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PROGRAMME NATIONAL D'OCEANOLOGIE COTIERE

MICROBIOLOGIE SANITAIRE

Valorisation des résultats du P.N.O.C. à travers la modélisation

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Le modèle "Adaptations Physiologiques" élaboré dans le cadre du P.N.O.C. – volet sanitaire a été développé dans le rapport 1996 du contrat n° 95243601 DEL. Dans le présent travail, nous présentons quelques exemples d'application de ce modèle.
Parmi ces exemples, certains ont déjà été exposés lors de réunions d'avancement des travaux ou du séminaire de Nantes (juin 1996). Les fiches les concernant sont présentées en annexe avec :

- Le devenir des catégories cellulaires et ses facteurs (annexe 1).
- La comparaison des processus physiologiques et de la prédation dans le devenir des cellules (annexe 2).
- L'étendue du risque sanitaire selon le modèle d'appréciation choisi (annexe 3).
- Des conséquences pratiques du modèle "adaptations physiologiques" avec les effets de la collecte et du traitement des eaux usées sur le devenir des E. coli d'un rejet en mer (annexe 4) et les effets sur la qualité d'une plage (annexe 5).
- Quelques conclusions issues de l'examen de ces exemples (annexe 6).

Plus récemment, les facteurs du comportement des catégories cellulaires ont été mieux analysés et l'on a développé un exemple d'application concernant le devenir des cellules dans un gradient fleuve–mer qui résume finalement plusieurs situations environnementales. Ces éléments ont été présentés au 5ème symposium Européen de microbiologie marine à Bergen, Norvège (août 1996). Cette communication (affiche) est rapportée ci–après.

TOWARDS A MATHEMATICAL MODEL OF CELLULAR BEHAVIOUR OF ENTERIC BACTERIA AS AFFECTED BY MARINE CONDITIONS

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ABSTRACT

A model of *E. coli* 's responses to marine environmental conditions and resulting cell behaviour has previously been developed. The model considers two kinds of antistress physiological responses and three induced cellular categories with different properties (culturable cells B_1, viable but definitively non-culturable cells B_2, resuscitable dormant cells B_3). Since previous simulations have shown a good correlation with experimental data, we analyse here the effects of some environmental parameters (organic matter, salinity, sunlight) on cellular category dynamics in sea water. Results show that sunlight is the most important factor affecting that dynamics. With the use of two *E. coli* inocula (log or stationnary phase cells), sanitary implications of the effects of these parameters are evaluated along a river-sea hypothetical gradiant. The fate of reversible dormant B_3 cells seems to depend not only on environmental conditions but strongly on the cellular state of the inoculum : stationary phase cells, with which a previously activated antistress response, are less sensitive to environmental stress leads to a high number of dormant cells.

If these model previsions are right, it would be highly important to develop suitable methodologies to detect and count *in situ* such dormant inculturable but resuscitable cells due to their sanitary significance

KEY WORDS : E. coli, sea water, marine stresses, survival, dormancy, cell behaviour, mathematical model.

INTRODUCTION

Adaptative responses of bacteria to adverse environmental conditions have been studied intensively during the last decade. The survival process under unfavourable environmental conditions depends on the ability of the cell to develop an anti-stress

response which induces "cross-protection" (HENGGE-ARONIS, 1993, GAUTHIER et *al*, 1993) through the production of "stress-proteins". Several studies have shown that adverse condition leads to the existence of different cellular states which exhibit different properties (ROSZAK and COLWELL, 1987, KAPRELYANTS et *al*, 1993,...). In sea water, enteric bacteria as well as many autochtonous bacteria enter into the viable but non culturable state (VNC) (XU et *al*, 1982, OLIVER, 1993).

In a previous paper we have proposed a general conceptual view of adaptative strategies of *Escherichia coli* in the marine environment and an associated mathematical model which allows us to simulate the behaviour of different cellular categories (MARTIN et *al*, in press).

Here, we develop, through model simulations, the effects of different marine environmental variables (salinity, organic matter, solar radiation) on three cellular state dynamics, and using two kinds of *E. coli* inocula (log or stationary phase preculture), their sanitary consequences in a river-sea hypothetical gradiant.

MODEL STRUCTURE AND HYPOTHESES

A complete description of the model has been previously described (MARTIN et *al*, in press). Principal hypotheses and equations used are summarized underneath.

Dynamic behaviour of bacteria cells was considered to dependent on (i) their energetic state (i.e., level of intracellular metabolic pool) and (ii) their ability to develop stress-responses.

Energetic model (fig. 1)

The conceptual model is based on the existence of an intracellular metabolic pool (q) where energy is stored and taken up.

Cell physiological status, from growth to lysis, was linked to three metabolic pool thresholds.

Anti-stress response model (Fig. 2)

