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ECOLE DOCTORALE « Géosciences marines »

par **COZIC AMANDINE**

pour obtenir le titre de

DOCTEUR

Spécialité : Géochimie Marine

DISTRIBUTION ET ROLE DES COMPOSES SOUFRES REDUITS VOLATILS SUR LA SPECIATION METALLIQUE

soutenue publiquement le 26 juin 2007

Devant la commission d'examen formée de :

E. Bucciarelli	Maître de conférence, Université de Bretagne occidentale, rapporteur
R. Cosson	Chargé de recherche, Université de Nantes, rapporteur
P. Le Corre	Professeur, Université de Bretagne occidentale, examinateur
J. Radford-Knoery	Chargé de recherche, Ifremer Nantes, co-directeur de thèse
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Proverbe chinois

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"Le vieil éléphant sait où trouver de l'eau"
Proverbe africain

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Résumé

Une méthode d'analyse sensible, précise et rapide a été élaborée pour le dosage simultané de 5 composés soufrés réduits volatils (CSRV) : le sulfure de dihydrogène (H_2S), le sulfure de carbonyle (OCS), le méthane thiol (MeSH), le diméthyle sulfure (DMS) et le diméthyle disulfure (DMDS). L'étude des CSRV en Baie de Quiberon met en évidence des variations saisonnières et interannuelles des concentrations en zone épibentique. L'H_2S analysé près de l'interface eau-sédiment (microzones anoxiques) montre des concentrations supérieures en été. La distribution d'OCS est cohérente avec une dégradation photochimique prédominante en hiver et une source sédimentaire en été. Les variations en MeSH, DMS et DMDS sont corrélées avec celles de la densité en Dinophycées. En estuaire de la Seine, l'absence d'H_2S volatil en zone de faibles salinités s'explique par la présence de Cu^{2+} et Zn^{2+} (complexation). En revanche, aucune interactions significatives n'a pas été mise en évidence entre Ag^{+} et OCS, et MeSH, et DMS. C'est l'abondance phytoplanctonique qui commande la distribution des MeSH, DMS et DMDS et la photodégradation de composés soufrés organiques et la diffusion à partir des sédiments celle d'OCS. Au sein d'une culture phytoplanctonique traitée à l'argent (photobioréacteur), la concentration en H_2S n'apparaît pas affectée par les ions métalliques. Des réactions de transformations du DMS pourraient expliquer l'augmentation des teneurs en MeSH et DMDS alors que la présence d'OCS serait davantage liée à la dégradation de composés soufrés biogéniques libérés par le phytoplancton.

Abstract

A sensitive, precise and rapid analytical method was elaborated to measure simultaneously 5 volatile reduced sulfur compounds (VRSC) ; hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS). The study of VRSC in the Bay of Quiberon highlights seasonal and interannual variations of concentrations in epibenthic zone. H_2S analyzed near the sediment water interface (anoxic microzones), is more elevated in summer. The OCS distribution is consistent with a predominant photodegradation in winter and a sedimentary source in summer. The variations of MeSH, DMS and DMDS are correlated with the Dinophyceae abundance. In the Seine estuary, the absence of volatile H_2S in the up estuary is explained by the presence of Cu^{2+} and Zn^{2+} (complexation). No significant interactions are observed between silver and OCS, MeSH, DMS. The distribution of MeSH, DMS and DMDS is linked to the phytoplanktonic abundance whereas the OCS's is governed by the organic sulfur compounds photodegradation and the sedimentary diffusion. In a phytoplanktonic culture submitted to increasing silver concentrations, the H_2S concentration not seem to be influenced by the metal ions. The DMS degradation may be explained the increase of MeSH and DMDS concentrations. The OCS analyzed may be produced by the degradation of biogenic (phytoplankton) sulfur compounds.

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Introduction générale

1. Origine et distribution des Composés Soufrés Réduits Volatils (CSRV)

1.1. Généralités

Le soufre (numéro atomique 16, masse atomique 32.06 $g.mol^{-1}$) est le deuxième élément du groupe VI de la classification périodique. Ce groupe comprend également l'oxygène, le sélénium et le polonium. Le Tableau 1 regroupe les propriétés physiques de cet élément. Parmi les dix isotopes connus, six sont radioactifs (^{29}S, ^{30}S, ^{31}S, ^{35}S, ^{37}S, ^{38}S ; Heslop et Jones, 1976) et parmi les quatre isotopes stables, les plus abondants dans la nature sont le ^{32}S et le ^{34}S qui comptent respectivement pour 95.1% et 4.2% du soufre total (Heslop et Jones, 1976). Les isotopes du soufre sont fréquemment utilisés par les biogéochimistes comme traceurs des transformations chimiques affectant les composés soufrés (Goldhaber et Kaplan, 1974). Le soufre peut exister sous différentes formes métastables à température ambiante mais l'unique forme vraiment stable à 100°C est la configuration α qui correspond à un arrangement d'anneaux octaédriques réunis dans un cristal. La formule chimique de cet α-soufre, également nommé soufre élémentaire, est S_8 (Heslop et Jones, 1976).

Tableau 1 – Propriétés de l'atome de soufre (Heslop et Jones, 1976)

Numéro atomique	16
Configuration électronique	$[Ne]3s^2 3p^4$
Rayon de la couche de covalence (pm)	104
Rayon ionique du tétraèdre (-II), (pm)	184
Rayon ionique du tétraèdre (+VI), (pm)	12
I(1), (kJ/mol)	1070
Affinité électronique (kJ/mol)	-353
Electronégativité (Allred-Rochow)	2.44
Etats d'oxydation	+VI, +IV, +III, +II, 0, -II

Sur Terre, les réservoirs géochimiques de soufre sont nombreux ce qui confère au cycle biogéochimique de cet élément une réelle complexité. Le cycle du soufre se déroule à la fois au sein des continents, des océans et de l'atmosphère (Kwint, 1997 ; Figure 1). De plus, des éléments chimiques majeurs, le cycle du soufre est l'un des plus perturbés par les activités humaines. En 2000, Kettle et Andreae a estimé que les émissions anthropiques de soufre dans l'atmosphère étaient équivalentes aux émissions naturelles. C'est la lithosphère avec $2x10^{10}$ Tg S qui en constitue le réservoir principal. Le soufre y est essentiellement présent sous forme solide et complexé à d'autres éléments (e.g., pyrite (FeS_2), évaporites ($Ca.SO_4$)). Par érosion, ce soufre sédimentaire se transforme en dioxide de soufre (SO_2) et s'ajoute au stock de soufre atmosphérique.
Chin et al. (2000) ont démontré que le soufre atmosphérique a essentiellement une origine anthropique (i.e., 80%). Cependant, des origines naturelles diverses (e.g., volcanique, marine) sont également prouvées (Brimblecombe et al., 1989). Dans l'atmosphère terrestre, le soufre est essentiellement présent sous forme oxydée (e.g., aérosols sulfatés, SO_2) alors que près de 90% du soufre mesuré dans l'atmosphère marine sont à l'état réduit (e.g., diméthyl sulfure DMS, sulfure de carbonyle OCS ; Kettle et Andreae, 2000). Les océans sont considérés comme une source majeure de composés soufrés puisque entre 26 et 74 Tg(S) dont 15 à 33 Tg(S) correspondent à du DMS, quittent les océans pour gagner l'atmosphère chaque année (Kettle et Andreae, 2000). Dans le milieu marin, les sulfates (SO_4^{2-}) constituent la forme dominante (i.e., 97% du soufre total) avec une concentration égale à 28 mM (0.9 $g(S).L^{-1}$). Cependant, du soufre réduit existe également dans la colonne d'eau sous forme de composés biogènes et de nombreux thiols (DMS, OCS, H_2S).

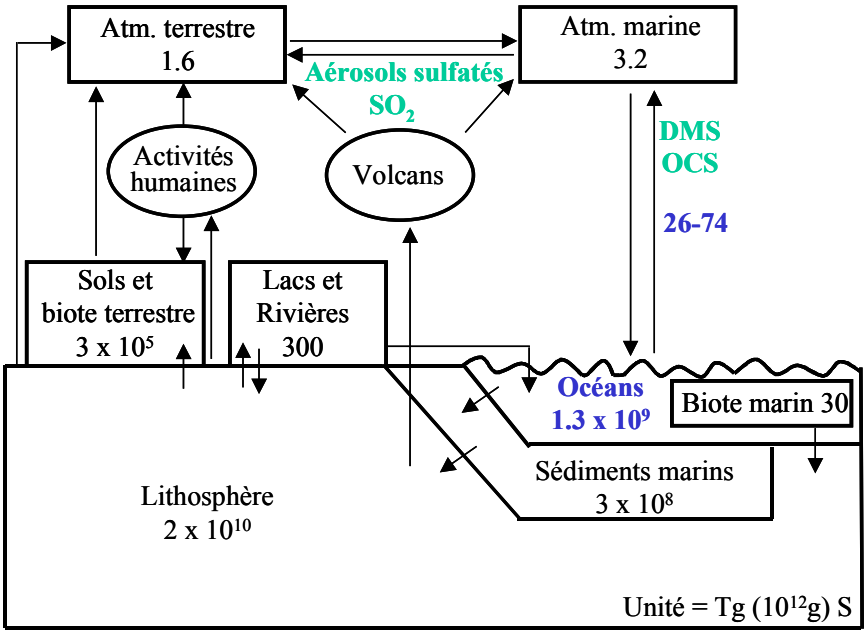

Figure 1 - Le cycle global du Soufre (d'après Global biogeochemical cycles, Butcher et al., 1992)

Les composés soufrés sont très réactifs et jouent un rôle essentiel dans la formation des pluies acides et la production d'aérosols atmosphériques (Andreae, 1986 ; Brandt et Van Eldik., 1995) capables d'affecter le climat global. Le soufre est un élément essentiel pour tout organisme vivant (e.g., plantes, animaux, microorganismes). Trois des acides aminés présents dans la plupart des protéines (i.e., cystéine, cystine et méthionine) contiennent du soufre. Le soufre entre également dans la composition des sulpholipides, de quelques vitamines, des esters de sulfates et dans de nombreux autres composés.

Le composé soufré océanique le plus étudié est sans contexte le diméthyl sulfure (DMS, "CH_3SCH_3") qui contribue à 50-60% des émissions biogènes (e.g., sources volcanique, végétale…) de soufre (Andreae, 1990 ; Bates et al., 1992 ; Spiro et al., 1992). Avec plus de 95% du flux de soufre vers l'atmosphère, ce sont les océans qui sont considérés comme la source principale de DMS avec des émissions estimées entre 15 et 33 Tg (S).y^{-1} (Kettle et Andreae, 2000). Dans les années 80, l'hypothèse que le DMS pourrait entrer dans la régulation biologique du climat terrestre a été avancée (Bates et al., 1987 ; Charlson et al., 1987) mais ce n'est que depuis très récemment que le DMS est inclus dans les modèles climatiques (Aumont et al., 2002 ; Bopp et al., 2003, 2004). Après émission dans l'atmosphère, le DMS est oxydé en sulfure de dioxyde (SO_2) et en d'autres composés (Charlson et al., 1987). Le SO_2 est transformé

en noyaux de condensation nuageux (CCN pour "cloud condensation nuclei" ; Charlson et al., 1987 ; Legrand et al., 1991 ; Mitchell et al., 1995). Le DMS est considéré comme le composé soufré volatil le plus abondant avec des concentrations de l'ordre de plusieurs nM (Stefels, 1997).

Cependant, la distribution et la biogéochimie d'autres composés soufrés réduits volatils ont également été l'objet de nombreux travaux scientifiques (e.g., Turner et Liss, 1985 ; Cutter et Krahforst, 1988 ; Cutter et Radford-Knoery, 1993) : ce sont le sulfure de dihydrogène (H_2S), le sulfure de carbonyle (OCS), le méthane thiol (MeSH, "CH_3SH") et le diméthyl disulfure (DMDS, "CH_3SSCH_3").

1.2. Méthane thiol (MeSH), diméthyl sulfure (DMS), diméthyl disulfure (DMDS)

Dans le milieu marin, le méthane thiol, le diméthyl sulfure et le diméthyl disulfure ont pour origine majeure le même composé biogénique, le dimethylsulfoniopropionate (DMSP ; Kelly et Baker, 1990 ; Kiene et al., 2000 ; Bouillon et al., 2002). Ce composé soufré est produit en quantité non négligeable (i.e., plusieurs dizaines de nM ; Iverson et al., 1989 ; Kiene, 1996) par certaines espèces phytoplanctoniques (Holligan et al., 1987 ; Turner et al., 1988) dans la zone euphotique des océans (Burgermeister et al., 1990 ; Turner et al. 1995). Le DMSP, principalement localisé dans le cytosol, montre une concentration intracellulaire régulée à la fois par une dégradation et une expulsion hors de la cellule mais également par une production intracellulaire (Stefels et al., 2007). Le transfert de DMSP hors de la cellule devrait alors être facilité par un clivage extracellulaire de DMSP (Stefels et al., 2007). Le DMSP est utilisé par le phytoplancton marin dans de nombreuses réactions physiologiques. Il participe notamment à l'osmorégulation et à la cryoprotection des algues et des bactéries (Ackman et al., 1966 ; Vairavamurthy et al., 1985 ; Ishida, 1996 ; Belviso et al., 1990). Le DMSP est également un donneur de groupement méthyl lors de certaines réactions métaboliques (Kiene et al., 1996). Enfin, des études plus récentes ont démontré l'utilisation, par les algues marines, du DMSP et de ses produits de dégradation (e.g., DMS, diméthyl sulfoxyde (DMSO)) comme antioxydants lors de phénomènes de stress oxydatifs (Sunda et al., 2002 ; Van Rijssel et Buma, 2002).

La production de DMS peut survenir lors de la croissance des cellules algales. Le phytoplancton excrète alors directement du DMS dans le milieu environnant suite à la conversion enzymatique du DMSP intracellulaire (Vairavamurthy et al., 1985 ; Belviso et al., 1990 ; Kwint et al., 1993). Mais la synthèse de DMS est essentiellement associée aux différents mécanismes entraînant une lyse cellulaire. Le DMSP est libéré dans le milieu extérieur et subit une conversion en DMS par la DMSP-lyase dont l'origine peut être bactérienne (Kiene, 1990 ; Kiene et Service, 1991 ; Kiene, 1992 ; Simo et al., 2002) ou algale (Cantoni et Anderson, 1956 ; Ishida, 1968). La formation de DMS à partir de DMSP s'accompagne également de la formation d'acrylate (cf. équation 1).

$$(CH_3)_2S^+CH_2CH_2COO^- \rightarrow CH_3SCH_3 + CH_2{=}CHCOO^- + H^+ \quad (1)$$

DMSP DMS Acrylate

Les processus accompagnant une lyse cellulaire sont la sénescence phytoplanctonique (Nguyen et al., 1988 ; Matrai et Keller, 1993 ; Stefels et Van Boekel, 1993), le broutage phytoplanctonique par le zooplancton (Wakeham et Dacey, 1989 ; Belviso et al., 1990 ; Leck et al., 1990 ; Belviso et al. 1993 ; Laroche et al., 1999) et l'infection virale des cellules phytoplanctoniques (Hill et al., 1998 ; Malin et al., 1998). Suite au broutage, le zooplancton ingère en partie, le DMSP algal (Tang et al., 1999 ; Archer et al, 2001) qui pourra ensuite être évacué, sans être métabolisé, dans les pelotes fécales (Kwint et al., 1996) et métabolisé par la DMSP-lyase bactérienne. Il est également vraisemblable que le DMSP soit directement converti en DMS à l'intérieur des organismes zooplanctoniques (Daly et Di Tulio, 1996) dans la mesure où la digestion phytoplanctonique favorise la réaction enzyme-substrat (Wolfe et Steinke, 1996).

Dans le milieu marin, le DMSP et le DMS subissent différents processus de dégradation. Le DMS est dégradé rapidement (i.e., quelques jours) dans la colonne d'eau (Kiene, 1993) et peut subir une photo-oxydation en diméthylsulfoxyde (DMSO ; Brimblecombe et Shooter, 1986). Par ailleurs, la sédimentation du phytoplancton et des pelotes fécales, sources de DMSP, est considérée comme un processus majeur de transfert du DMSP de la zone euphotique vers les couches inférieures de la colonne d'eau (Malin et al., 1992 ; Matrai et Vernet, 1997). La déméthylation et la déméthiolation par les bactéries sont également d'autres processus de dégradation des DMSP et DMS (Kiene et Taylor, 1988 ; Kiene et Lill, 2000). C'est ainsi que le méthane thiol (MeSH) est formé dans les sédiments anoxiques et dans la colonne d'eau (Kiene et Taylor, 1988 ; Taylor et Gilchrist, 1991 ; Lomans et al., 1997) ; le

groupement thiol de la méthionine des microorganismes anaérobies et aérobies est alors utilisé pour dégrader le DMSP et le DMS.
Les équations (2) et (3) indiquent les réactions de formation de MeSH à partir de la dégradation bactérienne du DMSP.

$$R\text{-}SH + (CH_3)_2S^+CH_2CH_2COO^- \rightarrow CH_3SCH_2CH_2COO^- + R\text{-}SCH_3 \quad (2)$$

DMSP 3-méthiolpropionate (MMPA)

$$CH_3SCH_2CH_2COO^- \rightarrow CH_3SH + CH_2{=}CHCOO^- \quad (3)$$

MMPA MeSH Acrylate

Comme le montre l'équation (4), le méthane thiol peut ensuite être hydrolysé en CH_4, CO_2 et H_2S. Ce phénomène est important dans les sédiments anoxiques (Zinder et Brock, 1978).

$$4\ CH_3SH + 3H_2O \rightarrow 3\ CH_4 + HCO_3^- + 4\ H_2S + H^+ \quad (4)$$

Un autre composé soufré volatil, le diméthyl disulfure, est également intimement lié au cycle du DMSP dans le milieu marin. En effet, le DMDS est issu de la dégradation bactérienne du DMSP mais également du MeSH. En effet, deux molécules de méthane thiol (cf., équations 2 et 3) peuvent se combiner pour former le DMDS (Gun et al., 2000).

Dans le milieu marin, les composés soufrés volatils biogènes (e.g., MeSH, DMS et DMDS) montrent des teneurs très variables selon l'environnement étudié (e.g., océan ouvert, milieu côtier…). De plus, les concentrations en chaque CSRV sont très différentes les unes des autres puisqu'elles varient de quelques nM à parfois plusieurs centaines pour un même environnement.
Premier produit de la dégradation du DMSP, le DMS est essentiellement synthétisé dans le milieu marin où il est le composé soufré réduit volatil le plus abondant (Stefels, 1997) avec des concentrations de l'ordre de plusieurs nM. Par exemple, le long du plateau continental européen (Uher et al., 2000), la concentration de DMS est comprise entre 0.6 et 38.5 nM. La concentration de DMS est généralement supérieure dans les zones côtières comme l'ont montré Yang et al. (2004) avec 11.3 nM mesurées dans la Baie de Funka (Japon) et 1.6 nM dans l'océan ouvert (NW de l'océan Pacifique). Des concentrations de DMS similaires sont observées au niveau de la marge continentale de Nouvelle-Zélande (Walker et al., 2000) avec des teneurs comprises entre 0.4 et 12.9 nM mais également au large du Maroc (Cap Ghir) avec jusqu'à 16 nM de DMS mesurées (Belviso et al., 2003).

Le méthane thiol et le diméthyl disulfure sont moins étudiés dans le milieu marin, en raison de leur plus faibles concentrations dans la colonne d'eau. Tanzer et Heumann (1992) ont déterminé une concentration en DMDS inférieure à 0.16 nM dans les eaux de surface de l'Océan Atlantique (30°S45°N) pour une concentration moyenne de DMS égale à 0.89 nM. Par ailleurs, quelques études ont mesuré des teneurs en MeSH et DMDS allant jusqu'à plusieurs nM dans des environnements anoxiques. Richards et al. (1994) ont déterminé, dans des lacs hypersalins et des étangs, des concentrations comprises respectivement entre 1.1 et 180 nM et entre 1 et 68 nM. A noter que les concentrations les plus élevées ont été observées dans les zones les plus anoxiques. Lomans et al. (1997) ont mis en évidence une production significative (>1.44 $\mu M.jour^{-1}$) de MeSH dans des sédiments de tourbières. Ces données suggèrent que le MeSH est préférentiellement produit ou éliminé moins rapidement dans les zones anoxiques.

1.3. Sulfure de carbonyle (OCS)

Avec un temps de résidence dans la troposphère estimé de 2 à 7 ans (Torres et al., 1980 ; Turco et al., 1980 ; Mihalopoulos et al., 1991 ; Chin et Davis, 1993), le sulfure de carbonyle joue un rôle essentiel dans le bilan radiatif de la planète puisqu'il est considéré comme une source majeure des aérosols sulfatés de la stratosphère (Crutzen, 1976 ; Servant, 1986). Aussi, en raison de cet important temps de résidence atmosphérique, même de très faibles variations des flux d'OCS peuvent induire des effets significatifs sur sa concentration. Par conséquent, afin d'évaluer les variabilités spatiale et temporelle des flux d'OCS vers l'atmosphère, il est nécessaire d'étudier le cycle du sulfure de carbonyle dans son ensemble. A l'échelle globale, les principales sources d'OCS sont *i)* la combustion de la biomasse (Crutzen et al., 1979 ; Andreae, 1993), *ii)* la combustion des énergies fossiles (Goldan et al., 1987 ; Fall et al., 1988 ; Staubes et al., 1989), *iii)* l'oxydation atmosphérique du carbone disulfure (CS_2 ; Wine et al., 1982 ; Jones et al., 1982) et du DMS (Barnes et al. 1994), et, *iv)* les émissions océaniques (Ferek et Andreae, 1983 ; Andreae et Ferek, 1992 ; Cutter et Radford-Knoery, 1993). Chin et Davis (1993) suggèrent que les océans seraient la première source d'OCS pour l'atmosphère (i.e., environ 30%).

Pourtant, dans un premier temps, les océans ont été considérés comme un puit d'OCS atmosphérique en raison de l'hydrolyse rapide du sulfure de carbonyle dans le milieu marin (Johnson, 1981). Mais, des études ultérieures ont prouvé que les eaux de surface

étaient supersaturées en OCS ce qui entraînait un flux d'OCS vers l'atmosphère (Andreae et Ferek, 1992 ; Johnson et Harrison, 1986 ; Mihalopoulos et al., 1992 ; Ulshöfer et al. 1996).

Le mécanisme majeur de production d'OCS dans les océans est la dégradation photochimique de composés soufrés organiques dissous tels que les acides aminés soufrés (e.g., méthionine, cystéine), le diméthylsulfoniopropionate (DMSP) (Ferek et Andreae, 1984 ; Zepp et Andreae, 1994 ; Flöck et Andreae, 1996 ; Uher et Andreae, 1997) ou issus de la matière organique dissoute et chromophorique (CDOM ; Zepp and Andreae, 1994). Par exemple, l'équation (5) résume la formation d'OCS via la dégradation du DMSP dans la colonne d'eau.

$$(CH_3)_2S^+CH_2CH_2COO^- + H_2O \rightarrow OCS + 2\ CH_4 + CH_2{=}CHCOO^- + H^+ \quad (5)$$

DMSP Acrylate

Cette production photochimique d'OCS est soumise à des variations saisonnières et quotidiennes avec des pics de production observés lorsque la concentration en composés soufrés organiques et l'intensité lumineuse sont maximales (Ferek et Andreae, 1984 ; Weiss et al., 1995 ; Ulshöfer et al., 1996 ; Kettle et al. 2001).

Flock and Andreae (1996) ont démontré une corrélation négative entre la production photochimique de l'OCS et la dégradation photochimique du méthane thiol (MeSH) ce qui tend à prouver que le méthane thiol serait un précurseur naturel du sulfure de carbonyle dans l'environnement marin (cf., équation 6).

$$CH_3SH + H_2O + CO_2 \rightarrow OCS + CH_3OH \quad (6)$$

MeSH Méthanol

De plus, des concentrations non négligeables d'OCS ont également été mesurées au-delà de la zone euphotique des océans. Il existerait donc une production non-photochimique (i.e., sans intervention des radiations lumineuses) d'OCS au sein des océans. ; on parle de production "noire" (Flock et Andreae, 1996 ; Ulshöfer et al., 1996 ; Von Hobe et al., 2001). Par ailleurs, des études (Cutter et Radford-Knoery, 1993 ; Zhang et al., 1998) ont mis en évidence des concentrations extrêmement élevées d'OCS dans les eaux interstitielles (i.e., 7 μM). Ces teneurs importantes résulteraient d'une synthèse de sulfure de carbonyle via la dégradation de la matière organique enfouie ou via la décomposition oxydative de la pyrite sédimentaire (Stedman et al., 1984).

Avec des teneurs en biomasse (e.g., phytoplancton) supérieure, les régions côtières et les estuaires possèdent des concentrations en OCS généralement supérieures à celles mesurées dans les zones océaniques. Cutter et Radford-Knoery (1993) ont estimé la concentration d'OCS à 0.4 nM dans les zones côtières de l'ouest de l'océan Atlantique et entre 0.3 et 12.1 nM dans deux estuaires américains. La mer des Sargasses, région oligotrophe, montre une teneur dix fois plus faible en sulfure de carbonyle (i.e., 0.09 nM ; Cutter et al., 2004). La concentration moyenne de sulfure de carbonyle dans les océans ouverts est d'environ 0.03 nM (Johnson et Harrison, 1986 ; Rasmussen et al., 1992). Aussi, avec des concentrations de l'ordre de quelques nM (e.g., Ferek et Andreae, 1983 ; Johnson et Harrison, 1986 ; Jorgensen et Okholm-Hansen, 1985), les environnements côtiers seraient responsables de 60% de la production océanique d'OCS atmosphérique (Andreae, 1986).

1.4. Sulfure de dihydrogène (H_2S)

Dans le milieu marin, le sulfure de dihydrogène est essentiellement produit *i)* dans des environnements anoxiques (e.g., sédiments, marais ; Luther at Tsamakis, 1989) via la réduction des sulfates et *ii)* dans les sources hydrothermales lors de processus similaires (Von Damm, 1990 ; Voordeckers et al., 2005).

Dans les sédiments côtiers anoxiques, la réduction bactérienne des sulfates (SO_4^{2-}) est la principale forme de dégradation de la matière organique enfouie (CH_2O ; Dyrssen et Kremling, 1990) comme le schématise l'équation (7).

$$SO_4^{2-} + 2\,(CH_2O) + 2\,H^+ \Leftrightarrow H_2S + 2\,CO_2 + 2\,H_2O \qquad (7)$$

Il en résulte la formation de sulfure de dihydrogène (H_2S) stocké dans les eaux interstitielles

Alldredge (1998 et 2000) a également mis en évidence la synthèse de sulfure de dihydrogène, selon le même processus, dans une colonne d'eau oxique lors des épisodes de neige marine. La matière organique sédimente sous formes de pelotes fécales à l'intérieur desquelles la teneur en oxygène est telle que des microzones anoxiques peuvent apparaître et donc induire la formation d'H_2S.

Cependant, à la surface des océans, la principale source de sulfure de dihydrogène semble être l'hydrolyse du sulfure de carbonyle (Elliot et al., 1987, 1989 ; Watts, 2000). Elliot et al. (1987) ont montré que la concentration de l'H_2S est du même ordre

de grandeur que celle de l'OCS en raison d'une vitesse d'hydrolyse de l'OCS et d'une vitesse d'oxydation de l'H_2S équivalente.
A pH acide (entre 4 et 7), l'hydrolyse de l'OCS est unimoléculaire :

$$OCS + H_2O \Leftrightarrow OCS.H_2O \rightarrow CO_2 + H_2S \quad (8)$$

Alors qu'à pH alcalin (entre 7 et 10), la réaction entraîne la formation de l'ion HS^- :

$$OCS + OH^- \Leftrightarrow OCS.OH^- \rightarrow CO_2 + HS^- \quad (9)$$

Une autre source d'H_2S dans le milieu marin peut être la production par le phytoplancton. En laboratoire, Davies et al. (1976) ont mesuré des concentrations en H_2S supérieures à 1 µM dans des cultures de *Dunaliella tertiolecta* (Chlorophycée) lorsque celles-ci étaient soumises à un stress métallique, en l'occurrence au mercure. La détoxification des cellules serait liée à une précipitation du métal par la synthèse de complexes insoluble HgHS. Walsh et al. (1994) ont démontré que cette synthèse phytoplanctonique de sulfure de dihydrogène, à des concentrations nanomolaires, existait chez diverses espèces algales : *Synechococcus sp* (Cyanobactérie), *Emiliana huxleyi* (Coccolithophoridée) ou *Thalassiosira oceanica* (Diatomée).

Par ses propriétés réductrice et volatile, le sulfure de dihydrogène H_2S est instable dans la colonne d'eau (oxique) ce qui explique les faibles concentrations habituellement rencontrées dans le milieu marin en opposition à celles mesurées dans les milieux confinés (e.g., sédiments, marais, vases). En effet, les concentrations d'H_2S varient considérablement puisque des teneurs de plusieurs µM voire mM sont très fréquemment mesurées dans les sédiments côtiers et les environnements sub- et anoxiques alors que dans les zones oxiques (i.e., océans, estuaires), des teneurs de quelques nM sont observées. Kuwabara et al. (1999) ont effectivement mesuré des concentrations d'H_2S de l'ordre de 100 µM dans les eaux interstitielles du bassin de Santa Barbara alors que dans la colonne d'eau, la concentration moyenne, entre 0 et 600 de profondeur, était de 3 nM. Luther et Tsamakis (1989) ont ainsi mesuré une concentration moyenne d'H_2S en Mer Méditerranée, de 2 nM dans la colonne d'eau oxique alors que Cutter et Krahforst (1988) ont déterminé des teneurs comprises entre < 0.1 to 1.1 nM dans les eaux de surface de l'océan Atlantique ouest.

1.5. Schéma récapitulatif des CSRV

Le sulfure de dihydrogène (H_2S), le sulfure de carbonyle (OCS), le méthane thiol (MeSH), le diméthyl sulfure(DMS) et le diméthyl disulfure (DMDS) apparaissent donc intimement liés dans l'environnement marin au vu des multiples interconversions possibles entre ces divers composés. Un schéma récapitulatif, présenté ci-dessous (Figure 2) et basé les connaissances actuelles, permet de mettre en exergue d'une part, les interactions entre les cinq composés soufrés réduits volatils (CSRV) étudiés au cours de ma thèse et d'autre part, le rôle de la biomasse marine (i.e., phytoplancton et bactéries) sur la production de ces CSRV.

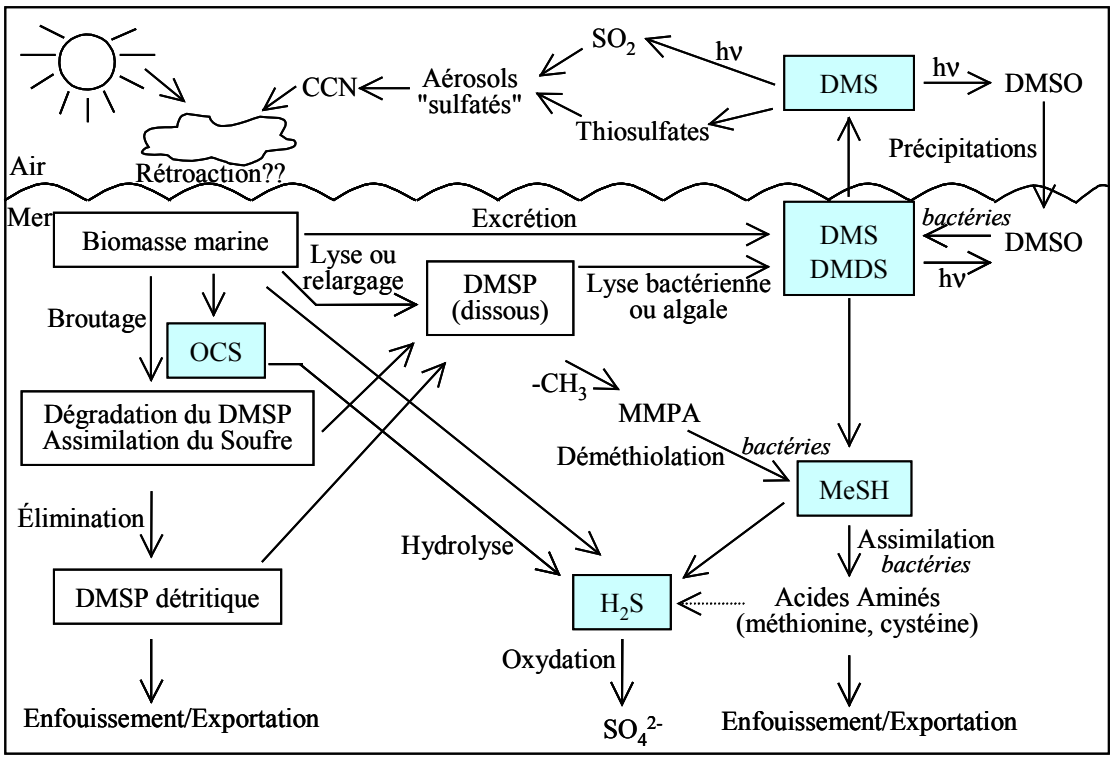

Figure 2 - Schéma conceptuel de la production des composes soufrés réduits volatils (CSRV) dans le milieu marin et des relations entre les CSRV et le phytoplancton.

Les CSRV présentent donc des mécanismes de production et de dégradation complexes et divers dans le milieu marin. Toutefois, un point commun entre ces composés est la présence de un ou deux atomes de soufre dont la configuration électronique de la couche périphérique permet de penser qu'ils puissent interagir avec des ions métalliques présent dans le milieu marin, et par conséquent affecter leur spéciation. La section suivante présente donc le rôle possible des CSRV sur la spéciation métallique dans un environnement aquatique.

2. Rôle des CSRV sur la spéciation métallique

Dans une solution aqueuse, un ion métallique M^{n+} n'est pas jamais ″libre″ mais est en permanence associé à des molécules d'H_2O. La vision schématique de l'arrangement moléculaire autour d'un métal dans un milieu aqueux est fondée sur l'existence de quatre zones concentriques (Figure 3 ; Morel, 1983) : *i)* une enveloppe primaire où les molécules d'eau sont chimiquement liées à l'ion métallique, *ii)* une enveloppe secondaire où les molécules d'eau sont ordonnées par l'influence électrostatique de l'ion ; le volume de cette couche augmente avec la charge ionique et est inversement proportionnelle à la taille du métal, *iii)* une zone ″transitoire″ séparant l'ion hydraté du milieu environnant ; c'est dans cette région que les molécules d'eau y sont le moins ordonnées, *iii)* le milieu aqueux *sensus stricto*.

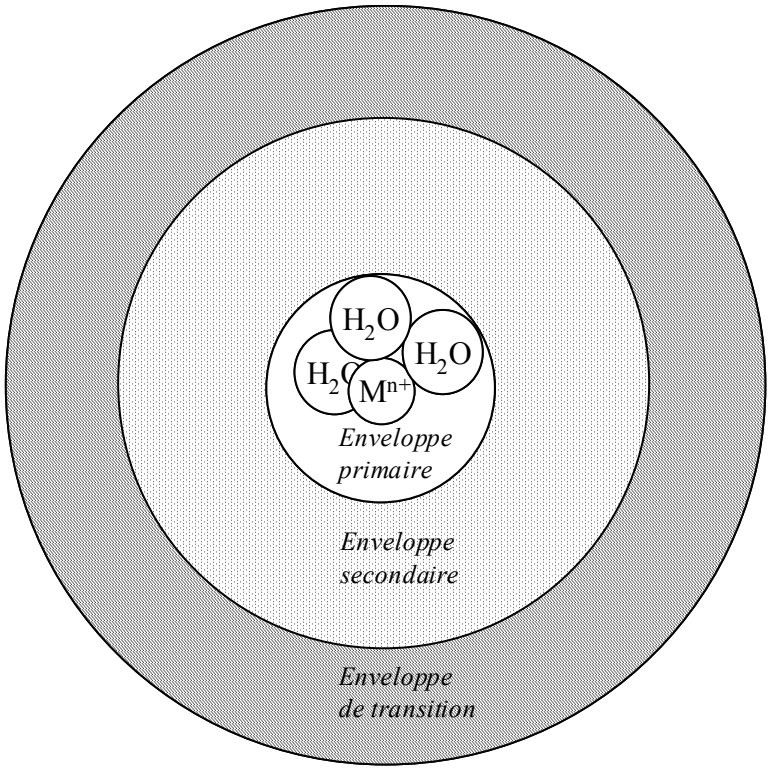

Figure 3 - Les différentes enveloppes autour d'un ion métallique (selon Burgess, 1978).

Les autres ligands qui peuvent remplacer les molécules d'eau autour de l'ion M^{n+} sont des espèces chimiques possédant une paire d'électrons libres à partager avec le métal (Morel, 1983). Ces composés peuvent être de simples anions (e.g., Cl^-, F^-, Br^-…) ou des espèces inorganiques plus complexes (e.g., NO_3^-, SO_4^{2-}, S^{2-}, CN^-…) mais également des molécules organiques contenant des groupements fonctionnels

possédant de l'oxygène, de l'azote ou des atomes de soufre qui vont jouer le rôle de porteurs d'électrons (e.g., R–OH, R–NH_3, R–SH ; Morel, 1983).
Un des mécanismes dominants dans le contrôle des concentrations en métaux dans le milieu marin (i.e., colonne d'eau oxygénée), résultent de l'adsorption physique et chimique des métaux avec la matière particulaire d'origine biogène ou détritique (Wangersky, 1986). La spéciation chimique des métaux traces dépend fortement des interactions avec les surfaces solides présentes dans l'environnement marin (Moffett et al., 1990 ; Gordon, 1992 ; Gledhill et Van den Berg, 1994 ; Ellwood et Van den Berg, 2001).
Les particules en suspension dans la colonne d'eau sont traditionnellement séparées des colloïdes et de la matière dissoute par une filtration à 0.45 µm. Ces particules, qui interagissent chimiquement avec les métaux, sont essentiellement issues de la biomasse phytoplanctonique produite dans la couche euphotique, et de sa décomposition par divers processus biologiques tel que le broutage par le zooplancton (Chester et Stoner, 1974 ; Tanoue et al., 1982). De plus, les interactions chimiques entre les particules en suspension et les métaux traces sont davantage liées à la disponibilité en matière organique qu'en particules en suspension inorganiques en raison des faibles concentrations de ces dernières dans l'océan ouvert et les régions côtières (Landing et Bruland, 1987 ; Martin et Gordon, 1988).

Parmi les ligands des métaux dans le milieu marin, on retrouve de nombreux composés soufrés comme les thiols (e.g., glutathion, phytochélatines ; Leal et Van den Berg, 1998 ; Laglera et Van den Berg, 2003 ; Young et al., 2003) et le sulfure de dihydrogène (Rozan et al., 2000). En effet, plusieurs métaux de transition (e.g., Cu^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}) et des métaux à sphère molle appelés ″acides faibles de Lewis″ (e.g., Ag^{+}, Cd^{2+}, Pb^{2+}) ont tendance à former des complexes stables avec des bases faibles (Smith et al., 2002) tels que les composés soufrés naturellement présents dans l'environnement marin (Stumm et Morgan, 1981). C'est pourquoi, la spéciation de ces métaux, dans les eaux suboxiques et anoxiques (e.g., hypolimnion, eaux interstitielles) est généralement dominée par la complexation avec l'H_2S (i.e., ions HS^{-}), les thiols et les polysulfides (Huerta-Diaz et al., 1998 ; Muller, 1999). Plus récemment, Rozan et al. (2000 et 2003) ont démontré que des espèces soufrées réduites pouvaient jouer un rôle dans la régulation de la biodisponibilité des métaux traces dans les eaux de surface (i.e., oxiques) des océans. La présence de composés soufrés réduits résistants à l'oxydation a effectivement été observée dans des environnements marins (Dyrssen,

1988 ; Luther et Tsamakis, 1989 ; Radford-Knoery et Cutter, 1993 ; Bowles et al., 2003). Aussi, si ces composés soufrés réduits sont ubiquistes et présents en quantité suffisante dans la colonne d'eau, ils devraient influencer le cycle, la spéciation et la biodisponibilité des métaux à sphère molle et de certains métaux de transition.

Les espèces H_2S-métal peuvent être présentes dans le milieu marin sous trois formes : complexes H_2S-métal, colloïdes et nanoclusters (Sukola et al., 2005). Les complexes sont essentiellement des espèces dissoutes (i.e., pour lesquelles un potentiel chimique peut être défini ; Stumm and Morgan, 1981) et sont présentes dans toute solution aqueuse où des ions métalliques et de l'H_2S (i.e., ions HS^- et S^{2-}) coexistent. Les colloïdes sont de petites particules (e.g., 1nm-1µm), dynamiques et qui ont tendance à s'agréger pour créer des particules de taille plus importante (e.g., précipitation possible ; Gianguzza et al., 1997). Sous certaines conditions, les espèces H_2S-métal peuvent également former des clusters (Johnston, 2002). Cependant, à l'heure actuelle, l'existence de clusters entre H_2S et certains métaux n'a pas été totalement établie dans l'environnement marin malgré la synthèse expérimentale de clusters (e.g., avec Cd, Cu, Pb, Zn ; Thompson et Helz, 1994 ; Korgel et Monbouquette, 1996 ; Dong et al., 2002 ; Luther et al., 2002).

Afin d'estimer plus précisément la force des interactions entre deux espèces chimiques, la constante de stabilité conditionnelle K′ est déterminée. La formation d'un complexe monodentate entre un ligand L et un métal M est mathématiquement identifiée au modèle de type Langmuirien d'absorption (Ruzic, 1996 ; Al-Farawati et Van den Berg, 1999) selon l'équation (8).

$$M + L \Leftrightarrow ML \quad (8)$$

La distribution des espèces labiles (métal et ligand) dans le milieu environnent répond simultanément aux équations (9) et (10).

$$Mt = M' + ML \quad (9)$$

$$Lt = L' + ML \quad (10)$$

avec Mt et Lt, les concentrations en métal total et en ligand total ; M′ et L′, les concentrations en composés libres (i.e., non complexés).

Par définition, la constante de stabilité conditionnelle K', aux conditions expérimentales, est le rapport de la concentration en complexe ML sur la concentration en éléments libres, M′ et L′.

$$K' = ML / [(M')(L')] \quad (11)$$

L'étude des constantes de stabilité conditionnelle de l'H_2S vis-à-vis des métaux a permis de classer par ordre d'importance ces interactions mais également de démontrer le rôle évident de l'H_2S dans la spéciation métallique. Al-Farawati et Van den Berg (1999) ont ainsi déterminé les constantes de stabilité conditionnelle dans le milieu marin (i.e., à pH 8, 25°C, 35 de salinité), entre le sulfure de dihydrogène et plusieurs métaux (Tableau 2). A noter que les constantes de stabilité conditionnelle K′ sont généralement exprimées sous forme logarithmique (i.e., log K′).

Tableau 2 - Constantes de stabilité conditionnelle de l'H_2S (sous forme d'ions HS^-) vis-à-vis d'ions métalliques (selon Al-Farawati et Van den Berg, 1999)(pH=8, 25°C, 35 de salinité).

Ion métallique	Constante de stabilité conditionnelle (log K′)
Cu^{2+}	12.9
Ag^{+}	11.6
Cd^{2+}	8.4
Pb^{2+}	8.0
Co^{2+}	6.8
Zn^{2+}	6.1
Ni^{2+}	5.1

Au vu de ces résultats, le sulfure de dihydrogène apparaît donc un complexant fort des ions Cu^{2+} et Ag^{+} dans le milieu marin alors que les distributions de Zn^{2+} et Ni^{2+} devrait être moins influencées par la présence d'H_2S à des teneurs nanomolaires.

A l'heure actuelle, les interactions entre les autres CSRV (i.e., OCS, MeSH, DMS, DMDS) et les métaux présents dans le milieu marin restent très peu étudiées et par là-même, les constantes de stabilité conditionnelle entre ces CSRVet les métaux n'ont pas encore été déterminées. Cependant, avec des teneurs généralement supérieures à celles en H_2S dans la colonne d'eau oxique, ces CSRV pourraient jouer un rôle non négligeable sur la spéciation métallique dans le milieu marin.

3. Influence des métaux sur le phytoplancton

Dans la partie précédente, le rôle de certains thiols (e.g., glutathion, H_2S) sur la spéciation métallique a été évoqué. Désormais, nous allons nous arrêter sur le rôle, positif ou négatif, joué par les métaux sur le développement de la biomasse marine. Comme les nutriments majeurs (i.e., azote, phosphore et silice), certains métaux traces

peuvent influencer la productivité et la distribution des communautés algales dans le milieu marin. Plusieurs métaux – fer, manganèse, cuivre, cobalt, molybdène, zinc et nickel – sont d'importants micro-nutriments (Sunda, 1988 ; Butler, 1998), alors que d'autres métaux (e.g., plomb, mercure, argent) sont des inhibiteurs biologiques et ne possèdent pas de fonction métabolique (Sunda, 1988 ; Okamoto et al., 2001). De plus, au moins quatre micro-nutriments métalliques – zinc, cuivre, fer et nickel – peuvent également être des inhibiteurs du métabolisme du phytoplancton lorsque leur concentration augmente significativement dans le milieu marin (Sunda, 1988 ; Pinto et al., 2003).

