assay. Water samples were filtered thorough 0.22 μm pore size 47 mm diameter membrane filter (Millipore GTTP). The filter was then aseptically placed in a 250 ml flask containing 13.5 ml sterile 0.05 M phosphate buffer (pH. 7.9) and 0.05 % sodium lauryl sulfate (SLS) (SigmaChemical Co). MUGal (Sigma Chemical Co.) was dissolved by heating (0.23 mg/ml in buffer + SLS heated to 70 °C) and 9 ml was added to each flash and to a sterile control flask containing 13.5 ml buffer + 0.05 % SLS. The flasks were incubated in a shaking water bath at 44.5 °C and fluorescence intensity of sample aliquots was measured every 5 min for 25 min with a SEQUOIA turner (excitation at 360 nm, emission at 450 nm), after addition of 100 μl 10 M NaOH to 2.5 ml sample in the kyvette. The enzymatic activity measured as production rate of MU (M min^{-1}), was determined by the least square linear regression.

β -D-glucuronidase (MUGluase) assay was performed as described in the MUGal-assay except that filters were placed in flasks containing 17 ml sterile 0.05 M phosphate buffer (pH 6.4) and the assay started by addition of 3 ml MUGlu-solution (50 mg MUGLu/50 ml sterile triton-water ; triton water was prepared by adding 1 drop of Triton X 100 to 50 ml distilled water).

MORLAIX 93

RESULTS

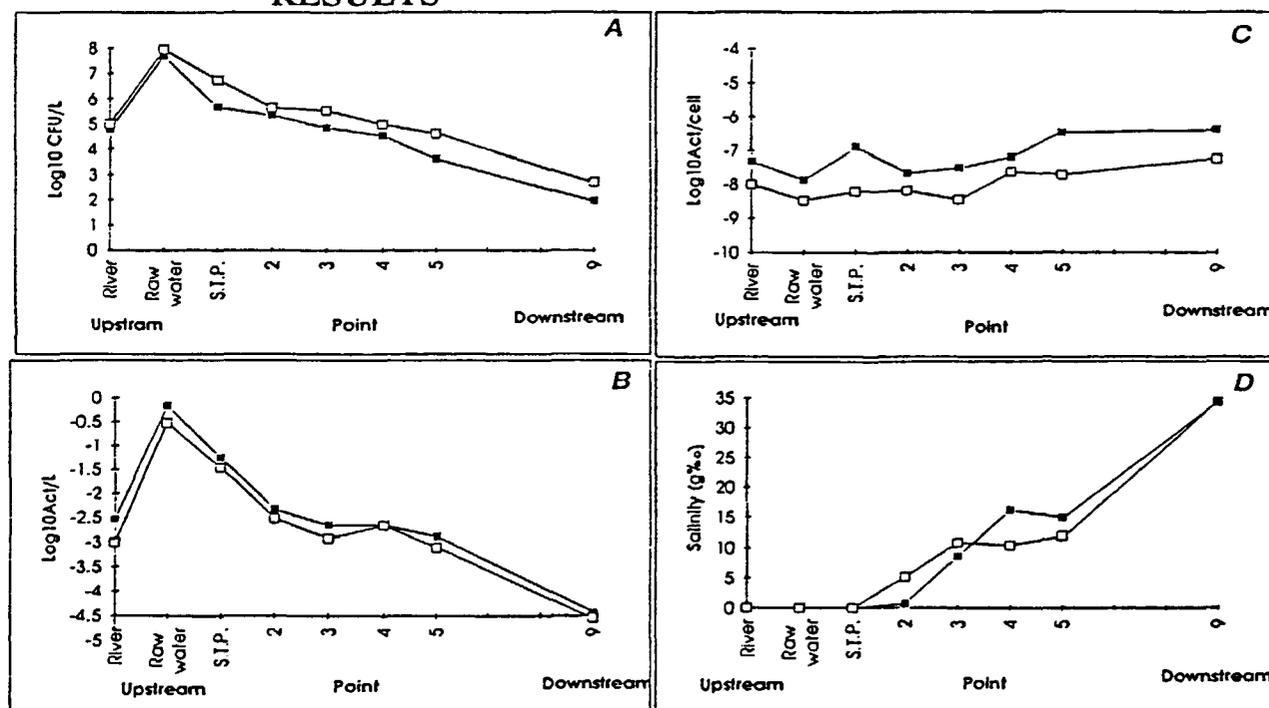


Fig. 2.
Estuary of Morlaix
(□ 11th Jan. 1993 -
■ 14th Jan. 1993)
A - Faecal coliforms
B - MUGglucuronidase
activities/L
C - MUGglucuronidase
activity /per cell
D - Salinities.