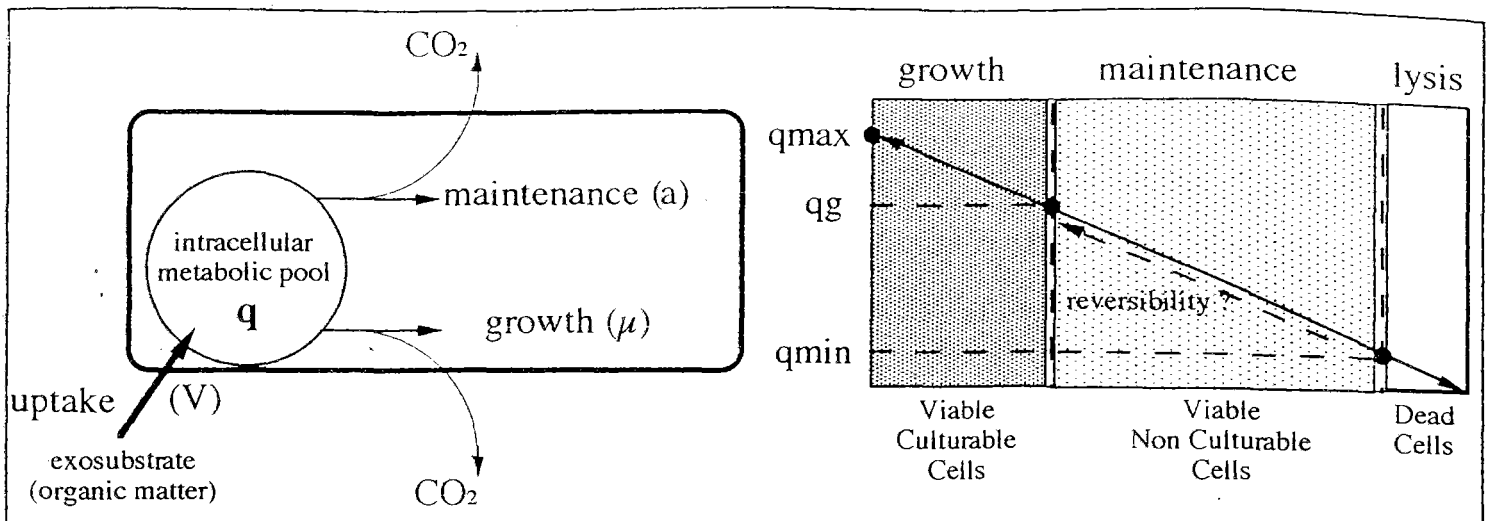


Fig. 1: Illustration of energetic model

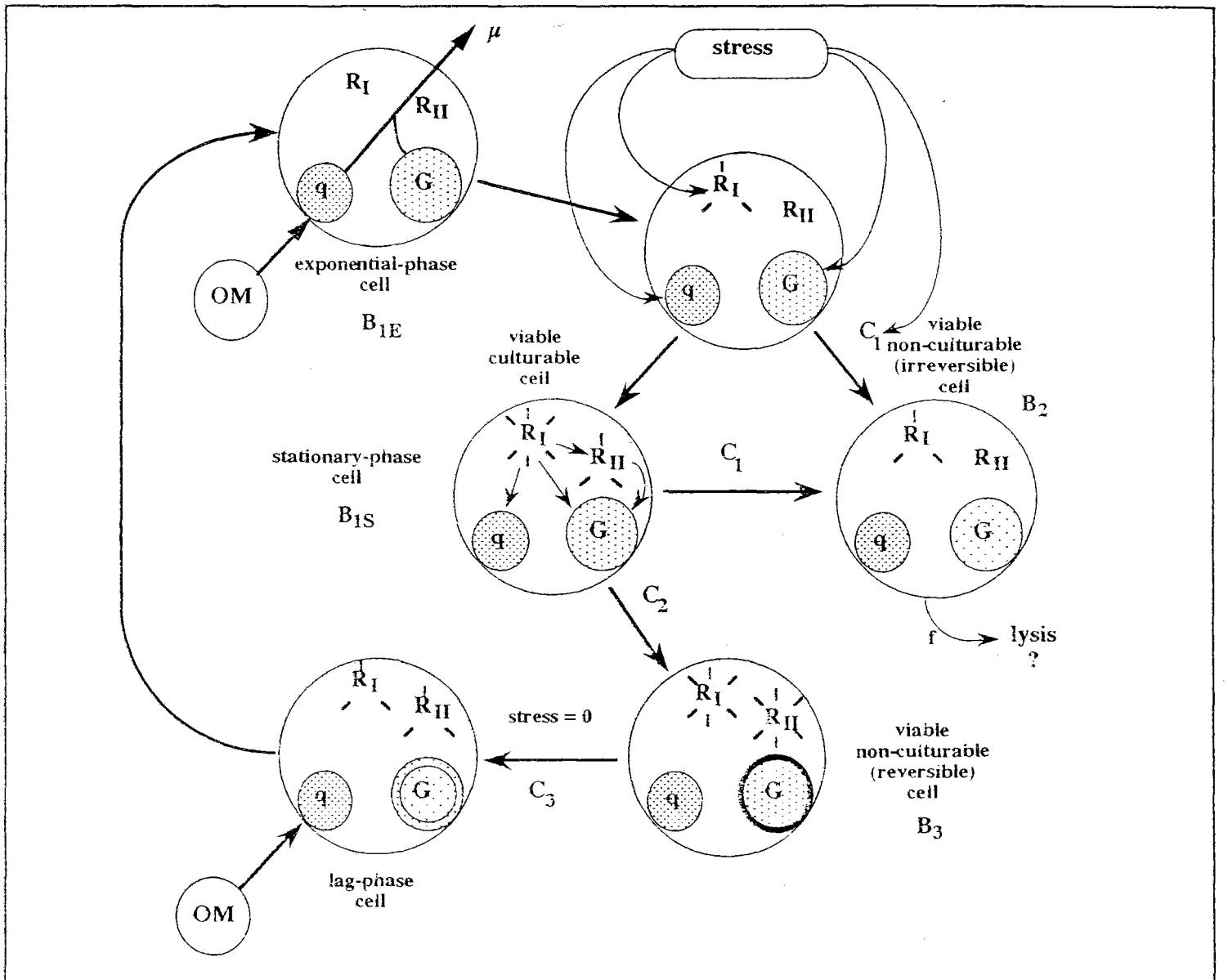


Fig. 2: Schematic representation of cellular behavior following the conceptual model of physiological adaptations.

We have assumed the following hypotheses : When cells taken from the exponential phase (B_1) are submitted to a stress, a portion of them may lose plateability altogether (B_2 cells). "Surviving" cells may develop successive adaptative strategies :

- a general or non specific antistress response (R_I) which will increase stress resistance in B_1 cells ("Rampart" strategy, enzymes destroying reactive oxygen species, osmoprotectant synthesis, ...)
- entry into "dormant" or VNC reversible state (B_3 cells), characterised by undefinitive partial plateability loss, DNA protection (e.g., DNA-binding protein), progressive decrease in maintenance requirements depending on metabolic pool level (Donjon" strategy) (R_{II}).

Three cellular categories results from these responses :

- B_{1S} : adapted viable culturable cells
- B_2 : viable but definitively non culturable cells
- B_3 : viable bur reversible non culturable cells.

Biological processes, equations and parameter values are summarized in tables 1, 2 and 3, respectively.

RESULTS :

Calibration of model parameters has been carried out from a lot of data collected during several microcosm experiments (TROUSSELLIER and *al*, in press). Most of the comparisons carried out in differents conditions (factorial multistress plans) have lead to a good correlation between observed and simulated data (MARTIN and *al*, in press). The validity of the model, thus allows us to analyse simulated cell population dynamics and to test the effects of environment parameters on the behaviour of cellular categories.

Table 1: Biological processes used in the model

1. **exosubstrate uptake (V)**: dependent on substrate concentration (saturation kinetics, A), inhibitory effects of salinity (B) and light (C) with eventual attenuation due to R_I response (K'_S and K'_L) and modulation by intracellular metabolic pool size (K_q)

$V = V_M\, A\, B\, C\, K_q$ where: $L = L_M \sin [\pi (t - t_0) / (t_f - t_0)]$

$A = OM / (K_{MO} + MO)$ $K'_S = A_S (R_I - R_{Im}) + 1$

$B = 1 / (1 + S / K_S\, K'_S)$ $K'_L = A_L (R_I - R_{Im}) + 1$

$C = 1 / (1 + L / K_L\, K'_L)$

$K_q = (q - q_m) / (q_M - q_m)$

2. **growth (μ)**:

$\mu = \mu_M [(q_1 - q_g) / (q_M - q_g)]$

where $q_1 - q_g$=available metabolic pool fraction for growth

3. **metabolic requirements for maintenance (a)**: dependent on q level (K_q)

$a = a_M K_q$

4. **RI and RII stress responses**:

$dR_i / dt = P_i R_i - P'_i R_i$

where P_i = activation rate (h^{-1}) and P'_i = repression rate (h^{-1})

R_I activation/repression by P_I: if OM = 0 or S ≥10 or L >4000 lux then P_I else P'_I

R_{II} activation by P_{II}: if $R_I \approx R_{IM}$ then P_{II}

R_{II} repression by P'_{II}: if OM > 0 and L < 4000 lux

5. **loss of culturability**:

a- transformation of B_1 cells into B_2 cells (viable but irreversible non culturable state)

$C_1 = C_0 + C_S + C_L + ß (C_S\, C_L)$ where:

$C_S = C_{SM}\, S / (K''_S\, K_S + S)$ and $K''_S = B_S [(R_I - R_{Im}) / R_I] + 1$

$C_L = C_{LM}\, L / (K''_L\, K_L + L)$ and $K''_L = B_L [(R_I - R_{Im}) / R_I] + 1$

transformation rate is limited by an instantaneous repair coefficient (δ) depending from R_I:

if $R_I > 0.8\, R_{IM}$ then $\delta\, C_1$ with $\delta < 1$

b- transformation of B_1 cells into B_3 cells (reversible dormant state):

$C_2 = C_{2M} [1 - ((R_{2M} - R_2) / R_{2M})]$

6. **B_3 cells dormancy exit**:

$C_3 = C_{3M} [1 - ((R_{2M} - R_2) / R_2)]$

activation of C_3 : if $R_{II} \le \alpha_2\, R_{IIM}$

repression of C_3 : if $R_{II} > \alpha_2\, R_{IIM}$ or if OM = 0 and L > 4000 lux

7. **Cell autolysis**:

if $q \le \alpha_3\, q_m$ then $f = f_M$

Table 2 : System of differential equations

variation of :

1. **exosubstrate (OM)** = autolysis gain - uptake losses

$dOM / dt = u\ f\ B_i - V_i\ B_i$

2. **intracellular metabolic pool (q)** = exosubstrate uptake - growth - maintenance - R_I activation - R_{II} activation + repression R_I and R_{II}

$dq / dt = V_i - (\mu_1 / Y_g)\ q_1 - a_i\ q_i - (P_I / Y_I)\ R_I - (P_{II} / Y_{II})\ R_{II} + P'_I\ R_I + P'_{II}\ R_{II}$

3. **stress response RI** = activation - repression

$dR_I / dt = P_I\ R_I - P'_I\ R_I$

4. **stress response RII** = activation - repression

$dR_{II} / dt = P_{II}\ R_{II} - P'_{II}\ R_{II}$

5. **B_1 cells** = growth - maintenance - autolysis - loss by entry into B_2 and B_3 states + gain from B_3 revival