3.1. Micro-nutriments métalliques

Les micro-nutriments métalliques sont indispensables aux mécanismes physiologiques du phytoplancton marin puisque ces métaux servent de cofacteurs à de nombreuses enzymes. Par exemple, le fer est nécessaire au cytochromes b et c et aux protéines soufrées (e.g., ferredoxine) pour permettre le transport d'électrons lors de la photosynthèse et de la respiration. Le fer est également un co-facteur de la superoxyde dismutase qui empêche l'attaque cellulaire par les molécules d'H_2O_2 (Geider et La Roche, 1994). Le zinc active les ADN et ARN polymérases qui induisent la réplication et la transcription des acides nucléiques (Sunda, 1989). Le manganèse sert d'accepteur d'électron dans l'oxydation d'H_2O au sein du photosystème II et avec le fer, il est essentiel dans la dismutation des radicaux libres (Sunda, 1989). Le molybdène et le fer sont indispensables à la fixation de l'azote et à la réduction des nitrates alors que le cuivre joue un rôle dans le transport d'électrons et l'oxydation de nombreuses molécules organiques (Sunda, 1989 ; Geider et La Roche, 1994). Cobalt et nickel servent d'activateurs respectivement à la vitamine B12 et à l'urée (Sunda, 1989). Si les métaux sont nécessaires à tous les organismes photosynthétiques, la concentration en micro-nutriments dans les cellules phytoplanctoniques dépend de la demande enzymatique qui est très variable d'une espèce à une autres. Sous des conditions stationnaires lors de la phase exponentielle de croissance, la concentration en métaux traces dans le milieu, la vitesse d'assimilation de ces métaux par le phytoplancton et la vitesse de croissance algale sont intimement liés les uns aux autres (Sunda et Huntsman, 1986 ; Morel, 1987).

3.2. Résistance du phytoplancton à une toxicité métallique

Certains métaux traces deviennent toxiques lorsque leur concentration augmente de manière significative dans le milieu environnant (Morel, 1983). C'est le cas, par exemple, du cuivre, qui en plus de son rôle nutritionnel, peut diminuer de 50% (i.e., EC^{50}) la croissance phytoplanctonique lorsque sa concentration dans le milieu marin est supérieure à 157 nM (Pinto et al., 2003). De plus, les atomes d'Hg, Ag et Pb qui ne sont pas indispensables aux mécanismes physiologiques des algues, peuvent, s'ils sont présents en quantité importante dans le milieu marin, induire des effets négatifs sur le métabolismes du phytoplancton.

Une autre voie de toxicité des métaux pour le phytoplancton est lorsqu'ils se complexent non-spécifiquement avec des molécules biologiques importantes telles que les enzymes et par conséquent, altèrent leurs fonctions métaboliques (Sunda, 1989). Souvent, ces sites de fixation sont normalement occupés par des métaux essentiels et lorsque ces métaux sont remplacés par d'autres, il en résulte une inhibition de l'activité biochimique cellulaire (Bruland et al., 1991). Celle-ci peut-être liée à des changements dans la configuration de la molécule, de sa charge mais également à une modification du potentiel d'oxydo-réduction. Par ailleurs, ce remplacement d'un micro-nutriment par un métal potentiellement toxique sur un site de fixation s'opère en raison de la non-spécificité de ce même site pour un métal particulier (Sunda 1989). Par conséquent, il existe en permanence une compétition entre les métaux micro-nutriments et les métaux toxiques pour se fixer sur les ligands biologiques (Harrison et Morel, 1983 ; Murphy et al., 1984 ; Bruland et al., 1991 ; Lee et al., 1996). Les effets notoires causés par des concentrations métalliques toxiques sur le phytoplancton incluent une inhibition de la croissance, une peroxydation des lipides membranaires, une dénaturation des protéines, une mutation de l'ADN et une mort cellulaire (Bowler et al., 1992 ; Bryan et Langston, 1992).

Afin de lutter contre un stress environnemental, la cellule phytoplanctonique a élaboré des mécanismes de détoxification métallique. Chez les algues, les métaux toxiques (e.g., Cd, Cu, Zn et Hg) sont fixés sur les phytochélatines, des polypeptides contenant deux ou plusieurs groupements cystéine et synthétisées en continu dans les cellules phytoplanctoniques (Ahner et al., 1995 ; Ahner et Morel, 1995 ; Ahner et al., 1997). Le phytoplancton est ainsi capable de lutter contre cette modification environnementale en stockant dans ses cellules, le métal et en le rendant non toxique pour son

développement physiologique (Sunda et Huntsman, 1998). Morelli et Scarano (1995) ont mis en évidence une synthèse supérieure de phytochélatines chez *Phaeodactylum triconutum* (Diatomée) lorsque celle-ci était soumise à un stress environnemental induit par une augmentation de la concentration en Cd. De plus, Lee et al. (1996) ont démontré que *Thalassiosira weissflogii* (Diatomée) avait la capacité d'expulser le métal hors de ses cellules lorsque celui-ci était complexé à des phytochélatines. Un même processus a également été observé par Leal et al. (1999), chez *Emiliana huxleyi* (Coccolithophoridée) lorsque celle-ci était soumise à des concentrations élevées en cuivre. Ces flux de métaux vers le milieu marin sont induits par des concentrations intracellulaires élevées et permettent ainsi de diminuer la toxicité en limitant l'accumulation de métal dans les cellules. De plus, Kawakami et al. (2006) ont mis en évidence la synthèse par le phytoplancton de glutathion (dissous) lorsque des concentrations élevées en métal (e.g., Cd, Pb, Zn) étaient mesurées dans le milieu environnant. Le glutathion est un tripeptide formé d'un acide glutamique, d'une cystéine et d'une glycine et est le principal groupement thiol produit par les organismes vivants (Giovanelli et al., 1980 ; Noctor et Foyer, 1998). En raison de la forte affinité du groupement thiol pour les métaux, le glutathion, comme les phytochélatines, est un puissant complexant intracellulaire des métaux tels que le cadmium et le plomb (Kawamaki et al., 2006).

Il apparaît donc que le phytoplancton est capable de lutter contre un stress métallique environnemental en synthétisant des composés soufrés organiques tels que les phytochélatines et le glutathion mais également en mettant en place des procédés d'expulsion dans le milieu environnant de ces complexes thiol-métal. Il peut donc exister dans le milieu marin, une relation directe entre la concentration de certains composés soufrés et celle de divers éléments métalliques.

4. Problématique et Objectifs de la thèse

En exploitant les ressources métalliques, les sociétés industrialisées ont significativement influencé les cycles biogéochimiques des métaux sur Terre (Butcher et al., 1992). D'une part, les flux de métaux entre les différents réservoirs ont été altérés et d'autre part, les formes chimiques sous lesquelles les métaux étaient présents ont été modifiées (Butcher et al., 1992). Les pollutions métalliques, phénomènes de plus en plus répandus et plus particulièrement dans les zones côtières, sont également

responsables d'importantes modifications de l'environnement (Ping et al., 2004 ; Viarolli et al., 2005). Aujourd'hui, l'étude de l'impact de ces pollutions métalliques sur la biomasse marine mais également sur les cycles biogéochimiques d'éléments chimiques (e.g., Carbone, Soufre) fait l'objet de nombreux travaux scientifiques (Butcher et al., 1992 ; Chiffoleau et al., 2005 ; Rees et al., 2005).

Ces recherches de thèse se dressent donc dans le contexte de suivi des contaminants métalliques dans les environnements côtiers et estuariens mais également dans le contexte plus intemporel de compréhension des cycles biogéochimiques dans le milieu marin. Il s'agit en effet, d'étudier les interactions possibles entre *i)* les métaux présents naturellement ou anthropogéniquement dans le milieu marin, *ii)* certains composés soufrés réduits volatils (CSRV ; H_2S, OCS, MeSH, DMS et DMDS) et *iii)* le phytoplancton qui synthétise naturellement des thiols (e.g., phytochélatines, glutathion, DMSP) lors de son développement cellulaire (e.g., croissance, sénescence) mais également lorsqu'il subit un stress environnemental (e.g., pollution métallique) (Figure 4).

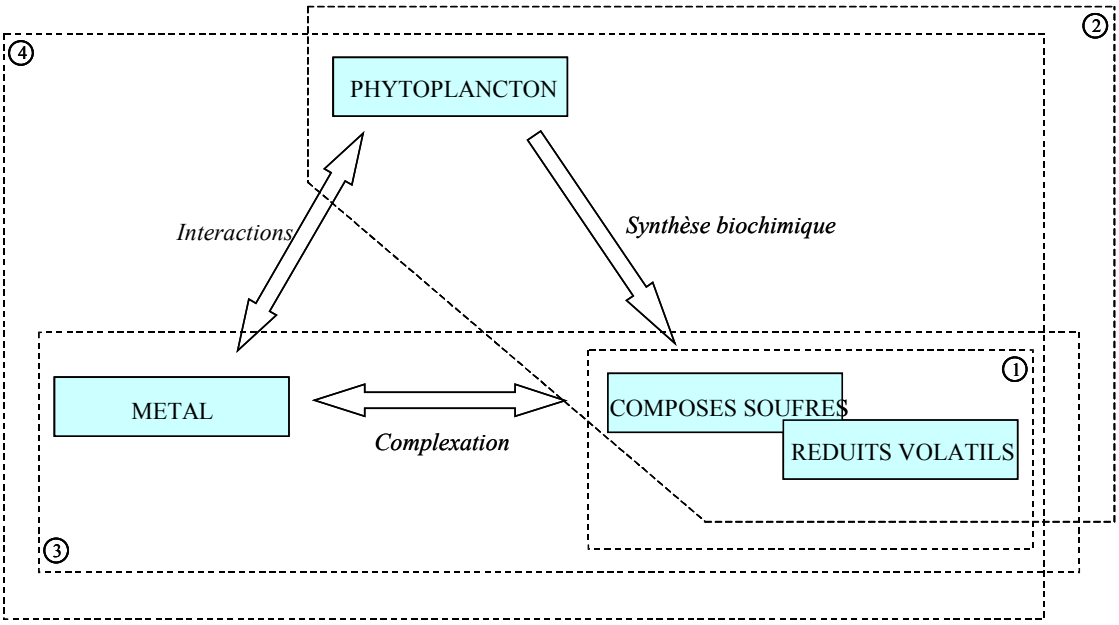

Figure 4 - Problématique et objectifs de la thèse. 1, Mise en place d'une méthode analytique des CSRV ; 2, Etude de la distribution des CSRV et du rôle du phytoplancton ; 3, Etude des interactions entre les CSRV et des métaux ; 4, Etude des interactions entre les CSRV, l'argent et le phytoplancton.

Ainsi, une première phase de travail a consisté à développer une méthode d'analyse sensible (i.e., limite de détection < 0.1 nM) et reproductible (i.e., déviation standard <

10%) des CSRV. La technique analytique choisie est la chromatographie gazeuse couplée à un système de piégeage cryogénique. L'optimisation de la méthode est détaillée dans l'**Article 1**.

Dans un second temps, il s'agissait de suivre la distribution de ces composés soufrés dans un environnement côtier non soumis à d'importantes pressions anthropiques : la Baie de Quiberon (Morbihan). Le suivi s'est déroulé de juillet 2004 à août 2006 afin de déterminer d'éventuelles variations inter-annuelles et saisonnières des concentrations en CSRV mais également le rôle joué par le phytoplancton sur la production de ces mêmes composés soufrés (**Article 2** ; Figure 4).

La troisième partie de cette thèse est basée sur l'étude, en laboratoire et sur le terrain, des interactions entre les CSRV et les métaux. Tout d'abord, les constantes de stabilité conditionnelle K′ entre certains CSRV (i.e., H_2S, OCS, MeSH, DMS) et l'argent (Ag^+) ont été déterminées expérimentalement. L'argent a été choisi comme "métal modèle" en raison de *i)* sa forte capacité à se complexer à l'H_2S (i.e., log K′=11.6) comparé aux autres métaux présents dans des environnements pollués (e.g., Cd, Hg… ; Al-Farawati et Van den Berg, 1999), *ii)* ses faibles interactions avec la matière organique à la différence du cuivre (Laglera et Van den Berg, 2003), *iii)* ses propriétés de contaminant en estuaire de la Seine (Chiffoleau et al., 2005). Ainsi, par la détermination des constantes de stabilité conditionnelle entre Ag^+ et OCS, MeSH et DMS et grâce aux séries de Irving-Williams (Morel, 1983), il a été possible de considérer les interactions de ces CSRV avec les autres métaux. Ensuite, une étude ponctuelle (23 mai–1er juin 2005), en estuaire de la Seine, a permis de déterminer l'évolution des concentrations en CSRV le long du gradient de salinité. Grâce au suivi simultané des teneurs en métaux (e.g., Ag, Cd, Zn, Cu…) dans l'estuaire, les interactions entre le sulfure de dihydrogène et certains métaux ont pu être quantifiées (**Article 3** ; Figure 4).

Enfin, dans une dernière phase de recherches, il s'agissait de s'interroger sur la réponse du phytoplancton à une contamination métallique (i.e., argent) en terme de synthèse de CSRV. En effet, il a été démontré que le phytoplancton était capable de tolérer une exposition métallique dans son environnement grâce à la synthèse de thiols qui vont agir comme des ligands et rendre le métal ″non toxique″. La question posée dans ce dernier chapitre est donc la suivante : ″le phytoplancton est-il capable de répondre à une contamination métallique par une synthèse supérieure en CSRV ? ″ Pour répondre à cette interrogation, nous avons donc élaboré un photobioréacteur afin de suivre en continu, une culture phytoplanctonique (e.g., *Isochrysis galbana affinis*

Tahiti, Haptophycée). Les résultats de cette expérience sont exposés dans la dernière partie du manuscrit (Figure 4). A noter qu'un effet compétiteur peut s'établir pour l'inertage du métal toxique entre le phytoplancton lui-même (i.e., fixation intracellulaire) et les CSRV présents dans le milieu environnant.

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Méthode d'analyse des CSRV

Article 1 – Analyse simultanée de cinq composés soufrés réduits volatils dans des échantillons naturels, par extraction et piégeage cryogénique couplés à une séparation par chromatographie gazeuse

Résumé

L'étude des composés soufrés réduits volatils (CSRV) dans un environnement côtier suscite l'intérêt des océanographes depuis plusieurs années. Jusqu'à présent, aucune méthode analytique n'était disponible pour le dosage simultané des CSRV suivants : sulfure de dihydrogène (H_2S), sulfure de carbonyle sulfide (OCS), méthane thiol (MeSH), diméthyl sulfure(DMS), diméthyl disulfure (DMDS). Afin de quantifier ces espèces soufrées dans divers environnements marins, cette méthode d'analyse a été développée. Elle possède une bonne précision pour chacun des CSRV puisque la déviation standard relative est respectivement de 6.0% pour l'H_2S, 4.1% pour l'OCS, 5.6% pour le MeSH, 4.9% pour le DMS and 8.4% pour le DMDS. Cette nouvelle méthode chromatographique présente également une limite de détection faible pour les CSRV étudiés, soit 67 pM pour l'H_2S, 33 pM pour l'OCS, 13 pM pour le MeSH, 100 pM pour le DMS et 33 pM pour le DMDS. Le protocole analytique présente trois phases successives *i)* une extraction à l'hélium des composés soufrés volatils d'un échantillon aqueux de 15 ml, *ii)* un piégeage cryogénique (i.e., azote liquide) des gaz extraits dans une boucle en Téflon® , *iii)* une séparation des CSRV par chromatographie gazeuse avec détecteur photométrique à flamme pulsée (PFPD). La vitesse d'analyse (i.e., 5 échantillons par heure) évite le stockage des échantillons et donc leur dégradation. Grâce à cette méthode analytique sensible et nouvelle, les concentrations en CSRV ont été déterminées dans deux environnements naturels : la Baie de Quiberon et l'estuaire de la Seine, France.

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Simultaneous analysis of five volatile reduced sulfur compounds by purge and cryogenic trapping / gas chromatographic separation in natural waters.

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Abstract

The detection of volatile reduced sulfur compounds (VRSC) has long held the attention of marine chemists. No method was presently available for the simultaneous analysis of the following volatile species ; Hydrogen sulfide H_2S, Carbonyl sulfide OCS, Methane thiol MeSH, Dimethyl sulfide DMS, Dimethyl disulfide DMDS ; from the same 15-ml natural water sample. In order to quantify these sulfur species in aquatic environments, a new sensitive method was developed featuring a good precision (i.e., 6.0% for H_2S, 4.1% for OCS, 5.6% for MeSH, 4.9% for DMS and 8.4% for DMDS) and low quantification limits (i.e., 67 pM for H_2S, 33 pM for OCS, 13 pM for MeSH, 100 pM for DMS and 33 pM for DMDS). The analytical protocol presented here is based on the stripping of a 15 ml-sample with helium and the liquid nitrogen-cooled trapping of extracted gases in a "purge and trap" apparatus made of Teflon® and inert polymers. Cryo-trapping is followed by capillary gas chromatographic separation, the quantification of VRSC amounts uses a pulsed flame photometric detection. The speed of analysis alleviates storage artifacts because sample turnaround time makes it possible to run 5 samples per hour. Thanks to this new analytical method, VRSC concentrations were determined in two different natural environments ; the bay of Quiberon and the Seine river, France.

Keywords : Gas chromatography - Volatile Reduced Sulfur Compounds – Natural water sample

1. Introduction

In recent years, there has been an increasing interest in estimating chemical species of marine sulfur. These compounds not only participate in the total atmospheric sulfur burden, they also play a role in climate regulation through the clouds formation (Charlson et al., 1987). In addition, they exert an influence on metal speciation and bioavailability via complexation reactions in the oceans (Al-Farawati and Van den Berg, 1999 ; Sukola et al., 2005). Apart from sulfate, a major ion in seawater, sulfur exists in various volatile reduced compounds in the oceans, particularly hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH, "CH_3SH"), dimethyl sulfide (DMS, "CH_3SCH_3") and dimethyl disulfide (DMDS, "CH_3SSCH_3"). All these volatile reduced sulfur species are extremely reactive and their analysis often raises many difficulties due to their low natural levels.

In anoxic marine environments such as marine sediments where water column ventilation is restricted, H_2S is produced from SO_4^{2-} reduction (Jacobs and Emerson, 1982 ; Dyrssen and Kremling, 1990). Owing to its low concentration (0.1 to 1.1 nM in surface seawater ; Cutter and Krahforst, 1988), is thought to be partially or totally complexed by trace metals. The concentration of OCS in surface seawater ranges from 0.01 to 0.1 nM (Rasmussen et al., 1982 ; Ferek and Andreae, 1983 ; Turner and Liss, 1985 ; Johnson and Harrison, 1986) and it is likely to be produced in marine waters by the photodegradation of natural organic matter by ultraviolet solar radiation (Zepp and Andreae, 1995). In addition, H_2S is produced by OCS hydrolysis (Ferek and Andreae, 1983 ; Watts, 2000).

The dimethyl sulfide is the result of the enzymatic cleavage of the dimethylsulfoniopropionate (DMSP) produced by marine biota (Keller et al., 1989 ; Simo et al., 2002) and released mostly due to phytoplankton grazing by zooplankton or phytoplankton senescence (Archer et al., 2002). Although anthropogenic emissions dominate the global sulfur budget, natural sulfur emissions continue to be a significant fraction (30-100%) of the total sulfur emissions in the tropical latitudes of the northern hemisphere and in all latitude belts of the southern hemisphere (Malin and Turner, 1992 ; Bates et al., 1992). Oceanic DMS emissions account for 2/3 of the natural sulfur flux into the atmosphere with 22 Tg/year (IPCC report, 2001). Atmospheric DMS is rapidly oxidized to acidic aerosols sulfates (via methanesulfonic acid and sulfur dioxide ; Malin, 1996) which contribute to the formation of cloud condensation nuclei

(Charlson et al. 1987), thus increasing the albedo of clouds and playing a significant role on climate change (Clarke et al., 1998). Other volatile reduced sulfur species exist in marine and estuarine environments, such as MeSH and DMDS that are bio-produced from methionine and DMSP (Kiene and Taylor, 1988). While the oceanic sources and sinks distribution of this compound are still largely unknown, DMDS in freshwaters come from the oxidative dimerization product of MeSH by polysulfides (Gun et al., 2000).

Given the great diversity in origin and behavior briefly summarized above, the possibility of interconversions and the general importance of sulfur species in marine and atmospheric biogeochemistry, it is essential to obtain numerous and accurate analyses of the concentrations of H_2S, OCS, MeSH, DMS and DMDS in the same sample aliquot. Since the common characteristic of these compounds is their volatility, simple or paired compounds analysis by stripping-cryogenic trapping-gas chromatography are the most frequently used procedures. For the last two decades, environmental chemists have increased the speed of analysis and the number of detectable species within the same run. Leck and Bägander (1988) used gas chromatography (GC) with a flame photometric detector (FPD) to determine reduced sulfur compounds in aqueous solution (i.e., 50-200 ml) but the duration of the stripping step was 20 minutes. Groene (1997) used cryogenic trapping coupled with gas chromatography to assess DMS, MeSH and DMDS concentrations in three northern Italian lakes. However, the technique used was not able to detect H_2S and OCS at low concentrations (i.e., <0.1 nM). Other scientists have simultaneously studied only one or two sulfur compounds (Johnson and Harrison, 1986 ; Radford-Knoery and Cutter, 1993). For example, Knoery and Cutter (1993) coupled the total dissolved sulfide and carbonyl sulfide analysis and devised a double cryogenic trapping apparatus to speed up the analysis. However, this technique was suitable only for H_2S and OCS. Simo et al. (1993) used an advanced method to determine volatile reduced sulfur compounds in water and wet sediment. The sulfur gases were concentrated by nitrogen purging and cryo-trap condensation. The trap was then connected on-line to a field-portable gas chromatograph provided with a secondary cryofocusing trap. But the detector used in all the previous works is a FPD which it was less specific for sulfur species and less sensitive (Cheskis et al., 1993) than the pulsed flame photometric detector (PFPD) used here, which is extremely sensitive to sulfur compounds and makes it possible to lower the sample volume to 15 ml and to speed up the analysis time to 10 minutes.

It should be noted that in seawater at pH 8, the predominant form of sulfide is bisulfide (HS^-). This is converted to H_2S at pH values below its pK_1 (6.52, 25°C, 35 of salinity ; Millero et al., 1988), and forms stable complexes with metal ions (Dyrssen, 1988). Several authors advise acidification (Leck and Bägander, 1988 ; Radford-Knoery and Cutter, 1993) which allows a quantitative recovery to analyze labile sulfide species of total (free plus complexed) dissolved sulfide by purge and trap. Electrochemical methods are also feasible (Al-Farawati and Van den Berg, 1999 ; Luther and Tsamakis, 1989), but this type of analysis requires a purging phase which may remove some of the volatile H_2S present and not detect other VRSC (e.g., OCS). For our purposes, we were interested in volatile species, hence chose not to treat the samples in anyway. Chromatographic interferences including lack of reproducibility and peak splitting were observed after sample acidification. Therefore, the concentrations reported here correspond to the free dissolved sulfide species and their complexes labile to a helium purging.

This article describes the optimization of the new analytical procedure and its application to the simultaneous determination of five reduced sulfur species (H_2S, OCS, MeSH, DMS, DMDS) in natural waters.

2. Experimental section

2.1. Apparatus

To avoid interactions of the analytical apparatus with volatile reduced sulfur species, all surfaces in contact with the sample were inert. Only Teflon® (PTFE) and Peek® (NAT) tubing were used. Peek® was used for Rotor and Stator valves, and all tubing connections (1/8"OD-1/16" upstream of trap, 1/16" OD -1 mm id for the trap, 1/16" ID -0.5 mm OD and Peek downstream of the trap). The gas extraction took place in a closed 50-ml Teflon® vessel (Savillex®). The vessel lid was drilled to fit three Teflon® tubes, i.e., one to introduce the sample into the vessel via a 3-way LuerLock valve and to monitor the vessel's internal pressure, the second one for the purging gas (helium) and the last one for the extracted gases to exit towards the trap.
The absence of anomalous overpressure (i.e., less than 0.5 bar above atmospheric pressure) in the vessel, was verified with a barometer comprising 20 cm of transparent clear Teflon® tube. It was capped at its distal end and filled with air and a drop of

deionized water at the other end near the 3-way valve connecting to the vessel. If pressure increases in the vessel, the liquid moves into the tube and away from the vessel, equilibrating pressure on both sides of the liquid drop.

A 40-cm length Teflon® tubing loop (1/16" ID) is used to cryogenically trap the extracted analytes. The loop was filled at its mid-section with 50 mg (7 cm) of Teflon® wool (Altech, part.n°4736). It was connected to a Valco Cheminent 6-way valve plumbed as shown in Figure 1. To prevent moisture build up in the chromatographic valve, the sulfur gases evolved from the vessel were be dried using a Nafion™ membrane (part Permapure n°MD05072F2) placed downstream the vessel. The 4-component analytical apparatus (stripping, drying, trapping and chromatographic analysis) is shown in Figure 1.

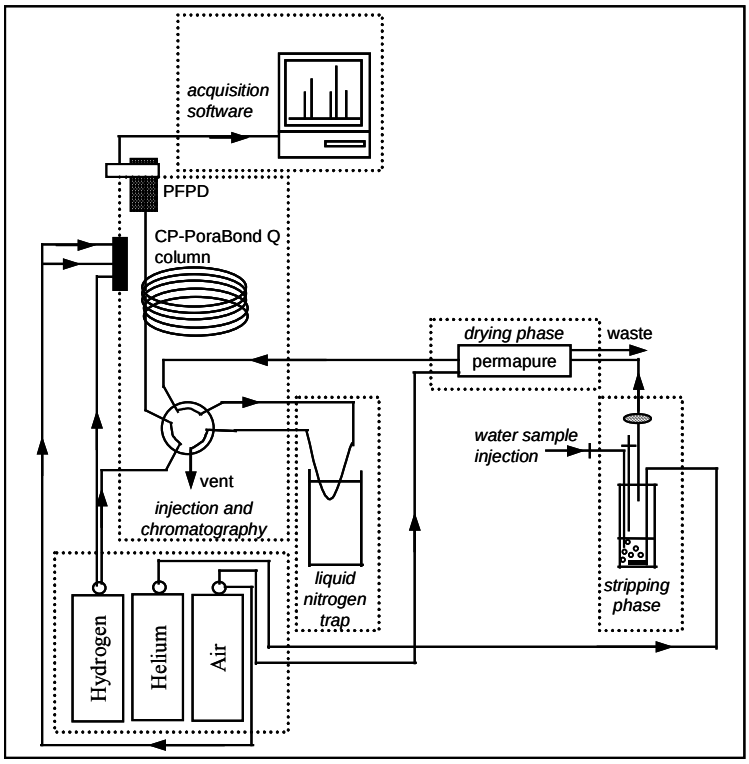

Figure 1 - Purge and cryogenic trapping/Gas Chromatographic separation apparatus with the four phases ; sample injection and stripping vessel, permapure dryer, liquid nitrogen trapping, gas chromatographic analysis.

The separation of the extracted gases was performed on a GC coupled with a PFPD (CP-3800, Varian corporation). The chromatographic column was a CP-PoraBond Q column (10-m length, 0.53 mm id, Varian) which provide a better separation of H_2S

and OCS because of a porous polymer stationary phase. Data acquisition software consisted of Varian's Star package running on a laptop computer.

2.2. Analytical procedure and parameters

The 15-ml samples were held in a 60-ml syringe (Codan®) and were injected without contact between sample and atmosphere into the vessel via the LuerLock 3-way valve. Helium flowed continuously, into the vessel to strip the sulfur gases from the liquid phase. Upstream of the cryogenic trap, a Nafion™ membrane (Permapure) was placed to avoid clogging of the trap and to prevent moisture in the chromatographic valve. After 10' of stripping, the valve was set to the injection "position" and the trap removed from liquid nitrogen. It was brought to above room temperature using a simple hair dryer placed to a fixed distance of the vessel (i.e., 20 cm) for 2 minutes. Each sulfur gas was released from the trap when its boiling point was attained by the trap ; -60.3°C for H_2S, -50°C for OCS, 5.95°C for MeSH, 37.3°C for DMS and 109°C for DMDS (Lewis and Fredericks, 1968 ; Andreae, 1980 ; Janicki et al., 1993 ; Figure 2).

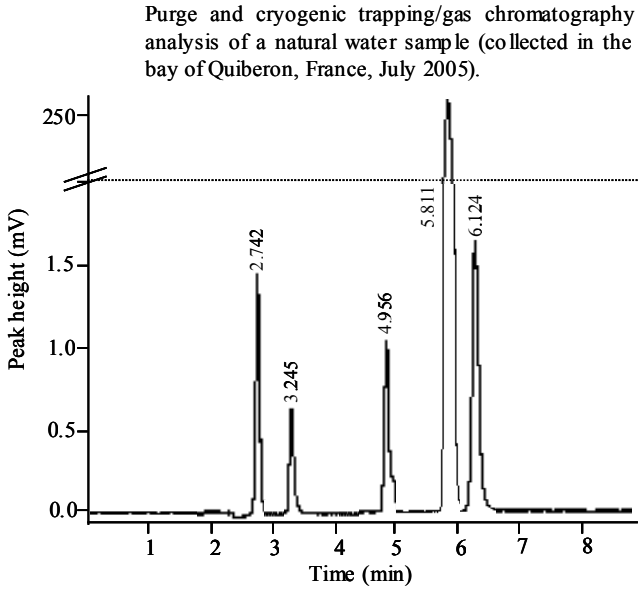

Figure 2 - Chromatogram obtained by the analysis of a natural seawater sample. VRSC retention times are highlighted (H_2S, 2.7±0.05 min ; OCS, 3.2±0.05 min ; MeSH, 4.9±0.05 min ; DMS, 5.8±0.05 min, DMDS, 6.1±0.05 min).

Following this, sulfur gases were swept into the chromatographic column by the carrier gas. Hydrogen was chosen as carrier gas to decouple the trapping system from the stripping system to avoid chromatograph damages (e.g., entry of water from the vessel in the valve). In addition, several chromatographic parameters were optimized (e.g., oven column temperature programming, air flow, hydrogen flow, helium flow) to reduce the analysis time to 10 minutes. Optimized parameters are given in Table 1.

Table 1 - Extraction and gas chromatography parameters.

Extraction	Time: 10 min Stripping gas flow (He, 3.0 bars, 99.999%): 75 ml/min, (fine bubbles)
Column	Carrier gas flow (H_2, 2.8 bars, 99.999%): 3.3 ml/min
	Oven temperature range : 45°C for 1.25 min from 45 to 60°C (60°C/min) from 60 to 180°C (80°C/min)
Detector	Detector gain 9 from 0 to 4 min and range 8 after 4 min Gate delay: 6.1 ms, 120 pmt Gate width: 1.9 ms Detector temperature: 250°C Detector H_2 flow: 12.5 ml/min Air1 flow (Air, 5.0 bars, 99.999%): 15.5 ml/min Air2 flow: 10.4 ml/min

2.3. Standards

Different types of standards were used in order to identify retention times of each VRSC. Certified sulfur gas mixes were obtained from Scott Gas (Scotty 58) with certified concentrations of DMS (1.1±0.01 ppm), OCS (1.0±0.06 ppm) and H_2S (1.1±0.04 ppm). For methane thiol (MeSH), following dilutions of a liquid standard solution (MeSNa, 21% w/w, Aldrich) were carried out in deionized water to obtain a picomolar standard solution. For dimethyl disulfide (DMDS), a permeation device (VICI-Metronics®) was used whose permeation rate and temperature dependence were gravimetrically determined. The DMDS was released from the permeation device at a constant rate and swept into a stream of helium. A given amount of DMDS can then be trapped by freezing the gas stream in liquid nitrogen. To avoid their degradation, all standards (Scott gas bottles, MeSNa solution, DMDS permeation tube) were stored at –18°C and were never used after the use by-date.

3. Results and Discussion

3.1. Stripping and Trapping

The "Purge and Trap" technique, also referred to as the "dynamic head space" technique, is often used to determine very low concentrations of free dissolved compounds in liquids (Wardencki, 1994 ; Careri et al., 1999).

Various adjustments are required to optimize the "purge and trap" system ; choosing the optimal purging gas flow (helium), optimizing the stripping efficiency, determining the analyzed sample volume and the optimal trap geometry.

3.1.1. Determination of the optimal purging gas flow

Knoery and Cutter (1993) have used a 75 ml/min helium flow whereas Belviso et al. (2003) opted for a flow of 70 ml/min. For this reason, several purging rates (i.e., 60 to 100 ml/min) were tested for reproducibility and efficiency with each VRSC. The best reproducibility (mean relative standard deviation below 10%) was obtained with a 75 ml/min helium flow whereas the detection limit was below the 0.1-nM level for only a helium flow included to 70 and 80 ml/min. So, these experiments highlighted that a 75 ml/min flow was optimal for the study of volatile sulfur compounds.

3.1.2. Optimization of the stripping efficiency

The size of helium bubbles in the vessel is also an essential parameter because the smaller they are the more efficient the extraction (Hassett and Milicic, 1985). To decrease the bubble size, a 3-cm long, porous polypropylene (100-µm pore diameter, only available) tube was connected to the Teflon® tube which released the helium in the stripping vessel (Figure 1). With this porous polypropylene tube, the helium diffused mostly and so, the bubbles were smaller and more numerous in the vessel. Unsuccessful results were obtained with an ultrasound bath (46 kHz, 50 W) to decrease the purging time.

Having optimized the purging conditions, it was necessary to find a compromise between the smallest dead volume (i.e., head-space) above the sample in the vessel and the entrainment of liquid aerosols that may be brought by the extracted gas flow into the cryogenic trap. For this reason and after several volumes samples tested, a 50-ml vessel (the only available Teflon® PTFE vessel) and 15-ml water samples were chosen to quantify the VRSC by stripping.

Experiments were carried out to determine the inertness of our system (Teflon® vessel, Nafion™ dryer, trap). Small amounts of each sulfur gas (9-45 pmol, n_i=4) were successively injected at several locations of the system : upstream of the empty vessel, upstream of the vessel filled by 15 ml of deionized water and downstream of the vessel (and upstream of the dryer). The aim was to verify whether the recovered amounts VRSC were the same wherever the gas injection took place. These experiments showed that there was no significant loss of signal between an injection immediately before the trap (empty or full) and injection downstream of the vessel (simplest possible analytical system). The variation of signal (i.e., value of the square root peak) was 1.6±1.6% for H_2S, 0.5±0.3% for OCS, 0.6±0.6% for DMS and 1.0±0.5% for DMDS. Methane thiol was not tested because of the lack of a gaseous solution. Consequently, the stripping vessel and the other parts of the analytical system were found to be relatively inert to the VRSC.

3.1.3. Optimization of the effectiveness of cryogenic trapping

The foremost objective was to reduce the trap volume and hence, the time required by the carrier gas (3.3 ml/min) to sweep the trap. To determine the best trapping efficiency, two diameters of tubing (1/16"OD and 1/8" OD), with and without 50 mg Teflon® wool plug (weight arbitrary chosen) were successively tested. Standard water samples (i.e., made from VRSC standards) were prepared and injected in the trapping vessel. For the same stripping time, the recovered gas area was 47 to 53% less with the 1/8" OD tubing which indicated that the thinner tube was more appropriate for VRSC cryogenic trapping. Moreover, for the 1/16"od tubing, whatever the VRSC considered, the trapping reproducibility was 22 to 27% higher with Teflon® wool added than without.

The tubing length was varied using lengths of 2, 5, 10, 15, 20 and 30 cm, for the same stripping time, to evaluate the optimal length. With only 10 cm of the loop dipped in liquid nitrogen, the optimal trapping efficiency for the five VRSC was reached. The signal was less than 57.8±2.4% (mean value between the five VRSC) for 5 cm immersed in liquid nitrogen, whereas up to 10 cm immersed, the signal was constant (2.3±1.2% ; mean value between the five VRSC). To facilitate the insertion of Teflon® wool and the analytical system configuration, the Teflon® cryogenic trap length is fixed to 40 cm with an 1/16"od and was filled with 50 mg of Teflon® wool. The total volume of the loop was about 300 µl and with this design, only 6 seconds were required for the carrier gas to sweep the loop.

3.2. Drying

The sulfur gases evolved from the vessel must be dried before reaching the cryogenic trap to avoid clogging and to prevent moisture build up in the valve. As a consequence, one 25-mm diameter, 0.45 µm pore size filter (i.e., hydrophobic Teflon®) was placed at the outlet of the vessel (Figure 1) which prevented larger aerosols leaving the vessel. Different drying systems were used for analyses of sulfur gases, particularly K_2CO_3, a cooled U-tube and Nafion™. Chemical drying agents like K_2CO_3, $CaCl_2$ and $CaSO_4$ are not suitable for low level sulfur gas analyses because they remove one or more of the analytes from the humid gas stream (Leck and Andreae, 1988 ; Andreae, 1980 ; Andersen and Bruno, 2003). The cooled U-tube is a cumbersome and mechanically fragile system with significant dead volumes and can induce gas losses, whereas Nafion™ (e.g., polymer fluorinated with grafted sulfonic acid groups) is compatible with all volatile gases analysis and is extremely practical due to its small size (Wardencki, 1994 ; Belviso et al., 2003). Therefore a 120-cm length tubular Nafion™ membrane (part Permapure n°MD05072F2) was included in the system (i.e., downstream of the trapping loop) to dry the helium stream. The device's efficiency is related to the gas flow outside the tubular membrane and removes the water which has crossed the membrane. Further to several experiments, we set a 200 ml/min airflow to prevent moisture trapping. Previous experiments to determine the best trap design were conducted with and without the Nafion™ membrane. No variations of the trapping efficiency were observed (i.e., less than 1.2±0.4% of RSD). So, Nafion™ membrane was not an issue to the VRSC measure.

3.3. Chromatographic analyses

3.3.1. Gas chromatograph

With a CP-PoraBond Q column, the sulfur gas separation depends on both its separation boiling point and the gas affinity with the porous polymer stationary phase. The column was connected to the injection valve with Peek tubing (20-cm in length, 1/16"od) to bypass the Silico-Steel™ transfer tube supplied with the instrument.

There are two types of sulfur specific detector : the SCD (Sulfur Chemiluminescence Detector) and the FPD (Flame Photometric Detector) with its variant, the Pulsed FPD (PFPD). The PFPD was chosen for several reasons including a more favourable price/performance ratio in terms of quantification and noise level (Andersen and

Bruno, 2003), despite the overall better sensitivity of the SCD (Lestremeau et al., 2004). Although the PFPD is more difficult to tune (i.e., gas flows, operating temperature and electronic settings), it provides better selectivity and sensitivity to sulfur species analysis than the FPD (Cheskis et al., 1993).

3.3.2. Detector settings

The instrument's manual advised a detector temperature of approximately 200°C to obtain sufficient selectivity. Maximum sensitivity and minimum temperature dependency of this sensitivity were determined for temperatures between 150 and 300°C, by repeated injections (n_i=4) of a mix of several sulfur gases (i.e., H_2S or H_2S+OCS+DMS) in a sample loop (Figure 3, curves are obtained combining the results observed for H_2S, OCS and DMS).

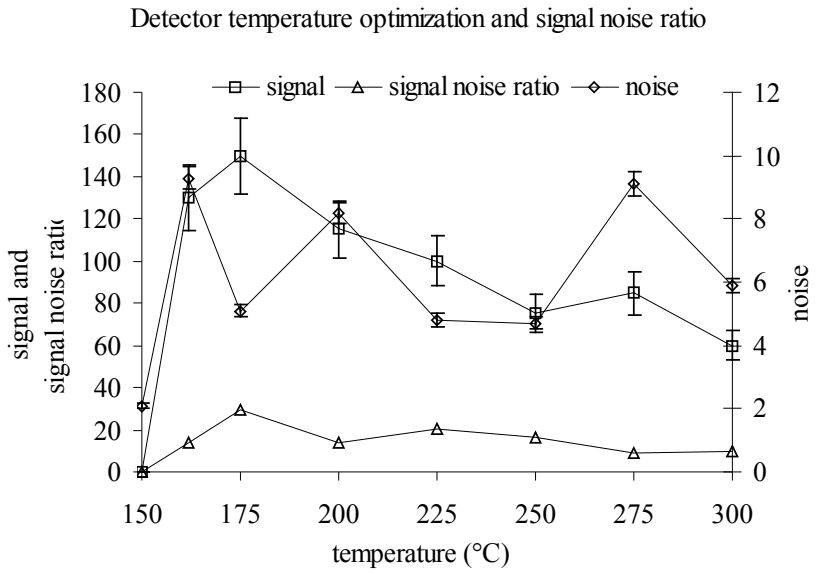

Figure 3 - Determination of the optimal detector temperature according to the ratio between the signal (arbitrary unit) and the noise (µV). Curves are obtained combining the results observed for H_2S, OCS and DMS.

For the three sulfur gases tested, the highest signal was obtained when the detector temperature was near 175°C. But, for the same temperature range, the signal/noise ratio is also highest which indicated a more unstable detector sensitivity. At 250°C, the signal is 56.7±1.4% lower than at 175°C but the signal noise ratio was also lower. So, a compromise was chosen to have both low signal noise ratio and low temperature sensitivity fixing the detector temperature at 250°C. Likewise, in order to maximize

sensitivity and reproducibility, the various gas flow rates of the chromatographic apparatus were also optimized (Table 1). Moreover, no degradation of PFPD performances (e.g., contamination of the combustor quartz tube) was observed for 6 months daily use with natural water samples.

3.3.3. Temperature programming (oven/integration)

Constant temperature program and variable oven temperature program were tested to determine the best analytical conditions for the five VRSC. Firstly, with constant temperature (e.g., 60°C, 120°C or 180°C) programs, compound elution was found to take too long or peak resolution was insufficient. In order to optimize the peaks separation, the oven temperature was therefore ramped up during the run (Table 1). These adjustments made it possible to obtain a better H_2S and OCS peak separation, methane thiol detection and also a better DMS and DMDS peak separation. In addition, the detector gain was changed during the run. At the beginning of the chromatographic analysis, it was set to 9, and before DMS elution the gain was set to 8. As a result, the sensitivity of the detector decreased by a factor of 10 before the DMS elution, avoiding its saturation by DMS, which often had a greater concentration by one or more orders of magnitude than those of the other volatile sulfur compounds.

3.3.4. Stripping and Trapping

This is one of the most important parameters in a "Purge and Trap" analysis. Hence, it was essential to determine the shortest, yet quantitative, extraction time for each sulfur gas for a natural water sample. As certified natural standards for VRSC did not exist due to the instability of these compounds, a seawater sample was collected in the Bay of Quiberon (France) in July 2005 and eighteen 15-ml aliquots were withdrawn from it. Each subsample was stored in a 60-ml syringe before to be injected in the stripping vessel. So various lengths of time (i.e., 2, 4, 8, 10 and 12 min) were tested in triplicate to determine when the VRSC extraction is complete. For H_2S and OCS complete extraction was found to be faster (8 min) than for MeSH, DMS and DMDS (10 min) (Figure 4).

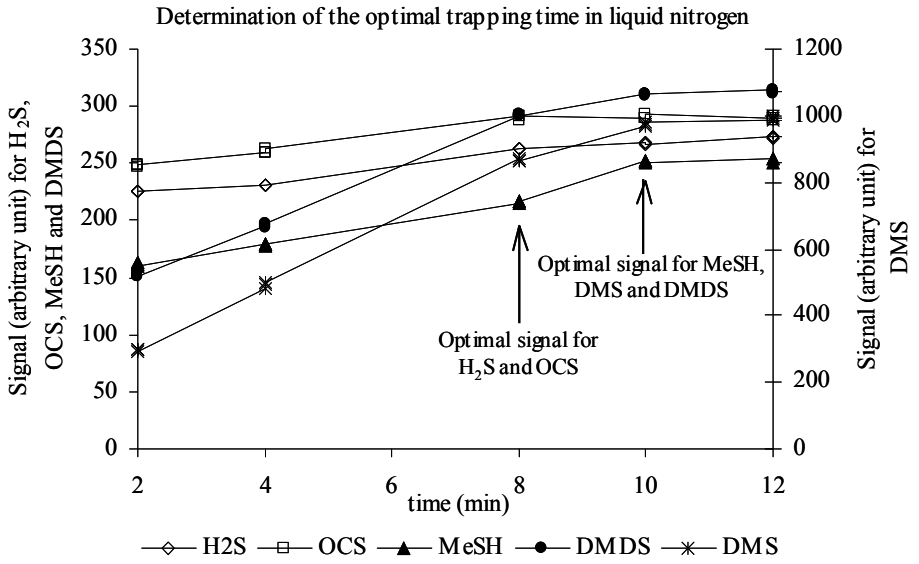

Figure 4 - Determination of the optimal stripping time for the five VRSC measured in a natural water sample. The error bars (relative standard deviation) are not shown to conserve the best graph clarity.

Between two analyses, it was necessary to wait for 2 minutes and to heat the trap with a hairdryer to prevent water vapor build up inside the trap and to quantitatively release high boiling compounds (DMS and DMDS) from the trap. Experiments were carried out to demonstrate the role of the trap warming. A given amount (45 pmol) of DMS and DMDS was injected (n_i=10) upstream of the vessel and the trap is warmed only for the first five injections. These experiments showed that the DMS and DMDS volatilization were incomplete if the trap was kept at ambient temperature. The mean amounts recovered were respectively 15.1±2.0% and 32.3±5.1% less than those obtained with warming the trap.