Some results are presented in fig. 2 ; the salinity at low tide on January the 11th and the 14th was less than 15 ‰ over the entire upper estuary (points 2 to 5), while at the entrance of the bay (point 9) salinity was closed to 35 ‰. Bacterial counts and glucuronidase activities describe the dilution of faecal coliforms (FC) from the STP outfall to the open sea : high contamination was measured at the STP ($5 \cdot 10^5$ FC/100 ml) and in raw water ($9 \cdot 10^6$ FC/100 ml) up to point 5, where contamination measured was highest then slowly decreased going downstream. At the mouth (point 9) faecal contamination was 50 FC/100 ml for the two hydrodynamic conditions and corresponded to very low glucuronidase activity ($3.5 \cdot 10^{-5}$ $\mu\text{mol/l}$). Enzymatic activity decreased on the same scale as FC all along the estuary. The mean cell activity (expressed in logarithm $\mu\text{mol/l}$) was 6.62 ± 0.52 (on Jan. 11th) and 7.84 ± 0.46 (on Jan. 14th).

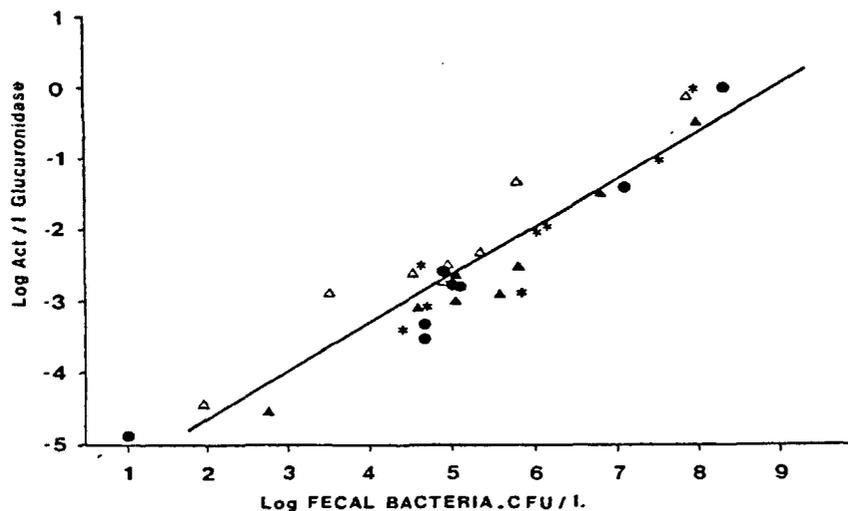


Fig. 3. Relationship between Faecal coliforms and MUGglucuronidase activity (● 27 March 1992, * 4 April 1992, Δ 11 Janv. 1993 ; \blacktriangle 14 Janv. 1993)

In fig. 3 the relationship is reported between FC counts and β glucuronidase activities : a strong correlation is obtained ($r = 0.93$ with $n = 32$; $y = 0.68 x - 6.07$) ; the relationship between β glucuronidase and β galactosidase activities is also very strong ($r = 0.97$; $n = 32$; $y = 0.96 x - 0.278$).

DISCUSSION

Freshwater discharged into the bay of Morlaix occasion low salinities in the upper estuary. The salinity increases towards the mouth as a result of mixing between estuary water and the open sea, enhanced by tidal action ; moreover, high bacterial contamination associated with low salinities was observed, as reported by Salomon and Pommepuy (1990). The impact of bacterial discharge of raw water is observed mainly in the upper estuary : in this area low mixing associated with high turbidity (100 mg l^{-1} and more) conditions required to maintain bacterial contamination. Pommepuy *et al.* (1992) observed low die-off rates due to water quality : organic matters which increase bacterial salt-tolerance and bring about light penetration enhance bacterial survival in coastal areas. At the mouth of the bay (pt. 9) the mixing of waters lowers contamination : physical mechanisms are much more effective in reducing bacterial sanitary risks, than is mortality (Salomon *et al.*, 1990).

Enzymatic assay is a sophisticated way to measure faecal contamination in coastal areas. While 24 hours or more are needed by conventional techniques, only 25 mn are necessary to obtain results with these rapid techniques. These substrates were used by several authors to recover faecal coliforms in food, water and clinical samples (Frampton and Restaino - Review 1993). McFeters *et al.* (1993) used it to recover coliforms and *E. coli* injured by chlorine in drinking water.

In the present study the technique from Berg *et al.* (1988) was used. The strong relationship between FC and enzymatic activities supports the fact that enzymatic assays give an excellent idea of faecal contamination ; however standard error is observed in this correlation. Glucuronidase activity - as galactosidase activity - is

inductive and its expression is affected by physiological factors or genetic differences between strains (Martins *et al.*, 1993) ; moreover some *Escherichia coli* do not encode the enzyme. Enzymatic activity by cell, slightly moving along the estuary suggests the same bacterial population is diluted along the estuary. Die-off rates are slightly the same for FC and *E. coli*. Difference between the main activity was observed between January the 11th and the 14th. One probable explanation is the difference in populations discharged in the bay by the STP one a given day of the week. This result is reinforced by the fact that β glucuronidase and β galactosidase activities are strongly correlated in this areas, with a very low standard error (0,12).

The detection limit is $10^2 - 10^3$ FC/100 ml that corresponding to EEC bathing standards. Nevertheless this technic can be applied today for rapid response about STP performance (disinfection) or about impact of bacterial discharge on coastal areas.

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