$dB_1 / dt = \mu_1\ B_1 - a_1\ B_1 - f\ B_1 - C_1\ B_1 - C_2\ B_2 + C_3\ B_3$

6. **B_2 cells** = gain from B_1 - maintenance - autolysis

$dB_2 / dt = C_1\ B_1 - a_2\ B_2 - f\ B_2$

7. **B_3 cells** = gain from B_1 - maintenance - autolysis - revival

$dB_3 / dt = C_2\ B_1 - a_3\ B_3 - f\ B_3 - C_3\ B_3$

Table 3 : Numerical values adopted for model variable and parameters

variable/parameter	symbol	value	unit
exosubstrate concentration	OM	x	mg C l^{-1}
maximum uptake rate	V_M	0.2	h^{-1}
half saturation constant for OM	K_{OM}	4	mg C l^{-1}
salinity	S	x	g l^{-1}
S half rate constant	K_S	20	g l^{-1}
maximum light intensity	L_M	x	lux
L half rate constant	K_L	35000	lux
maximum growth rate	μ_M	0.32	h^{-1}
q maximum level	q_M	0.5	-
q minimum level	q_m	0.001	-
q growth threshold	q_g	0.475	-
growth yield	Y_g	0.40	-
maximum specific maintenance rate	a_M	0.01	h^{-1}
R_I activation rate	P_I	0.19	h^{-1}
R_I repression rate	P'_I	0.25	h^{-1}
R_{II} activation rate	P_{II}	0.167	h^{-1}
R_{II} repression rate	P'_{II}	0.13	h^{-1}
R_I maximum level	R_{IM}	0.05	-
R_I minimum level	R_{Im}	0.005	-
R_{II} maximum level	R_{IIM}	0.005	-
R_{II} minimum level	R_{IIm}	0.0005	-
R_I production yield	Y_I	0.80	-
R_{II} production yield	Y_{II}	0.90	-
minimum V I N C transformation rate	C_0	10^{-5}	h^{-1}
maximum V I N C transformation rate due to S	C_{SM}	0.115	h^{-1}
maximum V I N C transformation rate due to L	C_{LM}	1.115	h^{-1}
S L interaction coefficient	ß	7.5	-
instantaneous repair coefficient	δ	0.01	-
maximum V R N C transformation rate	C_{2M}	0.016	h^{-1}
"revival" maximum rate	C_{3M}	0.064	h^{-1}
R_{II} action threshold coefficient	α_1	0.8	-
R_{II} revival threshold coefficient	α_2	0.2	-
q_M autolysis threshold coefficient	α_3	0.01	-
maximum autolysis rate	f_M	0.005	h^{-1}
assimilable lysed cellular fraction	u	0.3	-

1. Effects of the sea water environment on cellular state dynamics

1.1. General response to stress conditions.

Simulation results of the fate of log phase cells incubated in oligotrophic marine water (no organic matter, salinity = 38) with or without sunlight exposure are shown in fig. 3. Apart from differences in velocity of occuring between dark and light conditions, three stages may be identified :

- Stage 1 (initial injury)

  . Large increase of B_2 cells (B_1 transformation), especially with sunlight which strongly affects the culturability loss rate of B_1 cells.

  . No B_3 cell formation.

- Stage 2 (R_I response installation : "Rampart strategie")

  . Decrease of B_1 transformation rate also occuring with light.

  . No B_3 cell formation.

- Stage 3 (R_{II} response installation : "Donjon strategy"

  . Formation of B_3 cells, particulary with no sunlight (starvation and hyperosmotic stress).

1.2. Effects of environmental parameters.

We have simulated the effect of organic matter, salinity and sunlight intensity on cellular dynamics.

When cells are incubated in sea water and exposed to nycthemeral light cycle, an increase in organic carbon concentration leads to an increase of B_1 and B_3 cells (fig. 4). However, after 48 h incubation, these cells represent less than 1% of the population even at the highest simulated organic carbon concentration.

Under starvation conditions and darkness, an increase in salinity leads to a slow diminution of B_1, B_3 cells and a slow increase of B_2 ones. (Fig. 5). In any case, the proportion of B_1 cells remains higher than the proportion of B_3 cells, but differences