3.4. Retention time and Calibration

Experiments were carried out to define the retention time of volatile sulfur gases. With the capillary column, the order of elution was H_2S (2.7 min), OCS (3.2 min), MeSH (4.9 min), DMS (5.8 min) and DMDS (6.1 min) (Figure 2).

A calibration factor was used to link the square root of the peak area to the amount of sulfur gas. The square root was used instead of the peak area because the detector response to the sulfur amount injected was based on an arbitrary quadratic model with a linear response. The calibration factor for each volatile sulfur compounds was experimentally determined for this analytical system. It has been shown before that the

calibration factor in GC-PFPD is different for each sulfur compound (Lestremeau et al., 2004 ; Mestres et al., 1997).

To determine the calibration factor for H_2S, OCS and DMS, aqueous solutions of increasing concentration (up to 10 nM) were prepared from the various standards (i.e., certified Scott gas bottle, MeSNa solution, DMDS permeation tube). The values of the calibration factors with a CP-PoraBond Q column, were 13.7±0.1 (H_2S), 24.5±0.3 (OCS), 9.8±0.3 (DMS), 2.0±0.1 (MeSH) and 4.3±0.1 (DMDS). These calibration factors were acquired over a zero to 10 nM range using slopes of the best fit line (for all cases ; n>10 and r^2=0.99). It is important to note that the value of these various calibration factors varied with different parameters like oven temperature and the time of elution. For example, other calibration factors were obtained with another capillary chromatographic column (i.e.,CP-SIL5CB).

3.5. Precision and detection limits

The analytical precision (standard deviation as a percentage of the average concentration) was determined from natural water samples (collected in the bay of Quiberon and the Seine river, France) which were subsampled in four 15-ml aliquots and each subsample was analyzed 6 following times. The relative standard deviation (RSD) was found to vary between the five VRSC : 6.0% for H_2S, 4.1% for OCS, 5.6% for MeSH, 4.9% for DMS and 8.4% for DMDS (Table 2).

Table 2 - Analytical precisions for measurements of the concentrations of volatile reduced sulfur compounds in natural waters.

		Average concentration (nM) and standard deviation	Relative Standard Deviation (%)
Bay of Quiberon (June 2005)	H_2S	0.30 ± 0.02	6.0
	OCS	0.18 ± 0.01	4.4
	MeSH	2.18 ± 0.12	5.5
	DMS	7.27 ± 0.31	4.3
	DMDS	0.20 ± 0.02	8.8
Seine River (May 2005)	H_2S	0.83 ± 0.05	6.1
	OCS	0.61 ± 0.02	3.9
	MeSH	2.17 ± 0.13	5.8
	DMS	12.11 ± 0.67	5.5
	DMDS	1.27 ± 0.10	8.1

The quantification limits (10 times standard deviation of the blank) for the analysis of 15-ml natural water samples, were 1 pmol for H_2S (66.7 pM), 0.5 pmol for OCS (33.3 pM), 0.2 pmol for MeSH (13.3 pM), 1.5 pmol for DMS (100 pM) and 0.5 pmol for DMDS (33.3 pM).

3.6. Analytical method application to a survey of VRSC in natural water samples

Ocean margins, coastal areas and estuaries have been identified as significant sources of DMS (Andreae, 1986 ; Iverson et al., 1989 ; Turner et al., 1996). In addition, estuaries have also been identified as a significant source of various sulfur compounds and especially OCS (Radford-Knoery, 1993 ; Watts, 2000). H_2S whose main ocean source is the hydrolysis of OCS, is also a significant compound of the marine sulfur budget (Turner et al., 1996). The new analytical method has therefore enabled the quantification in a single run of five VRSC in coastal and river waters. The method was applied to samples from the bay of Quiberon (interannual and seasonal study ; Cozic et al., 2007a) and the Seine river along the salinity gradient (Cozic et al., 2007b).

4. Conclusion

A sensitive chromatographic method is described for the determination of five volatile reduced sulfur compounds concentrations in various natural waters (e.g., marine and estuary waters, rain waters). The method has several advantages including simplicity of use, high sensitivity and the possibility to quantify rapidly (5 samples per hour can be analyzed) and simultaneously five VRSC. Using the adjustments detailed here, it is possible to achieve better sensitivity and precision in order to obtain low detection limits (i.e., below 100 pM). The use of CP-PoraBond Q chromatographic column increases the sensitivity to VRSC. The small volume necessary for the analysis and the absence of previous treatment allow the study of various natural samples and decreases consequently the time of analysis. The developed method could be satisfactorily applied as a routine procedure to identify and quantify the VRSC in natural water samples. Moreover, this analytical apparatus is portable and robust and can therefore be taken on board a research vessel.

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Distribution des csrv dans un environnement côtier

Article 2 – Etude saisonnière et interannuelle des composés soufrés réduits volatils (CSRV) en zone épibentique dans un environnement côtier, la Baie de Quiberon (Bretagne, France).

Résumé

Les variations saisonnières et interannuelles des concentrations en sulfure de dihydrogène (H_2S), sulfure de carbonyle (OCS), méthane thiol (MeSH), diméthyl sulfure(DMS) et diméthyl disulfure (DMDS) ainsi que des paramètres ancillaires (e.g., l'abondance phytoplanctonique) ont été suivis dans un environnement côtier, la baie de Quiberon (Bretagne, France). La station échantillonnée, Men Er Roué, est localisée au centre de la baie et l'étude s'est déroulée de juillet 2004 à août 2006. l'échantillonnage a été mené dans la colonne d'eau et dans les deux premiers mètres au-dessus de l'interface eau-sédiment (IES). Pour chaque journée d'échantillonnage, 8 à 16 échantillons d'eau ont été prélevés simultanément à des hauteurs d'eau différentes afin de mettre en évidence d'éventuels gradients verticaux et par conséquent, de définir le rôle de l'IES sur la distribution des composés soufrés volatils réduits (CSRV) étudiés. Les concentrations varient respectivement entre 0 et 1.6 nM pour l'H_2S, 0 et 4.2 nM pour l'OCS, 0 et 7.8 nM pour le MeSH, 0.1 et 17.5 nM pour le DMS et entre 0 et 1.7 nM pour le DMDS. La distribution de l'OCS selon la profondeur montre des variations saisonnières significatives qui suggèrent un changement des sources en OCS au cours de l'année. En hiver, la source principale est la production photolytique alors qu'en été, les sédiments deviennent une source importante d'OCS pour la colonne d'eau. La distribution verticale en sulfure de dihydrogène pourrait être influencée par la présence de microzones anoxiques créées par la dégradation de la récente matière organique. De plus, un relargage d'H_2S par les sédiments n'est pas mis en évidence et donc ne peut expliquer les concentrations non négligeables mesurées dans les eaux proches de l'IES. La probable influence des Dinophycées sur les concentrations en MeSH, DMS et

DMDS est démontrée au cours de toute la période d'échantillonnage (i.e., 3 étés). Le décalage temporel (i.e., 2 mois) entre les maxima de densité en Dinophycées et concentrations en MeSH et DMDS pourrait être expliqué par une plus lente transformation du DMSP en ces deux CSRV par rapport à une production de DMS, via le DMSP, plus rapide.

Statut : en préparation

Seasonal and interannual study of epibenthic volatile reduced sulfur compounds (VRSC) in coastal environment : the Bay of Quiberon (Brittany, France).

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Abstract

Seasonal and annual variability of hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) concentrations and supporting parameters (e.g., phytoplanktonic cells abundance) were investigated in a coastal marine environment, the Bay of Quiberon (Brittany, France). The sampling station (Men Er Roué) was located in the middle of the Bay and was monitored from July 2004 to August 2006. The sampling was conducted in the water column, within two meters immediately above the sediment water interface (SWI). In that vertical range, 8 to 16 samples were simultaneously collected on each sampling day to investigate possible vertical gradients and hence, evidence of a role of the SWI in the distribution of these sulfur compounds. Minimum and maximum values were <0.1-1.6 nM for H_2S, <0.1-4.2 nM for OCS, <0.1-7.8 nM for MeSH, <0.1-17.5 nM for DMS and <0.1-1.7 nM for DMDS. Vertical carbonyl sulfide distribution showed clear seasonal variations which suggests a change of its sources through the year ; in winter, the major source was the photolytic production and in summer, bottom enrichments suggest that sediments became a significant source. Vertical sulfide distribution might be influenced by the presence of anoxic microzones from the decay of extremely fresh organic matter, but sediments (i.e., release of sulfide) did not appear to influence the H_2S concentration in the bottom waters. The likely influence of Dinophyceae abundance on the MeSH, DMS and DMDS concentrations was evident for the 3-summer monitored period. The evidence of a 2-months time span between the highest Dinophyceae density and the maximum concentrations of MeSH and DMDS might be explained by a slower transformation of DMSP (not determined here) in these sulfur compounds compared to a faster DMS production.

Keywords : Sulfur – Coastal environment – Sediment Water Interface – Phytoplankton

1. Introduction

Over the last decades, the distribution and biogeochemistry of sulfur compounds such as hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH, CH_3SH) dimethyl sulfide (DMS, CH_3SCH_3) and dimethyl disulfide (DMDS, CH_3SSCH_3) in the marine environment have received growing attention (Cutter and Radford-Knoery, 1993 ; Dacey et al., 1998 ; Zhang et al., 1998 ; Despiau et al., 2002 ; Yang et al., 2005). Interest in these chemical compounds is linked to their important reactivity and their significant contribution to the atmospheric sulfur budget. Kettle and Andreae (2000) showed that DMS may be responsible for up to 60% of the biogenic sulfur emissions at 15 to 33 Tg (S).yr^{-1} which leave the oceans to the atmosphere. Presently, its effect on the climate change and global warming are only suggested but are not proven (Bopp et al., 2004). Indeed, DMS is though to affect the Earth's radiation balance by forming cloud condensation nuclei (CCN ; Charlson et al., 1987). Moreover, sulfur compounds like thiols and H_2S may complex various soft sphere and transition metals (Sunda and Huntsman, 1998 ; Al-Farawati et al., 1999 ; Bell and Kramer, 1999 ; Smith et al., 2002 ; Sukola et al., 2005).

Even if the common characteristic is a sulfur atom with valence less than zero, these sulfur compounds show different properties (e.g., production, consumption mechanisms…).

In anoxic marine environments like marine sediments or in a water column with restricted ventilation, dissolved hydrogen sulfide is produced by bacterial sulfate reduction which remineralizes organic matter. In such anoxic environments and sediments, the sulfide concentration can be in micromolar levels, whereas in oxic areas (e.g., open oceans), H_2S always occurs at lower levels (ca. few nanomolar). In the open ocean, the first identified source of H_2S appears to be the hydrolysis of carbonyl sulfide (Elliot et al., 1989 ; Watts, 2000). Phytoplankton was also a source of hydrogen sulfide because it is able to produce directly H_2S when it is submitted to an environmental stress (Walsh et al., 1994). Hydrogen sulfide is also a significant compound of the marine sulfur budget (Andreae, 1990) with average coastal concentrations about 0.4 to 2.5 nM (Table 1).

Carbonyl sulfide is the most abundant and probably the most long-lived sulfur gas in the atmosphere (Ulshöfer and Andreae, 1998). Dissolved OCS is produced by a number of processes : photochemical degradation of dissolved organo-sulfur

compounds (Zepp and Andreae, 1994), non-photochemical production from dissolved organo-sulfur compounds (e.g., methane thiol degradation ; Ulshöfer et al., 1996) or diffusion out of sediments (Flock and Andreae, 1996). Its concentration decreases due to hydrolysis of dissolved OCS (Johnson and Harrison, 1986) and air-sea exchange (Ulshöfer et al., 1996). The concentration of OCS in the surface waters of the open ocean averages 0.03 nM (Johnson and Harrison, 1986) whereas coastal concentrations range from 0.07 nM (Rasmussen et al., 1992) to 1.2 nM in a eutrophic estuary (Jorgensen and Okholm-Hansen, 1985).

In term of sea-air exchange, dimethyl sulfide (DMS) is thought to be the major sulfur gas released from the oceans (Andreae, 1990). It is converted by enzymatic cleavage, from dimethylsulfoniopropionate (DMSP), a regulator of the internal osmotic pressure produced by phytoplankton. DMSP is one of the most abundant forms of reduced sulfur found in the euphotic zone of oceans, with concentrations (dissolved plus particulate forms) ranging from 5 to 50 nM. During blooms of DMSP-phytoplanktonic producers, the concentration can be higher than 100 nM (Malin et al., 1993). This molecule is released during phytoplanktonic grazing by zooplankton, phytoplanktonic viruses infection and phytoplanktonic cells senescence. In marine waters, it is converted to DMS by enzymatic cleavage (Keller et al., 1989 ; Simo et al., 2002). In marine environments, DMS concentration range is between 0.4 and 16 nM (Turner et al., 1988 ; Moret et al., 2000 ; Amouroux et al., 2002 ; Andreae et al., 2003).

Studies suggest that only a relatively small portion (<30%) of DMSP degradation is converted to DMS (Belviso et al., 1990 ; Kiene, 1992). Thus, the major part of DMSP is demethylated and further degraded to methane thiol (Kiene and Taylor, 1988). Moreover, the methane thiol is also produced from DMS (Kiene et al. 2002). Another sulfur compound, dimethyl disulfide, is synthesized from the DMSP (Tanzer and Heumann, 1992) but it also results from the oxidative dimerization of the methane thiol by polysulfides (Gun et al., 2000). In this paper, we collectively call these five compounds VRSC for Volatile Reduced Sulfur Compounds according to their common properties of volatilisation and oxidation.

We examined these VRSC in a coastal environment because of the complex relationships between these sulfur species. Also, the proximity of the sediment-water interface (SWI), a significant zone of enhanced organic matter (OM) degradation, may be a source of these compounds. The SWI is the locus where enhanced chemical and microbiological transformations are responsible for cycling biogenic constituents

between water and sediments (Ni et al., 2002 ; Viollier et al., 2003). Given that coastal sediments are only 10% of the total oceanic surface yet account for 80% of marine OM remineralization (Wollast, 1991), this is pertinent on the global scale. Although reduced sulfur compounds and principally H_2S, have been studied in porewaters (Jorgensen, 1977 ; Klump and Martens, 1989), their distribution at the cm to dm scale above the SWI and into the bottom water column is yet unknown in the nearshore environments. Fuelled by OM supply, bacteria activity there causes chemical interactions between water column and sediments (Luther et al., 1997 ; Anschutz et al., 2000). Thus, the SWI which plays a significant role on the distribution of chemical compounds (e.g., sulfur compounds) in the sediment, may also influence the water column just above it.

Over a 26 month-sampling period of the 2-m water column above the SWI, we describe the seasonal and interannual variations of VRSC concentrations and test two hypotheses. Firstly, does the SWI play a role in the temporal sulfur species distribution in the 2-m water column above the seabed ? The second hypothesis to check is the following ; what are the effects of the phytoplankton density variations on the distribution of VRSC ?, and does phytoplankton distribution clearly modify the SWI properties ?

Our purpose was to survey these five volatile reduced sulfur compounds during several months (i.e., from July 2004 to August 2006) in a temperate coastal marine environment, the Bay of Quiberon and to test the previous hypotheses. A part of the originality of this work was to study simultaneously H_2S, OCS, MeSH, DMS and DMDS.

2. Materials and Methods

2.1. The Sampling area : the Bay of Quiberon

The Bay of Quiberon was a semi-closed Bay in the south-west of Brittany (Morbihan) which opened onto the Bay of Biscay at 47°32N. The Western Bay of Quiberon covered an area of 150 km^2 with a 7-m average depth and was regularly exposed to waves and tidal action. Dominant winds were S-SW and N-NW onto an annual scale but between the end of winter and spring, they were NE or S. The swell was residual and came into the Bay with a 15 km-fetch. This coastal zone was also subject to tidal

currents whose maximum speed was between 0.1 (neap tide waters) and 0.2 (spring tide waters ; source : www.shom.fr) knot in the middle of the Bay (47°29N, 3°02W) while the spring tidal range was about 4.6 meters (source : www.shom.fr). The major part of sediments are sandy muds (63-80 µm ; Lemoine, 1989, unpubl.). The water was saturated with oxygen throughout the entire water column, there was no anoxia.

Sampling was conducted over an 24-month period (n=11) except for the winter months because of adverse weather conditions. The monitored station (Men Er Roué, 47°32N, 3°05W) was considered the best representative zone of the whole Bay of Quiberon. The station was near the Bay mouth and had a depth about 7.5 meters with sandy muds sediments (Figure 1).

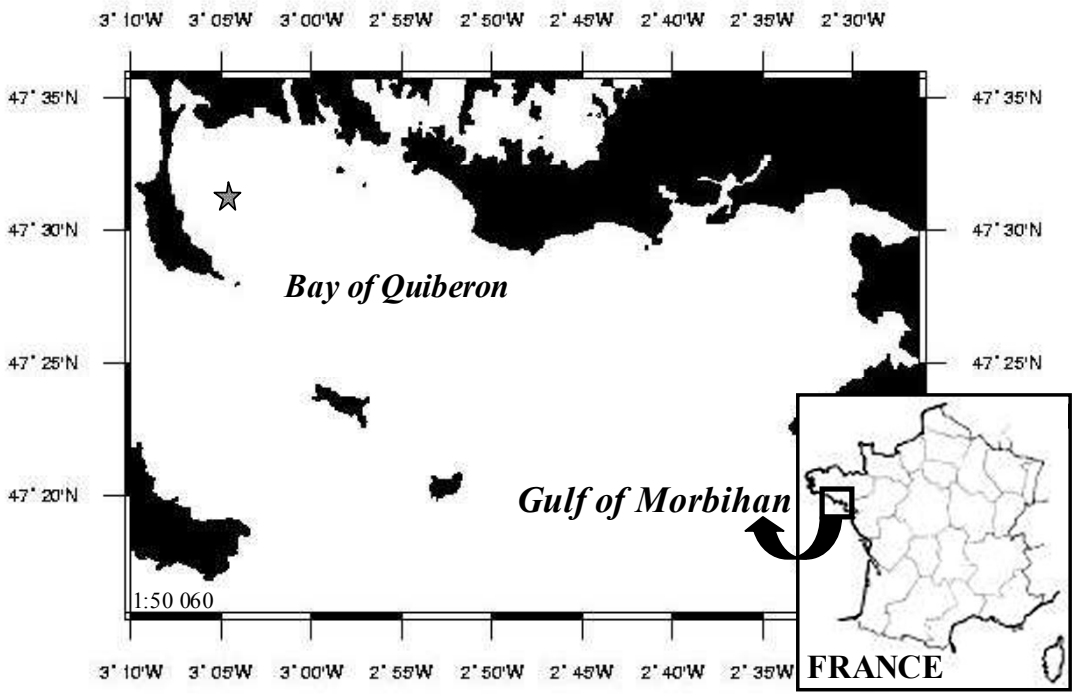

Figure 1 - Site of study, the Bay of Quiberon and the Men Er Roué station (located by the grey star).

2.2. Ancillary parameters

The phytoplankton density for our sampling period was monitored weekly through the REPHY (i.e., French network to survey the phytoplankton and phycotoxins densities on coastal environments). Assessments were carried out as follows ; one liter of water was taken from the sea surface at Men Er Roué and immediately, an acid lugol (i.e., 2 to 10 ml/L according to the phytoplankton density) solution is added to fix the algal

cells. Less than 36 hours later, the cell density of each phytoplanktonic species was determined using a Malassez cell and an inverted microscope.
Hydrographic parameters were also monitored from June 2004 to August 2006. Temperature and salinity were measured continuously (i.e., Micrel probe) at 50-cm immersion. Turbidity was daily *in situ* measured with a specific probe (WTW Turb 550IR). Precipitations (mm per month) and insolation (hours per month) were also available for the whole sampling period (www.meteofrance.com). Unfortunately, data concerning the wind strength were unavailable in the Bay of Quiberon for the May'04 to August'06 period.

2.3. Sampling, conservation and analyses

The epibenthic sampler, Susane (Radford-Knoery et al., 2007) was used to acquire water samples in order to reveal concentration gradients in the water column above the SWI. Briefly, it is a simple, lightweight and relatively inexpensive syringe sampler with fine scale and high vertical resolution. The Susane was put down on sediments by a scuba diver, and using a vertical rod, up to sixteen samples could be simultaneously collected at altitudes ranging from 1 cm to 200 cm above the seabed. For proper water column sampling, collection syringes thoroughly cleaned. To minimize sample degradation and for example, the production of OCS via photolysis (Ferek and Andreae, 1983), subsampling into transfer syringes was done as quickly as possible (less than five minutes) on the deck of a small boat. Water samples were refrigerated in the dark until analysis and less than 2 hours to prevent a possible DMSP degradation (Jean et al., 2004). The analytical technique used to determine the VRSC concentrations in the water column samples was a purge and trap extraction, followed by gas chromatography separation and pulsed flame photometric detection. The detection limit of this method was 0.07 nM for H_2S, 0.03 nM for OCS, 0.01 nM for MeSH, 0.1 nM for DMS and 0.03 nM for DMDS. Precision values were 6.0% for H_2S, 4.1% for OCS, 5.6% for MeSH, 4.9% for DMS and 8.4% for DMDS (Cozic et al., 2007).

One 30 cm-sediment core was taken, near the location where the water samples were collected, in order to analyse hydrogen sulfide concentrations in the top 10-cm of porewaters. To avoid degradation, sediment cores were also refrigerated in the dark (i.e., icebox) until analysis (i.e., < 2h). Extraction of H_2S from the porewaters was

based on the use of rhizons (Seeberg-Elverfeldt et al., 2005) connected to syringes and a colorimetric analysis (i.e., methylene blue method). Its detection limit was about 0.32 µM for hydrogen sulfide.

For each sampling day, height water altitudes above the SWI were generally sampled from the seabed to 200 cm above it. For some sampling days, more water heights were sampled (e.g., 15 water heights in June 2006). The sampling step was smaller within the 10 first centimeters above the SWI because we wanted to show preferentially the VRSC concentrations variations close to the SWI. In the upper column (i.e., above +70 cm), the sampling resolution was smaller because the water column was expected to be more homogeneous (Lemoine, 1989).

3. Results

3.1. Hydrography

Temperature, salinity turbidity, precipitations and insolation results for the whole study period are detailed in Figure 2. The seawater temperature reached 5-6°C during winter. From March on, it increased progressively to reach a maximum value in August (ca. 20°C). Interannual variations were not significant between the three summers sampled. The summer mean temperatures were 17.6°C ± 1.6 (n=7974) in 2004, 17.6°C ± 0.9 (n=8835) in 2005 and 16.6°C ± 0.4 (n=721) in 2006 (Figure 2A).

The salinity was relatively constant over the 26-months sampling. The summer mean salinity was 35 ± 0.9 (n=7974) in 2004, 34.9 ± 0.7 (n=8835) in 2005 and 33.9 ± 0.2 (n=721) in 2006 (Figure 2A). Rapid and short-time (i.e., less than one day) decreasing events of the salinity occurred during spring and summer 2005 during which the salinity could decrease to 20 (e.g., 23th of September). The opposite happened the previous winter when the salinity increased to > 36 (e.g., 4th of February ; Figure 2A).

The turbidity showed seasonal variation with higher values during the winter. Between October and March, the mean value of turbidity was 12.6 ± 1.9 mg/L (n=15) in 2004 and 12.5 ± 2.2 (n=10) in 2005 (Figure 2A) whereas in spring and summer, the mean value was 10.5 ± 0.6 mg/L (n=24) in 2004, 10.6 ± 0.6 mg/L (n=24) in 2005 and 10.3 ± 0.4 mg/L (n=121) in 2006.

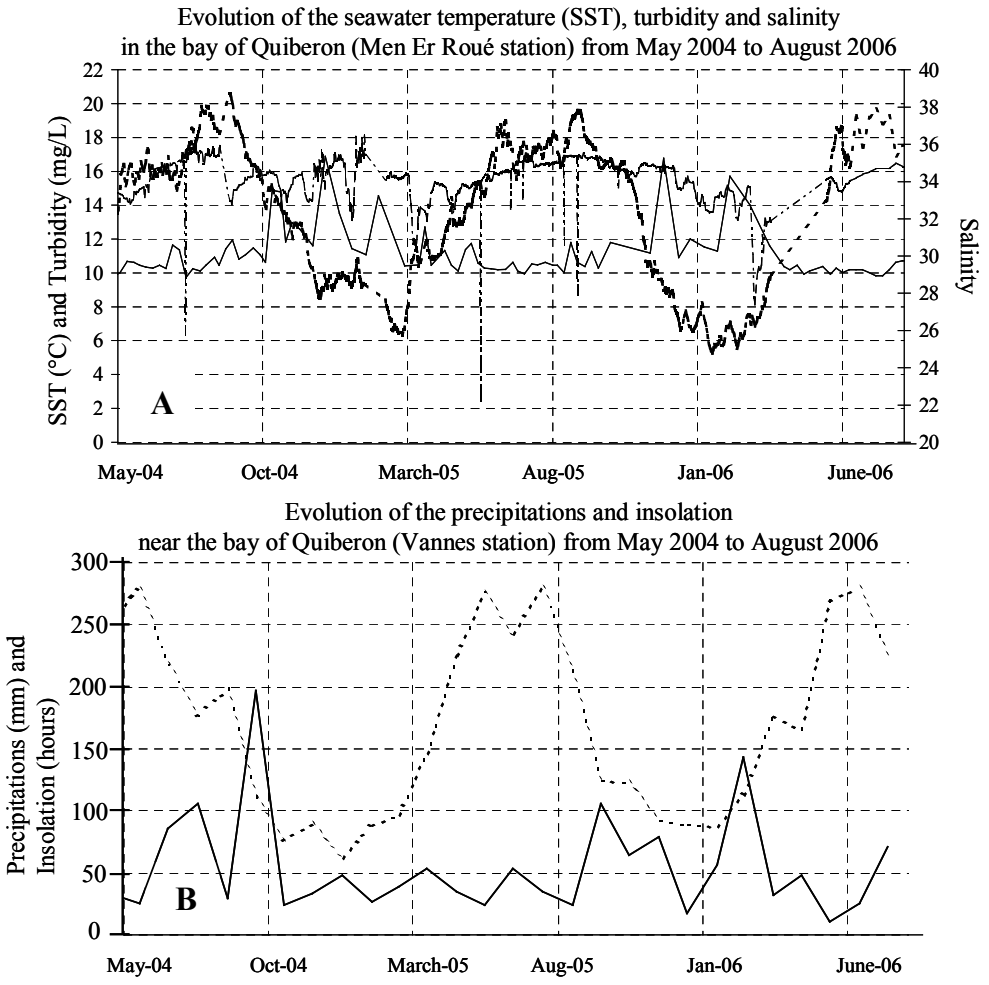

Figure 2 –Evolution of hydrographical parameters in the Bay of Quiberon from May 2004 to August 2006 ; A - Temperature (regular dotted line), Turbidity (filled line), Salinity (irregular dotted line) ; B – Precipitations (filled line) and Insolation (regular dotted line).

Precipitations showed seasonal variations with an increase in autumn period (Figure 2B). For example, in 2004 and 2005, precipitations were, respectively 29 mm and 24 mm in September and 197 mm and 105.4 mm in October. However, from November 2004 to September 2005, the precipitations were relatively constant with 36.1 ± 11.4 mm (n=11).

The insolation occurred clear variations through the year with a consistent increase from the winter to the summer period (Figure 2B). The mean summer value (June to August) was respectively 224.5 ± 51.5 (n=3) in 2004, 265.7 ± 20.3 (n=3) in 2005 and 258 ± 28.9 (n=3) in 2006. During the cold period (from October to February), the insolation decreased with 85.2 ± 18.4 (n=5) in 2004 and 103.4 ± 19.1 (n=5) in 2005.

3.2. Phytoplankton

In order to describe the role of phytoplankton species variability for the VRSC distribution, the variations of the two main algae families (i.e., Dinophyceae and Diatomae) were monitored for the sampling period. Dinophyceae and Diatomae accounted for more than 92% of the phytoplankton cells present at all seasons. Dinophyceae are different from the Diatomae because the former one known to synthesize significant amounts of DMSP (Holligan et al., 1987 ; Turner et al., 1988). As expected, the algal density varied seasonally and interannually (Figure 3). Two annual phytoplankton blooms were observed in 2004 (June and September), 2005 (March and May) and 2006 (May and July).

The sampling period was divided in several subperiods (Figure 3). The subperiods A, C and E corresponded to bloom events whereas the subperiods B and D corresponded to winter, on the basis of phytoplankton density. In order to clarify the description, the weekly survey of densities is presented by lines whereas the monthly survey (i.e., monthly mean value) is presented by shaded areas (Figure 3).

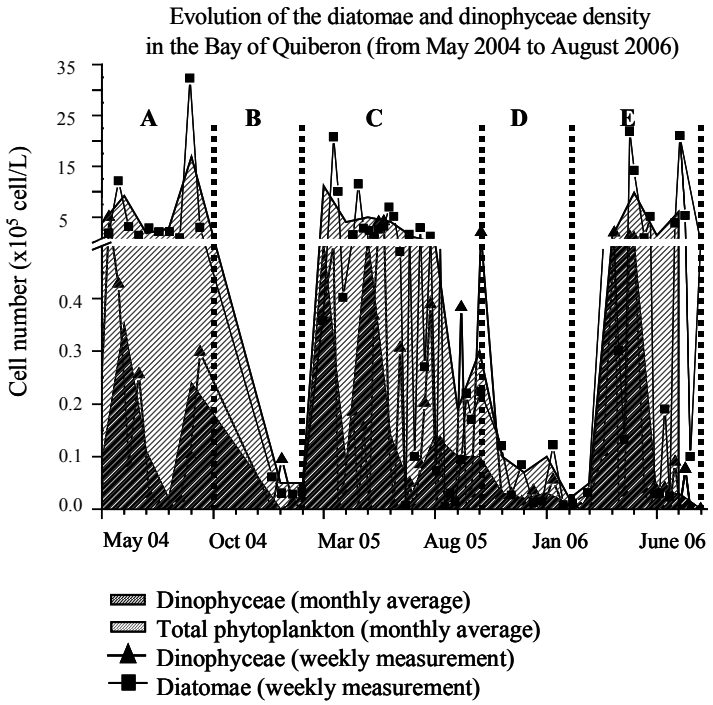

Figure 3 - Evolution of phytoplanktonic cells density from May 2004 to August 2006. (Total phytoplankton is the sum of Dinophyceae cells density and Diatomae cells density). The sampling period is divided in several subperiods (A to E).

3.2.1. Diatomae

More specifically, Diatomae distribution showed a seasonal feature (Figure 3) with higher algal density for the warm period (i.e., May to August). For example, from March to August 2005, the mean value of the Diatomae density was $(3.3 \pm 5.1)x10^5$ (n=23) cell.L^{-1} with a maximum ($20.7x10^5$ cell.L^{-1}) in the end of March. In the winter, the algal density decreased considerably to low values (e.g., $0.04x10^5$ cell.L^{-1} in 2005). Summer abundances were highly dymanic with large density swings ; the mean summer values of density were $(3.0 \pm 3.5)x10^5$ cell.L^{-1} (n=9) in 2004, $(2.4 \pm 2.9)x10^5$ cell.L^{-1} (n=18) in 2005 and $(5.2 \pm 7.9)x10^5$ cell.L^{-1} (n=14) in 2006. In 2004, the Diatomae density was maximum in the beginning of June ($12.1x10^5$ cell.L^{-1}) and September ($32.3x10^5$ cell.L^{-1}) whereas in 2006, it was maximum in May ($21.9x10^5$ cell.L^{-1}) and July ($21.0x10^5$ cell.L^{-1}). In 2005, after the maximum observed in march, the density decreased until $0.5x10^5$ cell.L^{-1} in the end of June. Later, it decreased again to reach only $0.01x10^5$ cell.L^{-1} in the beginning of September.

3.2.2. Dinophyceae

The Dinophyceae cells density was usually lower than that of the Diatomae but seasonal variations also occurred with high values during the summer period. During 2004, the Dinophyceae cells density showed the same features as the Diatomae density with two maxima, in the beginning of June ($0.4x10^5$ cell.L^{-1}) and in the end of September ($0.3x10^5$ cell.L^{-1}). In 2005, the cell density increased by a factor of fifteen between March and May reaching $4.0x10^5$ cell.L^{-1}. In 2006 only one maximum was noted in April with a monthly mean value of $(0.7 \pm 1.1)x10^5$ cell.L^{-1} (n=3). The summer period mean values (i.e., from June to August) were $(0.1 \pm 0.2)x10^5$ cell.L^{-1} (n=7) in 2004, $(0.4 \pm 1.1)x10^5$ cell.L^{-1} (n=13) in 2005 and $(0.03 \pm 0.03)x10^5$ cell.L^{-1} (n=10) in 2006. Moreover, at least two Dinophyceae blooms occurred per year with a time span between the blooms of 2 to 3 months ; June and September 2004, March and May 2005, June 2006 (no available data from September). During the winter period (i.e., from the end of September 2005 to the end of March 2006), the dinophyceae mean density was about $(0.2\pm 0.5)x10^5$ cell.L^{-1} (n=13).

3.2.3. Comparison with other phytoplancton data

In Bay of Quiberon, the Diatomae density was included in $0.01x10^5$ cell.L^{-1} and $16.6x10^5$ cell.L^{-1} and the Dinophyceae density was between zero and $0.9x10^5$ cell.L^{-1}. Jean et al. (2005) determined a Diatomae abundance below $0.01x10^5$ cell.L^{-1}

(maximum value in December) in the little Bay of Toulon (France) whereas zero to $0.07x10^5$ cell.L^{-1} of Dinophyceae were measured. In the English channel, higher abundance of Dinophyceae were occurred by Turner et al. (1988). Maximum cell number of *Gyrodinium aureolum* and *Ceratium lineatum* were respectively $40x10^5$ cell.L^{-1} and $1x10^5$ cell.L^{-1} in winter period. So, the phytoplankton abundances can occur significant variations according to the environment studied and the sampling period.

3.3. Suprabenthic distribution of VRSC

The VSRC profiles collected in the suprabenthic layer (i.e., from zero to ca. 200 cm above the SWI) are presented based on the subperiods described in Figure 3. Because sampling was not conducted during the winter, there were no water samples for the subperiod D and only one (1^{st} of February) for the subperiod B. But, within this important limitation, it was attempted to describe seasonal variations in VRSC concentrations (Figure 4).

Most of the H_2S profiles showed a clear trend of concentration increase close to the SWI. In addition, a layer exhibiting a relative concentration minimum was present at 30 to 60 cm above the SWI. With smoother concentration increase close to the bottom, OCS showed a nearly identical trend. MeSH profiles did not show evidence of concentration increase at the SWI but rather a zone of concentration maximum between 10 and 60 cm above the sediment-water interface.

The DMS profiles occurred a higher concentration in the top of water column sampled (i.e., beyond one meter above sediments). A feature of concentration increase into the first centimeters above the SWI was apparent for DMS in some summer profiles. In addition, some DMS profiles also showed a minimum concentration at 30 or 50 cm above sediments.

DMDS, like DMS, showed a higher concentration above 1-m altitude and did not occur clear vertical variations except in some summer profiles (subperiods C and E).

Weighted average were calculated to describe the seasonal and interannual variations of VRSC concentrations in order to obtain values more accurate in the 2-m water column monitored. For that, 10-cm heigth intervals were created and their weighted average were calculated. But, to highlight the vertical variations of VRSC concentration near the SWI, Figure 4 gives the values measured in the 2-m water column and no the mean values.

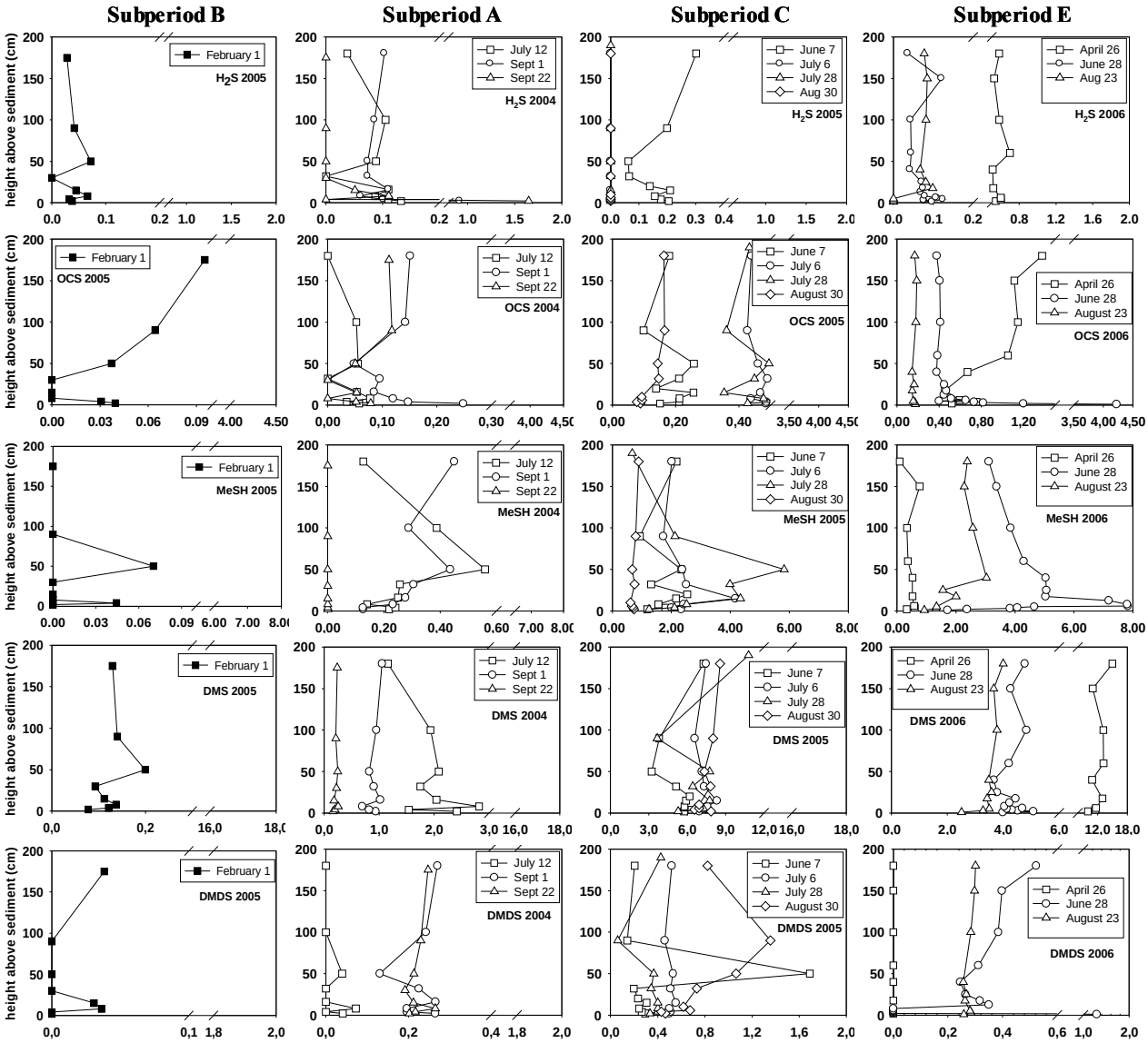

Figure 4 - Evolution of the VRSC concentrations in the Bay of Quiberon (Men Er Roué station) from July 2004 to August 2006. All concentrations are given in nM.

3.3.1. Seasonal variations of VRSC concentrations

The hydrogen sulfide concentration was maximum at the beginning of spring (April 2006, subperiod E) with about 0.57±0.06 nM (n=8, depth-weighted average over the entire profile) and was generally higher during the summer (ca. 0.10 nM except in 2005). Moreover, vertical variations in the 2 m-water column were greater for the spring (e.g., June 2005) and beginning of summer. From the end of summer into winter, H_2S concentration started to decrease to below the detection limit (0.07 nM, Cozic et al., 2007) above +30 cm (22th of September 2004). In winter, (February 2005, subperiod B), it was less than the summer period and never greater than to 0.07 nM.

Carbonyl sulfide showed a stronger vertical concentration gradient than H_2S for the summer periods, and exhibited larger variations in the 50-cm layer above the SWI. During the early summer period (e.g., subperiod C), the maximum concentration was often observed at 30-50 cm altitude which varied through the summer. The maximum was at +50 cm (0.26 nM) in June, at +32 cm (0.49 nM) at the beginning of July and at +50 cm (0.49 nM) at the end of July. At the end of summer (i.e., August), the concentration increased from 4 cm to reach 0.17 nM at 180 cm. In addition, its summer concentration (e.g., subperiod E) was from twice to twenty times (e.g., 4.20 nM observed in June 2006) greater than observed during the winter (subperiod B). The OCS concentration (i.e., weighted average) decreased progressively during the winter. At the end of subperiod A (i.e., 22th of September), there is 0.03 nM OCS below +50 cm and 0.10 nM OCS above +50 cm. For the subperiod B, OCS was below the detection limit (0.03 nM ; Cozic et al., 2007) between 8 and 30 cm whereas the mean concentration was 0.04 nM in the 0-8 cm altitude layer and 0.06 nM above 30 cm.

The MeSH concentrations showed significant seasonal variations with summer values ten times greater than winter values (e.g., subperiods B and C) and also clear variations in the 50-cm layer above the SWI. It was undetectable levels in the winter (subperiod B) except at +4 cm (0.04 nM) and at +50 cm (0.07 nM). The MeSH concentration increased during the summer period (e.g., subperiod C) and maximum values occurred between +10 and +50 cm above sediments. At the end of summer 2005, there was five times less MeSH (0.78 nM, weighted average over the entire profile) than in June and the vertical variations were less important. During the last warm sampled (subperiod E), MeSH concentration began to increase (0.47 nM, weighted average on the entire profile) to reach until 4.15 nM in the end of June.

Dimethyl sulfide concentrations were uniform (summer 2006) in the 2 m-water column sampled, or very variable (summer 2005). Concentrations were lowest in the winter (subperiod B) with 0.14 nM (weighted average over the entire profile) and increased substantially during the summer period, like MeSH, to reach several nanomolar (1.82 nM in July 2004, 7.90 nM in August 2005 and 4.34 nM in June 2006). The highest concentration was measured in spring (April 2006) with about 12.44 nM (weighted average over the entire profile).

For DMDS, no clear repeated, vertical variations were apparent in winter and spring. There was only in June and August 2005 (subperiod D) and in June 2006 (subperiod E) where a clear maximum value was observed. It was respectively 1.69 nM (at +50 cm), 1.36 nM (+90 cm) and 1.29 nM (+1cm). Thus, like for DMS a global increase in

concentration was noted from the spring to the end of summer. For an example, in winter 2005 (subperiod B), the DMDS concentration (weighted average over the entire profile) was 0.04 nM, and yet in summer it reached 1.02 nM (August, subperiod C).

3.3.2. Interannual variations of VRSC concentrations

During the 3 summer-sampling campaign, interannual variations were obtained for each sulfur gas. It was interesting to highlight similarities between the summers but also variability which could be linked to those of other parameters.

No clear interannual sulfide variations (i.e., statistical tests occurred, $p<0.05$) were apparent through the 3-summers survey with 0.07 nM in 2004, undetectable levels in 2005 (except in June) and 0.07 nM in 2006 (weighted averages over the entire profile). The summer hydrogen sulfide concentration was often more elevated near the SWI than in the upper water samples. This feature was apparent for summer 2004, June 2005 and summer 2006 with significant variations of the sulfide concentration in the 20-cm layer above the SWI. The maximum values usually occurred near sediments and they were followed by a rapid decrease, itself followed by another increase. The best example of this behaviour is observed in June 2005 with a minimum value (0.06 nM) detected from +32 to +50 cm. The opposite trend was recorded in winter (subperiod B) and spring (April, subperiod E) with a maximum value between two minimum zones in the 20-cm layer above the SWI.

No interannual variations (i.e., statistical tests occurred, $p<0.05$) were apparent in the concentration of OCS between the summers of 2005 and 2006 whose the carbonyl sulfide concentrations were respectively, 0.34 nM and 0.31 nM (depth-weighted average over the entire profile). In the summer of 2004, the OCS concentration was lower with 0.08 nM. During the summer period, the OCS concentration varied with an increase from the beginning of summer (e.g., subperiod A) reaching in the middle of summer (e.g. subperiod C) a maximum which was followed by a decrease. In the 2-m water column sampled, clear variations were observed only in the 50 cm immediately above the while sediments ; in the top of water column sampled, OCS concentrations were relatively constant. Some profiles (e.g., 1st of September 2004, July 2005, June 2006) showed an increase in OCS concentration just above the SWI and a minimum value near ca. +15 cm. The July 2004 and June 2005 profiles showed another trend in the 2-m water column sampled. From the SWI, the OCS concentration increased until ca. +10 cm and then, it decreased rapidly until a given altitude (+32 cm in 2004 and +20 cm in 2005) before to increase again.

Three different features were also observed for methane thiol concentration during the 3 summers surveyed. Interannual summer variations were detected ; 0.34 nM (non-detectable levels in the end of September) in 2004, 1.91 nM in 2005 and 3.28 nM in 2006 (weighted averages over the entire profile). Moreover, all summer profiles showed a sharp concentration decrease immediately above the SWI, overlain by a clear maximum at +50 cm (0.56 nM) in July 2004, +15 cm (4.17 nM) in July 2005 and +8 cm (7.83 nM) in June 2006. Out of the summer period, MeSH concentrations slightly varied in the 2-m water column sampled (except in June 2005). Concerning the evolution of MeSH concentration during summer, profiles were consistent with an increase until the middle of summer followed by a decrease to winter values.

Dimethyl sulfide profiles occurred less depth variations than all VRSC but clear interannual summer variations with 0.99 nM in 2004, 7.23 nM in 2005 and 3.99 nM in 2006 (weighted averages over the entire profile). During the early summer, it was about nine times more concentrated (1.82 nM) at the beginning of summer than in September (0.23 nM). Next year (subperiod C), DMS concentration showed two increase periods ; one from June (4.96 nM) to the beginning of July (7.14 nM) and another from the end of July (6.65 nM) to August (7.90 nM). In summer 2006 (subperiod E), DMS concentration did not vary so much between June (4.34 nM) and August (3.65 nM). The maximum DMS concentrations were often observed above +100 cm and sometimes, higher DMS concentrations were also recorded near the SWI. For example, in summer 2005 (e.g., 28th of July), the DMS concentration was 7.09 nM (weighted average over the entire profile) in the 50-cm layer above the SWI, 3.65 nM (minimum value) at +90 cm and 10.80 nM (maximum value) at +190 cm.

Dimethyl disulfide also showed interannual variations with summer concentrations of 0.15 nM in 2004, 0.56 nM and 0.32 nm in 2006 (weighted average over the entire profile). For most of the profiles, the vertical DMDS distribution was uniform in the 2-m water column (e.g., July 2004, August 2006). But for some profiles, clear variations occurred. It was the case in the beginning of September 2004 (subperiod A) with a minimum (0.13 nM) at +50 cm whereas the DMDS concentration was ca. 0.24±0.03 nM (n=11) over the entire profile. It was important to note there also existed variations of DMDS concentration during a given summer with no clear trend for the date of the maximum.

3.4. Porewater sulfide concentration

In order to ascertain the presence of a permanent oxic sediment layer in this eutrophic Bay and negate the possibility of a seasonal sulfide sedimentary source, we examined porewater H_2S concentration in 30-cm long cores collected at the same time as the water column depth profiles. For this report, we presented only the evolution of the H_2S concentration near the SWI and in a ca. 3-cm layer of sediments (Figure 5).

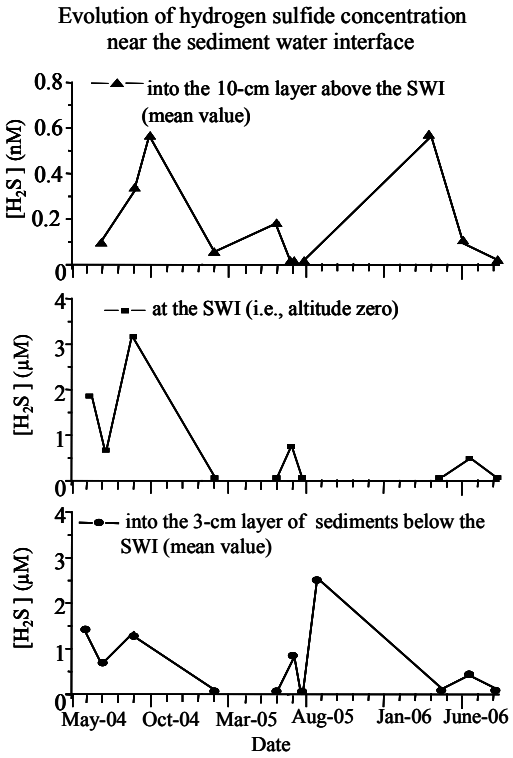

Figure 5 - Evolution of hydrogen sulfide concentration near the sediment water interface. The detection limit is 0.07 nM for the chromatographic method and 0.32 µM for the colorimetric method. The precision of these methods are respectively 10% and 6%.

Near the SWI (altitude zero), the sulfide concentration was often less than one micromolar but, given the analytical detection limit of 0.32 µM for the colorimetric method that was used, only few results were significantly different from non-detectable levels. Hydrogen sulfide concentrations were undetectable (<0.32 µM) in February, June and at the end of July 2005 and very low in spring and August 2006. The highest concentrations near the SWI, occurred at the beginning of summer ; 0.65

µM in July 2004, 0.73 µM in July 2005 and 0.46 µM in June 2006. The highest concentration (3.24 µM) at the SWI occurred in September 2004. Porewater sulfide concentrations showed relatively the same feature with higher values in summer and the highest in August 2005 (2.55 µM). Seasonal variations were greater than interannual variations with values measured in the upper 3-cm sediments remained two to three orders of magnitude greater than those recorded in the water column samples.

4. Discussion

There are many data available on the distribution of volatile reduced sulfur gases in the marine environment. Table 1 shows concentrations in different settings and highlights the increase of VRSC concentrations shoreward or in areas with increased productivity. The water column data we report here are consistent with the litterature data on dissolved H_2S, OCS, MeSH and DMS for comparable coastal environments (Table 1). Our 2-year sampling campaign gives the following concentration ranges ; up to 1.6 nM for H_2S, up to 4.2 nM for OCS, up to 7.8 nM for MeSH, from 0.1 to 17.5 nM for DMS and up to 1.7 nM for DMDS (Figure 4).

Table 1 - Comparison between seawater concentrations of H_2S, OCS, MeSH, DMS and DMDS and the values observed in the Bay of Quiberon.

Volatile Reduced Sulfur Compound	References	Bay of Quiberon Concentration range
Hydrogen Sulfide H_2S	0.4 – 2.5 nM Cutter and Krahforst 1988 ; Luther and Tsamakis 1989 ; Knoery and Cutter 1994	0 – 1.6 nM
Carbonyl Sulfide OCS	0.08 – 0.73 nM Mihalopoulos et al. 1992 ; Ulshöfer et al.1996 ; Cutter and Knoery 1993 ; Von Hobe et al. 2001 ; Cutter et al. 2004	0.02 – 4.2 nM
Methane Thiol MeSH	3 – 76 nM Lomans et al., 1997	0 – 7.8 nM
Dimethyl Sulfide DMS	0.4 – 16 nM Turner et al. 1988 ; Moret et al. 2000 ; Amouroux et al. 2002 ; Andreae et al. 2003	0.1 – 17.5 nM
Dimethyl Disulfide DMDS	>0.15 nM Tanzer and Heumann, 1992	0 – 1.7 nM

To simplify the discussion of the VRSC concentration evolution, the different sulfur species were placed in two groups. H_2S and OCS are studied together because they are directly issued from compounds as sulfate or dissolved organo-sulfur compounds (Dyrssen, 1985 ; Elliot et al., 1989 ; Zepp and Andreae, 1994). Methane thiol, DMS and DMDS, on the other hand, have all the same origin, DMSP (Kiene and Taylor, 1988 ; Dacey et al., 1998). The first hypothesis was whether the SWI plays an important role on the VRSC distribution in the bottom water column. The second hypothesis was the following ; does the phytoplankton distribution in the water column influence the VRSC distribution ?

4.1. Hydrogen sulfide (H_2S) and carbonyl sulfide (OCS)

For several vertical profiles, H_2S and OCS show the same global trend, a increase of concentrations toward the seabed which suggests a higher production near sediments than in the upper water column sampled (Figure 4, subperiod A). Moreover, H_2S was sometimes measured in porewaters near the SWI (Figure 5). Therefore, water column sulfide could have a sedimentary origin or it could be produced in the bottom water column. No diffusive gradients were calculated on account of the lack of data. But, for the following years sampled, a temporal decoupling (e.g., summer 2005) appears to exist between H_2S presence in the water column and sediments suggesting that the sediment was not essential as a source for the H_2S. By default, sulfide may likely have originated from the water column itself.

The lower water column could be favourable to H_2S production because of special conditions. Alldredge et al. (1998) showed that the phytoplanktonic cells are present in marine snow which is exported to the seafloor. These marine snow aggregates are enriched in microbial communities taking important part in phytoplankton degradation (Alldredge, 2000). Therefore, the important phytoplankton sedimentation (i.e., degradation of organic matter) may create anoxic microzones near the seabed and thus, sulfide may be produced in the first centimeters above sediments and remain undetected either by sediment porewaters studies or water column studies conducted using pumps or large bottles. We also note that the maximum H_2S concentration in the 10-cm layer above the SWI (Figure 5 ; September 2004, June 2005 and April 2006) occurred during blooms (Figure 3 ; subperiods A, C and E). This relation between the high density of phytoplankton cells and high sulfide levels encountered near the SWI, is consistent with high organic matter flux (i.e., post bloom event) and subsequent

rapid degradation releasing directly or inducing anoxic microzones where sulfate reduction may occur.

The hypothesis that direct release from phytoplankton cells is responsible for the higher H_2S concentration near sediments, can be evaluated as follows. Wollast et al. (1993) showed that the elemental composition of the particulate organic matter (POM) is $C_{106}H_{263}O_{110}N_{16}S_{1.7}P_1$. Thus, the sulfur content in POM (e.g., phytoplanktonic cell) is not negligible (i.e., 0.34% S). Considering a spherical phytoplanktonic (from $1.4x10^{-5}$ to $3.4x10^{-5}$ µl with a ratio of 0.1 between the dry weight and the fresh weight), we obtained a phytoplanktonic sulfur concentration between 6.2 $x10^2$ and $2.5x10^2$ nM during September 2004 (i.e., bloom with $16.9x10^5$ cell.L^{-1}). Therefore, the concentration of reduced, intracellular phytoplanktonic sulfur determined in the water column is one order of magnitude greater than the hydrogen sulfide concentration into the 10-cm layer above the SWI (0.24 nM ; Figure 5). Thus, a modest turnover of phytoplankton cells may become a significant source of reduced sulfur and its decay in the water column could contribute to the increasing of sulfide concentration near the SWI.

Cutter and Knoery (1993) studied OCS concentrations surface waters on the shelf of the western North Atlantic. They showed that porewaters are 200 times enriched compared to the water column. Thus, OCS produced in marine sediments, may diffuse through the SWI. Cutter and Zhang (1997) studied the OCS sediment-water fluxes in the Chesapeake Bay during 3 years. They showed highest values during the summer periods because the sedimentary OCS production (i.e., dark production) was coupled to a higher rate of microbial sulfate reduction, more important for summer. In the 3-years sampling in the Bay of Quiberon, the OCS concentration was twice as elevated near the SWI as in the shallow water column for every summer period (Figure 4). However, the opposite trend (i.e., an increasing with the altitude) was recorded for the winter period, spring and sometimes at the end of summer (Figure 4). For example, the 1st of February 2005, OCS concentration is 0.01 nM (weighted average) into the 50-cm layer above the SWI and 0.07 nM above +50 cm.

The principal source of OCS in oceans is photochemical production from chromophoric dissolved organic matter (CDOM ; Ferek and Andreae, 1984 ; Kettle et al., 2001). The magnitude of the photoproduction is related to the irradiance, seawater absorption and CDOM content (Ulshöfer and Andreae, 1998). In the euphotic zone, the DOM concentration is often correlated with the phytoplanktonic cells density.

Ferek and Andreae (1984) highlighted the photochemical production by a daily survey (springtime) of OCS concentration in surface seawater (e.g., Chesapeake Bay). Peaks of the OCS concentration were always observed during mid to late afternoon when the insolation was maximal. Mihaloupoulos et al. (1992) showed a positive correlation between the monthly average oceanic OCS concentration and the monthly average of the daily insolation period. Therefore, the higher OCS concentration analysed in Bay of Quiberon during the warm periods may be explained by a higher sun insolation (Figure 2) and an increase of phytoplanktonic cells density (Uher and Andreae, 1997).
So, sediments appear to be a source of OCS or neutral, given the shape of the water column gradient. During winter (Figure 4, subperiod B), photolytic production was the major source of OCS in the water column (Cutter and Zhang, 1997) whereas sediments were neutral. On the contrary, in summer (Figure 4, subperiods A, C and E), sediments appeared to be a OCS source (Kettle et al., 2001) which it explains the highest concentration observed near the SWI.

4.2. Dimethyl sulfide (DMS), methane thiol (MeSH) and dimethyl disulfide (DMDS)

DMS, MeSH and DMDS are produced directly or indirectly by bacterial degradation of DMSP (Kiene and Taylor, 1988 ; Tanzer and Heumann, 1992 ; Simo et al., 2002) and a significant production is confined to few classes of marine phytoplankton, mainly the dinophyceae (Keller et al., 1989). Therefore, a strong correlation may exist between the taxonomic position of the phytoplankton and the abundance of these VRSC in the Bay of Quiberon.
The dinophyceae cell density showed seasonal and annual variations with blooms (i.e., generally two per year) during the warm period (Figure 3 ; subperiods A, C and E). For winter (subperiods B and D), the dinophyceae concentration was much lower (on the order of 0.01×10^5 cell.L^{-1}) than during the summer periods. The higher concentrations of DMS, MeSH and DMDS were always recorded during the subperiods A, C and E (Figure 4) but various features existed for each of these VRSC during each summer monitored. Dinophyceae blooms were recorded in May 2005 (0.91×10^5 cell.L^{-1}) and April 2006 (0.67×10^5 cell.L^{-1}), two months before the highest summer MeSH concentrations which occurred at the end of July (2.76 nM, weighted average over the entire profile) in 2005 and in June 2006 (4.15 nM). A time span between Dinophyceae cells abundance and DMDS maximum occurred with higher values observed in the

beginning of July 2005 (0.50 nM) and in June 2006 (0.35 nM). Concerning DMS, a 2-month time span was only observed during the summer 2005 with highest concentration analysed in July with 7.14 nM and 6.65 nM at the beginning and end of this month. In 2006, the maximum DMS concentration observed in April is contemporaneous with the dinophyceae bloom (Figure 3 ; subperiod E). Therefore, in 2006, the production of DMS is faster than the previous year and than the MeSH and DMDS productions. The DMS concentration is always higher than MeSH and DMDS concentrations even in winter (Figure 4). The absence of MeSH (except at +50 cm) and DMDS in winter 2005 (subperiod B) can be explained by a lower dinophyceae density $(0.02\pm 0.5)x10^{5}$ cell.L^{-1} (n=2). Indeed, there is as much DMDS (0.23 nM, weighted average over the entire profile) as DMS (0.23 nM) in the end of September but five months later, there is about ten times more DMS than DMDS (0.01 nM). This is consistent with an additional winter production of DMS whereas methane thiol and dimethyl disulfide may be only produced for the warm period.

So, there exists a correlation between the time series of phytoplankton density and the levels of MeSH, DMS and DMDS. The DMSP-producers and VRSC synthesizers abundance may explain the distribution of these VSRC in the 2-m water column sampled and near the SWI. Methane thiol and DMDS concentrations are twice to four times higher in the 50-cm layer above the SWI in summer, whereas the DMS profiles show this feature only in the middle of summer. An hypothesis is purposed to explain the different features observed for these VRSC, in the 2-m water column, through the summer period. This increase of VRSC concentrations near the SWI may be linked to more abundant decomposing fragments of dinophyceae cells (Sorensen, 1988). The DMS synthesis appears faster than those of MeSH and DMDS because in June (Figure 4, subperiod C), there is always more DMS in the upper water column than in the 50-cm layer above the SWI. The DMS may be produced by the decay of dinophyceae coming from the first bloom. These algae cells fall into the water column and are degraded near the SWI to give MeSH and DMDS in the beginning of summer. Along the summer period, the VRSC synthesis may continue into the 50-cm layer above the SWI. At the end of summer (i.e., August), the opposite trend (i.e., highest concentration above +50 cm) observed for the three VRSC, may indicate a moving towards the upper water column of the DMSP-producers. During, the spring period, no gradients are observed in the 2-m water column sampled and the concentrations are 0.47 nM for MeSH (weighed average over the entire profile), 12.44 nM for DMS and

non-detectable levels for DMDS. These absence of vertical gradients may be linked to the mixing of the water column (i.e., non-stratified water column) according to the possible strong winds affecting the Bay and the 7-m depth (Lemoine, 1989).

Considering MeSH concentrations in the 2-m water column more in detail, a maximum is measured at a given altitude ; it is +20 cm in the spring period (2.6 nM), +15 cm at the beginning of summer (4.2 nM) and +50 cm for the middle of summer (5.8 nM ; Figure 4, subperiod C). This same trend is also observed for DMDS at the end of summer 2005 with a maximum concentration at +90 cm (1.4 nM) whereas the MeSH concentration is constant over the entire profile. Lomans et al (1997) showed MeSH can be produced in sediments when hydrogen sulfide is present in significant quantity. For example, in July 2005, H_2S shows a concentration markedly above the detection limit near the SWI and in the 3-cm layer beneath it (respectively, 0.73 µM and 0.76 µM ; Figure 5) whereas no sulfide is analysed near the SWI in June. Thus, a sedimentary origin of MeSH may be possible in July 2005 but it not appear to exist in June. The opposite phenomena (i.e., a clear minimum concentration depth) is observed for DMS in June 2005 with 5.1 nM measured at +32 cm (Figure 4). The hypothesis advanced to explain this minimum DMS concentration layer is the following. The high concentration (5.7 nM) observed near the SWI (+2 cm) may be induced by the decay of the first dinophyceae bloom (March, subperiod C) and the higher concentration (7.3 nM) measured on the top of water column (+180 cm) may be linked to the second bloom (May). Concerning DMDS concentration, in September 2004 and June 2006, there is also a given altitude where it is minimum. Moreover, in June 2006, the altitude of the lowest DMDS concentration corresponds to the maximum of MeSH concentration.

These variations of VRSC concentrations onto the 2-m water column are very complex and a unequivocal link between these three sulfur compounds is not really established on the base of our data. We can just conclude there exist discrete altitudes where higher VRSC concentrations are more favoured and that these altitudes vary during the warm period. Decay of Dinophyceae cells in the 2 m above the seabed may exist at various altitudes according to the DMSP-producers abundance.

5. Conclusion

This 3-summer survey of the volatile reduced sulfur compounds concentrations in a marine coastal environment highlighted interactions between the water column, the sediments, the phytoplankton and the VRSC distribution. All these interactions are schematically represented in the Figure 6.

The very tight sampling in the first centimeters above sediments made it possible to demonstrate that the SWI can play a key role on the VRSC distribution. Concerning OCS, its seasonal concentration variations are linked to the balance between its sinks and sources. During winter, the major source of OCS appears to be the photolytic production from CDOM (vertically uniform in the water column), whereas in the summer, sediments appear to be the main OCS source which explains highest concentrations measured near the seabed. This sedimentary source had already been showed by Cutter and Zhang (1997).

The variations of MeSH, DMS and DMDS concentrations may be directly linked to the seasonal variations of Dinophyceae density because blooms increase the available organic matter to the DMSP-producers and so, the production of these biogenic sulfur compounds. The observations of a 2-month time span between Dinophyceae density and MeSH and DMDS maximum may be explained by a slower transformation of DMSP in these sulfur compounds in opposite to the DMS production which appears faster. The vertical variations of MeSH, DMS and DMDS concentrations may be linked to the spatial repartition of DMSP-producers in the 2-m water column.

Concerning the sulfide inventory that is greater near the SWI, it is likely linked to anoxic microzones from the decay of organic matter (e.g., phytoplanktonic cells). These zones may be found above and below the SWI and so, H_2S analyzed does not seem to have a consistent sedimentary origin. Another processes of sulfide could be the direct release by phytoplanktonic cells in the first meter above the SWI.

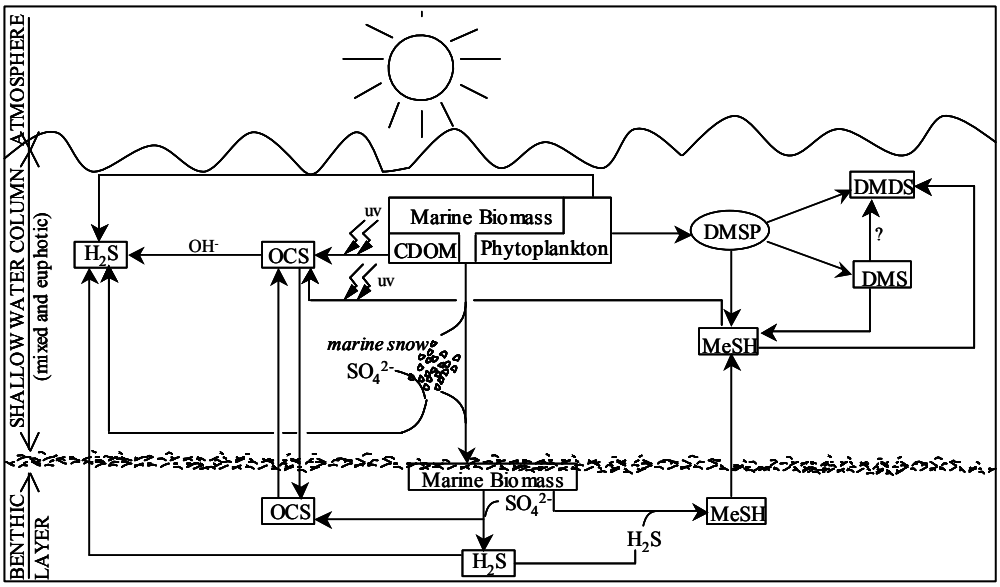

Figure 6 - Conceptual model illustrating the interactions between volatile reduced sulfur compounds, phytoplankton (e.g., dinophyceae) and sediment-water interface. This figure is based on the present knowledge on the VRSC production in the water column and sediment (i.e., Kiene and Taylor, 1988 ; Sorensen, 1988 ; Keller et al., 1989 ; Tanzer and Heumann, 1992 ; Radford-Knoery and Cutter, 1994 ; Cutter and Zhang, 1997 ; Lomans et al., 1997 ; Alldredge et al., 1998 and 2000 ; Kettle et al.,2001;Simo et al., 2002).

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Rôle des CSRV sur la spéciation métallique

Article 3 – Interactions entre les Composés Soufrés Réduits Volatils et les métaux en estuaire de la Seine (France).

Résumé

Pour la première fois, les concentrations de sulfure de dihydrogène (H_2S), sulfure de carbonyle (OCS), méthane thiol (MeSH), diméthyl sulfure (DMS) et diméthyl disulfure (DMDS) dissous en estuaire de la Seine (France) ont été mesurées. Plusieurs métaux dissous (Ag, Cd, Cu, Zn, Ni, Co, Pb) et divers paramètres ancillaires (e.g., matière en suspension, carbone organique particulaire) ont également été suivis. La campagne SILVER-2 s'est déroulée du 23 mai au 1er juin 2005 à bord du navire océanographique Thalia (Ifremer). Les échantillons d'eau ont été prélevés à l'aide d'une bouteille étanche et stérile, dans le premier mètre au-dessus de la surface et selon le gradient de salinité. D'importantes variations des concentrations en composés soufrés ont été observées dans l'estuaire et divers profils de distribution ont été déterminés pour les cinq composés soufrés réduits volatils (CSRV) suivis. Les teneurs maximales rencontrées sont très variables entre les espèces soufrées : 0.80 nM pour l'H_2S, 0.64 nM pour l'OCS, 3.06 nM pour le MeSH, 11.06 nM pour le DMS et 1.18 nM pour le DMDS.

Une étude expérimentale a également permis de déterminer les constantes de stabilité conditionnelle des CSRV vis-à-vis de l'argent (choisi comme "métal modèle"). Comme démontré dans la littérature, le sulfure de dihydrogène montre une forte affinité pour l'Ag (log $K'(Ag^+)$ H_2S>12) mais également pour divers métaux. Il est donc envisageable de considérer l'H_2S comme un important ligand métallique en estuaire de la Seine. Aussi, l'absence d'H_2S volatil dans la zone des basses salinités pourrait s'expliquer partiellement par de fortes interactions avec l'argent. En revanche, les autres composés soufrés (i.e., OCS, MeSH et DMS) montre des interactions avec l'argent non significatives avec log $K'(Ag^+)$OCS≈0, log $K'(Ag^+)$MeSH≈0 et log

$K'(Ag^{+})DMS \approx 0$. Par conséquent, ces CSRV ne peuvent être considérés comme des ligands métalliques en milieu marin.

Statut : en préparation, soumission prévue à "Estuaries"

Interaction between Volatile Reduced Sulfur Compounds and Metals in the Seine estuary (France).

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Abstract

The concentrations of volatile hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH), dimethyl sulfide (DMS) and dimethyl disulfide (DMDS) dissolved in the Seine estuary (France) were investigated for the first time. Several dissolved metals (Ag, Cd, Cu, Zn, Ni, Co, Pb) and supporting parameters (e.g., suspended particulate matter, particular organic carbon) were also monitored. A cruise (SILVER-2) was carried out from the 23rd of May to the 1st of June 2005 aboard the R/V Thalia (Ifremer). Water was collected from a depth of 1 meter beneath the sea surface and samples were distributed according to the salinity gradient. Large variations in the concentrations of the sulfur compounds were found in the estuary and different features were occurred for the five reduced volatile sulfur compounds (VSRC) studied. Maximal values measured were 0.80 nM for H_2S, 0.64 nM for OCS, 3.06 nM for MeSH, 11.06 nM for DMS and 1.18 nM for DMDS.

Further experiments were carried out to determine the conditional stability constants of VRSC to silver (chosen as a "model metal"). Strong complexes were found to exist between hydrogen sulfide and silver such it was already demonstrated by several authors. So, the data support H_2S may be an efficient ligand in the Seine estuary. The absence of volatile H_2S in the lowest salinities may be partially caused by strong interactions with silver. The other VSRC tested (i.e., OCS, MeSH and DMS) were found to no interact with silver according to their K′ values (i.e., log K′(Ag^+)OCS≈0, log K′(Ag^+)MeSH≈0 and log K′(Ag^+)DMS≈0). Therefore, these VRSC may not be significant ligands for soft sphere metals and transition metals.

Keywords : Seine estuary – Volatile reduced sulfur compounds – Metal – Sulfur silver complexation

1. Introduction

1.1. The Seine estuary : properties, characteristics and silver contamination

Estuarine systems, which depend largely on mixing between coastal marine waters and rivers as well as on internal processes (e.g., sedimentary, bacterial, chemical), are often complex (Cloern and Nichols, 1985 ; Nowicki and Oviat, 1990). Initially, studies considered the estuaries as separate entities with a high primary production due to an abundant organic matter supply which is rapidly transformed and possibly trapped (Nixon and Pilson, 1984 ; Jordan et al., 1991). Over time, with increasing awareness of coastal zone eutrophication, estuaries became considered as part of an interconnected system comprising the entire river network and the coastal zone (Garnier et al., 2001). As such, the estuarine ecosystem can play an important role in the characteristics of marine coastal environment.

The Seine river begins in Burgundy on the Langres plateau. Its upper part crosses agricultural and forested areas. Its has two main tributaries, the Marne and Oise rivers, which flow into the Seine in the greatly industrialized region of Paris. The Seine flows into La Manche at Le Havre, at the mouth of a 160-km long estuary (Figure 1). The upper limit of the Seine estuary is marked by the Poses Dam whose construction in 1860 permitted to control water levels and flooding. Over the last century, the Rouen and Le Havre regions have become heavily industrialised. With about 40% of french industry in its drainage bassin, the Seine receives inputs from the dense river network (e.g., waste treatment plants from urban areas) but also continental inputs from rainy periods.

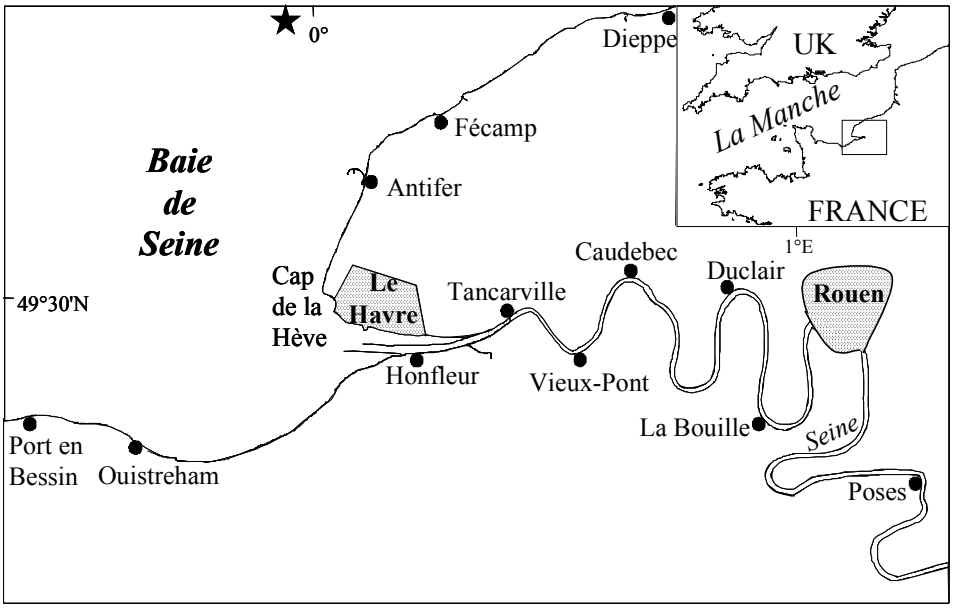

Figure 1 - Site of study, the Seine estuary and La Manche coasts. Marine stations are from Le Havre harbour (26-salinity) to off Dieppe (33-salinity). Estuarine stations are from Caudebec (0-salinity) to off Le Havre (30-salinity). The black star represents the sample station far away from urban inputs (33.5-salinity).

The national monitoring program (RNO) showed significant anthropogenic metal contamination of mussels along the Norman coasts with silver and cadmium concentrations several order of magnitude greater than lowest concentrations recorded in France at the same period (Chiffoleau et al., 2001 ; Chiffoleau et al., 2005). Furthermore, this metal contamination was also observed in molluscs from the bay of Seine (Miramand et al., 1998 ; Santini, 2004) and in *solea solea* (i.e., within liver) collected at the out of Seine estuary. According to the high toxicity of these metals, the observations pointed to silver and cadmium as major contaminants of Seine river (Chiffoleau et al., 2001 and 2005). The major sources of these metals to aquatic environments are the urban inputs related to the photography plants and medical imagery laboratories for silver and the phosphate fertilizer industry for cadmium (Chiffoleau et al., 2001 and 2005). Harbouring also about 30% of french population, the Seine basin is thought to be an important coastal source of contamination (Chiffoleau et al., 2001). With this knowledge, the Seine Aval program, which is a comprehensive research program, was launched to determine the possible origins of metals into the Seine river, to evaluate their fluxes into the marine environment, to measure their concentrations along the salinity gradient and to model their biogeochemistry in this estuary. Previous studies showed the remobilisation of

particulate silver and particulate cadmium in the mixing zone (i.e., freshwater to seawater ; Thouvenin et al., 2004) and so, the availability for the dissolved phase in salt waters of this particulate phase coming from freshwaters (Thouvenin et al., 2005). Other metals (Cu, Pb, Ni and Co), already observed by the RNO, were also monitored during the SILVER-2 cruise in order to determine their distribution along the salinity gradient in the Seine estuary.

In the aquatic environment, the distribution of a given metal is characterized by its concentration but also by its speciation. Indeed, the metal can be associated with various ligands which in turn, can modify the chemical state of metal. Sunda (1989) showed that the bioavalaibility of metal is directly linked to its speciation by ligands. Therefore, with the knowledge of the speciation of a given metal, it is possible to highlight the interactions between itself, the marine biota and suspended inorganic solid.

1.2. The Volatile Reduced Sulfur Compounds (VRSC) : origin, fate and properties

In aquatic environments, the biogeochemistry and distribution of volatile reduced sulfur compounds (VRSC) such as hydrogen sulfide (H_2S), carbonyl sulfide (OCS), methane thiol (MeSH, "CH_3SH") dimethyl sulfide (DMS, "CH_3SCH_3") and dimethyl disulfide (DMDS, "CH_3SSCH_3"), has received growing attention over the last decades (Turner and Liss, 1985 ; Cutter and Krahforst, 1988 ; Cutter and Radford Knoery, 1993). These sulfur chemical compounds are very reactive and they play a significant role for the atmospheric sulfur budget (Andreae, 1986) and could influence the global warming (Bopp et al., 2004).

Hydrogen sulfide is produced by anaerobic decomposition of organic matter via sulfate reduction (Dyrssen and Kremling, 1990). So, it is a constituent of anoxic marine environments like restricted ventilation water column or marine sediments with a concentration up to micromolar level. However, H_2S also exists in photic zones of oligotrophic to eutrophic waters (Luther and Tsamakis, 1989 ; Cutter and Krahforst, 1998). Walsh et al. (1994) also showed phytoplankton species can produce hydrogen sulfide in oxic waters. Nevertheless, an important source of this H_2S in the oceans appears to be the hydrolysis of carbonyl sulfide (Elliot et al., 1989 ; Watts, 2000). Moreover, the hydrogen sulfide is a significant compound of the marine sulfur budget

(Andreae, 1990) with average coastal concentrations about 0.1 to 1.6 nM (Cozic et al., 2007a).

Carbonyl sulfide is the most abundant and probably the most long-lived sulfur gas in the atmosphere (Ulshöfer and Andreae, 1998). In open ocean, its concentration averages 0.03 nM (Johnson and Harrison, 1986) whereas in coastal zone, it is between 0.07 nM (Rasmussen et al., 1992) and 12.1 nM (Jorgensen and Okholm-Hansen, 1985 ; Radford-Knoery and Cutter, 1994). Production of OCS is based on two principal processes ; the photochemical degradation of dissolved organo-sulfur compounds (Zepp and Andreae, 1994) and the dark production (i.e., non-photochemical) from dissolved organo-sulfur compounds or sediments (Flock and Andreae, 1996). Its consumption is due to hydrolysis of dissolved OCS (Johnson and Harrison, 1986) and air-sea exchange (Ulshöfer et al., 1996).

Dimethyl sulfide, methane thiol and dimethyl disulfide are synthesized from the same precursor, the dimethylsulfoniopropionate (DMSP ; Kiene and Taylor, 1988 ; Keller et al., 1989 ; Simo et al., 2002 ; Tanzer and Heumann, 1992). This molecule is released in water column during phytoplankton degradation through grazing by zooplankton, senescence and cell leaking (Archer et al., 2002). DMSP is subsequently converted to sulfur compounds by enzymatic cleavage or demethylation (Kiene and Taylor, 1988 ; Keller et al., 1989 ; Simo et al., 2002). In marine environments, the DMS concentration range is between 0.4 and 16 nM (Turner et al., 1988 ; Moret et al., 2000 ; Amouroux et al., 2002 ; Andreae et al., 2003) whereas the concentration of MeSH and DMDS is usually lower (Cozic et al., 2007a).

So, with diverse origins and fates, the study of these five VRSC in natural environment, seems to be complex, yet clearly linked to the biological productivity.

1.3. Interactions between sulfur compounds and metals

The soft sphere metals (e.g., Ag^{+}, Cd^{2+}, Pb^{2+}) and some transition metals (e.g., Cu^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}) tend to coordinate and complex with soft bases (Smith et al., 2002) and consequently exhibit an affinity for sulfur compounds (Stumm and Morgan, 1981) such as hydrogen sulfide and thiols (R-SH) whose MeSH and DMS are often the most abundant in aquatic environments (Laglera and Van den Berg, 2003).

The conditional stability constant K′ for Ag(I) organosulfur complexes (e.g., thiols) is about log K′=13 (Martell and Smith, 1977 ; Bell and Kramer, 1999) and it is much higher compared to those of Ag(I) carboxylate complexes (monodicarboxylic acids,

log K′=2-4 ; EDTA, log K′=7). Al-Farawati and Van den Berg (1999) determined (in seawater, pH 8, 25°C) several conditional stability constants of metal-hydrogen sulfide. From these experiments, the following conditional stability constants of H_2S by the following metals were obtained ; log K′(Cu^{2+})= 12.9, log K′(Ag^{+})=11.6, log K′(Cd^{2+})=8.4, log K′(Pb^{2+})=8.0, log K′(Co^{2+})=6.8 (at pH 9), log K′(Zn^{2+})= 6.1 and log K′(Ni^{2+})=5.1. These results confirm the possible interactions between these metals and H_2S in the marine environment.

Until now, the interactions between the other volatile reduced sulfur compounds (i.e., OCS, MeSH, DMS and DMDS) and metals (i.e., soft metal and transition metal) are totally unknown yet these compounds exhibit similar complexing properties. Thus, we have determined experimentally the VRSC conditional stability constants for one of the soft sphere metals, silver. The aim of these experiments was to determine the possible interactions between OCS, MeSH, DMS and DMDS and silver in aquatic environments. Silver was chosen as the first candidate according to its significance with respect to the Seine estuary contamination. According to the Irving-Williams (1953) series which classify metals among their affinity (i.e., $Co^{2+}<Ni^{2+}<Cu^{2+}>Zn^{2+}$) and the knowledge of the conditional stability constants with Ag^{+}, it should be possible to derive the possible interactions between these VRSC and metals in the Seine estuary may be appraised. To achieve this goal, the VRSC and metal concentrations were determined along the salinity gradient with the same sampling range during the SILVER-2 cruise, and a first approach to the determination of conditional stability constants between VRSC and silver was attempted.

2. Material and Method

2.1. Sampling and *in situ* measurements

The SILVER-2 cruise was carried out from the 23rd of May to the 1st of June 2005 on the R/V Thalia equipped with a clean van. Sampling was carried out along the salinity gradient to determine the evolution of VRSC and metals concentrations in the Seine estuary. During the 10-day cruise, each estuary station (i.e., from Caudebec to off Le Havre) was sampled twice ; one for a spring tide (23rd and 24th of May) and another for a neap tide (30th and 31st of May). Marine coastal water samples (i.e., from Le Havre harbour to off of Dieppe) were collected between the 25th and 29th of May (Figure 1).

One sample (called L station) was collected more off the shore (noted by a black star in the Figure 1) in order to have VRSC and metals concentrations in an area far away from urban inputs.

In order to complete the study of the VRSC distribution along the salinity gradient, various parameters (conductivity, sea surface temperature SST, particulate organic carbon POC, suspended particulate matter SPM) were also determined. Conductivity and SST were measured by a polarographic probe and so, are available for all stations surveyed (Chiffoleau et al., 2001). Suspended particulate matter (SPM) was collected by filtration of water samples (one filter available per sample, no replicates) through a pre-weighted membrane (0.45-µm Nuclepore®) under a low pressure (i.e., 1 bar) to avoid cell degradation (Chiffoleau et al., 2001). Filtration of another aliquot of water samples through precombusted glass fiber (0.7 µm GF/F Whatman) filters was carried out onboard. These filters were used to measure POC at the shore lab after individual storage in glass Petri dishes and immediately frozen and kept in the dark until analysis (Chiffoleau et al., 2001). The POC was analysed with a CHN autoanalyzer (Carlo Erba model) after decarbonation by HCl vapor (Chiffoleau et al., 2001). Triplicates filters were prepared to improve accuracy of POC measurements.

2.2. Volatile Reduced Sulfur Compounds analysis

To avoid the samples degradation for the VRSC, analyses were carried out on board immediately after collecting (i.e., less than 15 min). The water samples were collected using a clean (i.e., with HCl 0.5N) polycarbonate bottle dipped one-meter beneath the sea surface. The subsample was immediately drawn into a clean syringe to avoid the contact with atmosphere air and the sample contamination. The sample syringes were stored in a dark and cold place (i.e., icebox) until duplicate analysis.

The analytical method used here is fully described by Cozic et al. (2007b) which determines simultaneously the concentration of five reduced naturally volatile (i.e., free) sulfur compounds in a water sample. This method is based on a gas chromatography coupled with a purge and trap system. Briefly, the water sample (i.e., 15 ml) is injected via the syringe, into the stripping vessel. There, the sulfur gases are exsolved from the liquid phase and swept into a cryogenic preconcentrated trap. When extraction is complete, carrier gas (helium) sweeps the concentrated gases into the chromatographic column. With this analytical method, up to 30 water samples can be analysed per day (e.g., less than 12 min per sample). Moreover, every sample was

subsampled into a filtered subsample (0.45 µm, Teflon® PTFE) and a unfiltered subsample in order to know the sulfur ligands speciation (i.e., particular or dissolved). To minimize cell damage during the filtration, a gentle positive pressure (i.e., <0.1 kg/cm²) was applied by hand.

2.3. Metal analyses

The water samples for metal analyses downstream from the Tancarville bridge, were collected with an all-Teflon® pump (Chiffoleau et al., 1994). Additional water samples were taken between Vieux Port and Poses, again using clean bottles. Each sample was immediately filtered in the onboard laboratory van, using trace metal-cleaned pre-weighted filters (0.45-µm Nuclepore®; Chiffoleau et al., 1994). These filtered water subsamples were preserved in acid-cleaned 250-ml polyethylene bottles and stored at pH<1.6 until further analysis (Chiffoleau et al., 1994).

A preconcentration by a liquid/liquid extraction of the water samples (Danielsson et al., 1982) was occurred on the one hand, to eliminate the salted matrix which disturbed the signal and on the other hand, to concentrate the metals in the sample. This technique was based on the production of organic-metal complexes, the extraction of these complexes by an organic phase within complexes were very soluble and the destruction of these complexes by acidification of the organic phase. An ICP-MS was used to determine metal elemental concentrations (i.e., Ag, Cd, Pb, Cu, Co, Zn, Ni ; dissolved phase). Quality control and determination of accuracy were performed by analysis of certified international reference material of estuarine waters (SLEW-3). Analytical results obtained for the dissolved reference material always differed by less than 10% from the certified values and reproducibility was generally better than 6% for every metal examined. Quantification limits (i.e., 10 times the standard deviation of the blank) were 1 pM for Ag, 2 pM for Cd, 1 pM for Pb, 94 pM for Cu, 3 pM for Co, 123 pM for Zn and 34 pM for Ni (Chiffoleau, pers. comm.).

2.4. Study of silver complexation by sulfur compounds

The experimental protocol is based on the mixing of metal (i.e., silver) with a single volatile sulfur compound (H_2S, OCS, MeSH or DMS ; DMDS standard not available) dissolved in deionized water to keep the chemical system as simple as possible. Al-Farawati and Van den Berg (1999) showed the conditional stability constant of silver

for HS^- ions was not significantly modified with the salinity. The pH is fixed ca. 7.7 (with KOH added to the distilled water) for H_2S and DMS experiments and ca. 6.5 for OCS and MeSH. For example, it is essential that pH is greater than the H_2S's pKa (6.98 at 25°C ; Goldhaber et al., 1975) to have predominantly HS^- ions, which dominate in the sample ; HS^- is the chemical form of bisulfide, which reacts the most with metals. But the first reason of this increase of pH is to be nearly the seawater pH (ca. 8 ; Al-Farawati and Van den Berg, 1999) to reproduce a natural environment.
The chemical speciation experiments were conducted as follows. Firstly, from an initial and pure solution (99.99%) of $AgNO_3$ at 1 g/L, 10-ml solutions with Ag concentration ranged from zero to 2 or 20 nM, were drawn in acid-cleaned (HCl, 0.5 N) syringes. To avoid the photodegradation of metal solution, the syringes are placed in the dark at ambient temperature. Secondly, the volatile reduced sulfur liquid solutions are prepared (constant concentration, either 2 or 20 nM) from the gaseous or liquid standards. Gaseous standards were from custom-made sulfur gas bottles (Scott Gas®) with certified concentrations of H_2S (1.1±0.04 ppm) and DMS (1.1±0.01 ppm). The solubility, depending on pH, is 0.1 M/atm for H_2S (Boffi et al. 2000) and 0.63 M/atm for DMS (Barcellos da Rosa et al., 2003) in deionized water (pH<6, 25°C). Thus, according to the Henry's law, the gas quantity to inject into the acid-cleaned syringe is calculated in order to obtain equivalent targeted VRSC concentration in the liquid phase (i.e., deionized water). For OCS, a calibrated-release permeation device (VICI-Metronics) was used. Thanks to the OCS solubility (K_H=2.08 M/atm in distilled water ; Elliot et al., 1989), the air stream flux is calculated to obtain a 2-nM final liquid OCS concentration in the syringe. For methane thiol (MeSH), a liquid standard solution (21% w/w, Aldrich) is diluted to acquire a final solution at 2 or 20 nM. The last stage of the experiment is the mixing of silver and VRSC. So, to each syringe containing a given silver concentration (e.g., 0 nM, 0.25 nM…), an additional 10 ml of sulfur-containing solution were added. Each syringe is shaken energically for 2 minutes to mix the solutions of metal and sulfur compounds and equilibrate liquid and gas phases, and it is replaced in the dark waiting the chromatographic analysis. Care was taken to always maintain the same "contact" time between the VRSC and silver before analysis. The final pH is ca. 7.7 for the solutions with H_2S and DMS and ca. 6.5 for the solutions with OCS and MeSH.

Models of binding strength are based on the multicomponent version of the single 1:1 complex formation mechanism, which is mathematically identical to the model of the

Langmuirian type of submonolayer adsorption (Ruzic, 1996 ; Al-Farawati and Van den Berg, 1999) :

$M + S \Leftrightarrow MS$ (1)

with S, an individual binding site. For the following equations, the silver will be noted M (for metal) and the single VRSC will be noted L (for ligand).

The distribution of labile species (silver and VRSC) in the experiment gives the mass balance for the equation (1) :

$Mt = M' + ML$, $Lt = L' + ML$ (2)

with Mt and Lt, the total concentrations of metal and ligand, M′ and L′, the corresponding concentrations of unreacted compounds (i.e., free active sites), and definition of this apparent conditional stability constant :

$K' = ML/[(M')(L')]$ (3)

With the equations (2) and (3) and a simple mathematical adjustment,

$K' = Lt/[(Mt \times L') - L' (Lt - L')]$ (4)

Thanks to the equation (4) set up between the conditional stability constant K′, the concentration of free sulfur active sites L′, the total concentration of sulfur compound Lt and the total concentration of silver Mt, it is possible to determine graphically the conditional stability constant between silver and the VRSC studied.