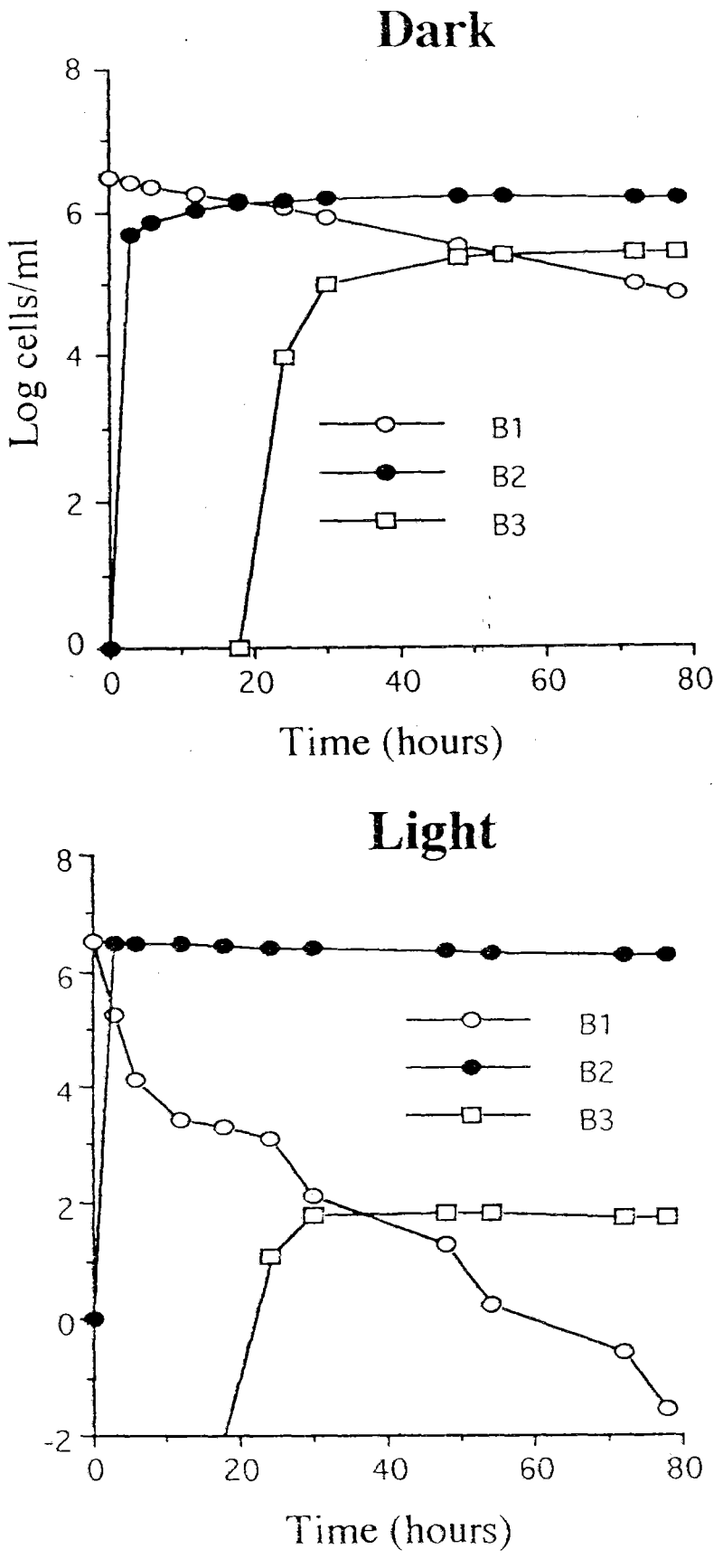
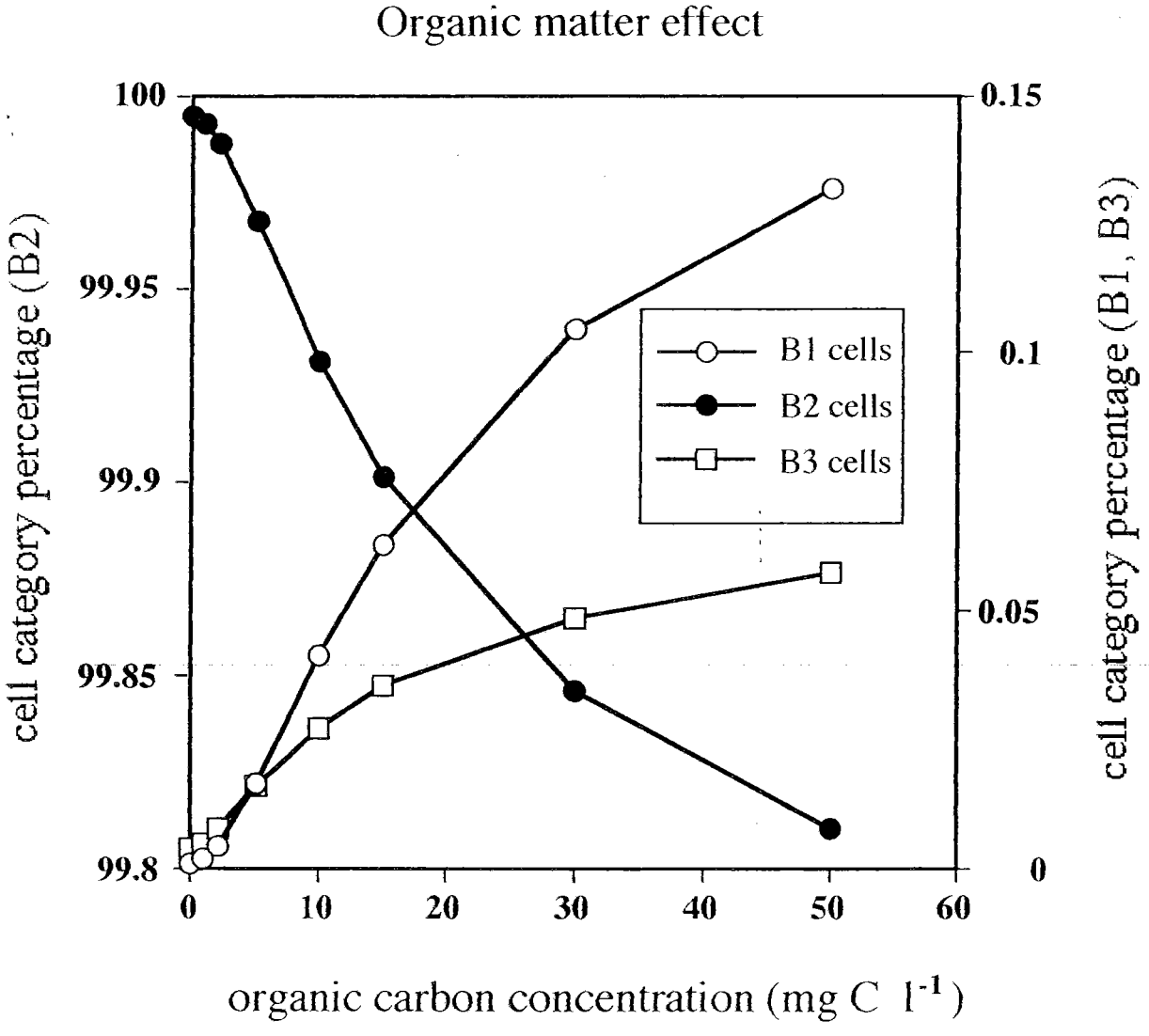
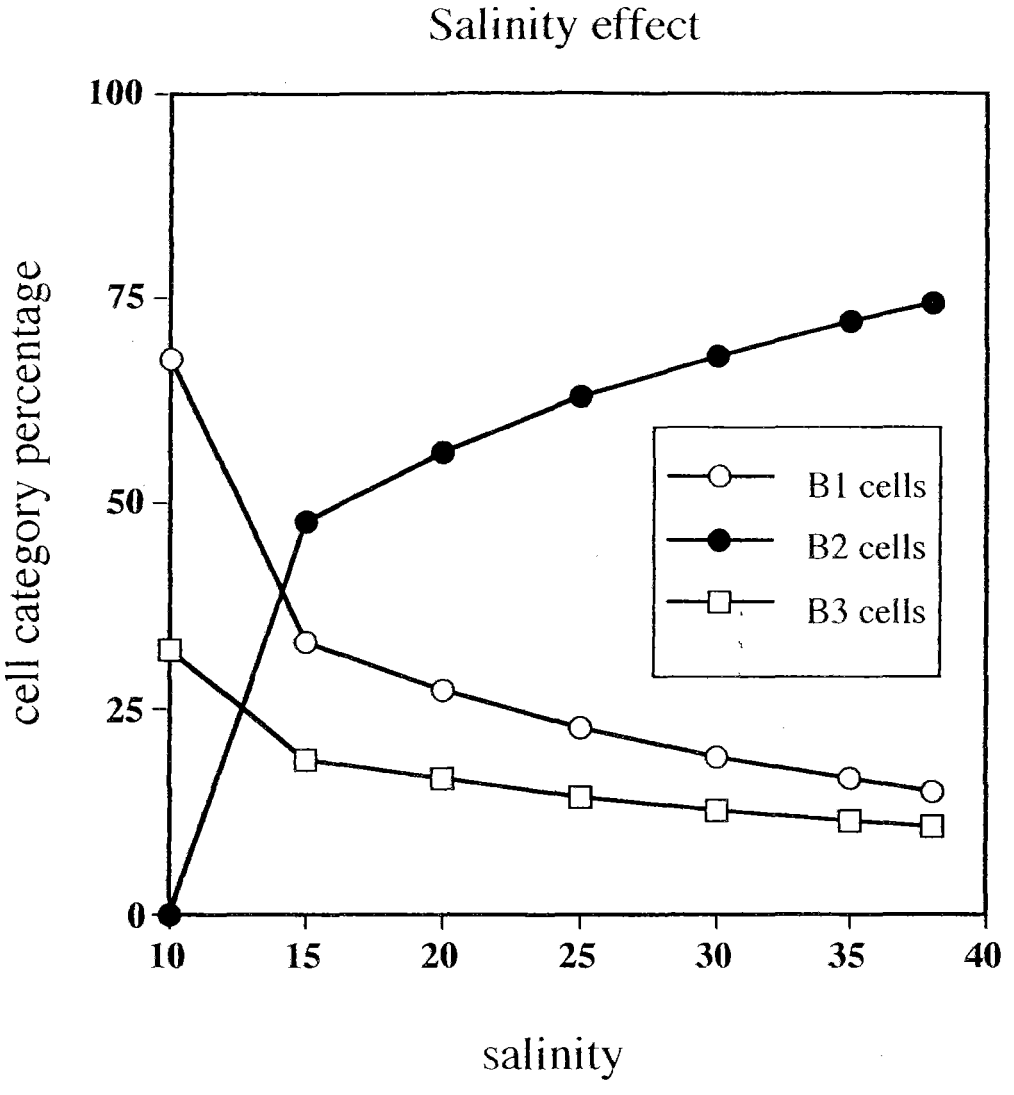


Fig. 3 : Incubation of log phase cells in oligotrophic marine water (salinity = 38) with or without sunlight : effects on succession of cellular categories (simulation results).