The equation (4) becomes a second-degree equation :

$L'^2 + (1/K' + Mt - Lt) \times L' - Lt/K' = 0$ (5)

Thus, the concentration of the free sulfide active sites is uniquely determined for one conditional stability constant and for the total concentrations of silver and VRSC chosen (equation 5). Comparing the theorical curves obtained (i.e., for a K′ fixed) to the experimental data (i.e., speciation experiment results between silver and one VRSC), we obtained a value for the conditional stability constant. To facilitate the discussion, the conditional stability constants will be noted by its logarithm value (log K′).

3. Results

3.1. Supporting parameters

3.1.1. Suspended particulate matter (SPM) distribution along the salinity gradient

According to a comparison (i.e., χ^2 test, p=0.05) of the SPM distribution along the salinity gradient, a clear difference was observed (Figure 2) between the two sampling periods in estuary.

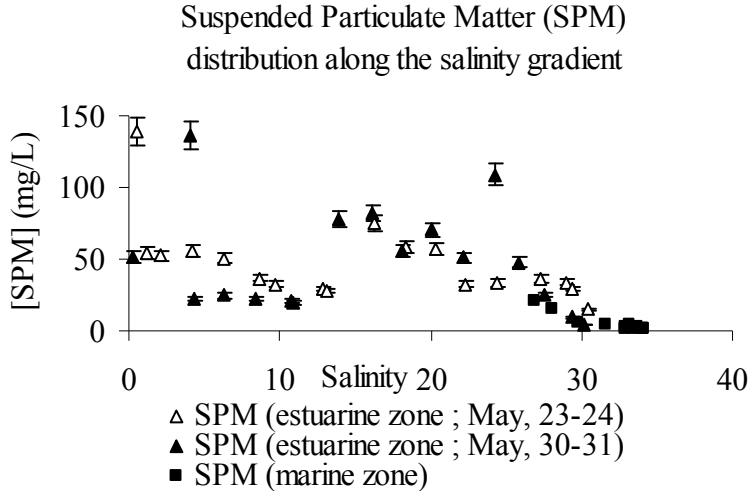

Figure 2 - Distribution of Suspended Particulate Matter (SPM) along the salinity gradient from May 23 to June 1, 2005.

The highest values were recorded for the spring tide sampling period (May, 23-24) but the maximum of SPM concentration was always occurred in the lowest salinities with 139 mg/L at 0.6 of salinity for the spring tide sampling and 136 mg/L at 4.1 of salinity for the neap tide sampling. Below ca. 10 of salinity, the SPM concentration decreased for the two estuarine sampling periods. It was 54.3 mg/L at the salinity 1.1 for the spring tide salinity and 51.3 mg/L (0.3-salinity) for the other period. Near 10, the SPM concentration decreased from the 23th of May (32.3 mg/L) to the 31st of May (21.3 mg/L). A second maximum value (78.2 mg/L) was measured at 13.9 of salinity and it was followed by a new decrease in the upper estuary. For the spring tide period, at the salinity ca. 30, the SPM concentration reached 14.6 mg/L, whereas it was one third this value during the neap tide period. In marine zone (i.e., up to 30 of salinity), the SPM concentration was ten times less that of the estuary. The same trend was observed with

a clear decrease of concentration from 26.9 of salinity (20.5 mg/L) to 34.1 of salinity (1.2 mg/L).

The SPM profile may be used to conclude the localisation of the maximum turbidity zone (MTZ). By definition, the highest SPM concentrations are observed in the MTZ and so, the MTZ should be located in the lowest salinities (i.e., 0 to 5 of salinity ; Figure 2 ; Van den Berg, 1993 ; Martino et al., 2002). The clear increase of SPM concentration near 14-18 of salinity shows the localisation of the mid estuarine maximum zone (MEZ), which is an area where the trace element mobilization and fluxes from sediments are higher (Gerringa et al., 2001 ; Robert et al., 2004 ; Buggy and Tobin, 2006).

3.1.2. Particulate organic carbon (POC) distribution along the salinity gradient

No significant variation of the POC distribution was observed (i.e., χ^2 test) in the estuarine zone, between the spring tide and the neap tide sampling periods (Figure 3).

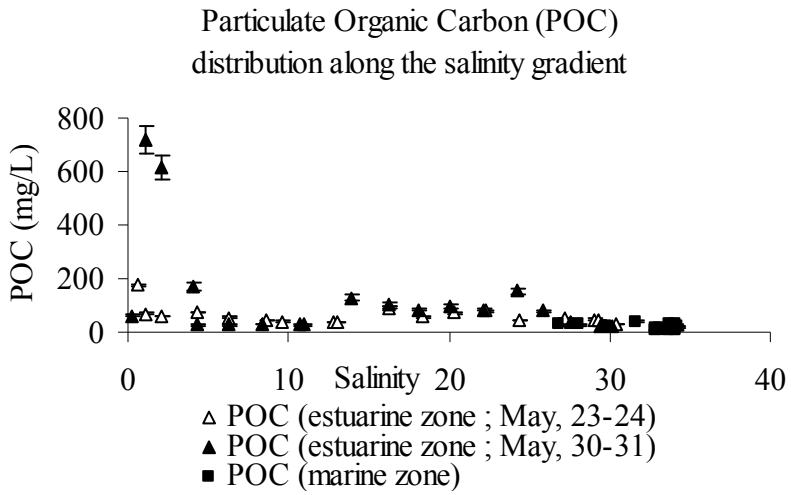

Figure 3 - Distribution of particulate organic carbon along the salinity gradient from May 23 to June 1, 2005.

The highest values were occurred, like SPM distribution, in the lowest salinités with up to 600 mg/L measured (Figure 3). But, the POC distribution was relatively constant from zero to ca. 14 of salinity with values between 174.3±4.3 mg/L (0.6 of salinity) and 36.8±0.4 mg/L (13.6 of salinity). This amount increased in the mid estuarine part with 90.5±23.3 mg/L (n=7) from 14 to 20 salinity range. In the marine zone, POC concentration slightly decreased with 18.1±10.1 mg/L (n=15). The relative standard deviation (RSD) for these measurements was included in 1.4% and 11.4%.

3.2. Distribution of VRSC along the salinity gradient

Before to comment the sulfur compounds profiles, we verified if there exists or not a difference between the distribution of VRSC from the filtered subsamples and the one from the unfiltered. According to a χ^2 test (p=0.05), it was concluded that no significant difference was between the filtered subsamples and the unfiltered ones. Moreover, no significant difference (χ^2 test) of VRSC concentrations was noted between the spring tide sampling and the neap tide but, different features for the volatile sulfur compounds distribution along the salinity gradient, were apparent. To more clarity, analytical precisions (6.0% for H_2S, 4.1% for OCS, 5.6±% for MeSH, 4.9% for DMS and 8.4% for DMDS ; Cozic et al., 2007b) were not noted on the Figure 4.

The concentration of hydrogen sulfide was below the detection limit (0.07 nM ; Cozic et al., 2007b) until 17.9 of salinity. In the mid estuary, there was a clear increase of H_2S concentration with 0.80±0.16 nM (n=23) in the 18.7 to 29.8 salinity range. In the higher salinities, a clear decrease occurred with 0.73±0.04 nM (n=4) at the salinity 25.2 and only 0.06±0.01 nM (n=3) at 33.5 (L station) (Figure 4A).

Carbonyl sulfide concentration showed two trends along the salinity gradient (Figure 4B). Firstly, it was constant in the upper estuary (i.e., from zero to 17.9-salinity) with ca. 0.64±0.26 nM (n=22). Upward 18.7-salinity, OCS concentrations began to decrease slightly to reach 0.61±0.07 nM (n=4) at 25.19 of salinity and half that at 29.8 of salinity (0.36±0.02 nM ; n=4). The marine end-member sampling confirmed this decrease in the high salinities with 0.43±0.10 nM (n=4) measured at the salinity 25.2 and the half (0.26±0.12 nM ; n=3) at the L station.

Methane thiol, dimethyl sulfide and dimethyl disulfide exhibited similar profiles along the salinity gradient ; an increase of concentration until a maximum value at mid-salinity range, followed by a decrease, which was even more pronounced for DMS and DMDS. The MeSH concentration clearly increased between 0.61 of salinity (0.22±0.07 nM ; n=4) and 17.9 (3.06±0.33 nM ; n=4) (Figure 4C). Above this salinity and in the estuarine zone, its concentration decreased slightly to reach 2.17±0.33 nM (n=4) observed at the salinity 25.2. This slow decrease was observed in the downer estuarine zone with 1.54±0.17 nM (n=3) recorded at 25.2 of salinity and 1.33±0.42 nM (n=2) at 33.5.

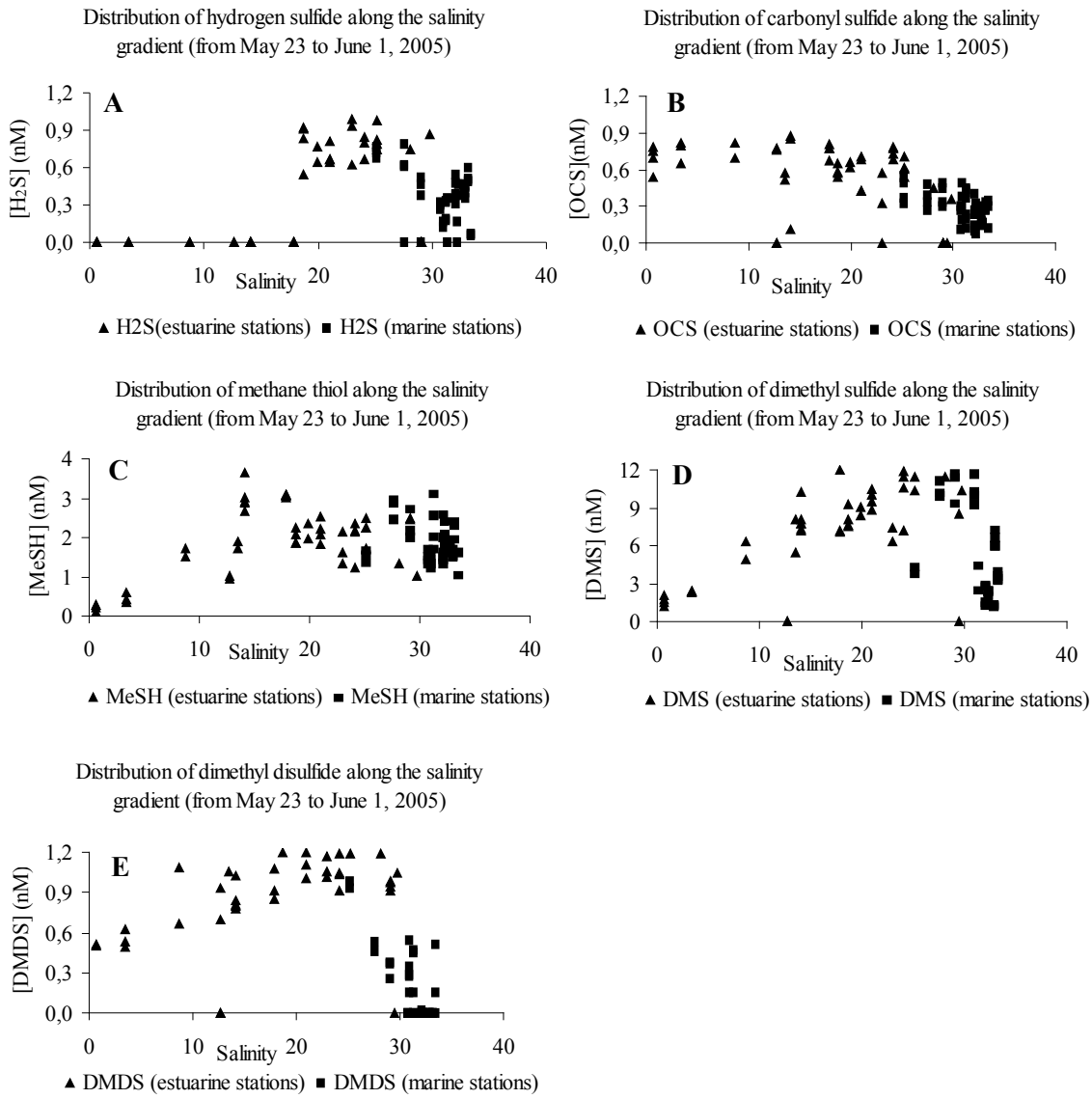

Figure 4 - Distribution of VRSC along the salinity gradient from the May 23 to June 1, 2005. Estuarine stations have a salinity in the range 0.6-29.5 of salinity. Marine stations have a salinity in the range 25.2-33.5 of salinity. A, H_2S ; B, OCS ; C, MeSH ; D, DMS ; E, DMDS.

The maximum of dimethyl sulfide concentration was observed in the 24.1 to 29.8 salinity range in estuarine and marine zones (10.81±2.36 nM ; n=32 ; Figure 4D). In the upper estuary (i.e., below 20 of salinity), DMS concentration was lower with 1.63±0.33 nM (n=4) at 0.6 but increased to reach 8.05±1.13 nM (n=6) at the salinity 14.1. In the downer estuarine zone, the lowest value of concentration was observed near 32.3 (2.35±0.20 nM ; n=4), which indicated a clear decrease of DMS concentration with the salinity.

The DMDS concentration doubled between 0.6-salinity (0.51±0.01 nM ; n=2) and 17.9-salinity (1.18±0.10 nM ; n=5) and it was uniform (1.15±0.30 nM ; n=29) in the

18.7 to 29.8 salinity range for the estuarine sampling (Figure 4E). Looking the distribution of DMDS in the oceanic zone, a clear decrease was observed with increasing salinity. Indeed, at the salinity 25.2, the concentration was 0.95±0.03 nM (n=2) and it was three times less (0.32±0.14nM ; n=5) at 31-salinity. From 32.1, it was minimum with 0.03±0.10 nM (n=25).

3.3. Determination of Silver-VRSC conditional stability constants

The results of the silver speciation experiments with sulfur compounds are presented in Figure 5. The speciation experiments with H_2S were carried out with initial sulfide concentration and initial silver concentration equal to 2 and 20 nM (Figure 5, A-B).

Comparing the experimental data with the theorical curves, conditional stability constants was determined. Its log K′ value was greater than 12. This result is coherent the one obtained by Al-Farawati and Van den Berg (1999) at seawater pH, lending confidence in our method used for the determination of conditional stability constants of VRSC for a metal.

The other reduced sulfur compounds tested (OCS, MeSH and DMS) did not seem to show a strong affinity for silver. The speciation experiments with the carbonyl sulfide were carried out with an initial sulfur concentration equal to 2 nM according to the OCS concentration range recorded in the Seine and others estuaries (Sciare et al., 2002). Looking at the evolution of free OCS (i.e., purgeable with helium) with increasing silver concentration, this compound did not exhibit strong interactions (Figure 5C). From zero to 2 nM of silver added, the volatile OCS concentration decreased less than 14.5%. Figure 5D shows more precisely the lowest value of the conditional stability constant coherent with the experimental data, K′ between 0.1 and 0.01 and so, log K′ is very low (≈0). For methane thiol, the same trend was observed with a very low conditional stability constant. Indeed, the same value was measured for an initial MeSH concentration of 2 nM (Figure 5E) or 20 nM (Figure 5F) and it is about 0.05 (log K′≈0).

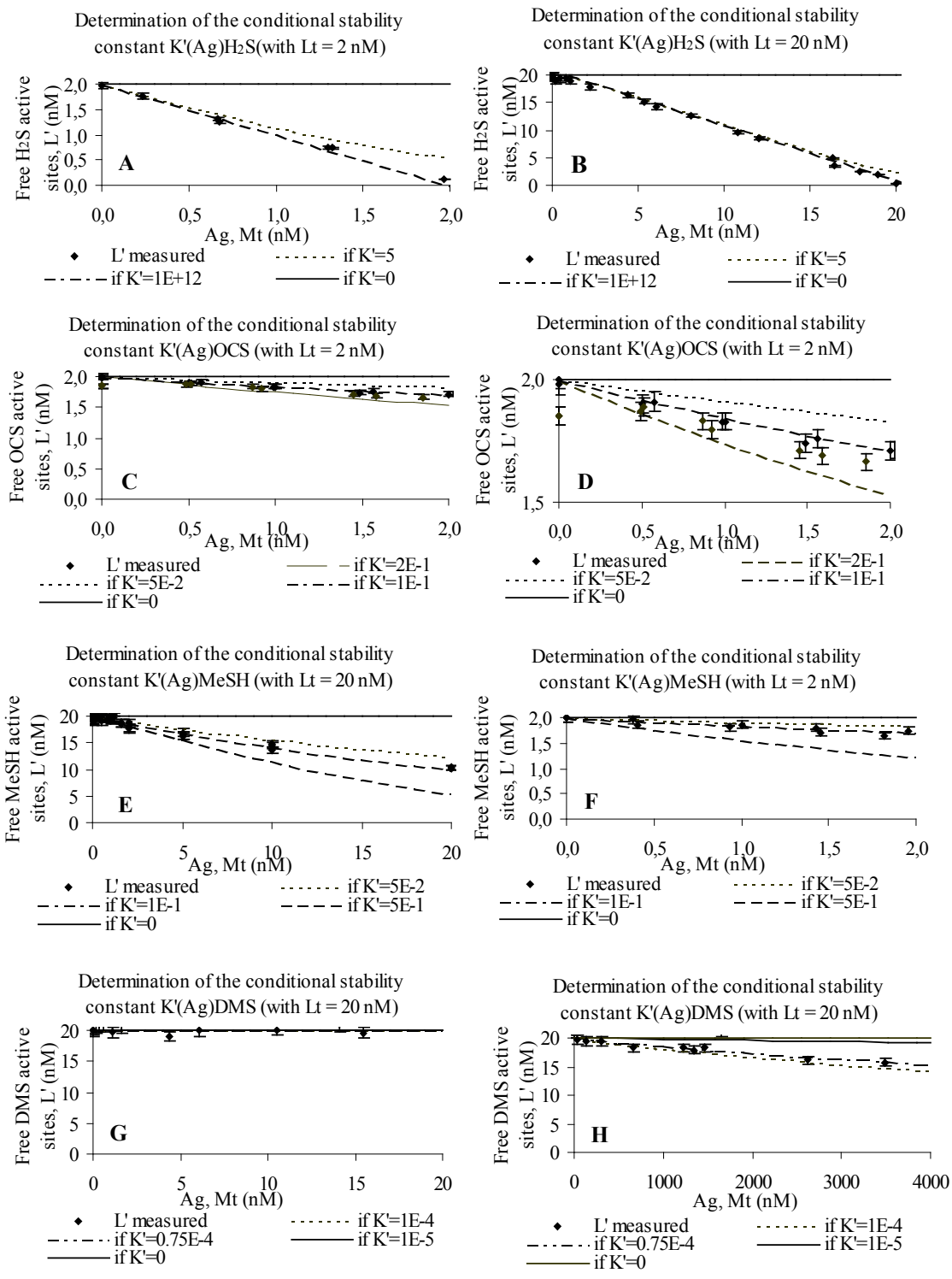

Figure 5 - Graphic determination of the conditional stability constants between the volatile reduced sulfur compounds and silver. A, K'(Ag)H_2S with Lt = 2 nM ; B, K'(Ag)H_2S with Lt = 20 nM ; C, K'(Ag)OCS with Lt = 2 nM ; D, zoom of Figure C ; E, K'(Ag)MeSH with Lt = 2 nM ; F, K'(Ag)MeSH with Lt = 20 nM ; G, K'(Ag)DMS with Lt = 20 nM ; H, K'(Ag)DMS with Lt = 20 nM and increase of Mt.

The DMS concentrations observed in natural waters are often higher than those of the other VRSC and so, the speciation experiments were carried out with an initial sulfur concentration of 20 nM. Figure 5G shows the absence of interactions between DMS and Ag ions for a metal concentration included in zero and 20 nM. But, if the silver concentration was increased until 4 µM (experimental value), a slight decrease of the free DMS concentration was observed (Figure 5H). Comparing this decrease to the theorical curves calculated, it was concluded that the conditional stability constant K′ (Ag)DMS is 0.75×10^{-4} (log K′≈0) which is a non-significant value given the observed silver levels.

Therefore, with these experiments, it was demonstrated that only the hydrogen sulfide may strongly interact with silver in aquatic environments. Carbonyl sulfide and methane thiol had weakly affinity for silver whereas the dimethyl sulfide did not interact with it.

3.4. Metals distribution along the salinity gradient

Metals surveyed for the SILVER-2 cruise were silver (Ag^{+}), cadmium (Cd^{2+}), lead (Pb^{2+}), copper (Cu^{2+}), cobalt (Co^{2+}), zinc (Zn^{2+}) and nickel (Ni^{2+}). Only silver concentration was surveyed for the two estuarine transects (i.e., May 23-24 and May 30-31 ; Figure 6A) ; the other metals were measured only for the second estuarine sampling period (Figure 6B-C).

Firstly, various concentration ranges occurred between metals. Zinc had the highest concentration with 84.0 nM at 6.3 of salinity (Figure 6C). Copper and nickel concentrations were within the 10 to 30 nM range (Figure 6C), whereas cadmium, lead and cobalt concentrations were lower and never greater than ca. 1.5 nM for Co, ca. 1 nM for Cd and Pb (Figure 6B). Silver was the lowest concentrated with less than 0.06 nM measured (Figure 6A).

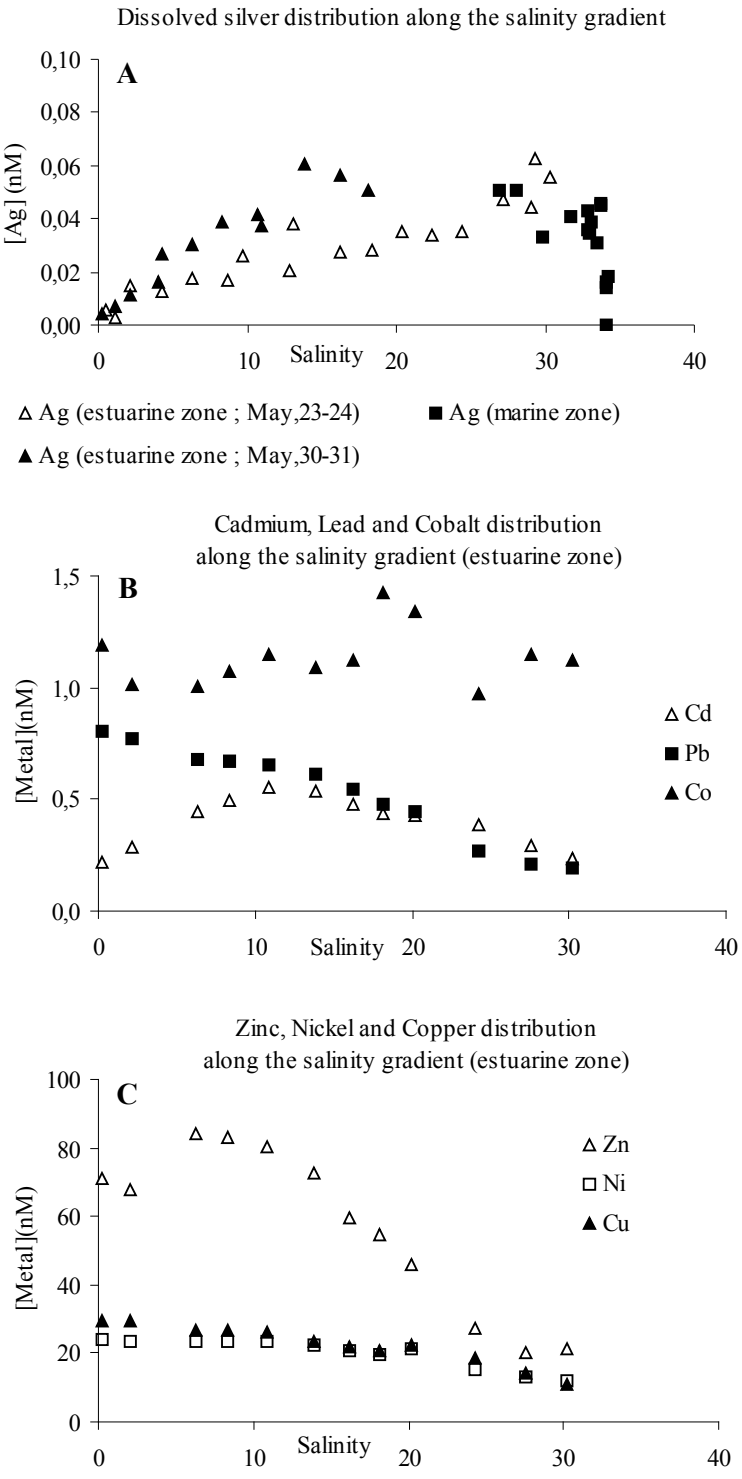

Figure 6 - Distribution of dissolved metals along the salinity gradient from May 23 to June 1, 2005. A, dissolved silver ; B, Cd, Pb and Co ; C, Zn, Ni and Cu.

Various distribution trends were occurred along the salinity gradient. Cadmium and silver concentrations increased in estuarine zone, from zero to ca. 13-15. At the lowest

salinity, the Cd concentration is 0.2 nM ; it doubled (ca. 0.5 nM) at 8.3 of salinity (Figure 6B). The maximum (0.5 nM) was reached near 14, followed by a decrease in the highest salinity sampled (0.2 nM at 30.2). Concerning silver distribution, an increase occurred whetever the estuarine sampling period (Figure 6A) with a concentration above 0.01 nM at 0.6-salinity and at ca. 8.5 of salinity, 0.02 nM for the spring tide sampling (23-24th of May) and 0.04 nM for the neap tide period. From 29.7 of salinity, the silver concentration decreased rapidly. Cobalt occurred a relatively constant concentration (1.1±0.2 nM, n=12) along the salinity gradient (Figure 6B).

Nickel and copper distribution in the Seine estuary owned the same trend with a slight decrease along the salinity gradient (Figure 6C). Indeed, at 0.3-salinity, the concentration was 24.2 nM for Ni and 29.4 nM for Cu whereas at 30.2-salinity, it was respectively 11.9 nM and 10.8 nM. After the maximum (84.0 nM) reached near 6 of salinity, Zn concentration clearly decreased with 59.6 nM at 16.2 of salinity and 21.5 nM at 30.2 (Figure 6C).

4. Discussion

The VRSC studied in this article show various trends in their distribution along the salinity gradient but regardless of the considered compound, a decrease of concentration is observed in marine zone. To facilitate the explanation of these variations, DMS, DMDS and MeSH are regrouped because all of them show a progressive increase of concentration until the mid estuarine zone. Otherwise, another trend is observed for carbonyl sulfide and hydrogen sulfide with constant levels at low salinities.

Moreover, since only hydrogen sulfide has significant conditional stability constants for metals surveyed, it is the only sulfur gas which may influence the metal distribution. So, in turn, with important metals concentrations, the sulfide distribution may be significantly modify whereas the distribution of other VRSC not be linked to the presence of metals in estuary according to the very low K′ values.

4.1. Distribution of DMS, DMDS and MeSH along the salinity gradient

Dimethyl sulfide, dimethyl disulfide and methane thiol show a clear increase of concentration from the lowest salinities to respectively 24.1, 17.9 and 17.9 of salinity

(Figure 4C-E). The phytoplankton density and the POC distribution are positively correlated in the Seine estuary (Chiffoleau et al. ; 2001). So, with a relatively constant POC value from zero to ca. 24 of salinity (Figure 3), the phytoplankton density should be constant along the salinity gradient (below 24). Thus, the increase of VRSC levels with salinity which is frequently observed in other estuaries could be explained by change in phytoplankton species assemblages along the salinity gradient (Muylaert and Sabbe, 1999) rather than abundance changes. In fact, Ahel et al. (1996) showed that freshwaters phytoplankton species dominate estuarine environments for salinities not exceeding 5 whereas marine species develop for salinities exceeding 15 whatever the tidal range. Indeed, the amplitude and specific shape of the tidal curve combined with relatively low river inputs to give the estuary essentially marine dynamic characteristics (Salomon, 1988).

Moreover, this increase of concentration with salinity was also showed for DMS by Sciare et al. (2002) in the Gironde estuary (France) with maximum concentration recorded at salinity 22.5. Studying the distribution of phytoplanktonic pigments, they highlighted the more important were 19'-HEX and peridinin which are specific indicators of Phaeocystis and Dinophyceae, both known to be high producers of DMSP (Liss et al., 1997). Lemaire et al. (2002) studying the distribution of pheopigments in nine European estuaries, showed that the mid estuarine maximum zone is a highly reactive region for the phytoplankton (i.e., organic matter) degradation. Indeed, Cunha et al. (2000) occurred the highest bacterial abundance and enzymatic activity were measured near in the mid estuarine part. So, the hypothesis of a shift in the phytoplanktonic population from the lesser producers like diatoms in up estuary to high DMS producers more downstream, may be accepted to explain the distribution of DMS, DMDS and MeSH in the Seine river. Unfortunately, pigments data are not available for the SILVER-2 cruise.

Comparing to the other European estuaries (Sciare et al., 2002), the DMS concentration in Seine river is very high with maximum values greater than 10 nM near 25-salinity (Figure 4D). The mean estuary concentration (8.2±4.5 nM ; n=52) measured in Seine is twice that of the Rhine (Germany) and about ten times more elevated than in the Gironde (France, 0.7 nM ; Sciare et al., 2002). These results suggest a significant production of DMS in the estuarine plume but the absence of quantitative data on DMSP and phytoplankton species make it impossible to explain more precisely these high DMS concentrations. In the marine zone (above 30-salinity), the DMS concentration (3.2±1.8 nM ; n=22) is similar to the values determined by

Turner et al. (1988 ; 3.6 nM, n=198) in seawater around mainland Britain and by Walker et al. (2000 ; 0.4-12.9 nM) over the continental shelf. Presently, no DMDS estuarine measurements were carried out in other estuaries and so, no comparison with our data were possible.

Compared to DMS and DMDS, the slower decrease of MeSH concentrations in marine waters may be explained by its possible consumption by bacterioplankton which use MeSH for their amino acid synthesis (Kiene et al., 2000). The hypothesis advanced to explain the constant MeSH concentration above 25 of salinity (Figure 4C) is the increase of the bacterial growth rates which induces preferentially the DMSP degradation in MeSH (Kiene and Taylor, 1988). The clear decrease of DMS and DMDS concentrations into the shelf waters may also be linked to either a dilution from the upper estuarine waters either by the mixing with an older oceanic water mass which contains less DMS and DMDS than MeSH (Figure 4D-E). Thus, additional work appears to be clearly needed to better understand on the one hand, the increase of DMS and DMDS in the plume compared to the marine zone and on the other hand, the high estuary values measured for DMS.

4.2. Distribution of OCS along the salinity gradient

Higher levels of OCS occurred in the estuarine waters (mean value, 0.5±0.3 nM, n=50) compared to the shelf waters (mean value, 0.3±0.1 nM, n=47) (Figure 4B). These results are consistent with those obtained for by Sciare and al. (2002) in the Rhine estuary (Germany, 0.50 nM), Watts (2000) in Yarmouth estuary (UK) (0.77 nM) and Cutter and Knoery (1993) in two estuaries in the eastern United States (0.3 nM). Concerning the OCS concentration in marine zone, Ulshöfer et al. (1995) estimated it at 0.005-0.019 nM in the North Atlantic ocean and Mihalopoulos et al. (1991) found less than 0.02 nM of OCS in Mediterranean Sea. Thus, with values often greater than in oceanic regions, the estuaries can be considered as a significant source of carbonyl sulfide to the atmosphere. In fact, Zhang et al. (1998) demonstrated the sea-air flux of OCS in Chesapeake bay was over 50 times more than those from the open ocean, because of the higher production of OCS in this type of environment.

The production of OCS in surface waters is thought to be dominated by photochemical processes, which depend on the concentration of the photosensitizers (i.e., chromophoric dissolved organic matter, CDOM ; Zepp and Andreae, 1994), which is in turn, related to phytoplankton concentration (Kettle et al., 2001). Andreae and Ferek

(1992) suggested that higher concentrations of a precursor, dissolved organic sulfur compounds in the water column, may result from increased of eutrophication by nutrient runoff from land. The Seine estuary is a eutrophied region and receives significant inputs (i.e., humic acids, fulvic acids…) from the continent because 40% of the french industry and 25% of the french population are in its drainage basin (Chiffoleau et al., 2001). So, the high concentration (0.64±0.26 nM, n=22) observed in the lowest salinities (i.e., up to 17.9) could be linked to the microbial degradation of organic sulfur compounds such as cysteine (Cooper, 1983) whose the concentration is higher in the upper estuarine part (Andreae and Ferek,1992). The photo-destruction of methane thiol could also be another source of carbonyl sulfide in the Seine river (Flock and Andreae, 1996).

Moreover, with the increase of SPM concentrations near 14-salinity to ca. 60 mg/L, the light penetration depth should decrease (Smith and Baker, 1979) and therefore, it the OCS production by photodegradation should be decreased. However, in the mid estuarine part, the prevalence of the sedimentary source is greater (Van den Berg, 1993 ; Robert et al., 2004). Indeed, Cutter and Radford-Knoery (1993) showed that the concentration of carbonyl sulfide in porewaters (less than 1 cm depth) of Chesapeake Bay, is up to 7500 nM. For an example, sediments of the Chesapeake Bay account for up to 75% of the total OCS sources to the water column (Zhang et al., 1998). Thus, diffusion of OCS from sediments to the overlying waters may be another important source in the estuarine environment and preferentially in the mid estuarine part. The surface water distribution of OCS in the Seine river is likely to be influenced by the flux of sulfur gas from sediments because of the relatively shallow estuary (10-m mean depth) comparable to Chesapeake Bay and the important difference of concentration between water column and sediments. This source of carbonyl sulfide may also explain the constant concentration observed in the upper estuary (i.e. up to 17.9 of salinity), although no porewater data are available to estimate the sedimentary OCS concentration.

Like DMS and DMDS, the clear decrease of concentration from ca. 18 of salinity, could be likely due to a simple dilution of high CDOM concentration in estuarine waters with lower CDOM concentration in seawater (i.e., less organisms).

4.3. Distribution of H_2S along the salinity gradient

In the late of 1980s, Cutter and Krahforst (1988) successfully measured total sulfide (< 0.1 to 1.1 nM) in surface waters of the western Atlantic. Subsequent studies (Luther and Tsamakis, 1989) in marine systems made similar findings with 2 nM of sulfide analysed in the eastern Mediterranean Sea surface waters and ca. 0.9 nM along the East Coast of the United States. Concentrations in freshwaters are less well known according to the few studies carried out, but the limited data set suggest concentrations less than nanomolar range or up to several nM (Bianchini and Bowles, 2002). In the Seine river, the mean sulfide concentration is 0.80±0.16 nM (n=23) from 18.7 of salinity whereas in the oceanic zone (station L), it is minimum (Figure 4A).

First, an explanation will be proposed to understand the absence of free sulfide in the upper estuary and then, looking the possible role of some trace metals on the sulfide distribution. Secondly, the possible origins of H_2S above 17.9-salinity will be successively discussed.

The absence of volatile sulfide (i.e., the chemical form detected by gas chromatography) occurred in the lowest salinities (i.e., up to 17.9 , Figure 4A), while significant concentrations of carbonyl sulfide and others VRSC are measured, suggests significant removal mechanisms of H_2S which govern the sulfur gas behavior. Oxidation induced by higher oxygen levels in the upper estuarine part (Chiffoleau et al., 2001 ; Garnier et al., 2001), complexation with particulate trace metals, or metal sulfide precipitation may explain the absence of free H_2S from zero to 17.9 of salinity.

Andreae et al. (1991) performed speciation measurements and showed that only 12% of sulfide was free. Like in the Seine river, Rozan et al. (1999) found no free sulfide in four southern new England rivers and they postulated that metal sulfide complexes were the cause. Moreover, Elliot et al. (1989) have pointed out that complexation of trace oceanic metals with the bisulfide (S^{2-}) and sulfide ions (HS^-) should impact the amount of volatile H_2S in the water column whereas Bruland (1983) and Dyrssen (1988) showed clear metal-sulfide interactions in aquatic environments with metal concentrations in the pico to nanomolar range.

With a strong conditional complexation constant with sulfide (log K′(Ag)=11.6 ; Al-Farawati and Van den Berg, 1999), silver likely influences the percentage of free sulfide in the Seine estuary (Figure 4A). However, its concentration (0-0.06 nM ; Figure 6A) is very low compared to the sulfide concentration (Figure 4A) and the strong interactions of silver with organic ligands (Luoma et al., 1995 ; Bruland, 1992)

or chloride highlighted do not support the silver as a metal responsible for the absence of free HS^- in the upper estuarine part. Copper may also influence the sulfide distribution in the estuary because its concentration is important (25.6±3.2 nM, n=8 ; Figure 6C) and its conditional complexation constant with bisulfide ions is high (log K′=12.9 ; Al Farawati and Van den Berg, 1999). Like the H_2S-Zn complexes, the H_2S-Cu complexes are kinetically inert to dissociation (Luther et al. ; 1996). Thus, the absence of free sulfide (HS^- ions) in the upper estuarine part may be explain by important interactions with copper. Otherwise, several authors (Cutter and Krahforst, 1988 ; Dyrssen, 1988 ; Rozan et al., 1999) demonstrated the key role of copper in natural water sulfide distribution and concluded that it was the most abundant of the strongly sulfide interactive metals. So, copper may be the metal which plays the most important role onto the absence of H_2S below ca. 18 of salinity. With weak interactions with sulfide (Al-Farawati and Van den Berg, 1999) and low concentrations measured (Figure 6B), cadmium, lead and cobalt metals do not appear to play a significant role on the H_2S distribution in the Seine estuary. Although zinc and nickel are high in the upper estuarine part (respectively, 71.7±10.2 nM (n=8) and 22.6±3.2 nM ; Figure 6C), they do not influence the H_2S distribution because of their very low conditional complexation constants for bisulfide ions (Al-Farawati and Van den Berg, 1999).

The presence of strong “metal-ligands” likely explains the absence of free sulfide in the upper part of the Seine. This hypothesis was already advanced by Cutter and Krahforst (1988) who showed that with a concentration less than 1 nM, sulfide may affect the cycling of several trace metals via the formation of stable complexes and in turn, the free sulfide concentration may be considerably decreased in the water column.

Thanks to the chemical equilibrium program MINEQL+, the hydrogen sulfide speciation was calculated in order to determine the role of copper and silver as ligands. Salinity from 0.3 to 30.2 and total H_2S concentration from 0.1 to 1 nM were tested. The pH was set at 8.2 and all metals measured during the oceanographic campaign were added in the model. The result occurred the major part of hydrogen sulfide was free (41% to 93% of total H_2S) although the presence of cooper and silver. The lowest percentage of free H_2S was obtained for a 0.3-salinity and a 0.1-nM of total H_2S. The bisulfide ions were only complexed with silver to form $AgHS_{aq}$. With the chemical equilibrium program MINEQL+ and the conditional stability constants used (Al-Farawati and Van den Berg, 1999), no complexation with copper was determined although the log K′ between HS^- and copper is higher than log K′$(Ag)HS^-$ (Al-Farawati and Van den Berg,

1999). Moreover, the increase of free sulfide percentage with the salinity seem to be linked to an increase of silver complexation with chloride ions.

The increase in SPM concentration in the middle of estuary (Figure 2) coincides with the abrupt increase of hydrogen sulfide (ca. 0.8 nM) from 18.7 (Figure 4A). The mid estuarine maximum zone is characterized by sediment mobilization (Gerringa et al., 2001 ; Robert et al., 2004 ; Buggy and Tobin, 2006). So, the presence of H_2S from the mid estuarine part could be linked to a released of sedimentary sulfide (Luther at Tsamakis, 1989). Moreover, sediments have an ability to scavenge metals (Xue and Sigg, 1999 ; Kogut and Voelker, 2001) which interact with sulfide and therefore, increase the free sulfide concentration in the surface waters. Other sources of hydrogen sulfide in an oxic water column are the hydrolysis of carbonyl sulfide, the phytoplanktonic production and the sulfate reduction. With no difference observed between filtered and unfiltered samples for the H_2S concentrations, it is concluded that SPM does not play a key role for H_2S distribution along the salinity gradient. At the higher salinities (24.1 to 29.8), where phytoplankton activity could be induced the highest observed levels of dimethyl sulfide, dimethyl disulfide and methane thiol (Figure 4C-E), it can also play a significant role in the hydrogen sulfide production (Walsh et al., 1994). The hydrolysis of carbonyl sulfide may be highest in the mid estuarine maximum zone (MEZ) and may explain the presence of H_2S from 18.9 of salinity (Figure 4A-B). Moreover, the highest degradation of organic matter (e.g., phytoplanktonic cells) in the MEZ may create anoxic microzones, which may induce the sulfate reduction and so, the hydrogen sulfide production. Presently, with available data, the quantitative part of these H_2S sources is unknown.
The clear decrease of sulfide concentration in the marine zone is related, like for the other VRSC, to a dilution of freshwaters into La Manche.

5. Conclusion

An experimental process has permitted to determine the conditional stability constants of four volatile reduced sulfur compounds for silver ; log $K'(Ag^+)H_2S$=12, log $K'(Ag^+)OCS\approx0$, log $K'(Ag^+)MeSH\approx0$, log $K'(Ag^+)DMS\approx0$. So, no significant interactions were shown between OCS and silver, MeSH and silver and DMS and silver. Therefore, the silver distribution in the estuarine waters is not influenced by the presence of these three VRSC. In turn, the distribution of these sulfur compounds may

be not modified by a silver contamination. However, strong interactions should exist between sulfide and silver according to the high value of the conditional stability constant.

Simultaneous measurements of H_2S, OCS, MeSH, DMS and DMDS concentrations permitted to survey the VRSC distribution along the salinity gradient in the Seine estuary during a 10-days cruise. The concentration ranges observed were 0-0.98 nM for H_2S, 0.07-0.87 for OCS, 0.11-3.65 nM for MeSH, 1.19-12 nM for DMS and 0-1.19 nM for DMDS. The variations of biogenic sulfur (i.e., MeSH, DMS and DMDS) compounds distribution may be linked to a speciation of phytoplankton with an increase of high DMSP-producers abundance near 20 of salinity. The clear decrease of all VRSC concentrations in "the high salinity end" of the estuary (above 30 of salinity) was coherent with a dilution of freshwaters into La Manche. The significant OCS concentration observed in Seine estuary was explained by various processes ; dissolved organic sulfur compounds photodegradation, methane thiol degradation and OCS diffusion from sediments (in the mid estuarine part).

The efficient interactions between trace metals and sulfide were highlighted. Although its low concentration, silver was the unique metal to interact with bisulfide ions but did not explain the undetectable levels of H_2S occurred below 14 of salinity. The clear increase of sulfide concentration in the highest salinities was related to the increase of sulfide sources (i.e., flux from sediments, hydrolysis of OCS, phytoplankton degradation) but also to the presence of others metal complexation mechanisms (e.g., sedimentary scavenging) which may free the sulfide previously complexed. Moreover, the possible oxygen depletion should stabilize H_2S in the MEZ.

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Interactions CSRV – Argent – Phytoplancton

Le phytoplancton est-il capable de répondre à une exposition métallique par une synthèse supérieure en composés soufrés réduits volatils (CSRV) ?

1. Introduction

1.1. Synthèse de composés soufrés réduits volatils par le phytoplancton

Les océans sont une source majeure des composés soufrés organiques qui sont impliqués dans la formation des pluies acides (Kennedy, 1986) mais également dans la production d'aérosols atmosphériques capables d'influencer le climat global (Charlson et al., 1987 ; Clarke et al., 1998). Avec près de 26 à 74 $Tg(S).an^{-1}$ qui quittent le milieu marin pour gagner l'atmosphère (Kettle et Andreae, 2000), la connaissance du cycle du soufre (e.g., production, interactions avec les métaux) dans les océans, est essentielle à une meilleure compréhension de la régulation du système terrestre. Le phytoplancton, par sa biomasse importante (Pena et al., 1990 ; Maranon et al., 2000), est considéré comme l'une des principales sources de ces composés soufrés biogènes. En effet, les cellules phytoplanctoniques contiennent divers composés soufrés comme par exemple, *i)* des acides aminés : méthionine, cystéine et glycine (Matrai et Keller, 1994), *ii)* des phytochélatines qui sont des polypeptides contenant des résidus de cystéine (Ahner et Morel, 1995 ; Ahner et al., 1997), *iii)* du glutathion, un tripeptide formé d'un acide glutamique, d'une cystéine et d'une glycine (Noctor et Foyer, 1998 ; Kawakami et al., 2006).

Dans le milieu marin, afin d'assurer le maintien d'une osmorégulation au sein de leurs cellules, de nombreuses familles phytoplanctoniques synthétisent un composé soufré organique, le dimethylsulfoniopropionate (DMSP ; Vairavamurthy et al., 1985). Ce composé est totalement ubiquiste dans la zone euphotique (Burgermeister et al., 1990 ; Turner et al. 1995) où il est considéré comme le composé soufré réduit le plus abondant avec des concentrations totales comprises entre 5 et 50 nM (Kiene, 1996).

Des teneurs supérieures à plusieurs dizaines de nM sont parfois rencontrées lors des périodes de blooms phytoplanctoniques (Malin et al., 1993). De plus, dans une cellule phytoplanctonique, le soufre organique particulaire est majoritairement (50 à 100%) sous forme de DMSP (Matrai et Keller, 1994). Comme tout osmolyte intracellulaire, le DMSP est libre (sous forme dissoute, dDMSP) dans le cytoplasme des cellules phytoplanctoniques et peut donc être libéré par divers processus dans le milieu environnant. De très petites quantités de dDMSP sont libérées dans le milieu marin lors de la période de croissance des cellules phytoplanctoniques. En revanche, lors de stress ou de sénescence cellulaire (e.g., action du zooplancton ou infection virale), des quantités non négligeables (i.e., plusieurs dizaines de nM) de dDMSP sont mesurées dans le milieu (Hill et al., 1998 ; Malin et al., 1998 ; Laroche et al., 1999 ; Simo et al., 2002). Une fois libéré dans le milieu environnant, le DMSP est un composé labile qui peut être utilisé par les microorganismes marins tels que les bactéries qui vont alors le transformer en divers composés soufrés réduits.

Le diméthyl sulfure (DMS) est le produit le plus important en concentration, de la dégradation du DMSP (Andreae and Raemdonck, 1983 ; Bates et al., 1987 ; Charlson et al., 1987 ; Turner et al., 1988). Il est issu du clivage enzymatique du DMSP lors de la lyse cellulaire ou de l'attaque bactériale cellulaire (Keller et al., 1989 ; Archer et al., 2002). De plus, des études in *situ* ont prouvé le rôle significatif de certaines familles phytoplanctoniques comme les Haptophycées (Turner et al., 1988 ; Holligan et al., 1987) et les Dinophycées (Holligan et al., 1987) sur la synthèse de DMSP. La distribution du DMSP serait donc davantage corrélée à la spéciation phytoplanctonique (Bates et al., 1985) qu'à la production primaire (Andreae and Barnard, 1984).

Kiene et Taylor (1988) ont également mis en évidence la synthèse d'autres composés soufrés biogéniques, le méthane thiol (MeSH) et le diméthyl disulfure (DMDS) par dégradation bactérienne du DMSP. Le méthane thiol peut également être indirectement produit par une déméthylation du DMS et induire par sa propre déméthylation, une synthèse de sulfure de dihydrogène (H_2S ; Kiene et al., 2002). Une autre voie de formation du DMDS est la dimérisation de deux molécules de MeSH (Gun et al., 2002). A noter que l'H_2S est également produit directement par les cellules phytoplanctoniques marines (Walsh et al., 1994). Mais son origine majeure reste l'hydrolyse du sulfure de carbonyle (OCS ; Elliot et al., 1989 ; Watts, 2000) issu

essentiellement de la dégradation photochimique de composés organo-soufrés (Zepp et Andreae, 1994) et de la dégradation du méthane thiol (Ulshöfer et al., 1996).
Par conséquent, les concentrations des divers composés soufrés (e.g., H_2S, OCS, MeSH, DMS, DMDS) mesurés dans le milieu marin sont intimement liées les unes aux autres et directement corrélées à la concentration en DMSP.

1.2. Interactions entre les CSRV et l'Argent

Dans le milieu marin, la plupart des métaux à sphère molle (e.g., Ag^+, Cd^{2+}, Pb^{2+}) tendent à se complexer avec des bases faibles (Smith et al., 2002) et donc à montrer une réelle affinité pour les composés soufrés tels que le sulfure de dihydrogène et les thiols (e.g., MeSH, DMS ; Stumm and Morgan, 1981).
Concernant l'argent (Ag^+), de nombreuses études ont effectivement démontré les valeurs élevées des constantes de complexation conditionnelles (K') des composés soufrés vis-à-vis de l'ion Ag^+. Martell et Smith (1977) ont mis en évidence de très fortes interactions (i.e., log K′=13) entre l'argent et des composés organo-soufrés. Al-Farawati and Van den Berg (1999) ont déterminé dans le milieu marin (pH 8, 25°C) la constante de complexation conditionnelle entre l'H_2S et Ag^+ (i.e., log K′=11.6) et ont donc mis en évidence de très fortes interactions entre ce métal et l'H_2S. Les constantes de complexation conditionnelles d'autres composés soufrés volatils (i.e., OCS, MeSH, DMS) vis-à-vis de l'argent (Ag^+) ont été déterminées expérimentalement au cours de cette thèse (Cozic et al., 2007a ; Chapitre 4, Article 3). Elles ont permis de conclure à l'existence d'interactions très faibles à nulles entre Ag^+ et OCS (log K′≈0), entre Ag^+ et MeSH (log K′≈0) mais également à leur absence entre Ag^+ et DMS (log K′≈0).

1.3. Problématique

Dans le milieu marin, des métaux peuvent influencer la distribution des espèces phytoplanctoniques mais également leur productivité (Sunda, 1989). En effet, plusieurs métaux comme le fer, le manganèse, le cuivre, le cobalt, le molybdène, le zinc et le nickel, sont considérés comme des micro-nutriments essentiels au métabolisme des cellules phytoplanctoniques (Sunda, 1989 ; Butler, 1998). En revanche, d'autres ions métalliques tels que le plomb, le mercure et l'argent sont des inhibiteurs biologiques et ne possèdent pas de fonction métabolique connue (Sunda, 1989 ; Okamoto et al., 2001). Afin de résister à une contamination métallique environnementale, la cellule

phytoplanctonique a élaboré différents processus de détoxification. Tout d'abord, elle est capable de fixer les ions métalliques sur ses phytochélatines membranaires (Ahner et Morel, 1995 ; Ahner et al., 1997) afin de fixer le métal et de le rendre non-toxique pour son développement physiologique (Sunda et Huntsman, 1998). De plus, le phytoplancton serait capable de synthétiser davantage de phytochélatines et de glutathion lorsqu'il serait soumis à un environnement défavorable (Morelli et Scarano, 1995 ; Kawakami et al., 2006).

C'est donc à partir de ces connaissances concernant *i)* l'induction de la synthèse de composés soufrés organiques par les cellules phytoplanctoniques lorsqu'elles sont soumises à des concentrations en métaux supérieures de plusieurs ordres de grandeur aux concentrations naturelles, et *ii)* les fortes interactions entre le sulfure de dihydrogène et l'argent (Morel, 1983 ; Al-Farawati et Van den Berg, 1999), que se dresse la problématique de ce dernier chapitre. Celle-ci peut se résumer ainsi : le phytoplancton serait-il capable de répondre à une exposition à l'argent par une synthèse supérieure en composés soufrés réduits volatils (CSRV) ?

Afin de résister à cette contamination métallique, le phytoplancton induirait au sein de ses cellules, une production supérieure de thiols (Ahner et al., 1997 ; Sunda et Huntsman, 1998 ; Noctor et Foyer, 1998) et de DMSP. Ce dernier serait alors libéré dans le milieu environnant en quantité plus importante et se transformerait en CSRV (e.g., MeSH, DMS ; Kiene et Taylor, 1988 ; Keller et al., 1989 ; Archer et al., 2002). Le méthane thiol serait ensuite dégradé en sulfure de carbonyle (Flock et Andreae, 1996) qui lui-même s'hydrolyserait en H_2S (Elliot et al., 1989 ; Watts, 2000 ; Figure 5). Par le biais de ces diverses réactions, la concentration en H_2S pourrait donc augmenter dans le milieu environnant ce qui permettrait, en retour, la complexation des ions métalliques ajoutés dans le milieu de culture et donc l'inertage du métal toxique. La concentration en H_2S sera également mesurée avant l'injection de métal afin d'estimer d'éventuelles interactions avec les micro-nutriments composant le milieu de croissance.

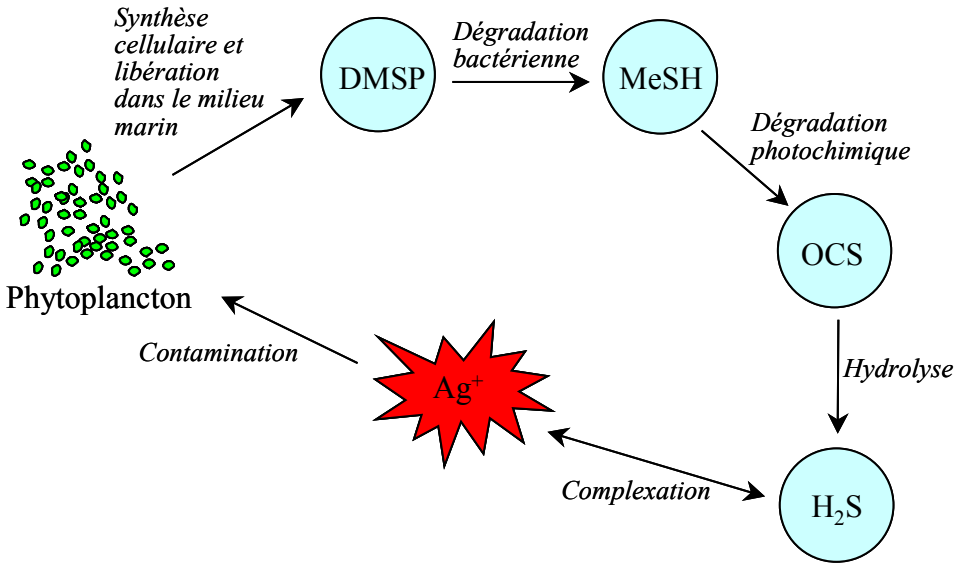

Figure 5 - Problématique de l'expérience : ″le phytoplancton est-il capable de répondre à une exposition à l'argent par une synthèse supérieure en CSRV ? ″

2. Protocole expérimental

Afin de vérifier cette hypothèse, un suivi quotidien (i.e., 25/07 au 18/09) d'une culture phytoplanctonique monospécifique soumise à une exposition à l'argent a été menée en laboratoire. Le protocole expérimental a donc été établi après avoir choisi une espèce phytoplanctonique synthétisant des quantités significatives de DSMP. Un photobioréacteur a ensuite été élaboré pour permettre un suivi continu de la culture tout en contrôlant des paramètres physico-chimiques (e.g., pH, température…). La contamination du milieu de culture, par une concentration croissante d'Ag, a débuté quelques jours après que l'état stationnaire a été atteint par la culture.

2.1. Choix de l'espèce phytoplanctonique

Bien que le DMSP ne soit pas la source exclusive de CSRV (Davies et al., 1976 ; Ferek et Andreae, 1984 ; Walsh et al., 1994 ; Zepp et Andreae, 1994), il est la plus importante en concentration (Matrai et Keller, 1994). C'est pourquoi, nous avons décidé de nous intéresser principalement à cette voie de synthèse des CSRV et par là-même de choisir une espèce phytoplanctonique grande productrice de DMSP. En effet, les teneurs en DMSP varient considérablement d'une espèce phytoplanctonique à une autre. De nombreux auteurs (Holligan et al., 1987 ; Turner et al., 1988) ont démontré que les Haptophycées étaient d'importants producteurs de DMSP. Cette classa

phytoplanctonique compte pour environ 45% de la biomasse algale aux latitudes moyennes (Lee, 1980).

Par ailleurs et pour des raisons pratiques, l'espèce phytoplanctonique cultivée doit atteindre son état d'équilibre rapidement, être suffisamment résistante pour accepter une modification régulière de son milieu, et si possible non toxique pour le manipulateur. *Isochrysis galbana affinis Tahiti* (Haptophycée ; Tableau 3) a donc été choisie pour cette expérience car elle possède toutes les propriétés requises et est étudiée depuis plusieurs années par le laboratoire Physiologie et Biotechnologie des Algues d'Ifremer Nantes (Bougaran et al., 2003).

C'est une micro-algue unicellulaire marine, brune (Yap et al., 2004) et solitaire. Son diamètre est de l'ordre de 1 µm et elle est communément appelée T-Iso. Elle possède également deux flagelles de dimension inégale lui permettant de se déplacer très rapidement dans le milieu marin (Figure 6).

Tableau 3 - Systématique de *Isochrysis galbana affinis Tahiti* (Haptophycée).

Règne	Végétal
Division	Haptophyta
Classe	Haptophyceae
Ordre	Isochrysidales
Famille	Isochrysidaceae
Genre	Isochrysis
Espèce	*Isochrysis galbana affinis Tahiti*

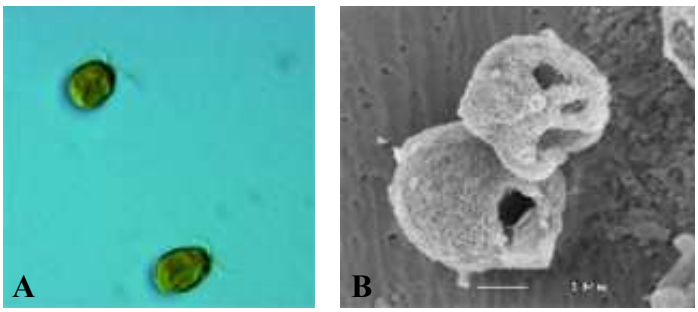

Figure 6 - Cellule de *Isochrysis galbana affinis Tahiti* (Haptophycée), A, en microscopie optique (x100), B, en microscopie électronique.

2.2. Mise en place d'un photobioréacteur (PBR)

Le choix d'une culture en continu s'est rapidement imposée en raison des nombreux avantages qu'elle offre par rapport à une culture en série (e.g., série d'erlenmeyer). Tout d'abord, elle permet de maintenir une culture en phase stationnaire et le phytoplancton en phase exponentielle permanente (i.e., turnover de $1j^{-1}$). De plus, le suivi des densité cellulaire et densité optique permet de déceler toute modification de la physiologie des algues liée à un changement expérimental (i.e., exposition métallique). Par ailleurs, une culture en continu dans un photobioréacteur, relativement étanche aux gaz, contrôle les échanges avec le milieu extérieur et donc empêche tout perte de composés volatils contenus dans le milieu de culture.

Divers paramètres ont guidé l'élaboration puis la construction du photobioréacteur. Tout d'abord, le choix d'un photobioréacteur (PBR) de type torique est lié au fait que la répartition du rayonnement lumineux (i.e., 110 µmol photons. s^{-1}. m^{-2}) doit se faire sur la surface de l'appareil (Csôgôr et al., 1999) et emprunter un trajet optique constant afin d'assurer une croissance optimale de la culture phytoplanctonique. Par ailleurs, pour éviter toutes interactions avec les composés soufrés qui seront synthétisés, tous les éléments en contact avec la culture phytoplanctonique sont non-métalliques (e.g., Téflon®, PMMA…). Le PBR est constitué d'un assemblage de deux pièces en polyméthyle de méthacrylate (PMMA, Plexiglas® ; Figure 7) ; ses dimensions sont les suivantes : 36 cm de long, 38 cm de haut et 8 cm d'épaisseur pour un volume de 1.1 L.

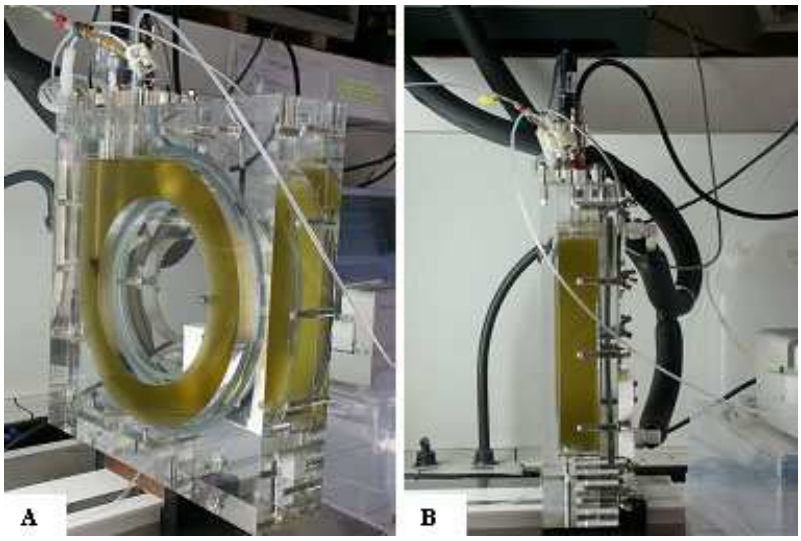

Figure 7 - A, Vue de ¾ arrière du PBR ; B, Vue de profil du PBR

Le PMMA absorbe les ultraviolets, la lumière visible et les infrarouges (Bougaran, comm. pers. ; Hahn et al., 1999 ; Figure 8).

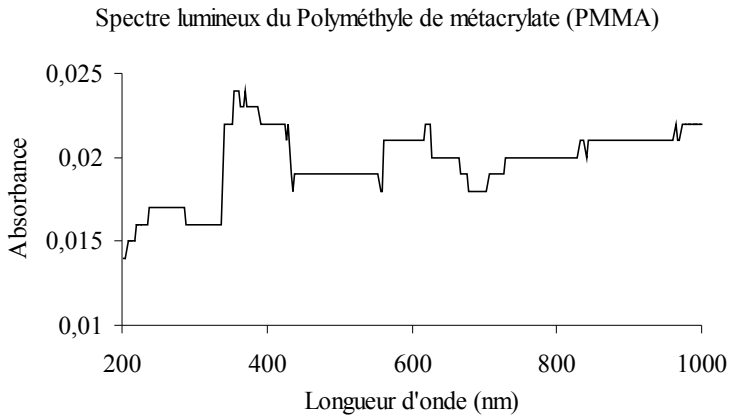

Figure 8 – Spectre lumineux du PMMA.

Pour éviter toute perte de composés soufrés volatils et l'entrée de contaminants (e.g., gaz, bactéries…), l'assemblage du PBR se fait avec des joints toriques. L'expérience se déroulant à l'état d'équilibre dynamique, les fuites de gaz éventuelles sont maintenues constantes ; ceci permet de suivre les variations des concentrations en CSRV au cours du temps. Afin de connecter divers éléments (e.g., sonde pH, arrivée de CO_2, hélice…), plusieurs ports sont prévus sur la partie supérieure du PBR (Figure 9). Le prélèvement de la culture (i.e., dosage des CSRV et Ag) se fait dans la partie inférieure du PBR par un tube Téflon® connecté à un robinet trois voies (LuerLock®). Il n'y a donc aucun échange avec l'air ambiant ce qui permet d'éviter toute contamination du prélèvement.

La température du milieu est régulée par un flux d'eau déionisée et thermostatée (i.e., env. 27°C ; Huber®) qui circule contre les parois du PBR et permet donc une atténuation des fluctuations quotidiennes de la température.

Dans le milieu marin, un équilibre carbonaté a tendance à s'établir (Sigg et al. 1992) :

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \quad (1)$$

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad pK_1=6.1 \quad (2)$$

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad pK_2=9.3 \quad (3)$$

Comme le milieu marin possède un pH de 8.2 (25°C, 35 de salinité ; Morel, 1983), c'est la forme bicarbonate (HCO_3^-) qui prédomine. De plus, lors de la photosynthèse, le phytoplancton consomme le CO_2 dissous pour former de la matière organique (équation 4 ; Ricklefs E. et Miller G.L., 2005).

$HCO_3^- + H^+ \Leftrightarrow CH_2O + O_2$ (4)

Il en résulte une augmentation du pH du milieu de culture par un déplacement de l'équilibre carbonaté et une consommation de protons. Afin de maintenir la concentration d'ions bicarbonate constante, une électrovanne (Figure 5) s'ouvre lorsque le pH augmente dans le milieu de culture, et remplace l'injection d'air par une injection de CO_2 gazeux jusqu'à ce que l'équilibre souhaité (et son pH) soit retrouvé.

Ces paramètres physico-chimiques (i.e., T°C, pH) sont contrôlés et enregistrés en permanence par un ordinateur équipé d'un logiciel spécifique développé par Visual Basic (Exploralgue®).

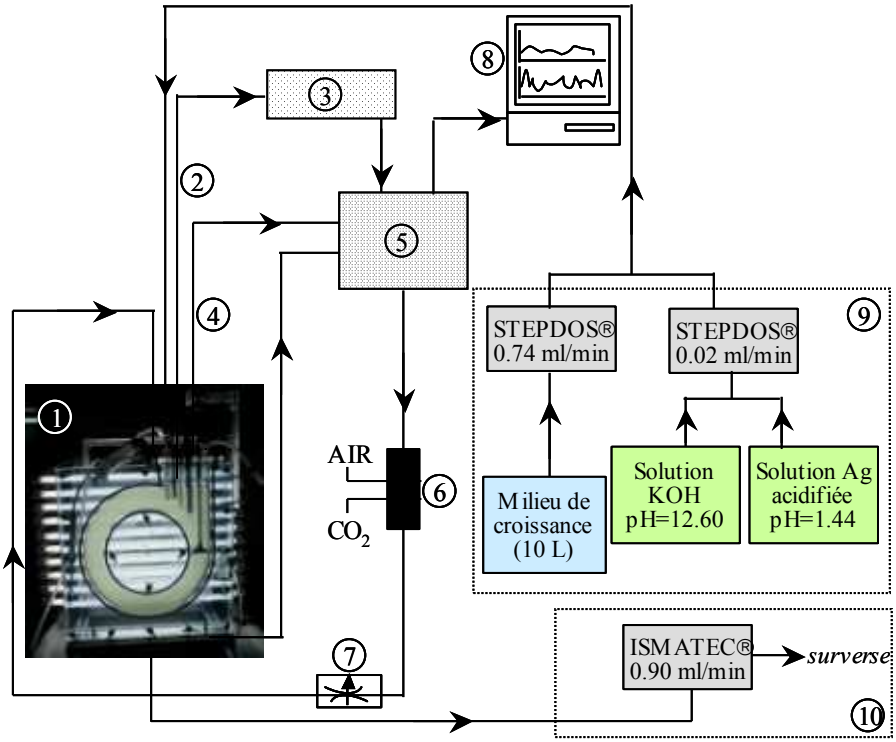

Figure 9 - Schéma du montage lors d'une expérience d'exposition métallique : 1, Photobioréacteur ; 2, sonde pH ; 3, pH-mètre ; 4, sonde température ; 5, système d'acquisition de données ; 6, électrovanne ; 7, régulateur du flux de gaz ; 8, traitement des données ; 9, milieu de croissance et système de contamination métallique ; 10, système d'évacuation de la surverse.

Le PBR est placé dans le laboratoire à l'abri de la lumière extérieure. L'intensité lumineuse est apportée à la culture par dix tubes fluorescents (13W/954, Osram). Le système est adapté à un flux continu de culture par l'ajout d'une pompe diaphragmatique KNF STEPDOS® qui apportent des nutriments (i.e., milieu de

croissance, 0.74 ml/min) en permanence et d'une pompe péristaltique ISMATEC® qui évacue la surverse (0.90 ml/min ; Figure 9).

2.3. Conditions de culture

Le milieu de croissance est préparé à partir d'eau marine filtrée (0.22 µm ; origine : St Malo, France) et enrichie de milieu de Conway (1ml.L^{-1} ; Walne, 1966) composé d'un mélange de sels nutritifs, vitamines et métaux (Tableau 4).

Tableau 4 - Composition du milieu de Conway utilisé pour la croissance de T-Iso (1 ml par litre d'eau de mer filtrée).

Elément	Concentration (g.L^{-1})
$MnCl_2$, $4H_2O$	0.36
H_3BO_3	33.60
Na_2-EDTA, $2H_2O$	49.80
NaH_2 PO_4, $2H_2O$	26.00
$NaNO_3$	100.00
$FeCl_3$, $6H_2O$	1.30
$ZnCl_2$	0.21
$CoCl_2$, $6H_2O$	0.20
$(NH_4)_6$ Mo_7 $(O_2)_4$, $4H_2O$	0.09
$CuSO_4$, $5H_2O$	0.20
B1 (Thiamine)	0.20
B12 (Cyanocobalamine)	0.01

Afin de préserver ses qualités nutritionnelles et d'éviter la contamination, le milieu de croissance (10L) est conservé dans une poche hermétique, stérile et inerte à tout métal (en film HyQ®CX5-14, Labtainer Bioprocess Container, Hyclone™). Le PBR et tous les éléments de connexion (e.g., tubes Téflon®, vis de fixation…) sont stérilisés à l'acide péroxyacétique 0.5%v/v pendant 20 min puis rincés trois fois à l'eau déionisée. Afin d'obtenir une croissance optimale de T-Iso, la température du milieu est régulée à 27±0.5°C et l'intensité lumineuse est maintenue constante (i.e., 110±10 µmol phot. $m^{-2}.s^{-1}$; Bougaran et al., 2003). Afin d'optimiser la croissance de T-Iso, le pH est fixé à 7.2±0.05 (Bougaran et al., 2003). Les gaz (CO_2 et air) sont filtrés à travers une membrane filtrante Téflon® (0.22 µm) et amenés dans le PBR avec un flux de 0.2±0.05 $L.min^{-1}$ (Bougaran et al., 2003). La vitesse de rotation de l'hélice d'agitation doit être

fixée à la valeur minimale permettant à la fois d'éviter du dépôt phytoplanctonique sur les parois et la destruction des cellules ; elle est régulée à 120 tours.min^{-1}.

L'inoculum est extrait d'une culture de T-Iso produite en continu et ayant atteint l'état stationnaire. La culture est lancée le 25/07/06 avec une densité cellulaire initiale dans le PBR, d'environ $1.50x10^6$ cellules/ml. La densité cellulaire est suivie quotidiennement par un comptage cellulaire (cellule de Malassez) après fixation au Lugol, à l'aide d'un logiciel d'analyse d'image (Samba Technologies™). La déviation standard relative (RSD) de cette méthode est inférieure à 6% (Bouragan et al., 2003). La densité optique est également mesurée à 680 nm de longueur d'onde (Sotin, 2006) avec un spectrophotomètre Milton Roy™ (Spectronic 401 ; cuve en quartz).

2.4. Injection de métal dans le système

La problématique repose sur l'injection d'une solution d'argent de concentration croissante dans la culture de T-Iso. Pour déterminer l'éventuelle capacité du phytoplancton à répondre à cette modification environnementale par une synthèse supérieure en composés soufrés réduits volatils (CSRV). Les concentrations en CSRV (i.e., H_2S, OCS, MeSH, DMS et DMDS) sont mesurées très régulièrement lors de la période d'expérimentation, en parallèle de la densité cellulaire et de la densité optique. Les concentrations en composés soufrés volatils (i.e., libres) sont déterminées par chromatographie gazeuse couplée à un système ″purge and trap″ (Chapitre 2 ; Cozic et al., 2007b). Ces mesures de concentrations en CSRV seront menées sur des échantillons (triplicats) non filtrés et sur des échantillons filtrés (filtre Téflon® PTFE 0.45 µm). La pression exercée lors de la filtration (i.e., <0.1 kg.cm^{-2}) devrait être suffisamment douce pour éviter un écrasement des cellules et une libération de matériel biologique.

Dans l'environnement côtier, la concentration en argent est généralement très inférieure à la nanomole. Par exemple, Smith et Flegal (1993) ont estimé que la concentration en Ag^+ comprise entre 0.01 et 0.25 nM dans la baie de San Francisco et d'environ 0.003 nM dans les eaux côtières adjacentes. Munoz-Barbosa et al. (2000) ont mesuré des teneurs de 0.5 à 14.3 nM dans la région côtière de Baja California (Mexique). Aussi, afin de déterminer l'effet sur la culture phytoplanctonique d'une exposition à l'argent, la concentration au cours de l'expérience sera comprise entre 0 et 10 nM et sera régulièrement augmentée (i.e., après stabilisation de la densité cellulaire).

Pour éviter une adsorption des ions Ag^+ sur les parois de la poche contenant la solution métallique, il est indispensable d'acidifier la solution métallique (i.e., pH≈1.4). Le choix de l'acide sulfurique s'est rapidement imposé comme le plus approprié. En effet, l'acide nitrique ne pouvait être utilisé au risque de modifier le milieu de croissance artificiel (Tableau 2) contenant une concentration significative en ions NO_3^-. L'acide chlorhydrique n'a également pas été choisi puisqu'en présence d'ions chlorures dans des solutions aqueuses de faible force ionique (e.g., solution d'eau distillée contenue dans la poche "Ag"), les ions Ag^+ forment instantanément des précipités $AgCl_s$ (Luoma et al., 1995). Comme le pH dans le photobioréacteur a alors tendance à diminuer très fortement, une solution basique (KOH, pH=12.60, exempt de traces d'argent) est simultanément injectée dans le PBR. La solution d'Ag acidifiée et la solution de base forte sont conservées dans des poches identiques à celle du milieu de croissance (Labtainer Bioprocess Container, Hyclone™). Le débit de la pompe Stepdos® amenant l'argent et la solution basique est fixé à environ 5% du débit total arrivant dans le PBR ; le débit est donc fixé à 0.02 ml/min (pour un débit total au niveau de la pompe de 0.04 ml/min car mélange de deux solutions). Il est en effet, indispensable de diminuer au maximum ce débit pour éviter une dilution du milieu de culture.

La solution d'argent est préparée à partir d'une solution étalon d'$AgNO_3$ à 1 g/L (9.3 mM, pureté 99.99%, Merk®) diluée dans de l'acide sulfurique (36.4 mM). Dans un premier temps, la solution d'argent est remplacée pendant 4 jours par une solution d'acide sulfurique (pH=1.44 ; 0 nM Ag) afin d'acclimater la culture à ce milieu de croissance légèrement modifié. La contamination débute lorsque les paramètres biologiques (e.g., densité optique, densité cellulaire) sont stabilisés. A l'aide d'une seringue stérile (Codan®), une solution d'Ag est injectée dans le PBR afin d'obtenir une contamination immédiate de la culture phytoplanctonique à la concentration désirée. Puis, la pompe STEPDOS® amenant l'argent et la solution basique est réactivée. La concentration en Ag est ensuite régulièrement augmentée et ce, lorsque la culture a recouvert un état quasi-stationnaire (Tableau 5).

Pendant la durée de l'expérience, 32 prélèvements du milieu de culture ont été menés afin de vérifier si la quantité d'Ag injectée est réellement présente dans le milieu de croissance. Pour cela, 10 ml de milieu ont été prélevés via le robinet 3 voies et immédiatement acidifiés avec du HNO_3 concentré (14.4 M) à raison de 1.5µl/ml. Il s'agit, par cette acidification d'éviter l'adsorption des ions Ag^+ sur les parois du tube. Téflon®. A noter que les jours d'augmentation de la concentration en métal dans le

PBR, le prélèvement se faisait quelques minutes (i.e., env. 10 min) après l'injection d'Ag. De plus, la poche contenant la solution métallique a été pesée avant le début de l'exposition de la culture et à la fin de cette même contamination. Il s'agissait de vérifier que le débit de la poche "argent" restait constant et égal à 0.02 ml/min lors de l'expérience.

Tableau 5 – Exposition métallique de la culture de T-Iso.

[$AgNO_3$] dans la poche	[$AgNO_3$] dans le PBR	Premier jour de contamination
4.72 nM	0.1 nM	13/08/06
23.58 nM	0.5 nM	21/08/06
47.17 nM	1 nM	29/08/06
235.85 nM	5 nM	04/09/06
417.70 nM	10 nM	10/09/06

3. Résultats

3.1. Densité cellulaire et densité optique

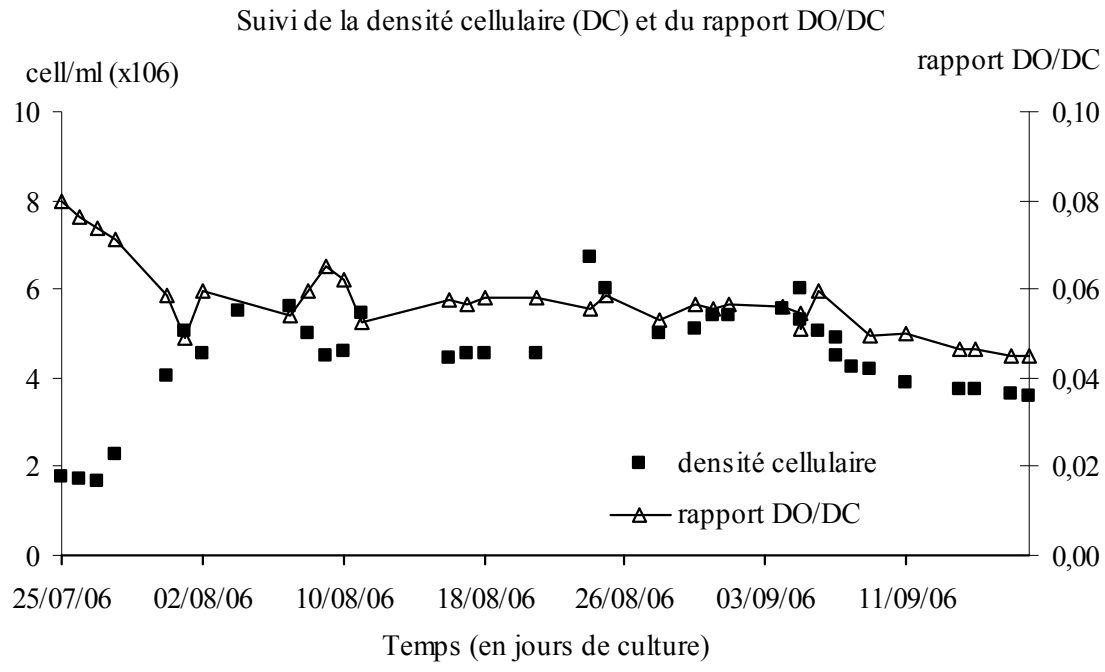

Figure 10 - Evolution de la densité cellulaire et du rapport densité optique/densité cellulaire.

Le suivi du rapport de la densité optique sur la densité cellulaire met en évidence l'état stationnaire de la culture entre le 04/08 et le 05/09 (0.06, n=18) succédant à la phase de croissance cellulaire (Figure 10). Le phytoplancton est alors en phase exponentielle de développement (i.e., turnover de $1j^{-1}$; Bougaran et al., 2003). En fin d'expérience, le rapport tend à diminuer d'environ 25% entre le 06/09 et le 18/09.

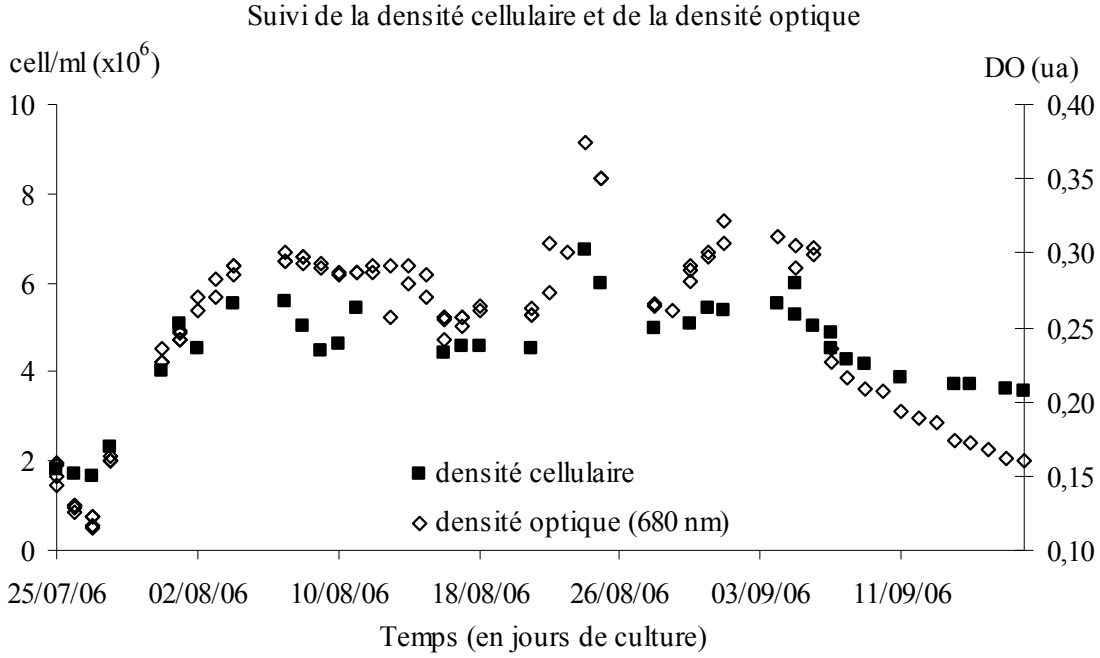

Figure 11 - Evolution de la densité cellulaire et de la densité optique de la culture de T-Iso (25/07-18/09/06).

La culture atteint donc son état stationnaire le 04/08, avec $5.5x10^6$ cell/ml soit une augmentation de 309% en 10 jours (Figure 11) alors que la densité optique (DO) montre une nette diminution (-21.7%) lors des deux premiers jours de culture (25-27/07). Les deux variables sont relativement constantes entre le 04/08 et le 15/08 avec respectivement $(5.6\pm0.2)x10^6$ cell/ml (n=6) et 0.3 ua (n=16). A noter que la densité cellulaire montre une courte phase de décroissance du 07/08 au 10/08 ($4.6x10^6$ cell/ml). La densité optique diminue ensuite jusqu'au 18/08 (0.26±0.07 ua ; n=6) alors que la densité cellulaire reste faible et constante ($(4.5\pm0.1)x10^6$ cell/ml ; n=4) du 16/08 au 20/08. Une rapide phase d'augmentation est ensuite observée jusqu'au 24/08 où les maxima des densités cellulaire (DC) et optique sont mesurés. Ils sont respectivement égaux à $6.7x10^6$ cell/ml et 0.37 ua. Puis, la DC diminue pour atteindre $5.0x10^6$ cell/ml le 28/08, avant de se stabiliser jusqu'au 04/09 $(5.3\pm0.2)x10^6$ cell/ml ; n=5). La DO

montre une dernière phase d'augmentation entre le 28/08 et le 01/09 où elle atteint 0.31±0.01 ua (n=2 ; Figure 11). A partir du 04/09, la DC diminue progressivement : $4.23x10^6$ cell/ml sont mesurées le 08/09 et $3.9x10^6$ cell/ml trois jours plus tard. Un dernier état stationnaire est observé entre le 14/09 et le 18/09 avec $(3.7±0.1)x10^6$ cell/ml (n=4). La DO montre, quant à elle, une nette diminution de la DO dès le 08/09 pour atteindre 0.16 ua le 18/09.

3.2. Vérification par ICP-MS de la concentration en argent dans le PBR

Afin de tester la possible adsorption d'argent sur le photobioréacteur (en PMMA), des prélèvements réguliers (n=32) du milieu de culture ont permis de doser *a posteriori* l'argent total réellement présent dans le PBR.

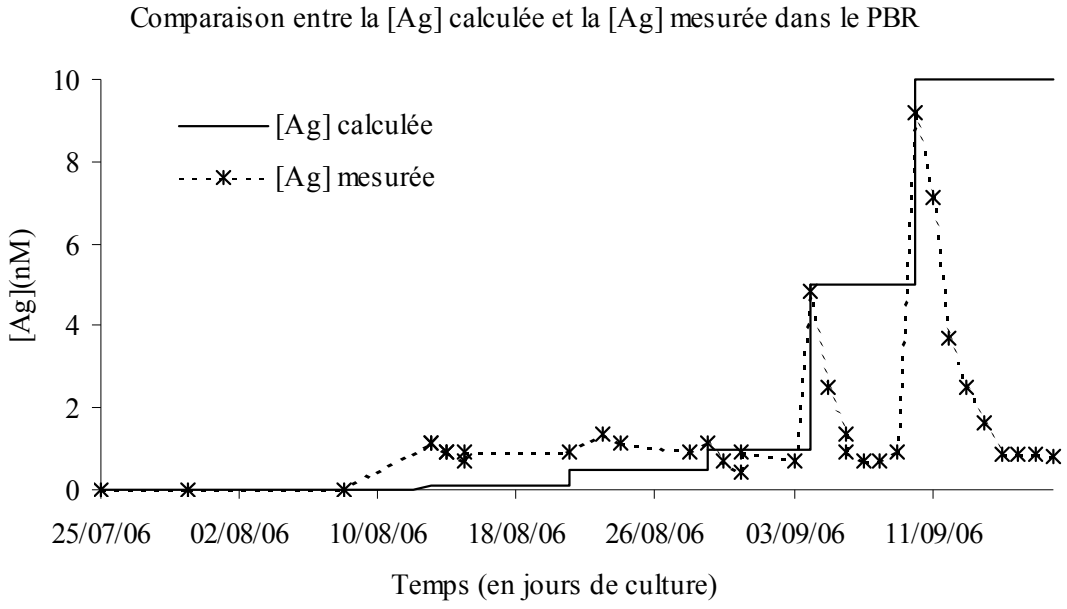

Figure 12 - Comparaison entre la concentration calculée en Ag et la concentration en Ag observée dans le PBR.

De cette étude, il resort que d'importants écarts existent entre la concentration prévue en métal et la concentration observée dans le PBR (Figure 12). En l'absence d'Ag dans le PBR, il n'existe aucune différence entre la concentration souhaitée et la concentration mesurée (n=3). En revanche, pour 0.1 et 0.5 nM de métal ajouté, des concentrations supérieures sont mesurées par ICP-MS. Pour une exposition à 0.1 nM

(13/08), la teneur moyenne en métal mesurée est égale à 0.9±0.2 nM (n=6) soit presque 10 fois supérieure à celle désirée. Lorsque 0.5 nM d'Ag est ajoutée dans le PBR (21/09), le double soit 1.1±0.2 nM (n=4), est mesuré par ICP-MS. Pour des concentrations plus élevées calculées (i.e., 1 à 10 nM), les valeurs mesurées par ICP-MS restent inférieures. En effet, pour une exposition à 1 nM d'Ag (29/08), seule 0.7±0.19 nM (n=4) est analysée ; 30% du métal ont donc été adsorbés (Figure 12). Un phénomène particulier est observé pour des ajouts de 5 (04/09) et 10 nM (10/09). En effet, l'échantillon correspondant au premier jour d'exposition ne montre pas de différence significative avec la quantité désirée en Ag et injectée dans le PBR (respectivement, 4.8 nM et 9.2 nM). Cependant, dès le second jour suivant ces ajouts, une nette diminution de la concentration d'argent présent dans le PBR est mise en évidence : -47.7% (i.e., 2.5 nM) pour une exposition à 5 nM et –22.5% (i.e., 7.1 nM) pour une exposition à 10 nM. Cette baisse se poursuit pour atteindre en fin d'expérience, respectivement 0.9 nM (soit –80.9%) le 09/09 et 0.8 nM (soit –90.9%) le 18/09 (Figure 12).

En résumé, quelle que soit la concentration en argent désirée dans le PBR, les mesures de métal dans les échantillons par ICP-MS donnent une teneur en Ag de l'ordre de la nanomole excepté lors des premiers jours d'exposition à 5 nM (04/09) et 10 nM (10/09) où concentration calculée et concentration mesurée sont identiques (i.e., 4.8 nM et 9.2 nM). On peut donc considérer que la concentration en Ag dans le PBR est restée quasiment la même au cours de l'expérience (i.e., env. 1 nM) exceptée le 04-05/09 et le 10-14/09.

3.3. Composés Soufrés Réduits Volatils (CSRV)

Le suivi des concentrations en composés soufrés débute le 07/08 lorsque la phase stationnaire est atteinte par la culture phytoplanctonique. En effet, dans un premier temps, il s'agit de suivre l'évolution des CSRV en l'absence d'ajout métallique puis de déterminer les effets possibles d'une exposition croissante à l'argent sur la synthèse de composés soufrés.

L'analyse concomitante d'échantillons non filtrés et d'échantillons filtrés pour chaque prélèvement va permettre de différencier la teneur totale en CSRV de celle en phase dissoute. Cependant, les concentrations en CSRV dissous sont fréquemment supérieures aux concentrations totales (Figure 13). Ce résultat suggère que la pression

exercée lors de filtration a été trop puissante (Sunda et al., 2002). Celle-ci entraînerait alors une libération des CSRV contenus dans les cellules phytoplanctoniques et par là-même induirait une augmentation des concentrations en CSRV dissous. Les données concernant les concentrations en CSRV dissous ne seront donc pas détaillées dans ce chapitre.

3.3.1. Sulfure de dihydrogène (H_2S)

Avant l'injection de métal (13/08/06), la concentration en H_2S reste relativement constante (0.20±0.06 nM (n=5) d'H_2S dissous ; Figure 13A). La présence de micro-nutriments n'influence donc pas la production d'H_2S.

De plus, il existe une variation du pourcentage d'H_2S dissous au cours du temps mais l'écart reste faible entre H_2S dissous et H_2S total (i.e., <25%) excepté entre le 22/08 et le 01/09 où l'H_2S dissous correspond à environ 50% et 66% de l'H_2S total. Le sulfure d'hydrogène est donc essentiellement sous forme dissoute dans le milieu de culture et cette proportion reste relativement constante.

La concentration en H_2S total est relativement stable entre le 08/08 et le 19/08 avec 0.19±0.02 nM (n=8). Une courte phase d'augmentation est observée entre le 21/08 et le 24/08 avec une concentration maximale enregistrée le 22/08 (0.66±0.27 nM ; n=3). Dès le 23/08 et jusqu'au 01/09, une diminution de la concentration est mise en évidence avec respectivement 0.46±0.11 nM (n=7) et 0.16±0.01 nM (n=3). La fin de l'expérience (06/09-18/09) est marquée par une stabilisation de la concentration en sulfure de dihydrogène (0.20±0.02 nM, n=18).