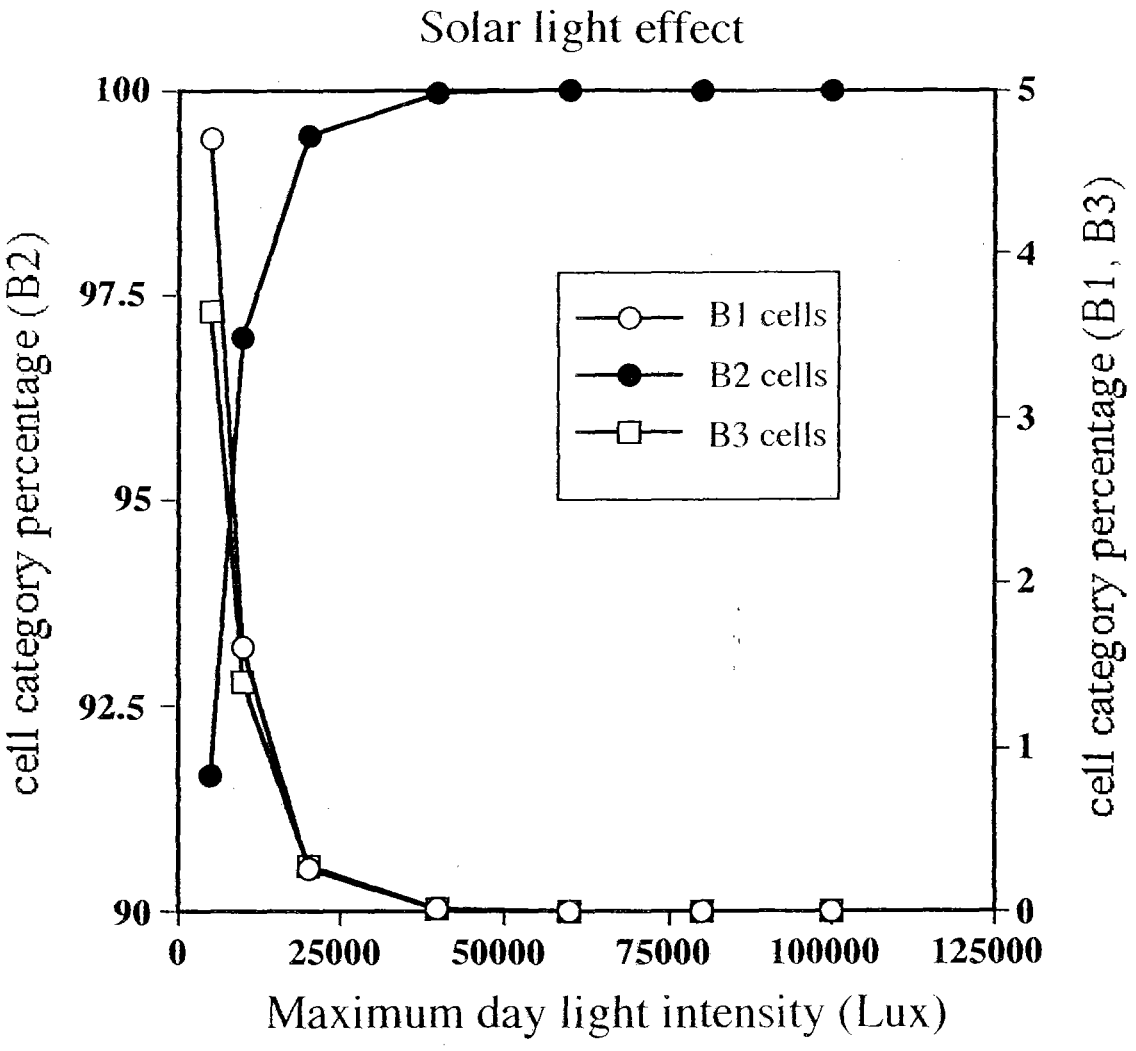
Expected proportions of different cellular categories resulting from 48h00 incubation with different initial organic carbon concentrations (Lmax = 65000 Lux, S = 38)

Fig. 4



Expected proportions of different cellular categories resulting from 48 h00 exposure at different salinities (no organic matter and dark conditions)

Fig. 5



Expected proportions of different cellular categories resulting from 48h00 nycthemeral exposure with different maximum day light intensity (no organic matter and salinity = 38)

Fig. 6

tend to decrease with increasing salt concentrations. At sea water salinity each of them represents about 10% of cells.

In marine oligotrophic waters, B_1 and B_3 cells are mainly affected by solar light (fig. 6). They exihibit an exponential decrease when light intensity increases (from 0 to 50 000 Lux) leading to undetectable levels after two days when maximum light intensity (at noon reaches 50 000 Lux or more. At the same time, B_2 cells increase to constitute the only detectable cells.

Obviously, in oligotrophic sea water conditions, sunlight is the main factor affecting the disappearance of B_1, B_3 cells. Even when the cells are in the presence of relativity large amounts of assimilable organic matter, viable bacteria represent a very small part of the total number of cells. In contrast, the effect of salinity appears to be quite limited.

2. Application of the model : simulation of cellular dynamics in an hypothetical gradiant of environmental conditions.

The model has been used to simulate the behaviour of cellular categories in a gradient from a river to the sea (fig. 7). Cell concentrations are those expected after 24 or 48 h incubation of log or stationary phase *E. coli* inocula, in batch microcosm. Environmental conditions are reported in tab. 4.

tab. 4

Values for assimilable organic carbon have been adapted from a realistic range. It takes into account an increase in carbon concentration occuring in the middle part of the estuary due to the death of non halotolerant organisms (coming from the river) and lower carbon concentrations in sea water. Salinity values represent a possible gradient

in such conditions. A nycthemeral cycle (sine curve) has been used to simulate height variation (L_{MAX} at noon). L_{MAX} values for different cases have been deduced from theoretical attenuation light coefficient values due to expected turbidity along such a gradiant (zero turbidity in sea water). The starting time of simulations has been fixed at 10 h 00 a.m. Simulation results are reported in fig 7.

As expected from previous considerations, we can observe a strong increase of B_2 cells in all cases with percentages generally greather than 90% of the total count.

With **log-phase cell inocula**, culturable B_1 cells show a slow decrease from the river to the sea, which is more important for the last two stations especially after 48 h 00 exposure. This is the consequence of more harmful stress conditions in the marine environment.

Dormant B_3 cells show a different pattern : undetectable in river and upper-part estuarine water, they appear in middle-estuarine conditions. Then, as B_1 cells, they decrease in marine waters. However, after 48 h exposure in the sea their proportion is higher than those of B_1 cells.

The absence of B_3 cells in river or low salinity water may be explained by night and day cycles, which leads to successive stress and non-stress conditions with activation and repression alternation of antistress responses. This is especially the case for the R_{II} response which requires a longer time to be completely activated.

Phase stationary inocula show a different pattern. These cells with a previously activated R_1 response are less sensitive to environmental stresses. B_1 culturable cells exhibit a slower decrease, particulary in the middle part estuary where organic matter concentration is high.

It is interesting to note the high B_3 cell percentages obtained from the phase stationary inocula. After 48 h 00 exposure, they are very close to B_1 cell percentages and more especially in sea water conditions. With that kind of pre-adapted inocula, which represent the most likely case, the number of dormant cells remains particulary high ranging from 10^4 to 10^5 ml^{-1} in all stations with an initial abundancy of 3 $10^6 ml^{-1}$

Tab. 4 : Hypothetical gradient of environmental conditions from a river to the sea.

	orgarnic matter (mg C/ml)	salinity	Lmax (lux)
I : river	5	1	32500
II : upper–part estuary	5	10	40625
III : middle–part estuary	10	20	48750
IV : lower–part estuary	5	30	56875
V : sea	0.5	38	65000

Simulation of the evolution of the different cell categories (B1, B2, B3) following an hypothetical gradient of environmental conditions from a river to the sea.