3.3.2. Sulfure de carbonyle (OCS)

La concentration maximale est observée le premier jour avec 1.02±0.02 nM (n=3) d'OCS total (Figure 13B). Il s'ensuit une rapide diminution jusqu'au 19/08 où 0.06 nM (n=3) est mesurée. Comme pour le sulfure d'hydrogène, une augmentation est observée le 22/08 avec 0.46±0.23 nM (n=3). Une phase de diminution progressive enregistrée entre le 24/08 (0.24±0.04 nM, n=3) et le 04/09 (0.15±0.01 nM, n=3) précède une période de stabilité du 05/09 au dernier jour de l'expérience (18/09) avec 0.17±0.01 nM (n=15) d'OCS total mesuré.

3.3.3. Méthane thiol (MeSH)

La concentration en méthane thiol montre de nombreuses fluctuations entre le 07/08 et le 18/09 avec de rapides augmentations les 16/08, 22/08 et 29/08 (Figure 13C). La

concentration en MeSH total est alors respectivement égale à 1.05±0.76 nM (n=3), 2.02±0.77 nM (n=3) et 0.34±0.24 nM (n=3). La première augmentation (16/08) succède une phase stationnaire avec 0.37±0.012 nM (n=3) de MeSH total et précède une diminution puisque le 17/08, 1.03±0.64 nM (n=3) de MeSH total est mesurée. La même tendance est observée après la seconde rapide augmentation (22/08) avec 8 fois moins (0.24±0.042 nM ; n=3) de MeSH total analysé le 23/08. La dernière augmentation (29/08) précède également une phase de diminution de la concentration qui tend à devenir constante jusqu'au 18/09 (0.39±0.12 nM, n=18).

3.3.4. Diméthyl sulfure (DMS)

Comme le méthane thiol, le DMS montre une concentration en dissous souvent supérieure à la concentration totale analysée : cette tendance est observée du 08/08 au 16/08 et du 24/08 au 18/09 (Figure 13D). Ce résultat suggère donc une pression trop importante exercée lors de la filtration des échantillons.

La concentration du DMS montre globalement trois phases entre le 07/08 et le 18/09 : une augmentation jusqu'au 22/08 pour le DMS total, une diminution progressive qui s'atténue le 04/09 suivie d'une ultime phase stationnaire (Figure 13D). Entre le 07/08 et le 24/08, la concentration en DMS total passe de 6.44±0.53 nM (n=3) à 11.29±0.97 nM (n=3) avec un maximum atteint le 22/08 (14.72±2.92 nM ; n=3). La diminution débute le 28/08, avec 9.90±0.97 nM (n=3) de DMS total pour se terminer le 04/09, avec 9.09±0.21 nM (n=3) de DMS total. Jusqu'à la fin de l'expérience, la teneur en DMS total reste relativement stable (5.41±0.05 nM, n=9).

3.3.5. Diméthyl disulfure (DMDS)

L'évolution de la concentration en DMDS total montre 4 périodes distinctes (Figure 13E). Tout d'abord, tout comme le DMS, une phase d'augmentation est observée entre le 07/08 et le 11/08 (+0.07 nM). Il s'ensuit une très lente diminution avec une valeur minimale atteinte le 01/09 (0.36±0.38 nM, n=3). La troisième phase est une nouvelle augmentation toute aussi rapide que celle de la concentration en DMS. La valeur maximale en DMDS total est mesurée le 08/09 et est égale à 0.70±0.99 nM (n=3). A partir du 09/09, la concentration en DMDS total reste stable (0.74±0.043 nM ; n=10) alors que celle en DMDS dissous entame une diminution progressive avec 0.37±0.01 nM (n=3) analysée le 18/09.

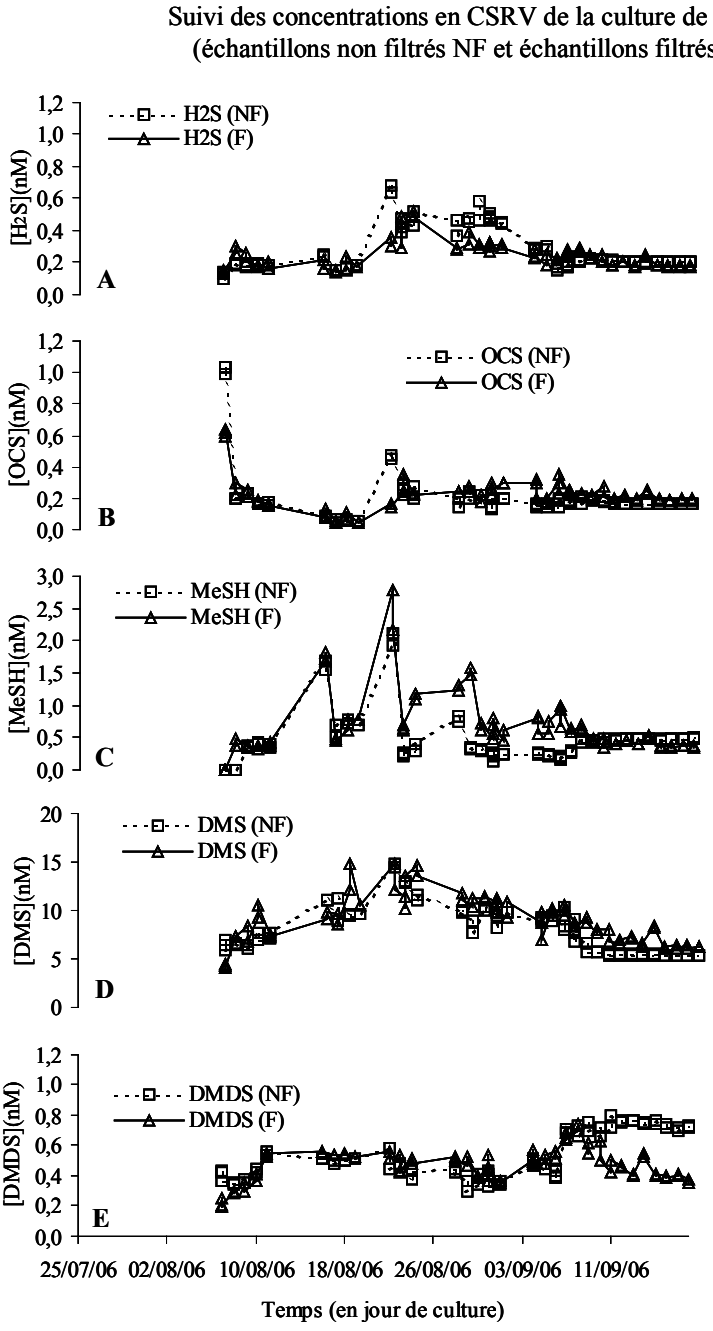

Figure 13 - Evolution des concentrations en composés soufrés réduits volatils (CSRV) (échantillons non filtrés et filtrés). A, H_2S ; B, OCS ; C, MeSH ; D, DMS ; E, DMDS.

4. Discussion

4.1. Concentration en Argent dans le photobioréacteur

Lors de l'expérience de contamination, la concentration en métal dans le PBR reste quasi-constante, de l'ordre de la nanomole par litre alors que la concentration dans la poche hermétique et stérile est augmentée de manière contrôlée. Des mesures d'Ag à partir d'échantillons (3 par périodes d'exposition ; duplicats) prélevés directement dans la poche contenant le métal permettent d'exclure une perte de métal à ce niveau. De plus, la vérification du débit de la poche permet également d'exclure une dilution de la solution métallique liée à une diminution du débit au cours de l'expérience. Le débit de 0.02 ml/min est conservé tout au long de l'expérience (i.e., 07/08-18/09).

La perte de métal se produit donc en aval de la poche contenant le métal toxique par une adsorption soit au niveau des connexions entre la poche et le PBR soit au sein du PBR lui-même (i.e., adsorption sur les parois du PBR ou/et complexation par des métaux présents dans le milieu de culture). Il est peu probable que les ions Ag^+ s'adsorbent sur les tubes connectifs entre la poche et le PBR en raison de l'acidification de la solution métallique qui empêche toute adsorption sur le Téflon®. L'adsorption de l'Ag pourrait donc avoir lieu au niveau des parois PBR ou alors via une complexation métallique, dans le milieu de culture. L'hypothèse d'une fixation des ions Ag^+ par les cellules phytoplanctoniques peut en effet être exclue puisque la mesure de l'argent s'est faite sur des échantillons non filtrés.

En effet, le milieu de Conway contient plusieurs éléments chimiques capables d'interagir avec les ions Ag^+. Par exemple, les ions chlorures, avec une concentration de 17.8 µM dans le milieu de croissance et une constante de stabilité conditionnelle K′ vis-à-vis des ions Ag^+ élevée (log K′=9.7 ; Morel, 1983) ont tendance à former des précipités avec l'argent ($AgCl_s$). D'autres éléments comme l'EDTA (127.4 µM) et PO_4^{3-} (158.7 µM) présentent également des constantes de stabilité conditionnelle vis-à-vis des ions Ag^+ significatives : log K′(Ag^+)EDTA=14.9 et log K′(Ag^+)PO_4^{3-}=17.6. Ces éléments pourraient donc également induire une diminution significative de la concentration en Ag libre dans le milieu de croissance. Cependant, les complexes formés entre Ag^+ et l'EDTA et entre Ag^+ et PO_4^{3-} sont neutres et donc rendent possibles la mesure de cet argent complexé par ICP-MS. Par ailleurs, cette hypothèse de complexation de tous les ions Ag^+ par des éléments chimiques, essentiellement par

les chlorures, n'est pas supportée par les mesures d'argent dissous menées dans divers environnements marins. En effet, des concentrations de l'ordre de quelques nM sont mesurées malgré la présence des ions chlorures (0.56 M ; Squire et al., 2002 ; Chiffoleau et al., 2005). De plus, le phénomène de perte d'argent dans le PBR est progressif (e.g., après ajout de 5 nM) et donc n'appuie pas l'hypothèse d'une complexation de tous les Ag^+ par des composés chimiques tels que Cl^- et PO_4^{3-}, présents en permanence dans le milieu de culture.

Par conséquent, l'hypothèse la plus plausible pour expliquer une diminution de la concentration en argent dans le PBR par rapport à la quantité présente dans la poche, est une fixation du métal sur les parois du PBR. A l'heure actuelle, aucune expérience n'a été conduite pour examiner si le polyméthyle de méthacrylate (PMMA) était effectivement capable de fixer les ions Ag^+ de manière irréversible, et par là-même d'en empêcher la détection par ICP-MS. Si cette hypothèse est vérifiée, il faudra alors changer de matériau pour réitérer les expériences d'exposition métallique sur une culture phytoplanctonique.

4.2. Variations des concentrations en CSRV au cours de l'expérience

Avec une concentration en argent relativement stable (i.e., env. 1 nM) au cours de l'expérience (exceptée les 04/09 et 10/09 où 4.8 nM et 9.2 nM sont mesurées) (Figure 12), les variations des concentrations en CSRV ne sont ne sont donc pas explicables par une exposition métallique croissante. La densité cellulaire et la densité optique montrent toutefois des variations temporelles imputables à une modification de l'état physiologique de la culture phytoplanctonique. On se propose donc de vérifier si un lien significatif existe entre l'évolution des concentrations en CSRV et celle des paramètres phytoplanctoniques (i.e., densité cellulaire et densité optique) mais également de vérifier l'absence d'interactions directes entre le métal et ces CSRV. En effet, Cozic et al. (2007a) ont déterminé des constantes de stabilité conditionnelle très faibles entre Ag et certains CSRV puisque $\log K'(Ag^+)OCS \approx 0$, $\log K'(Ag^+)MeSH \approx 0$ et $\log K'(Ag^+)DMS \approx 0$. Ces résultats tendent donc à exclure toute interaction significative de ces CSRV avec l'argent. Seul le sulfure de dihydrogène avec un $\log K' \approx 12$ (Morel, 1983 ; Al-Farawati and Van den Berg, 1999) peut être considéré comme un ligand significatif des ions Ag^+.

4.2.1. Sulfure de carbonyle

Le sulfure de carbonyle ne montre pas de réelles variations de sa concentration au cours de l'expérience mis à part le 22/08 où une nette augmentation est observée : 0.46±0.23 nM (n=3) soit +0.40 nM en 3 jours d'OCS total (Figure 13B). La densité cellulaire et la densité optique montrent également une nette augmentation avec respectivement +44% et +42% entre le 21/08 et le 24/08 (Figure 11). Par ailleurs, lorsqu'on étudie l'évolution de la concentration en OCS à la fin de l'expérience (i.e., après le 04/09), on constate qu'elle se stabilise. Les importants ajouts d'Ag détectés les 04/09 (4.82 nM) et 10/09 (9.17 nM) n'induisent pas de modification de la concentration en OCS (Figure 13B) ce qui est cohérent avec l'absence d'interactions établie par la valeur de la constante de stabilité conditionnelle (Cozic et al., 2007a). En revanche, la diminution de la densité cellulaire observée dès le 04/09 (mesure faite quelques minutes après l'injection de métal) pourrait s'expliquer par l'augmentation rapide de la concentration en Ag dans le milieu de culture. La culture réagirait à cette exposition d'Ag (4.8 nM) et la diminution progressive de la densité optique indique clairement une modification de l'état physiologique de la culture phytoplanctonique (Figure 11). L'augmentation instantanée de la teneur en métal (le 10/09) n'induit pas une diminution plus rapide des densité cellulaire et densité optique ce qui prouve que la réponse du phytoplancton à l'exposition métallique s'est produite dès l'ajout de 4.8 nM d'argent. En revanche, la concentration en OCS reste stable jusqu'à la fin de l'expérience ce qui est cohérent avec une synthèse permanente de ce CSRV liée à la dégradation photochimique de composés organo-soufrés biogènes (Zepp and Andreae, 1994) présents dans le milieu de culture.

4.2.2. Méthane thiol

La concentration en MeSH montre trois augmentations rapides les 16/08, 22/08 et 29/08 (Figure 13C). La contamination métallique restant constante (1.0±0.2 nM, n=11) entre le 13/08 et le 29/08 et la constante de stabilité conditionnelle étant si faible (log $K'(Ag^{+})MeSH \approx 0$) que des interactions entre le métal et le CSRV sont probablement non significatives. De plus, lorsque les pics de métal sont mesurés (i.e., 04/09 et 10/09), aucune augmentation instantanée de la concentration en MeSH n'est enregistrée. Les variations de MeSH seraient donc davantage liées à sa synthèse, plus ou moins importante, à partir du DMSP libéré par les cellules phytoplanctoniques (Kiene and Taylor, 1988) mais également à une dégradation variable du DMS (Kiene et al. 2002). Par exemple, l'augmentation de la concentration en MeSH est

observée le 16/08 lorsque la densité cellulaire et la densité optique diminuent, suggérant des modifications physiologiques du phytoplancton (e.g., stress). Il pourrait donc y a une libération plus importante de DMSP dans le milieu de culture (Malin et al., 1998 ; Simo et al., 2002) et donc une augmentation de la synthèse de MeSH (Kiene et Taylor, 1988). Les concentrations maximales en méthane thiol dissous (i.e.,2.49±1.62 nM, n=3) et diméthyl sulfure dissous (i.e., 13.52±1.88 nM, n=3) sont observées le 22/08 ce qui tend à admettre une dégradation de DMS en MeSH (Kiene et al. 2002).

4.2.3. Diméthyl sulfure

Le diméthyle sulfure, principal composé soufré synthétisé par le phytoplancton via le DMSP (Keller et al., 1989), montre une augmentation progressive de sa concentration entre le 07/08 et le 24/08 lorsque la concentration en métal est constante (i.e., env. 1 nM) dans le milieu de culture (Figure 13D, Figure 12). La teneur en DMS commence à diminuer dès le 25/08 alors que la concentration en Ag reste la même ce qui semble indiquer l'absence d'interactions entre ce CSRV et le métal. Cette conclusion est également appuyée par l'évolution de la teneur en DMS à partir du 04/09 ; la concentration reste stable et n'est en rien modifiée par l'ajout de métal les 04/09 et 10/09. La constante de stabilité conditionnelle entre Ag et DMS déterminée par Cozic et al. (2007a) étant très faible (i.e., log $K'(Ag^{+})DMS \approx 0$), il ne devrait pas exister d'interactions significatives entre ces deux éléments chimiques. L'augmentation de la concentration en DMS dans le PBR pourrait s'expliquer par une synthèse permanente à partir du DMSP libéré par les cellules phytoplanctoniques (Keller et al., 1989 ; Archer et al. 2002) et une dégradation en d'autres CSRV (i.e., DMDS, MeSH) (Kiene et Taylor, 1988) plus lente. La diminution de la teneur en DMS enregistrée à partir du 25/08 pourrait être liée soit à une synthèse supérieure de MeSH à partir du ″pool″ de DMSP disponible au détriment de la synthèse de DMS soit à une vitesse de dégradation du DMS plus importante. La stabilisation de la concentration en DMS mesurée à partir du 04/09 pourrait être liée aux changements physiologiques qui s'opèrent chez le phytoplancton (i.e., chute de la densité optique ; Figure 11) et induite par l'exposition à 4.8 nM d'Ag (i.e., stress). Le phytoplancton, stressé par cette exposition métallique, libérerait davantage de DMSP (Matrai et Keller ; 1994) et permettrait ainsi de maintenir une certaine concentration en DMS mais également en MeSH et DMDS dans le milieu environnant (Figures 13C-E). En effet, Il est reconnu que la plupart du DMSP phytoplanctonique peut être libéré lors d'un stress

environnemental (Hill et al., 1998 ; Simo et al., 2002) pour être transformé via des dégradations bactériennes en CSRV (Kiene and Taylor, 1988). Cependant, Yoch et al. (1997) ont montré la présence de DMSP lyases intracellulaires qui pourraient donc expliquer une synthèse de DMS (dissous) à l'intérieur des cellules. Une libération de DMS intracellulaire lors d'un stress oxidatif a également été mise en évidence par Sunda et al. (2002) ce qui appuie une dégradation intracellulaire de DMSP.

4.2.4. Diméthyl disulfure

Le diméthyl disulfure montre, comme le MeSH et le DMS, une augmentation de sa concentration dans les premiers jours de l'expérience avec une concentration en DMDS dissous doublée entre le 07/08 et le 11/08 alors que les concentrations en MeSH et DMS dissous augmentent, respectivement de 0 à 0.39 nM et de 4.26 à 7.47 nM (Figures 13C-D). A la même période, une diminution de la densité cellulaire (Figure 11) est observée ce qui tend à indiquer que le phytoplancton subirait un stress temporaire. Aussi, nos résultats apparaissent-ils en cohérence avec une augmentation de la libération de DMSP observée en période de stress environnemental (Kiene et Taylor, 1988 ; Keller et al., 1989 ; Archer et al. 2002). La concentration en DMDS dissous entame une diminution pour atteindre sa valeur minimale (0.36±0.28 nM, n=3) le 01/09 alors que la densité cellulaire reste stable entre le 28/08 et le 04/09 (Figure 10). Les observations tendent à émettre l'hypothèse que la synthèse de DMDS via la dégradation de DMSP ne se ferait pas préférentiellement entre le 11/08 et le 01/09 et pourrait être supplantée par la synthèse de DMS (Figures 13D-E). Le phénomène inverse (i.e., synthèse de DMDS majoritaire) est mis en évidence du 01/09 au 09/09 avec une augmentation de la concentration en DMDS alors que la concentration en DMS diminue. Au vu de ces résultats, il apparaît donc que la synthèse de DMS et celle de DMDS seraient intimement liées au sein de la culture phytoplanctonique. Un phénomène particulier et actuellement inexpliqué, est enregistré à la fin de l'expérience avec une chute de la concentration en DMDS dissous (i.e., -50% entre le 09/09 et le 18/09) alors que la concentration en DMDS total se stabilise (0.74±0.043 nM ; n=10). La teneur en DMDS particulaire augmenterait donc lorsque la culture phytoplanctonique est affaiblie par l'ajout brutal de métal (4.8 nM, le 04/09).

4.2.5. Sulfure de dihydrogène

Contrairement aux autres CSRV, le sulfure de dihydrogène est considéré comme un ligand fort de l'argent étant donné la valeur de la constante de stabilité conditionnelle (i.e., log $K'(Ag+)H_2S \approx 12$; Morel, 1983 ; Al-Farawati and Van den Berg, 1999). Cependant, la concentration en H_2S total reste stable entre le 08/08 et le 19/08 alors que la concentration en métal passe de 0 à ≈1 nM le 13/08 (Figure 13A, Figure 12). De plus, aucune modification rapide de la teneur en sulfure de dihydrogène total n'est observée lorsque les deux pics d'ajout d'Ag sont mesurés (i.e., 04/08 et 10/09). L'hypothèse envisagée est que les ions Ag^+ non adsorbés sur les parois du PBR, seraient rapidement complexés par des composés chimiques possédant une constante de stabilité conditionnelle supérieure à celle de l'H_2S vis-à-vis du métal ou présents en quantité significativement plus importante que l'H_2S (EDTA, PO_4^{3-}). La nette augmentation observée entre le 20/08 et le 24/08 pourrait être à rapprocher d'une augmentation des densité cellulaire et densité optique (respectivement, +44% et +42%) indiquant une hausse de la croissance phytoplanctonique. En effet, Walsh et al. (1994) ont mis en évidence une augmentation simultanée de la densité phytoplanctonique et de la concentration en H_2S en réponse à un stress métallique. Aussi, même si la concentration en métal reste constante (i.e., ≈1 nM) dans le milieu, le phytoplancton semble être affecté par cette contamination. La diminution de la teneur en sulfure de dihydrogène observée du 24/09 au 06/09, pourrait être lié à une diminution de la densité cellulaire. Il semblerait donc que les variations d'H_2S se calent relativement bien sur celles de la densité phytoplanctonique ce qui tend à prouver la production directe de sulfure de dihydrogène par la culture de T-Iso et l'absence de lien direct entre l'H_2S et la contamination métallique.

5. Conclusion

L'élaboration d'un photobioréacteur torique sans pièce métallique et donc le choix d'une culture en continu, ont permis de suivre l'évolution des concentrations en CSRV issus de la dégradation de composés soufrés d'origine phytoplanctonique (*Isochrysis galbana affinis tahiti*). Les teneurs (i.e., totales) mesurées en CSRV sont comprises entre 0.14 et 0.64 nM pour H_2S, 0.04 et 1.03 nM pour OCS, 0 et 2.11 nM pour MeSH, 5.31 et 14.88 nM pour DMS et 0.30 et 0.76 nM pour DMDS. A noter que ces valeurs sont cohérentes avec celles rencontrées en Baie de Quiberon (Chapitre 3 ; Cozic et al., 2007c) et estuaire de la Seine (Chapitre 4 ; Cozic et al., 2007a).

En raison d'une plausible adsorption des ions Ag^+ sur les parois du photobioréacteur, il a été impossible d'étudier les effets sur une culture phytoplanctonique d'une contamination métallique progressive. Cependant, des variations des concentrations en CSRV ainsi que des modifications de l'état physiologique de phytoplancton (i.e., taux de croissance modifié) ont été observées lors de l'expérience. Le sulfure de carbonyle serait produit à partir de la dégradation de composés soufrés biogènes libérés par le phytoplancton dans le milieu de culture et ce, quelle que soit la concentration en métal. De plus, il apparaît que la concentration en DMS diminue lorsque les concentrations en MeSH et DMDS augmentent ce qui tendrait à prouver que des réactions de transformations du DMS ont effectivement lieu dans le PBR. Malgré une constante de stabilité conditionnelle élevée pour l'argent (log $K' \approx 12$; Morel, 1983 ; Al-Farawati et Van den Berg, 1999), la concentration en H_2S ne semble pas être affectée par les deux pics de contamination métallique mesurés. Cette observation tend à appuyer l'hypothèse que les ions Ag^+ ajoutés (i.e., non adsorbés sur les parois du PBR) sont rapidement complexés par des ligands (i.e., EDTA, PO_4^{3-}). Des expériences ultérieures devraient permettre d'affirmer ou d'infirmer la fixation sur le photobioréacteur des ions argent mais également les effets réels, sur les concentrations en CSRV, d'une exposition métallique. Dans un premier temps, le PBR sera rempli d'eau milliQ et de l'argent sera injecté à des concentrations variables. Les teneurs en Ag mesurées par ICP-MS seront comparées aux teneurs théoriques initialement injectées dans le PBR. Le comportement des ions Ag^+ vis-à-vis de ligands comme PO_4^{3-} et EDTA sera également étudié en utilisant le logiciel MinEql+.

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Conclusion générale

1. Rappel des objectifs

L'intérêt des océanographes pour le soufre est lié au fait que certains composés dont le DMS, sont capables d'influencer le climat global de la Terre (Andreae, 1986 ; Bopp et al., 2004) mais également de modifier la spéciation d'éléments chimiques comme les métaux (Leal et Van den Berg, 1998 ; Laglera et Van den Berg, 2003 ; Young et al., 2003 ; Sukola et al., 2005) dans les océans. Aussi, les problématiques liées au soufre sont nombreuses et souvent intimement liées.

Cette thèse s'inscrit donc dans le cadre d'une meilleure compréhension du cycles biogéochimique du soufre dans le milieu marin. Elle est basée sur l'étude de cinq composés soufrés réduits volatils (CSRV) à savoir le sulfure de dihydrogène (H_2S), le sulfure de carbonyle (OCS), le méthane thiol (MeSH), le diméthyl sulfure(DMS) et le diméthyl disulfure (DMDS).
L'objectif principal de ces recherches est de déterminer précisément le rôle des métaux et du phytoplancton sur la distribution des CSRV dans des environnements marins. Au terme de ce travail, les réponses attendues doivent permettre de fournir des connaissances sur les interactions entre les CSRV, les métaux et le phytoplancton.

Objectifs de la thèse - Les recherches développées dans le cadre de cette thèse avaient pour but d'apporter des éléments de réponse aux questions suivantes :

- Comment mesurer simultanément ces cinq CSRV présents à des niveaux de traces dans un échantillon aqueux ?
- Existe-il des variations interannuelles et saisonnières dans la distribution des CSRV dans un environnement côtier ? Quels sont les paramètres physiques, chimiques et/ou biologiques capables de modifier la distribution de ces CSRV ?
- La distribution des CSRV peut-elle être influencée par la présence de métaux dissous par exemple, dans un milieu anthropisé ? Quelles sont les interactions entre ces CSRV et les métaux ?

- Le phytoplancton, source de certains composés soufrés volatils, est-il capable de répondre à une exposition métallique par une synthèse supérieure en CSRV ?

Ces problématiques constituent l'ensemble du travail de thèse. Les trois premières interrogations ont été présentées et discutées successivement dans les trois premiers chapitres (articles) alors que le dernier axe de recherche est développé dans la dernière partie de ce manuscrit. L'objectif final de cette thèse est d'enrichir les connaissances du lecteur sur le cycle du soufre dans le milieu marin par le biais d'une meilleure compréhension des interactions entre les CSRV, certains métaux et le phytoplancton.

2. Rappel des principaux résultats

Les résultats obtenus au cours de ces trois ans de thèse développés dans quatre chapitres distincts se résument à :

Article 1 - Simultaneous analysis of five volatile reduced sulfur compounds by purge and cryogenic trapping/gas chromatography separation in natural waters.

Une méthode chromatographique a été élaborée pour l'analyse simultanée de cinq CSRV dans des échantillons aqueux (i.e., eaux marines, eaux estuariennes, précipitations…) de faible volume (i.e., ca. 15 ml). Elle possède une haute sensibilité aux CSRV avec une limite de détection de 0.01 nM pour le MeSH, 0.03 nM pour l'OCS et le DMDS, 0.07 nM pour l'H_2S et 0.1 nM pour le DMS. La reproductibilité de cette méthode est relativement bonne pour des échantillons naturels relativement instables (i.e., en raison du caractère volatil des composés soufrés étudiés) puisqu'elle atteint 6.0% pour l'H_2S, 4.1 % pour l'OCS, 5.6% pour le MeSH, 4.9% pour le DMS et 8.4% pour le DMDS. De plus, cette méthode présente un caractère réellement novateur avec une vitesse d'analyse de 10 min et la possibilité, par sa robustesse, d'être embarquée sur un navire océanographique (caractère indispensable pour l'étude des CSRV en estuaire de la Seine par exemple).

Article 2 - Seasonal and interannual study of epibenthic volatile reduced sulfur compounds (VRSC) in coastal environment : the Bay of Quiberon (Brittany, France).

L'étude interannuelle et saisonnière des CSRV dans un environnement côtier, la Baie de Quiberon, a permis de mettre en évidence une variabilité temporelle des concentrations en composés soufrés en zone suprabenthique grâce à l'échantillonneur Susane (Radford-Knoery et al, 2007). La distribution annuelle d'OCS serait directement liée à une balance entre les sources et les puits d'OCS avec une dégradation photochimique apparemment prédominante en période hivernale et complétée par une hypothétique source sédimentaire en été. Les variations des concentrations en MeSH, DMS et DMDS sont davantage à corréler aux changements saisonniers de la densité en Dinophycées. Le décalage temporel (i.e., 2 mois) observé entre le maximum d'abondance phytoplanctonique et les maxima en MeSH et DMDS tend à suggérer une vitesse de dégradation plus lente du DMSP en ces composés soufrés par rapport à une production en DMS plus rapide. Concernant le sulfure de dihydrogène, une source sédimentaire n'est actuellement que suggérée en Baie de Quiberon. D'autres hypothèses sont donc avancées pour expliquer ces teneurs supérieures en H_2S près de l'interface eau-sédiment. Par exemple, la formation de microzones anoxiques, induites par la dégradation de matière organique, pourrait éventuellement expliquer la présence d'H_2S près de l'interface eau-sédiment. Une libération directe d'H_2S, par les cellules phytoplanctoniques, dans la colonne d'eau inférieure, pourrait également être considérée comme une source significative de sulfure de dihydrogène en Baie de Quiberon.

Article 3 - Volatile Reduced Sulfur Compounds : Distribution and Ligands properties in the Seine estuary (France).

Dans un premier temps, les constantes de stabilité conditionnelle de quatre CSRV (i.e., H_2S, OCS, MeSH et DMS) vis-à-vis de l'argent (Ag^+) sont déterminées : log $K'(Ag^+)H_2S$=12, log $K'(Ag^+)$OCS≈0, log $K'(Ag^+)$MeSH≈0, log $K'(Ag^+)$DMS≈0. Ces expériences permettent ainsi de confirmer l'existence de très fortes interactions entre l'Ag et l'H_2S (Morel, 1983 ; Al-Farawati et Van den Berg, 1999) mais également de conclure à l'absence d'interactions significatives entre le métal et OCS, et MeSH, et DMS. Par conséquent, la distribution en OCS, MeSH et DMS dans l'estuaire de la Seine n'est en aucun cas influencée par la présence d'argent dissous.

Le suivi des concentrations en CSRV dans l'estuaire, met en évidence d'importantes variations de distribution le long du gradient de salinité avec des teneurs comprises entre zéro et quelques nM. L'abondance phytoplanctonique semble être le facteur responsable de l'augmentation des concentrations en MeSH, DMS et DMDS vers les

fortes salinités alors que la répartition en OCS peut s'expliquer par divers processus tels que la photodégradation de composés soufrés organiques, et hypothétiquement la diffusion à partir des sédiments. Les variations de la concentration en H_2S semblent davantage liées à *i)* la présence d'argent dont la constante de stabilité conditionnelle vis-à-vis du sulfure de dihydrogène est élevée (log K′=12.9 ; Al-Farawati et Van den Berg, 1999).et *ii)* une modification des sources d'H_2S dans l'estuaire (i.e., source sédimentaire, hydrolyse de l'OCS, dégradation phytoplanctonique).

Le phytoplancton est-il capable de répondre à une exposition métallique par une synthèse supérieure en composés soufrés réduits volatils (CSRV) ?

Ce dernier axe de recherche a pour objectif de déterminer la capacité d'une espèce phytoplanctonique à lutter contre une contamination à l'argent de son milieu de culture. Pour cela, un photobioréacteur (PBR) adapté au dosage de composés volatils a été élaboré et a permis le suivi en continu d'une culture d'*Isochrysis galbana affinis Tahiti* (Haptophycée). En début d'expérience et malgré des ajouts réguliers et croissants d'argent, la concentration en métal reste relativement constante (ca., 1 nM) dans le milieu de culture. L'hypothèse avancée est que les ions métalliques seraient adsorbés sur les parois du PBR. Aussi, cette première expérience ne permet pas de conclure sur les effets de la contamination métallique sur la culture algale. Cependant, des variations des concentrations en CSRV ainsi que des modifications de l'état physiologique de phytoplancton (i.e., taux de croissance modifié) sont observées. Seules des hypothèses peuvent être avancées pour expliquer ces variations. L'OCS analysé serait issu de la dégradation de composés soufrés biogènes libérés par le phytoplancton dans le milieu de culture alors que l'augmentation des teneurs en MeSH et DMDS tendraient à prouver que des réactions de transformations du DMS ont effectivement lieu dans le PBR. Concernant le sulfure de dihydrogène et malgré une constante de stabilité conditionnelle élevée pour l'argent (log K′≈12 ; Morel, 1983 ; Al-Farawati and Van den Berg, 1999), la concentration en H_2S ne semble pas être affectée par la présence de métal ce qui tend à appuyer l'hypothèse que les ions Ag^+ ajoutés (i.e., non adsorbés sur les parois du PBR) sont rapidement complexés par des ligands naturellement présents dans le milieu de culture (e.g., EDTA, PO_4^{3-}).

3. Perspectives de recherche

Un large spectre de recherche concernant les CSRV a été abordé (i.e., distribution, spéciation métallique, production par le phytoplancton) au cours de cette thèse et au fur et à mesure de son avancement, de nouvelles interrogations se sont posées. Cette dernière partie de la conclusion soulève les "mystères" non élucidés lors de ces trois années de thèse concernant les CSRV et oriente vers de nouveaux axes de recherche qu'il serait intéressant de développer afin de mieux appréhender la distribution et le rôle des CSRV dans l'environnement marin.

- Est-ce que la zone de production de sulfure de carbonyle se situe dans les sédiments ou la colonne d'eau elle-même ? Quelle est la part de la production noire sur la synthèse d'OCS dans un environnement côtier ? Existe-t-il des variations journalières et/ou saisonnières de cette production ?
- Quelles sont les conséquences en terme de synthèse de CSRV, d'une exposition métallique (e.g., Ag) sur une culture phytoplanctonique considérée comme grande production de DMSP ?

Concernant la première interrogation, afin d'appuyer ou d'exclure l'existence d'une source sédimentaire, des mesures de la concentration en sulfure de carbonyle dans les eaux interstitielles seraient à mener en utilisant la méthode chromatographique élaborée au cours de cette thèse. En cas de production noire avérée, un suivi saisonnier précis (e.g., 1 prélèvement/mois) mais également journalier (e.g., 1 prélèvement/4 heures) permettrait de suivre l'importance de cette voie de synthèse d'OCS par rapport à la production photochimique.

Afin de répondre à la seconde interrogation, il est essentiel, dans un premier temps, d'exclure toute fixation des ions métalliques sur les parois du photobioréacteur (PBR) ou toute complexation du métal par le milieu de culture. Pour cela, le PBR en PMMA, sera rempli d'eau milliQ et d'une solution d'ions métalliques (e.g., Ag^{+}) de concentration constante. Régulièrement, des prélèvements seront effectués afin de mesurer par ICP-MS la concentration en métal dans le PBR. Si la cinétique montre au cours du temps, une diminution de la concentration en métal dans le PBR alors il sera envisageable de considérer qu'une fixation des ions métalliques sur le PMMA existe. Dans le cas contraire, on étudiera, à l'aide du logiciel MinEql+, les interactions possibles entre les ions métalliques (e.g., Ag^{+}) et certains ligands (e.g., PO_4^{3-}, EDTA) contenus dans le milieu de culture. Si aucune interactions significatives n'est mise en

évidence alors l'étude de la synthèse de CSRV via la dégradation du DMSP, par une culture phytoplancton soumise à une exposition métallique pourra débuter dans le PBR en Plexiglas®. Dans un premier, la teneur en métal sera proche de celle rencontrée dans un environnement non pollué puis elle sera augmentée progressivement jusqu'aux teneurs fréquemment mesurées dans un milieu contaminé. Les concentrations en CSRV seront mesurées régulièrement dans des échantillons non filtrés. De plus, il serait également intéressant de suivre la concentration en DMSP pour évaluer la production des CSRV à partir de ce précurseur. Aujourd'hui, un photobioréacteur totalement opérationnel est disponible au laboratoire et devrait permettre de mener les expérience d'exposition métallique d'une culture phytoplanctonique. Dans les prochains mois, plusieurs métaux seront testés – Ag, Cd, Hg – sur l'espèce phytoplanctonique *Isochrysis galbana affinis Tahiti.*

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Annexes

Annexe 1 - Article 4 - The suprabenthic sampler for nearshore environments (Susane): a new device to collect simultaneously closely-spaced water samples immediately above the sediment water interface in shallow, quiescent waters.

The suprabenthic sampler for nearshore environments (Susane): a new device to collect simultaneously closely-spaced water samples immediately above the sediment water interface in shallow, quiescent waters.

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Conflict of interest

No potential conflict of interest exists.

Abstract

This paper describes a small (40*40*40cm, 10kg), simple (all mechanical) and relatively inexpensive (3000€) sampler capable of acquiring simultaneously up to 16 discrete water samples at centimeter scale resolution immediately above the sediment, in shallow, quiescent environments (current speed <10cm s^{-1}). The lightweight (15kg) sampler has been designed to obtain representative, quality whole water samples, . To illustrate the usefulness of the device, this paper also presents original data showing the existence of chemical (dissolved trace sulfur gases and ammonia) gradients in this suprabenthic zone of the water column.

The device collects samples from up to sixteen different depth in an undisturbed water column by the simultaneous filling of 60-mL syringes. The depths are easily adjusted by positioning the remote tube inlets along a vertical mast offset (10cm) from the body of the sampler. In order to increase sample quality, dead-volume in this tubing is flushed in situ prior to filling the syringes.

Built with materials compatible with trace metal sampling and easily deployed by one person from a small boat, this device has been used to reveal concentration gradients in the water-column above the sediment water interface in the Baie de Quiberon and the tidal creek Rivière d'Auray (Morbihan, France). Obtained depth profiles provide direct evidence of significant concentration gradients at the sediment water interface. Eventually, sediment-water chemical fluxes could be quantified using these gradients coupled to measurements of bottom water diffusivity.

In addition to being a helpful new tool to investigate sediment-water interactions in the lower water-column, this device could also be used without modification for high resolution sampling of other interfaces like air/water or midwater oxic/anoxic transition zones.

Introduction

To chemically characterize the water column near farmed oyster beds and evaluate sediment water interface (SWI) fluxes in this anthropized environment, we needed to acquire water samples immediately around the oysters at centimeter-spacing from the sediment near which they are growing. While the former requirement is easily accommodated using standard oceanographic sampling bottles, we were unable to come across the description of a sampling device that could help us fill the high resolution sampling need. Hence, we designed a suprabenthic sampler for the nearshore environment (Susane) presented below. As an illustration of the usefulness of the sampler, we show here observed suprabenthic chemical gradients of gaseous sulfur species (dissolved hydrogen sulfide, carbonyl sulfide, and dimethylsulfide), and ammonium.

Materials

1. Previous sampler designs

For the mid-water-column stratified environments, a close-interval water sampler was first described by Broenkow (1969), and subsequently modified as described in Blakar, (1979), Grasshoff (1983) and De Lange *et al.* (1990). With these devices, samples are drawn into syringes whose spacing is determined at construction and which corresponds to the vertical sampling resolution (typically fixed at 10cm). This distance is more than adequate to characterize gradients in the midst of a stratified water column. The syringes can eventually be pre-filled with reagents to fix the samples, their cannulae remain opened to the environment after sampling. Still for upper open ocean work, but designed to collect larger samples in the Fine Scale Sampler (FSS; Lunven *et al.*, unpubl.) consists in a vertical, linear array of 15 Niskin-type bottles. They are positioned horizontally at 20 cm intervals with a CTD and held in a ca. 4.5 m chassis lowered, using the ship's crane to estuarine plume halocline. Here again, the resolution is decimetric and fixed at construction. These samplers were designed to sample gradients at below the air-sea interface or at mid-water transition zones, but not for use immediately above the SWI.

In order to sample suprabenthic gradients, several sizeable instrument packages have been devised and deployed on the seabed. For this purpose, Bale and Barrett (1995) designed a device that can be used to characterize suspended matter and phytoplankton species in the suprabenthic layer; it collects up to ten discrete, 4-L samples in the first two meters above the SWI. Likewise, Thomsen *et al.* (1994) devised an instrument cage about 2 m

high and 3 m wide which can pump 15L of water at up to four heights within the first meter of the water column. It is fitted notably with nephelometers, pumps, a compass, and linked to the surface vessel by a hydrowire. The sheer size of these samplers make them unsuitable at collecting samples from a small craft. Given these limitations, we had to design the sampler described below in order to fulfill our research needs and deployment requirements.

2. Functional design of the new sampler and principle of operation

The Susane device shown in Fig. 1 consists of a vertical, 180cm-tall sampling mast (only the bottom part is shown in both panels) supporting Teflon sampling tubes threaded through holes pre-drilled in the mast, and the syringe actuator (about 40x40x60cm). The actuator comprises two plates welded a spring-loaded jack, and a knee joint-like mechanism to trigger the sampler. One of the plates holds syringe barrels while the other is connected to their pistons. Samples are simultaneously drawn into the syringes via the sampling tubes when the two plates spread apart with the force applied by the jack. Fairing plates on the side of the actuator and behind the central suspension point act as vanes which pivot the sampler. Thus, the sampler's front and the forward-protruding mast always face upstream of any current there may be. Thus, sample inlets point into a water-column that is undisturbed by passage around the body of the actuator.

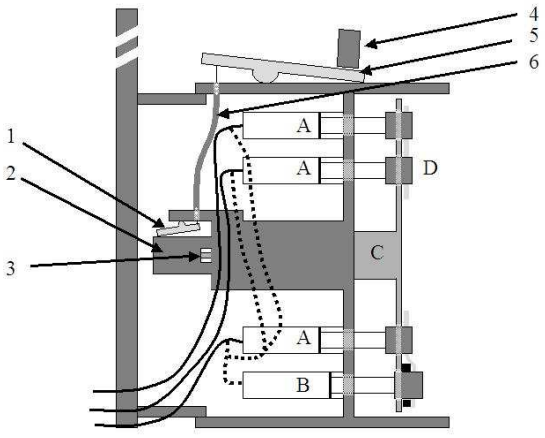


Fig. 1a

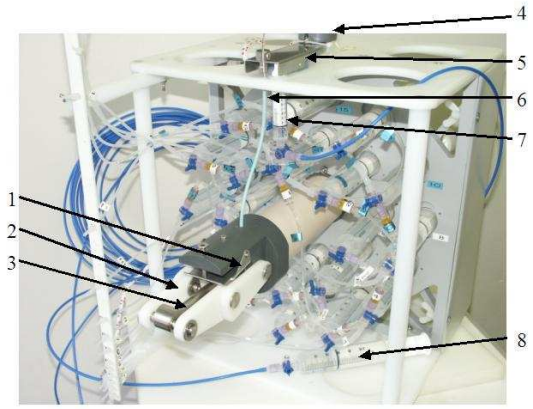


Fig. 1b

Figure 1 : Description of the suprabenthic sampler Susane. Fig. 1a : Schematic drawing the Susane showing the sampling mast and the actuator: three closely-spaced sampling tubes (black solid black curving lines), and four filled syringes. In the shown configuration, the three top syringes (A) are connected to a flushing syringe (B, see Fig. 3 for details) by a tubing network (dotted line). Syringes fill when the interdependent frame, female side of the jack, stationary plate (both dark gray), syringe barrels (C), and the interdependent male side of the jack, mobile plate (both light gray), syringe pistons spread apart. Connection between mobile plate and piston is afforded by syringe extensions (D) that can be traversed by a bendable PVC rod (light gray). The onset of plate spreading is triggered by rotating the slave lever (1) located atop the knee joint-like hinge (represented as a rectangle identified in (2)) into its "tripped" position (as shown in this panel). The smooth SS rod (3) linking the male side of the jack and front of the hinge is shown. Near the top face of the sampler, an oceanographic messenger (4) is drawn resting on the master lever (5), also in "tripped position". Master and slave levers are connected by a sheathed SS cable (6). An alternate way of pulling on the cable is rotation of the master lever using hydraulic force from the piston of a remote (master) syringe (not shown here for clarity, see Fig. 1b).
Fig. 1b : front photograph of the Susane with its knee joint-like hinge in extended position. The hinge's slave lever (1), white polyethylene structure (2) and smooth SS rod (3) are shown. Near the top face of the sampler, part of an oceanographic messenger (4) rests where it hits the master lever (5), thus pulling on the SS cable (6). An alternate way of pulling on the SS cable is by displacing upward the piston of the smaller, slave syringe (7) adjacent to the sheath using hydraulic pressure from a master syringe (8) and a 20m length of tubing (shown coiled behind the sampler).

3. Description of the actuator

Figure 1 is a schematic of the mechanism that locks the jack in compressed position and allows for its easy release. The critical part is a hinge functioning like a knee joint (2). When opened (extended) at an angle 183°, the hinge is inherently locked and the spring is compressed with a 500N force. When at rest (bent), the hinge angle is about 80° . When a cam (hereafter "slave lever", identified as (1) in Fig. 1) applies a small (10N) force at the axis of the hinge (at the "back" of the knee), it is brought from 183° to less than 180°, at which point, the knee-like hinge bends on its own and allows the jack to expand, pulling apart the two PVC plates. This is schematically represented in Fig. 2. One of the syringe bearing plates is welded to the stationary part of the jack and holds the syringe barrels, and the other plate is mobile and pulls out the syringes pistons. The jack's lateral rigidity ensures that stationary and mobile PVC plates remain parallel, thus always filling the syringes at the same rate.