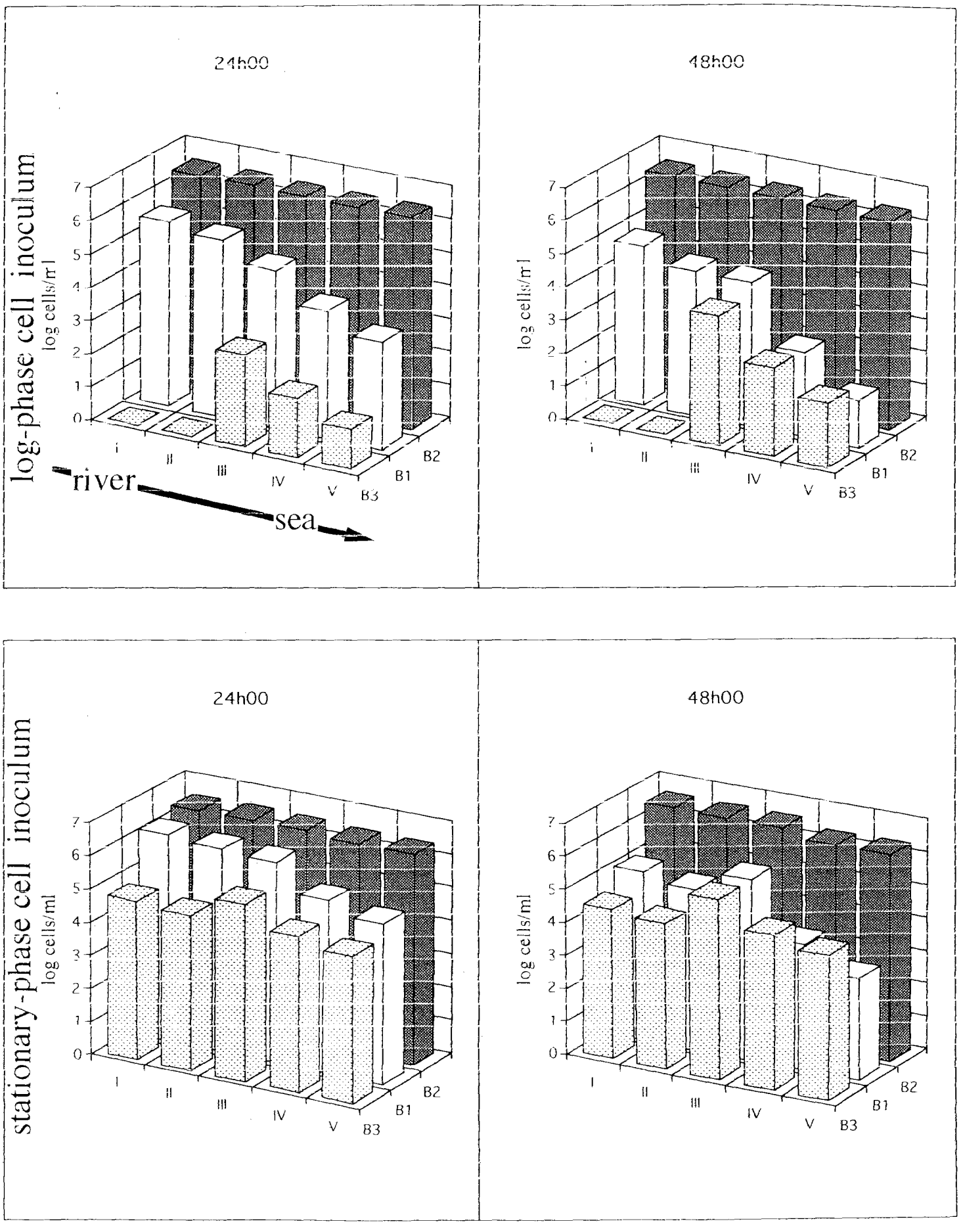


Fig. 7

CONCLUSION

While greatly perfectible, this model is to our knowledge, the first one proposed to simulate the behaviour of cellular categories in a stressed population.

For us, one of the main advantages of a modelling approach was to offer a formal framework to synthetize different pieces of knowledge on consequences of starvation and other stresses on cell dynamics.

Another important application of models is to help us to emphasize unanswered or unaddressed questions. For instance, our results highlight an important sanitary (if cells are considered as pathogens) and ecological (for all autochtonous and allochtonous species) question : if our model hypotheses are valid, these cells, and not all the VNC cells, would be capable of regrowth if they are placed in suitable conditions. So, they would be the cells that are able to recover pathogenic properties (pathogenic species) or biogeochemical activities (autochtonous species). But do these cells really exist in natural populations ? If this is the case our model allows us to predict typical environmental conditions where they can be found in detectable numbers. Of course, the answer depends on a suitable methodology to detect and count in situ such dormant "resuscitable" cells.

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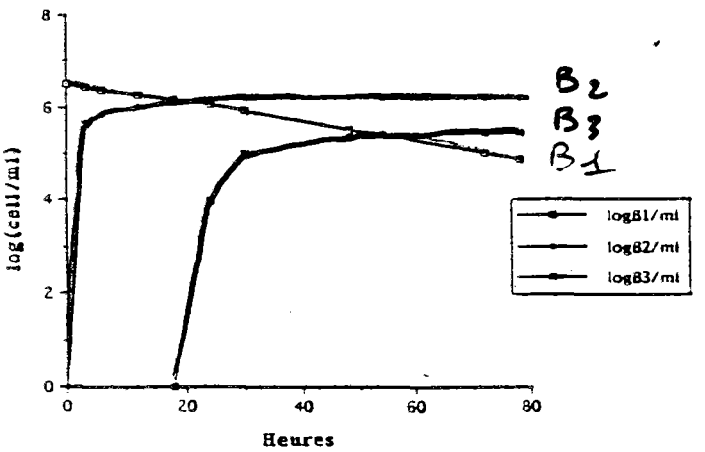
ANNEXES

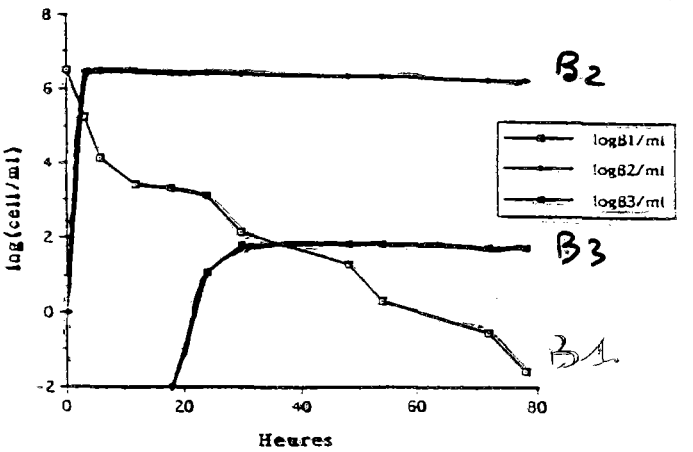
DEVENIR DES CATEGORIES CELLULAIRES

SIMULATIONS (eau de mer oligotrophe)

OBSCURITE



LUMIERE SOLAIRE



- ⇒ 3 phases + ou – marquées

1. Agression initiale :

- Forte croissance B_2 (transformation $B_1 \rightarrow B_2$)
- Absence de cellules B_3

2. Installation R_1 :

- Diminution du taux de formation des B_2 (B_1 stat)
- Absence de B_3

3. Installation R_2 :

- Apparition de cellules B_3 en dormance

APPROCHE EXPERIMENTALE

	Microcosmes							
	Obscurité				Lumière solaire			
On admet :	Δt	% B1	% B2	% B3	Δt	% B1	% B2	% B3
B_1 = UFC	0	100	0	0	0	100	0	0
B2 = DVC – UFC	24	14,1	85,9	0	6	1,8	98,2	0
B_3 = TC – (B_1 + B_2)	72	0,12	99,7	0,28	72	$1,6.10^{-4}$	99,9	0,098

- ⇒ Semble conforter les hypothèses du modèle

DEVENIR DES CATEGORIES CELLUAIRES

Il dépends :

- de la nature du stress et de son intensité

ex : devenir de 10^6 cellules en 48 h dans l'eau de mer

	L_{MAX}	B1	B2	B3	Fréquence B3
$MO^-S^+L^-$	0	151 400	741 400	107 200	$\approx 10^{-1}$
$MO^-S^+L^+$	20 KL	2 700	994 400	2 900	$\approx 10^{-3}$
$MO^-S^+L^+$	90 KL	2	999 988	10	10^{-6}

⇒ La fréquence des B3 diminue avec l'intensité lumineuse

- de la durée d'exposition et de la pré-adaptation

ex : $MO^-S^+L^+$ (L_{MAX} = 90 KL)

	Sans pré-adaptation (t0 = 10 h 00 HL)			Avec pré-adaptation (t0 = 20 h 00 HL)		
Δt en mer	% B1	%B2	% B3	% B1	%B2	% B3
12 h	0,04	99,96	0,00	1,06	98,89	0,05
24 h	0,02	99,98	0,0002	0,05	99,75	0,20
48 h	0,0002	≈ 100	0,001	0,003	99,78	0,22

⇒ Le devenir des bactéries émises en mer à 10 h ou à 20 h (pré-adaptation) sera différent au plan quantitatif et qualitatif : la préadaptation favorise les cellules B_3.

DEVENIR DES CELLULES :

⇒ Processus physiologiques et prédation

Taux de prédations Kp mesurés en 1995

	Kp (h^{-1})	
	Hiver	Printemps
Baie du lazaret	3.10^{-4}	$3,5.10^{-2}$
Ile des Embiez	3.10^{-5}	$3,5.10^{-3}$

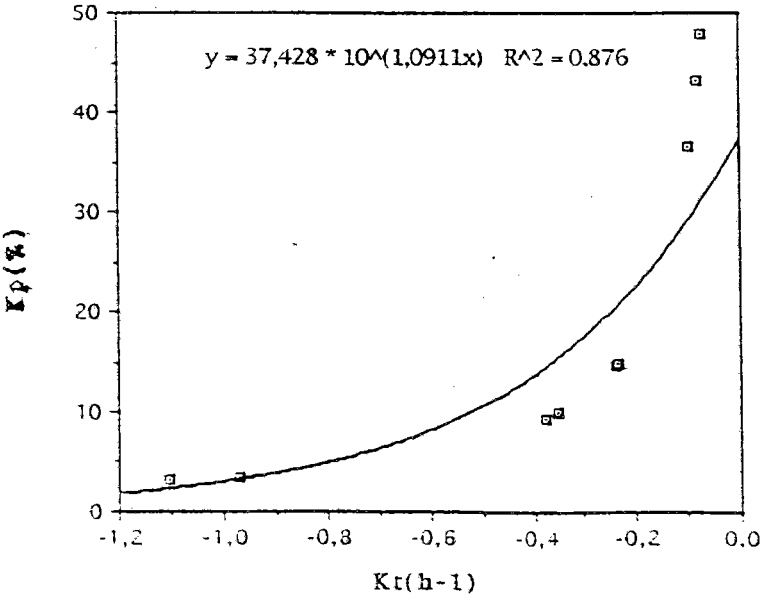
Contribution de la prédation à la décroissance :

Simulation : MO–S+L+, L_M = 90 KL, Kp = 0,035 h^{-1}

⇒ – Kp important pour la décroissance des effectifs totaux (T90 = 66 h) et des cellules B_3.

– moins efficace que la lumière pour B_1 :

Contribution de la prédation (Kp) au taux de décroissance total (Kt) .

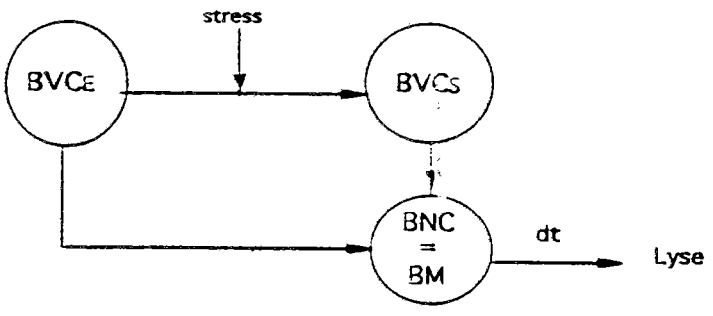


La contribution relative de la prédation augmente aux faibles taux de décroissance (3 à 48 % du taux de décroissance total ici)

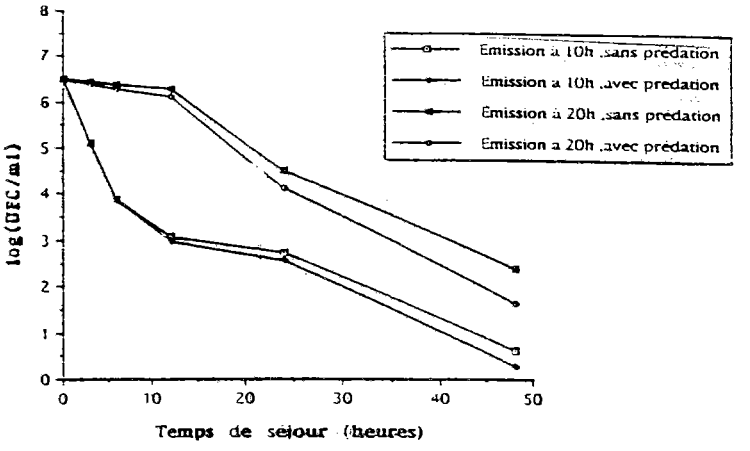
RISQUE SANITAIRE SELON LE MODELE CONSIDERE

ANNEXE 3

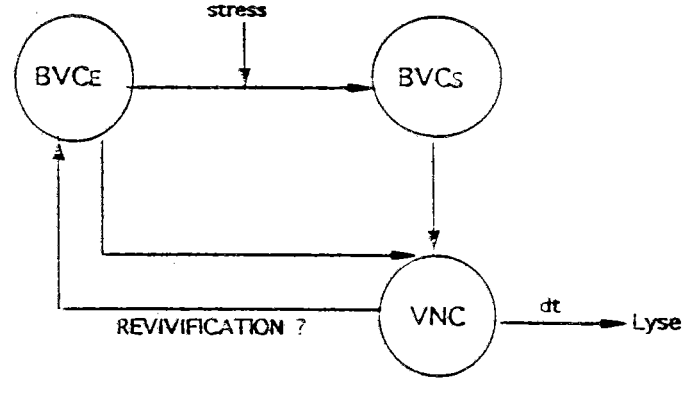
MOD T90 CFU : Risque Min



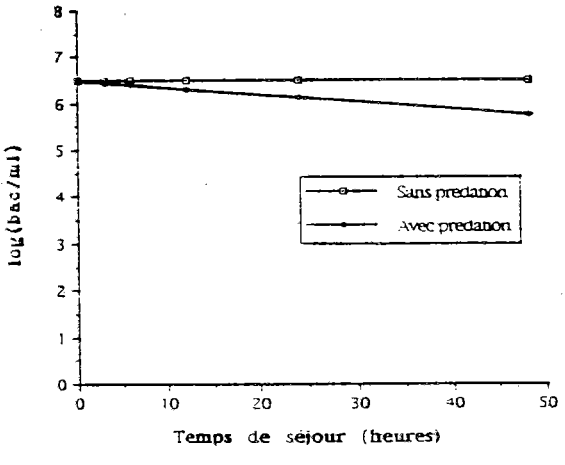
Evolution des UFC selon l'heure d'émission, avec ou sans predation (Lmax=90KJ)



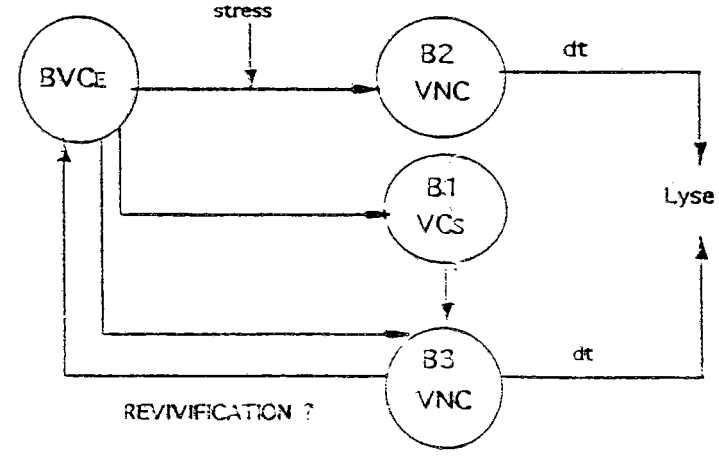
MOD VNC = DORMANCE : Risque Max



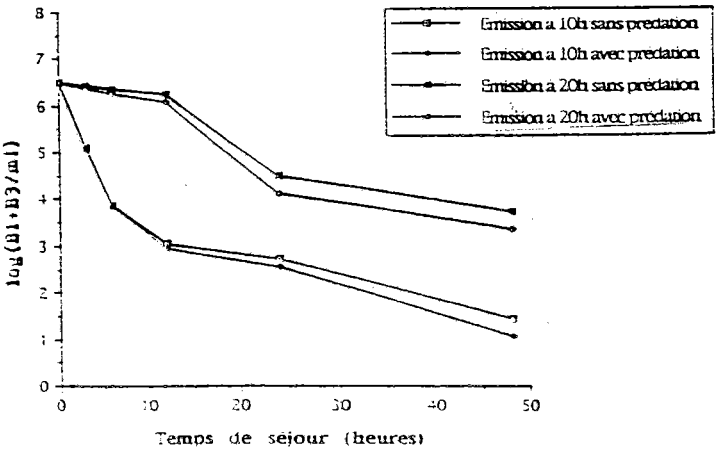
Evolution du Total Cougr avec ou sans prédation



MOD ADAP. PHYSIOL.



Evolution des bacteries B1(URC) et B3(VNCR) selon l'heure d'émission (Lmax=90KJ)



Conséquences pratiques (virtuelles).

Exemple : Effets de la collecte et du traitement des eaux usées sur le devenir des coli d'un rejet en mer.

Hypothèses.

- Bactéries émises en phase exponentielle (pas sûr) : R_1 et R_2 min

- Concentrations initiales : $B_1 = 10^{8,5}$ / 100 ml , $B_2 = B_3 = 0$

- Temps de séjours :

. réseau	0,5 à 8 h
. ST physico-chimique	2,5 h
. ST biologique	12 h
. émissaire	0,5 h

- Effets des traitements :

	Temps de séjour cumulés	Abattement log bactéries
Brut	1 à 8,5 h	0
T.P.C.	3,5 à 11 h	0,5
T.BIO	13,5 à 21 h	1

Conséquences pratiques (suite).

Résultats des simulations.

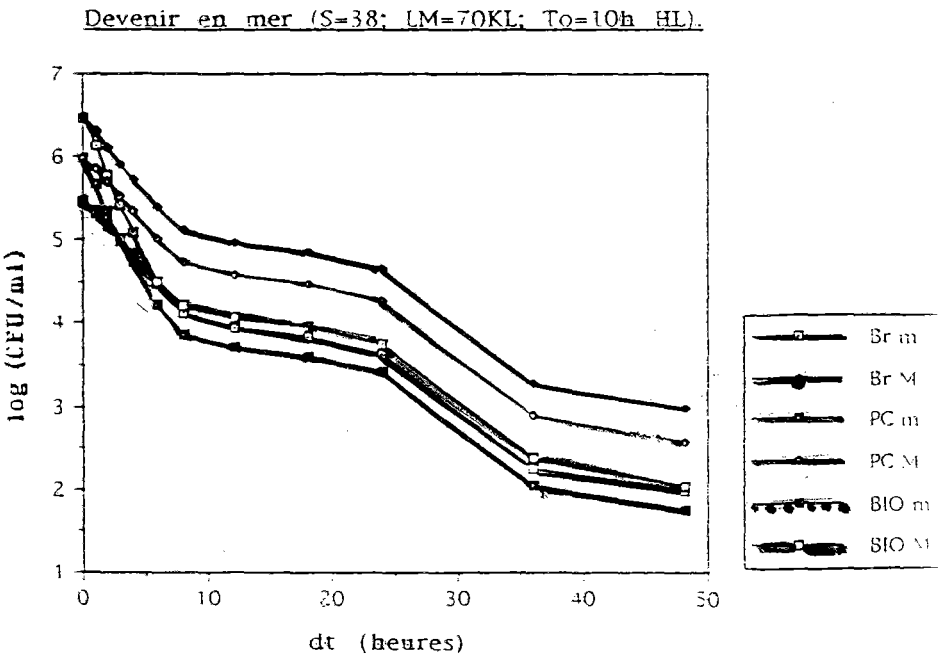
1. Caractéristiques des bactéries issues de l'émissaire.

(n = 9 ; α = 0,01).

	B1				B2		B3	
	log/ml	q_1	R_1	R_2	log/ml	q_2	log/ml	q_3
BRUT								
$\bar{X}$	6,49	0,451	0,0156	5×10^{-4}	2,175	0,484	absence	
Δm	6,475	0,425	0,0075	"	1,80	0,478		
ΔM	6,50	0,477	0,024	"	2,37	0,489		
TPC								
$\bar{X}$	5,98	0,425	0,0233	5×10^{-4}	1,84	0,475	absence	
Δm	5,97	0,396	0,0096	"	1,64	0,462		
ΔM	5,99	0,454	0,037	"	1,98	0,489		
BIO								
$\bar{X}$	5,448	0,371	0,05	$6,7.10^{-4}$	1,67	0,435	absence	
Δm	5,44	0,363	"	$5,8.10^{-4}$	1,60	0,426		
ΔM	5,454	0,380	"	$7,7.10^{-4}$	1,73	0,444		

2. Devenir en mer selon temps de séjour (S = 38 ; L_M = 70 KL T_0 = 10 h HL).

	T90	T90 M
BRUT	3,38	5,76
T PC	3,81	6,35
T BIO	6,45	6,45



CONSEQUENCES MOD. A.P. : un exemple

SCENARIO : (simple)

Soit une plage située à une distance d d'un émissaire.

On analyse la qualité de l'eau pendant 24 h avec un pas d'échantillonnage de 4 h.

On admet :

- Concentration CF/100 ml d'eau usée = cte = 10^7
- Dilution EFF/EM à la plage = cte = 1/10 000
- LMAX = 65 KL ; durée du jour = 12 h
- Transfert des CF : horizontal, surface
- Durée (temps de séjour en mer) :
  - . Variable selon la vitesse du vent
  - . Plage de variation : 4 à 32 h par sauts de 4 h.
- Critère de qualité de l'eau : Normes G et I
  - . A : CF/100 ml $\subseteq$ 100 Bonne qualité
  - . B : 100 < CF/100 ml $\subseteq$ 2000 Qualité moyenne
  - . C : CF/100 ml > 2000 Mauvaise qualité

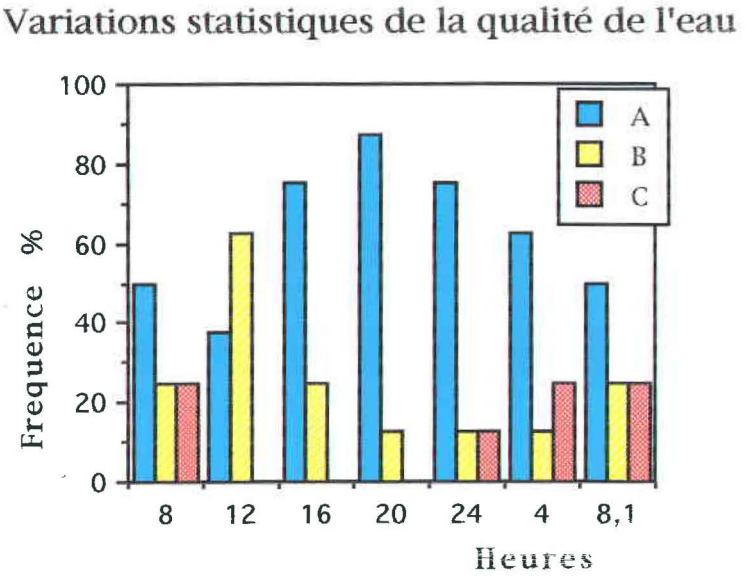
RESULTATS :

48 observations (6 échantillons avec 8 valeurs possibles selon le temps de séjour en mer et l'heure d'émission des CF).

Qualité globale

Classement		%
A	31	64,6
B	12	25,0
C	5	10,4

➡ ***Classement en C***



CONCLUSIONS :

- Qualité variable selon l'heure d'échantillonnage.
- Difficile de prévoir sauf bonne connaissance des facteurs physiques.

CONCLUSIONS

1. Le modèle permet de reproduire les phénomènes observés en différentes situations et de hiérarchiser les processus.

2. Il suggère que toutes les cellules issues d'une exposition à un stress n'ont pas les mêmes propriétés. La proportion de chaque catégorie cellulaire dépend de plusieurs facteurs : nature et intensité du stress, durée d'exposition, préadaptation (R_1)...

3. Cela conduit à des difficultés pour la prévision du devenir des cellules, qui dépend lui aussi de plusieurs paramètres aléatoires : heure d'émission, intensité lumineuse et durée du jour, turbidité, temps de séjour dans l'eau de mer lié à la vitesse du courant...

Il serait toutefois nécessaire de confirmer l'existence de ces catégories cellulaires avec des marqueurs discriminants. Il sera également nécessaire d'aborder les problèmes de revivification éventuelle des cellules dormantes (conditions) et du maintien de leur pouvoir pathogène, qui conditionnent tous deux leur intérêt sanitaire.