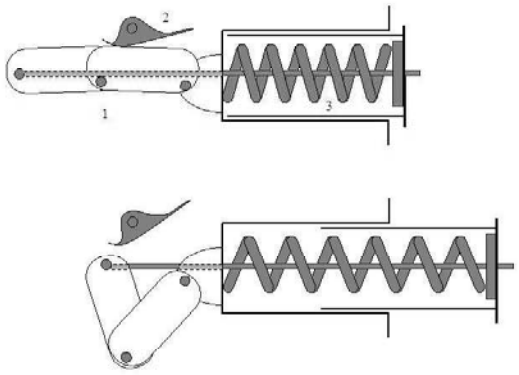


Fig. 2

Fig. 2: Schematic drawings of the Susane before and after sampling. Top panel: the jack is cocked position and ready to be tripped. (1) Knee joint-like hinge in locked position. The angle of "overextension" (183°) is limited by the machining of hinge parts. Rotation of slave lever (2) decreases the angle to less than 180°, at which point the force of the spiral spring (3) finishes to bend the knee-like hinge.
Bottom panel: the hinge has bent and has allowed the smooth SS rod to shift rightward and the jack to extend, pushing apart stationary and mobile plates.

The syringes barrels are bayonet-locked into through-holes with a side rabbet machined in the thickness of the stationary PVC plate. These holes are oblong to match the shape of the syringe barrels' finger rests. Each syringe piston is extended with a drilled cylindrical extension which protrudes from the mobile PVC plate. As shown in Figures 1 and 3, a removable PVC rod threaded into the piston extensions ensures that the mobile plate will pull all the pistons at the same time. A 3-way valve tipping each syringe connects it to a length of 1.5mm ID Teflon tubing whose inlet is at the selected height on the mast. This particular set-up physically separates the sampling position from the actual syringe location, although at the cost of introducing "dead volume" in the sample path.
This dead-volume and associated drawbacks with sample integrity are eliminated as follows. This volume is actually flushed before filling the sampling syringes by using a dedicated set of four of the sampler's twenty syringes. This is achieved by simply eliminating the travel play between the mobile PVC plate and the pistons of the flushing syringes, while maintaining it at 20-mm for the sampling syringes (Fig. 3).

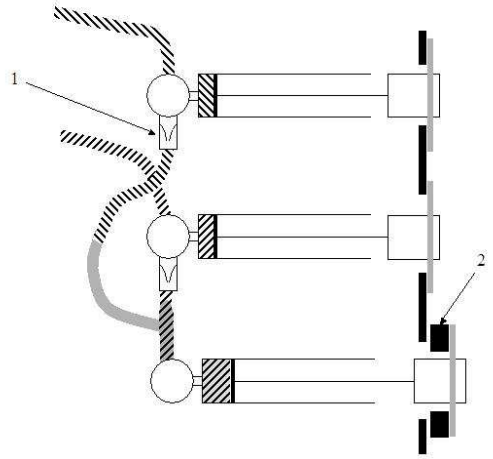


Fig. 3

Fig. 3 : Schematic drawing of the tubing manifold used to flush sampling tubes and the an example of two syringes being filled immediately after the start of the second drawing stage. The top two (sampling) syringes are collecting water from the prescribed depths (hatched areas) while the bottom (flushing) syringe is filling with both dead volume (gray) and sample (hatched) from the top syringe sampling tube. Once sampling is done, backflow out the flushing syringe is prevented by the LuerLock check-valve (1) connected to the 3-way-valve at the tip of the syringes. The shim (2) for the flushing syringe is a 20mm high, hollow cylinder placed around the syringe extension, which takes up the play between mobile plate and PVC rod.

This difference in travel play defines the two stages of the plates' motion. When the two plates are pushed apart by the jack, only the flushing syringes start to fill immediately. Each flushing syringe is connected to 4 other sampling syringes using tubing and Y-connectors, and the liquid drawn during the initial stage (20-mm travel) exceeds that of the dead volume. As the jack's extension continues beyond the first 20mm and into the second stage, sampling syringes begin to fill, while water is still being drawn into the flushing syringes. At the end of the stroke of the jack, pistons of the flushing syringes are thus 20mm further out of the barrels than those of the other syringes. Check valves placed between the sampling and the flushing syringes prevent back-flow into the sampling syringe (Fig. 3).

To release the jack, the slave lever shown on Figures 1 and 3 is pulled via a sheathed stainless steel cable, by another ("master") lever placed on top of the frame of the Susane. The master lever can be actuated several ways, one of which is an oceanographic messenger sliding down the hydrowire and hitting it. This configuration requires that the sampler is held in place by a taut cable (mid-water sampling). The second way is preferred when the sampler is used resting on the bottom and uses hydraulic transmission of force. This configuration is easily implemented in 20-m deep sampling locations and is conducted as follows. Two syringes are half filled with water and connected at both ends

of a length of a 2mm ID semi-rigid polyethylene tubing. The "lower" (i.e., in situ) syringe is positioned on the Susane frame, in a manner that pushing the piston on the upper syringe will apply pressure on the master lever. Use of this tripping mechanism is only limited by the practicality of paying out and spooling both the tubing and line to safely retrieve the sampler. It is worthy to note that this hydraulic trigger makes the Susane equally useable upside down, e.g., for air-water interface studies. This is achieved using small weight at the tip of the mast and a slightly bigger float fixed on the base of the sampler.

4. Construction materials

Materials used in the construction of the suprabenthic sampler are polyethylene, PVC and stainless steel (SS) which were chosen for their mechanical properties and resistance to seawater corrosion. The 316-SS spring was custom made to exert a force of 500N and 400N in the compressed and extended positions of the jack, respectively. This relatively large force requires the use of a threaded SS rod and a crank to easily compress the spring, and the use of the knee-like hinge to be able to reliably lock the jack, and yet to enable its consistent release using force in the 10N range. The spring is maintained compressed by a smooth SS rod between the hinge and the back plate, preventing possible accidental projection of sampler parts in case of structural failure, a notable safety feature. A glass-reinforced epoxy vertical sampling mast extends 180cm upward from the base of the Susane and is drilled at selected intervals to thread and hold the sample tubing inlets. Plastic parts are either welded together or fastened using stainless steel nuts and bolts. The jack provides alone the mechanical stiffness necessary for smooth spreading of the PVC plates. Other plastic parts essentially provide protection to the syringes during handling of the Susane on a typically crowded small boat.

Sample contacts only 2mm ID Teflon tubing, polycarbonate 3-way valves (Cole-Parmer), and the interior of the sampling syringes. We use 60mL, LuerLock, syringes with their silicone O-ringed piston which are made of polypropylene and polyethylene (Codan, ref 62.8426). They can withstand acid-cleaning and repeated uses while remaining water-tight due to their silicone O-ring. This latter feature also allows for minimal friction during piston travel (~15N or less at sliding speeds of 20cm/min, after removing silicone lubricant). Minimal piston friction and leak-tightness are important features when drawing 20 syringes simultaneously. Silicone LuerLock check valves (Cole-Parmer) and Tygon tube lengths connected using polyethylene barbed fittings (Cole Parmer) link sampling and flushing syringes.

5. Preparation and sampling using the Susane

At the laboratory, Teflon tubing, 3-way valves and syringes are cleaned using sequential isopropyl alcohol and 0.5 N HCl acid soakings and deionized water rinses. Then they are stored individually and filled with 0.01N HCl to keep the inside of the barrels as free from contamination as possible; the sampling tubes are then capped. Prior to deployment, sampling inlets are positioned appropriately on the mast and uncapped, pistons are pushed in to empty the syringes of their storage solution; possible leaks at valves, fittings and connectors are verified, and the spring jack is compressed using the threaded SS rod and crank until the hinge can be locked with a nudge from a finger. Shims are put in place at the purge syringes and the PVC rod is then threaded through the piston extensions and secured. After an ultimate and necessary verification of proper valve positions, the Susane is then lowered to its sampling location. When lowered from a small craft, one minute spent at 10-30cm altitude is sufficient to orient the sampling mast upstream of the weakest currents, before slowly bringing the Susane to the seabed. After an additional 5-8 minutes equilibration time at the deployment depth, The Susane is then tripped. This wait allows for the settling of resuspended particles, or for the dissipation of turbulence induced by lowering the instrument at this thepth.. The syringes fill in about 45 seconds, after which the sampler can be brought back on board.

Once on deck, the 3-way valves are closed. Then, the spring is slightly compressed again (SS threaded rod) to release tension on the syringes, the threaded PVC rod is removed, and subsampling of each syringe can then begin. For this purpose, it is possible to dispense sample directly into other containers, or to use another set of syringes fitted with 3-way valves to ensure hermetic transfer. After rinsing sampling syringes with deionized water or site water, the Susane can be readied for the next deployment. Turnaround-time between two deployments is about 45 minutes.

6. Unique characteristics

The most important and original feature of this sampler is its small dead-volume (<250 µL) for each 60-mL sample despite the presence of sampling tubes upstream of the syringes. This has been attained by configuring a tubing manifold which allows for a thorough flushing of the sampling tubes. Also, the integrity of the sample until subsampling is preserved by the check-valves. The length of the sampling tube (between 30 and 200cm) also isolates samples held in the syringes from the water column and atmosphere while the sampler is hoisted back on deck. These features prevent sample mixing with water from either the flushing syringe or the environment.

Drawing samples through flexible Teflon tubing allows to freely chose the exact sampling depth of each syringe. Furthermore, the tube inlet connections can be reconfigured to associate one sample tubing with several sampling syringes which will accordingly collect larger volumes of sample. This enables to collect replicate samples of larger volume.

Assessment

To validate the proper functioning of the Susane, laboratory experiments and repeated field deployments were conducted. Furthermore, construction of an earlier prototype allowed to improve its design.

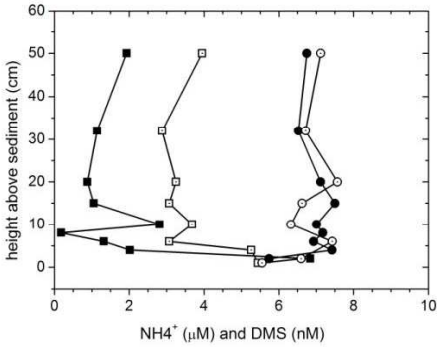
The actuator design is an evolution of an earlier design which used four springs and guide rods to ensure smooth spreading of stationary and mobile plates. Simultaneous compression of the springs (300N, supplied by hand) and sliding a locking pin in place required strength and boat stability, both of which could be lacking during deployment from very small craft. Furthermore, a General-Oceanics push-rod and pin trigger mechanism is inadequate to release a lanyard kept taut by a spring force much larger than 300N. Drawing from this experience, it was decided to use the knee-like joint and the single spring design described here. This triggering system has a perfect field record to date (37 deployements).

Another original feature of this sampler is the flushing of the sample paths, which complicates somewhat the tubing connections. Efficiency of sampling tube flushing, and of the overall leak-tightness of the syringes was evaluated on a lab bench as follows. The extremity of empty sampling tubes were immersed in a beaker of water and the Susane was tripped, filling its syringes in 15 seconds. The amount of air drawn in sampling syringes corresponds to the un-flushed/leak volume from the sampling tubes. This un-flushed volume was minimized by balancing between the flushing syringes the lengths of tubing directed to them. Also, the play between sampling syringe piston extensions and the mobile plate was increased from 10 to 20mm. In this configuration, the flushing volume drawn by each flushing syringe is double that of the sampling tubes upstream of the sampling syringes. In the field, the same experiment (trigger the Susane with sampling tubes initially full of air) was carried out. Upon recovery of the sampler, the sampling syringes had about 0.1 to 0.3mL of air in them, 45 seconds were necessary to fill the syringes in situ. Overall leak-tightness was assessed by closing the 3-way valve of several syringes and yet triggering the Susane while immersed in 1m of water. No water ingress was noted in the barrels of the closed syringes. Further tests were performed in large seawater tanks. The first one was to assess the time necessary to draw sample in the syringes, determined to be in the range of 35 to 45 seconds. It was measured

To verify that the Susane functions properly, it was deployed on the Morbihan coast (Western France) to collect samples in the suprabenthic zone. They were analyzed for the compounds shown here which were chosen for their range of composition and reactivity in the marine environment, and for their potential relevance for oyster bed well being.

The two sampling sites support oyster production and are underlain by organic rich sediments. The first sampling site is the Fort Espagnol (47°36.70'N, 2°57.33'W on the tidal Auray river). Sediment at the tidal river is very-fine sand to silt, and sulfide levels in the sediment were <0.3μM at 1 and 7cm, respectively in June 2005. Ammonia was 51 and 55μM at the same depths. Concentrations were determined using the Cline (1969) and Solorzano (1969) methods on hermetically extracted porewaters (Seeberg-Elverfeldt *et al.*, 2005). The sampling point is within the tidal range, and water depth was 55cm during sampling. The second site is at 47°32.1'N and 03°3.1W in the Baie de Quiberon (150km^2, 7m mean depth) where the water-column is mixed, according to temperature, salinity and dissolved oxygen measurements carried out with a CTD probe. The sediment is mostly sandy mud subjected to wave action (Lemoine, 1989). Dissolved porewater sulfide concentrations were characterized identically and were respectively <0.2μM less than 2cm from the SWI and reached 1μM at 7cm depth in the core collected at the sampling site.

After collection using the Susane according to the procedures described above, 8 to 10 water samples collected at each station were analyzed for the compounds shown here. Volatile sulfur species were determined by unacidified purge extraction and cryogenic trapping in an all-Teflon system, followed by semi-capillary gas chromatography separation and pulsed flame photometric detection (Cozic and Radford-Knoery, in prep 2006). Ammonium was determined spectrophotometrically using the indophenol blue method (Solorzano, 1969) on undiluted samples. The obtained results are presented in Figure 4.



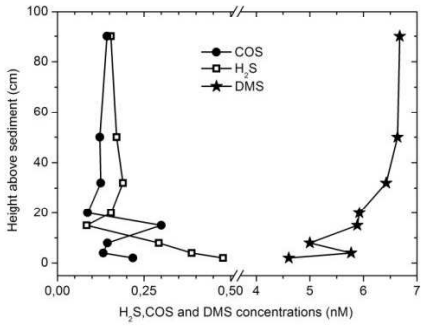


Fig. 4a

Fig. 4b

Fig. 4 : Vertical concentration gradients at two Morbihan sites. Samples were analyzed within 2 h of collection. a) Concentration profiles of ammonia (squares, filtered 0.4µm) and dimethylsulfide (circles, unfiltered) at Fort Espagnol. Filled and hollow symbols are for samples collected at 10h00 and 12h00, respectively. Low tide was at 12:06. The suprabenthic zone exhibits a significant increase in ammonia standing stock over time and sharp gradients, while DMS inventory is constant over time. Nevertheless, both compounds display significant concentration gradients with increasing distance from the sediment. At the time of sampling, current speed (=advection) was on the order of 1 to 2 m minute^{-1}, as estimated from observation of a cloud of resuspended of particles. b): H2S, COS and DMS concentrations in the Baie de Quiberon (11m depth). Shapes of the profiles suggest sedimentary input for H_2S and removal for DMS. The latter compound also seems to be removed at the SWI at Fort Espagnol (see panel a). COS gradient there is not as well defined, requiring further investigation.

In the Fort Espagnol (FE) tidal river, the Susane was deployed twice in a 2-hour interval during ebb tide. Water column ammonium levels decrease sharply in the first 5cm above the SWI from >5 µM to reach the "bulk" water concentrations of ~2µM at 40cm above the SWI (Fig. 4a). The second profile collected at the time of low tide shows greater concentrations in the lowest 45cm of the water column. The two dissolved dimethylsulfide (DMS) profiles are similar in shape and in concentrations (Fig 4a).

For the Western Bay of Quiberon (Fig. 4b), water-column levels of H_2S were minimum at heights greater than 100cm above the SWI. Closer to the SWI, H_2S concentrations increased linearly from 0.15nM at 16cm to a maximum of ca. 0.5nM at 2cm above the SWI. For COS, concentrations are uniform at heights greater than 20cm above the SWI and show greater variability near the bottom. For DMS, concentrations show a smooth increase from 2cm above the SWI (4.5nM) to nearly uniform levels of 6.8nM reached at 50cm above the SWI. The data show well-defined, although opposite concentration gradients. These data also show that the water column, even of relatively open embayment, is not as well mixed, chemically speaking, as could be inferred from the CTD probe data or any other bulk sampling method.

Discussion

There are many available data on the distribution of gaseous reduced sulfur species in the marine environment; for the purpose of this discussion, the levels of those reported in this paper reflect the role of biological activity in their respective biogeochemical cycles. Water column concentrations above the suprabenthic layer (here defined as less than 50cm above the sediment) reported here are consistent with previously published values of dissolved H_2S, COS, and DMS concentrations for comparable coastal environments (e.g., Radford-Knoery and Cutter 1994, and Sciare *et al.* 2002). Such convergence suggests that our sampling and analytical protocols yield internally consistent and accurate results. Yet, the features shown in our concentration depth profiles have not been reported previously for lack of comparable samples. However, for each profile reported here, samples collected using the Susane were treated identically (subsampling and analysis), and the observed differences indicate that the gradients are real and not sampling or analytical artifacts. Thus, observed concentration depth profiles reflect the balance of processes at the SWI which includes fluxes between the sediment and the water-column and transformations in the suprabenthos.

To explain the presence of (unstable) dissolved sulfide in the oxygenated water column of the Baie de Quiberon, two sources can be invoked. The least likely one is the sulfidic sedimentary layer buried under oxic sandy mud, because the thickness of this oxic layer effectively prevents significant sulfide flux from reaching the SWI. A second possible source is the in situ production of sulfide (i.e. within the water column above SWI) linked to sulfate-reducing micro-environments in the floc layer or to phytoplankton activity (e.g. Walsh *et al.*, 1994). For the DMS depth profiles, the limited dataset presented here suggests that the SWI is more likely a sink than a source of this compound.

A description of the complexity of ammonium cycling at the sediment water interface is beyond the scope of this paper since such cycling depends on the rather delicate balance between the supply of electron acceptors and their demand by microbial processes for the remineralization of organic nitrogen (e.g., (Klump and Martens, 1983). The first two steps in this cycle are the dissolution of particulate organic nitrogen to dissolved organic nitrogen, followed by its transformation into dissolved inorganic nitrogen through several interdependent reactions like nitrification, denitrification and ammonification (Henriksen and Kemp 1988, Hansen and Blackburn 1991, Sloth *et al.* 1995). For the purpose of this paper, it suffices to say that for organic rich coastal sites the sediment can often act as a source of ammonium to the overlying water column (e.g., Klump and Martens, 1981). Vertical concentration profiles in the water column have not been used to reveal the existence nor the intensity of large upward ammonium fluxes to coastal waters. In the

depth profiles presented here, dissolved ammonium concentrations increase toward the bottom, exhibiting distinctive upward gradients. In addition, an increase in ammonium standing stock can be observed, consistent with the shown gradient. Given the weak current speed at the times of sampling (1-2m minute^{-1}) and the rather homogenous nature of the sediment in the vicinity of the sampled area, the two depth profiles were acquired in essentially the same water mass. Taken together, observations of a vertical gradient and a temporal increase then converge and strengthen our contention that the gradient is itself direct evidence of upward sedimentary flux. To test the robustness of this finding, a rough calculation based on the variation of the standing stock of ammonium in indicates an upward flux on the order of 1mmol m^{-2} h^{-1} (from a concentration variation of ~1.5mmol m^{-3} observed in a 50cm-tall water column over a 2h time span). While high compared to values obtained using incubations, the value of this flux is nevertheless consistent with fluxes observed in other French Atlantic oyster-farming environments (Sornin *et al.*, 1990).

While fluxes of dissolved compounds between sediment and water column have been shown using sediment core modeling and benthic chamber incubations, to our knowledge this is the first example that gradients and probable fluxes can be directly revealed using discrete samples collected above the SWI.

Conclusion, comments and recommendations

The suprabenthic sampler for near-shore environments (Susane) now makes it possible to obtain discrete samples in the first centimeters above the sediment-water interface, and hence suprabenthic concentrations gradients. The Susane is easy to handle and a yet relatively inexpensive addition to the array of sampling tools used by marine scientists. For biogeochemistry, this device will help to bridge the gap between different methods used to determine fluxes at the sediment-water interface, e.g., sediment profile modeling, ex-situ core incubation, benthic chambers. Indeed for samples that we collected in different coastal environment like tidal river, barrier-island sound, or sheltered embayment, analyses systematically show suprabenthic concentration gradients. This has been observed for the other chemical species considered thus far and including methane thiol, and two dissolved mercury species. While more data is needed to better quantify the processes responsible for these observations, it is clear that the suprabenthic zone can now be sampled intensively enough to examine distributions with previously unattainable detail. This ability may reveal processes previously unrecognized. and ultimately lead to better understanding of the sediment-water interactions. Furthermore, coupling chemical observations near the SWI with measured or modeled transport coefficients (e.g. Hondzo *et al.*, 2005) may enable the direct determination of chemical fluxes at the sediment water interface.

Likewise for other aquatic sciences like microbiological or plankton dynamics the Susane could help in the description of concentrations and abundances. Indeed for some studies, sample volume requirements are in the 1 to 250mL range which can be accommodated with this sampler. Finally, there is no depth limitation to using Susane as it is entirely constructed of solid materials, hence enabling sampling well below the surface, e.g., at oxic-anoxic interfaces.

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Annexe 2 - Concentration en CSRV en Baie de Quiberon (12/07/04-23/08/06)

date	hauteur (cm)	m éch (g)	racine aire H2S	racine aire COS	racine aire MeSH	racine aire DMS	racine aire DMDS	H2S (nM)	COS (nM)	MeSH (nM)	DMS (nM)	DMDS (nM)
12/07/2004	2	19,3	45,83	20,00	75,50	834,57	14,14	0,132	0,058	0,218	2,412	0,041
12/07/2004	4	22,7	44,72	14,14	97,98	626,90	0,00	0,110	0,035	0,241	1,540	0,000
12/07/2004	8	20,3	31,62	28,28	50,99	1025,09	26,46	0,087	0,078	0,140	2,816	0,073
12/07/2004	16	20,8	41,23	20,00	93,27	761,91	0,00	0,111	0,054	0,250	2,043	0,000
12/07/2004	32	22,7	0,00	0,00	104,40	712,11	0,00	0,000	0,000	0,257	1,750	0,000
12/07/2004	50	20	31,62	20,00	200,50	747,53	14,14	0,088	0,056	0,559	2,085	0,039
12/07/2004	100	18,4	34,64	17,32	127,67	638,04	0,00	0,105	0,053	0,387	1,934	0,000
12/07/2004	180	25,7	17,32	0,00	58,31	534,88	0,00	0,038	0,000	0,127	1,161	0,000
01/09/2004	2	19,3	317,65	86,02	43,59	325,12	91,65	0,918	0,249	0,126	0,940	0,265
01/09/2004	4	22,7	41,23	60,00	50,99	334,07	80,00	0,101	0,147	0,125	0,821	0,197
01/09/2004	8	20,3	22,36	43,59	84,26	252,98	71,41	0,061	0,120	0,232	0,695	0,196
01/09/2004	16	20,8	41,23	31,62	102,96	380,92	98,99	0,111	0,085	0,276	1,021	0,265
01/09/2004	32	22,7	30,00	38,73	124,10	368,51	91,65	0,074	0,095	0,305	0,905	0,225
01/09/2004	50	20	26,46	17,32	156,20	294,79	46,90	0,074	0,048	0,436	0,822	0,131
01/09/2004	100	18,4	28,28	46,90	94,87	313,37	80,00	0,086	0,142	0,288	0,950	0,242
01/09/2004	180	14,3	26,46	38,73	115,33	270,74	69,28	0,103	0,151	0,450	1,056	0,270
22/09/2004	2	20,3	599,75	28,74	0,00	59,67	73,62	1,648	0,079	0,000	0,164	0,202
22/09/2004	4	20,7	0,00	19,39	0,00	74,36	80,50	0,000	0,052	0,000	0,200	0,217
22/09/2004	8	19,5	38,70	0,00	0,00	90,06	92,63	0,111	0,000	0,000	0,258	0,265
22/09/2004	15	20,5	18,79	19,47	0,00	66,71	78,04	0,051	0,053	0,000	0,181	0,212
22/09/2004	30	21	0,00	0,00	0,00	85,38	72,18	0,000	0,000	0,000	0,227	0,192
22/09/2004	50	20,7	0,00	18,84	0,00	92,30	79,18	0,000	0,051	0,000	0,249	0,213
22/09/2004	90	18,3	0,00	38,55	0,00	70,85	75,43	0,000	0,117	0,000	0,216	0,230
22/09/2004	175	17,5	0,00	35,31	0,00	75,43	77,72	0,000	0,113	0,000	0,240	0,248
01/02/2005	2	17,7	11,96	12,53	0,00	24,69	0,00	0,038	0,039	0,000	0,078	0,000
01/02/2005	4	18,38	10,63	10,15	14,63	40,07	0,00	0,032	0,031	0,044	0,122	0,000
01/02/2005	8	19,86	23,67	0,00	0,00	48,96	12,96	0,066	0,000	0,000	0,137	0,036
01/02/2005	15	19,72	15,78	0,00	0,00	39,71	10,91	0,045	0,000	0,000	0,112	0,031
01/02/2005	30	19,6	0,00	0,00	0,00	32,69	0,00	0,000	0,000	0,000	0,093	0,000
01/02/2005	50	17,24	22,36	11,49	21,70	61,68	0,00	0,072	0,037	0,070	0,200	0,000
01/02/2005	90	21,96	16,34	25,34	0,00	55,04	0,00	0,041	0,064	0,000	0,140	0,000
01/02/2005	175	20,05	10,05	34,25	0,00	46,61	13,75	0,028	0,095	0,000	0,130	0,038

07/06/2005	2	19,59	20,12	14,76	116,02	562,31	28,83	0,205	0,151	1,184	5,741	0,294
07/06/2005	4	17,72	15,72	18,73	158,82	560,78	23,52	0,177	0,211	1,793	6,329	0,265
07/06/2005	8	18,56	14,46	19,70	144,92	536,14	22,49	0,156	0,212	1,562	5,777	0,242
07/06/2005	15	19,65	20,69	25,22	212,52	579,95	30,08	0,211	0,257	2,163	5,903	0,306
07/06/2005	20	19,2	13,27	13,27	243,70	593,34	22,45	0,138	0,138	2,539	6,181	0,234
07/06/2005	32	19,62	6,40	20,69	129,01	502,15	19,39	0,065	0,211	1,315	5,119	0,198
07/06/2005	50	18,36	5,74	23,66	214,50	295,14	155,09	0,063	0,258	2,337	3,215	1,689
07/06/2005	90	19,65	19,54	9,70	91,91	373,55	13,96	0,199	0,099	0,936	3,802	0,142
07/06/2005	180	16,24	24,54	14,63	176,74	590,06	16,61	0,302	0,180	2,177	7,267	0,205
06/07/2005	2	18,4	0,00	44,62	214,66	638,24	45,99	0,000	0,485	2,333	6,937	0,500
06/07/2005	4	19,65	0,00	47,78	196,52	747,15	50,10	0,000	0,486	2,000	7,605	0,510
06/07/2005	8	19,99	0,00	43,75	241,27	757,18	49,91	0,000	0,438	2,414	7,576	0,499
06/07/2005	15	17,58	0,00	41,12	366,34	731,15	48,66	0,000	0,468	4,168	8,318	0,554
06/07/2005	32	18,92	0,00	46,37	236,79	691,69	48,11	0,000	0,490	2,503	7,312	0,509
06/07/2005	50	19,96	0,00	45,93	236,33	711,80	52,95	0,000	0,460	2,368	7,132	0,531
06/07/2005	90	20,58	0,00	43,92	177,77	677,30	47,28	0,000	0,427	1,728	6,582	0,459
06/07/2005	180	18,87	0,00	41,45	190,10	703,59	48,94	0,000	0,439	2,015	7,457	0,519
28/07/2005	2	19,79	0,00	42,36	123,02	523,03	33,18	0,000	0,428	1,243	5,286	0,335
28/07/2005	4	21,24	0,00	55,57	224,00	776,34	42,59	0,000	0,523	2,109	7,310	0,401
28/07/2005	8	20,52	0,00	49,04	258,52	709,47	41,21	0,000	0,478	2,520	6,915	0,402
28/07/2005	15	19,57	0,00	34,55	424,91	757,54	39,20	0,000	0,353	4,342	7,742	0,401
28/07/2005	32	25,83	0,00	58,06	515,44	829,25	44,51	0,000	0,450	3,991	6,421	0,345
28/07/2005	50	18,41	0,00	45,60	536,51	712,37	33,75	0,000	0,495	5,828	7,739	0,367
28/07/2005	90	19,14	0,00	34,55	203,06	349,45	5,92	0,000	0,361	2,122	3,652	0,062
28/07/2005	190	15,79	0,00	34,19	53,14	852,46	33,72	0,000	0,433	0,673	10,798	0,427
30/08/2005	2	10,76	0,00	8,77	73,07	778,53	46,21	0,000	0,089	0,737	7,853	0,466
30/08/2005	4	13,15	0,00	9,38	85,63	795,68	52,27	0,000	0,077	0,707	6,568	0,431
30/08/2005	6	11,26	0,00	9,75	68,93	719,13	69,99	0,000	0,094	0,665	6,932	0,675
30/08/2005	10	11,98	0,00	10,25	67,84	763,81	68,20	0,000	0,093	0,615	6,920	0,618
30/08/2005	32	11,49	0,00	15,56	80,11	827,14	77,48	0,000	0,147	0,757	7,814	0,732
30/08/2005	50	12,91	0,00	17,06	80,20	872,09	126,61	0,000	0,143	0,674	7,332	1,064
30/08/2005	90	10,69	0,00	16,28	78,38	789,98	133,51	0,000	0,165	0,796	8,021	1,356
30/08/2005	180	10,45	0,00	15,68	86,24	824,24	79,25	0,000	0,163	0,896	8,561	0,823

26/04/2006	2	17,41	74,51	33,32	728,61	750,57	0,00	0,545	0,519	0,327	10,049	0,000
26/04/2006	4	15,63	73,67	43,49	1200,97	774,82	0,00	0,600	0,754	0,600	11,555	0,000
26/04/2006	6	15,61	73,47	34,23	1161,77	781,48	0,00	0,599	0,594	0,582	11,670	0,000
26/04/2006	17,5	14,36	58,40	24,06	964,13	799,68	0,00	0,517	0,454	0,525	12,981	0,000
26/04/2006	40	16,51	66,28	40,72	1106,15	773,70	0,00	0,511	0,668	0,524	10,924	0,000
26/04/2006	60	14,53	79,73	56,43	687,05	822,70	0,00	0,698	1,052	0,369	13,198	0,000
26/04/2006	100	14,53	66,53	61,45	624,99	819,61	0,00	0,583	1,146	0,336	13,149	0,000
26/04/2006	150	16,5	68,21	67,71	1621,80	780,12	0,00	0,526	1,112	0,768	11,021	0,000
26/04/2006	180	14,37	65,71	72,97	173,60	923,83	0,00	0,582	1,376	0,094	14,986	0,000
28/06/2006	1	22,4	29,90	2316,51	103,28	870,31	122,30	0,098	4,224	1,714	3,981	1,294
28/06/2006	2	20	21,19	586,72	127,70	990,80	0,00	0,077	1,198	2,374	5,076	0,000
28/06/2006	3	21	22,00	420,35	216,45	926,26	0,00	0,077	0,818	3,832	4,519	0,000
28/06/2006	4	21,4	36,36	382,63	234,97	901,04	0,00	0,124	0,730	4,082	4,314	0,000
28/06/2006	5	24,1	36,52	235,64	301,88	970,76	0,00	0,111	0,399	4,656	4,127	0,000
28/06/2006	6	20,3	29,75	323,40	427,47	927,74	0,00	0,107	0,651	7,828	4,683	0,000
28/06/2006	8	22,4	24,27	281,50	470,37	883,12	0,00	0,079	0,513	7,806	4,039	0,000
28/06/2006	12,5	22	20,71	239,36	425,10	907,29	32,45	0,069	0,444	7,183	4,225	0,350
28/06/2006	17,5	21,1	21,54	241,27	285,90	914,41	28,21	0,075	0,467	5,037	4,440	0,317
28/06/2006	25	23	22,36	251,44	313,52	847,49	25,63	0,071	0,447	5,067	3,775	0,264
28/06/2006	40	24,6	14,11	226,40	333,63	878,27	25,44	0,042	0,376	5,042	3,658	0,245
28/06/2006	60	21,8	13,38	205,02	251,93	894,62	28,64	0,045	0,384	4,296	4,205	0,311
28/06/2006	100	19,8	11,70	199,35	205,05	932,96	32,11	0,043	0,411	3,850	4,828	0,384
28/06/2006	150	19,7	32,62	194,88	178,75	819,51	33,05	0,121	0,404	3,373	4,262	0,397
28/06/2006	180	20,6	10,20	189,99	172,58	959,46	45,46	0,036	0,377	3,114	4,772	0,523
23/08/2006	1	13,69	0,00	58,66	34,12	334,67	14,90	0,000	0,175	0,926	2,505	0,258
23/08/2006	3	15,39	0,00	63,80	54,81	492,28	18,47	0,000	0,169	1,324	3,277	0,284
23/08/2006	5	15,39	0,00	58,26	55,45	525,91	18,25	0,000	0,155	1,339	3,501	0,281
23/08/2006	17,5	16,85	22,78	59,26	90,70	563,09	18,68	0,099	0,144	2,001	3,424	0,263
23/08/2006	25	13,62	15,13	54,12	57,03	476,04	15,33	0,081	0,162	1,556	3,581	0,267
23/08/2006	40	15,3	14,00	53,62	124,87	520,72	16,52	0,067	0,143	3,034	3,487	0,256
23/08/2006	100	14,74	16,46	64,61	101,99	543,04	17,64	0,082	0,179	2,572	3,775	0,284
23/08/2006	150	14,85	17,26	68,17	91,09	530,27	18,63	0,085	0,188	2,280	3,659	0,297
23/08/2006	180	16,01	16,91	65,64	102,58	625,32	20,32	0,077	0,167	2,382	4,002	0,301

Annexe 3 - Concentration en CSRV en estuaire de la Seine (23/05/05-30/05/05)

* salinité

salinité labo = mesurée au labo par Chiffoleau et al.

salinité maître = mesurée par Chiffoleau et al.

salinité esclave = mesurée par Cozic et al.

salinité esclave corrigée = nouvelle calibration de la sonde au labo (juillet 2005) pour corriger les salinités mesurées pendant la mission (Cozic et al.)

			salinité*					H2S		OCS		MeSH		DMS		DMDS	
date plvt	station	type éch	maître	labo	esclave	esclave cor.	T°C	racine du pic	nM	racine du pic	nM	racine du pic	nM	racine du pic	nM	racine du pic	nM
23/05/2005	E1	F	0,0	0,6	0,4	0,6	19	0,00	0,000	76,51	0,753	22,65	0,223	173,55	1,708	90,17	0,887
23/05/2005	E1	F	0,0	0,6	0,4	0,6	19	0,00	0,000	79,67	0,784	28,14	0,277	206,21	2,030	95,04	0,935
23/05/2005	L3	F	33,2	33,5	31,1	31,3	16,4	32,50	0,320	44,12	0,434	258,89	2,548	2086,24	20,534	47,68	0,469
23/05/2005	L3	F	33,2	33,5	31,1	31,3	16,4	33,42	0,329	45,01	0,443	316,69	3,117	1848,49	18,194	46,25	0,455
23/05/2005	L8	F	32,5	32,9	30,8	31,0	16,1	17,26	0,170	54,72	0,539	148,17	1,458	937,13	9,224	15,56	0,153
23/05/2005	L8	F	32,5	32,9	30,8	31,0	16,1	12,49	0,123	50,35	0,496	169,72	1,670	1026,03	10,099	28,46	0,280
23/05/2005	E1	NF	0,0	0,6	0,4	0,6	19	0,00	0,000	54,65	0,538	11,27	0,111	126,06	1,241	50,71	0,499
23/05/2005	E1	NF	0,0	0,6	0,4	0,6	19	0,00	0,000	70,78	0,697	26,91	0,265	158,49	1,560	82,86	0,816
23/05/2005	E4	NF	4,0	4,3	3,2	3,4	17,7	0,00	0,000	66,36	0,653	60,84	0,599	233,87	2,302	54,29	0,534
23/05/2005	L3	NF	33,2	33,5	31,1	31,3	16,4	18,00	0,177	41,93	0,413	208,11	2,048	2036,66	20,046	0,00	0,000
23/05/2005	L8	NF	32,5	32,9	30,8	31,0	16,1	61,00	0,600	39,27	0,386	119,34	1,175	1022,92	10,068	71,85	0,707
23/05/2005	L8	NF	32,5	32,9	30,8	31,0	16,1	48,11	0,474	30,89	0,304	124,87	1,229	1023,03	10,069	29,61	0,291
23/05/2005	L9	NF	32,5	32,9	30,6	30,8	16,7	33,05	0,325	11,66	0,115	134,19	1,321	1331,69	13,107	0,00	0,000
23/05/2005	L9	NF	32,5	32,9	30,6	30,8	16,7	27,50	0,271	27,18	0,268	146,72	1,444	1358,32	13,369	0,00	0,000

24/05/2005	E4	F	4,0	4,3	3,2	3,4	17,7	0,00	0,000	80,80	0,795	34,38	0,338	247,06	2,432	50,34	0,495
24/05/2005	L9	F	32,5	32,9	30,6	30,8	16,7	30,36	0,299	31,00	0,305	174,65	1,719	1609,33	15,840	0,00	0,000
24/05/2005	L9	F	32,5	32,9	30,6	30,8	16,7	33,15	0,326	4,00	0,039	155,99	1,535	318,59	3,136	0,00	0,000
24/05/2005	L10	F	33,7	34,1	32	32,2	15,7	0,00	0,000	41,41	0,408	135,83	1,337	2227,31	21,922	0,00	0,000
24/05/2005	L10	F	33,7	34,1	32	32,2	15,7	68,26	0,672	27,78	0,273	297,77	2,931	2624,53	25,832	111,72	1,100
24/05/2005	E4	NF	4,0	4,3	3,2	3,4	17,7	0,00	0,000	82,64	0,813	43,63	0,429	241,39	2,376	63,62	0,626
24/05/2005	E6	NF	8,0	8,7	8,5	8,7	17,1	0,00	0,000	71,18	0,701	154,52	1,521	501,36	4,935	67,51	0,664
24/05/2005	E6	NF	8,0	8,7	8,5	8,7	17,1	0,00	0,000	83,10	0,818	174,34	1,716	637,52	6,275	110,21	1,085
24/05/2005	E9	NF	14,0	13,1	13,9	14,1	17,1	0,00	0,000	102,02	1,004	305,21	3,004	743,60	7,319	81,64	0,804
24/05/2005	E9	NF	14,0	13,1	13,9	14,1	17,1	0,00	0,000	88,63	0,872	292,76	2,882	729,04	7,176	82,32	0,810
24/05/2005	L10	NF	33,7	34,1	32	32,2	15,7	17,12	0,168	35,87	0,353	145,88	1,436	2657,96	26,161	0,00	0,000
24/05/2005	L10	NF	33,7	34,1	32	32,2	15,7	32,68	0,378	29,78	0,345	49,59	0,574	2358,97	27,316	2,00	0,023
25/05/2005	E9	F	14,0	13,1	13,9	14,1	17,1	0,00	0,000	86,10	0,847	271,94	2,677	787,08	7,747	79,57	0,783
25/05/2005	E9	F	14,0	13,1	13,9	14,1	17,1	0,00	0,000	110,00	1,083	412,12	4,056	1041,95	10,255	104,16	1,025
25/05/2005	E9	F	14,0	13,1	13,9	14,1	17,1	0,00	0,000	11,22	0,110	371,37	3,655	821,15	8,082	14,76	0,145
25/05/2005	E10	F	16,0	16,2	12,5	12,7	17,3	0,00	0,000	0,00	0,000	32,19	0,317	0,00	0,000	0,00	0,000
25/05/2005	E10	F	16,0	16,2	12,5	12,7	17,3	0,00	0,000	0,00	0,000	33,94	0,334	0,00	0,000	0,00	0,000
25/05/2005	E12	F	20,0	20,3	17,7	17,9	17,4	0,00	0,000	79,12	0,779	374,75	3,689	734,51	7,229	109,16	1,074
25/05/2005	E12	F	20,0	20,3	17,7	17,9	17,4	0,00	0,000	68,71	0,676	378,09	3,721	725,12	7,137	92,85	0,914
25/05/2005	L12	F	33,7	34,1	32,8	33,0	15,5	46,30	0,456	17,38	0,171	156,09	1,536	745,91	7,342	0,00	0,000
25/05/2005	L12	F	33,7	34,1	32,8	33,0	15,5	45,19	0,445	31,18	0,307	175,87	1,731	613,11	6,035	0,00	0,000
25/05/2005	L11	F	32,8	33,1	31,9	32,1	15,8	31,62	0,311	10,15	0,100	204,56	2,013	1310,98	12,903	0,00	0,000
25/05/2005	L11	F	32,8	33,1	31,9	32,1	15,8	54,95	0,541	12,45	0,123	263,40	2,593	1301,79	12,813	0,00	0,000
25/05/2005	L13	F	33,3	33,7	32,6	32,8	17	46,24	0,455	18,73	0,184	175,61	1,728	134,77	1,326	0,00	0,000
25/05/2005	L13	F	33,3	33,7	32,6	32,8	17	58,73	0,578	18,63	0,183	183,52	1,806	136,89	1,347	0,00	0,000

25/05/2005	E9	NF	14,0	13,1	13,9	14,1	17,1	0,00	0,000	97,54	0,960	296,10	2,914	785,87	7,735	0,00	0,000
25/05/2005	E10	NF	16,0	16,2	12,5	12,7	17,3	17,29	0,170	78,65	0,774	96,24	0,947	313,72	3,088	94,89	0,934
25/05/2005	E10	NF	16,0	16,2	12,5	12,7	17,3	9,75	0,096	77,05	0,758	103,68	1,020	291,30	2,867	71,13	0,700
25/05/2005	E12	NF	20,0	20,3	17,7	17,9	17,4	0,00	0,000	82,52	0,812	107,25	1,056	384,38	3,783	86,57	0,852
25/05/2005	E12	NF	20,0	20,3	17,7	17,9	17,4	0,00	0,000	78,71	0,775	395,54	3,893	1220,12	12,009	191,51	1,885
25/05/2005	E18	NF	30,0	30,4	28,9	29,1	16,5	0,00	0,000	0,00	0,000	157,75	1,553	1170,73	11,523	52,40	0,516
25/05/2005	E18	NF	30,0	30,4	28,9	29,1	16,5	0,00	0,000	0,00	0,000	194,98	1,919	1267,77	12,478	59,14	0,582
25/05/2005	L12	NF	33,7	34,1	32,8	33,0	15,5	36,14	0,356	3,74	0,037	98,90	0,973	607,91	5,983	0,00	0,000
25/05/2005	L12	NF	33,7	34,1	32,8	33,0	15,5	41,09	0,404	9,85	0,097	153,92	1,515	680,86	6,701	0,00	0,000
25/05/2005	L11	NF	32,8	33,1	31,9	32,1	15,8	39,38	0,388	14,66	0,144	154,67	1,522	1144,10	11,261	0,00	0,000
25/05/2005	L11	NF	32,8	33,1	31,9	32,1	15,8	48,89	0,481	24,04	0,237	166,01	1,634	1167,37	11,490	0,00	0,000
25/05/2005	L13	NF	33,3	33,7	32,6	32,8	17	47,81	0,471	24,45	0,241	148,55	1,462	149,10	1,467	0,00	0,000
25/05/2005	L13	NF	33,3	33,7	32,6	32,8	17	45,41	0,447	14,42	0,142	162,97	1,604	120,82	1,189	0,00	0,000
25/05/2005	L14	NF	33,8	34,2	33	33,2	15	51,91	0,511	27,17	0,267	199,24	1,961	346,08	3,406	0,00	0,000
25/05/2005	L14	NF	33,8	34,2	33	33,2	15	50,48	0,497	33,63	0,331	236,44	2,327	394,79	3,886	0,00	0,000
26/05/2005	E18	F	30,0	30,4	28,9	29,1	16,5	0,00	0,000	0,00	0,000	251,10	2,471	1267,98	12,480	58,46	0,575
26/05/2005	E18	F	30,0	30,4	28,9	29,1	16,5	0,00	0,000	0,00	0,000	282,60	2,782	1438,09	14,154	45,27	0,446
26/05/2005		F			17,7	17,9	17,4	87,93	0,865	138,03	1,359	176,19	1,734	378,51	3,726	96,64	0,951
26/05/2005		F			17,7	17,9	17,4	132,71	1,306	95,80	0,943	255,72	2,517	423,05	4,164	106,85	1,052
26/05/2005	L1	F	26,0	26,9	25	25,2	17,7	0,00	0,000	55,70	0,548	137,75	1,356	439,24	4,323	0,00	0,000
26/05/2005	L1	F	26,0	26,9	25	25,2	17,7	0,00	0,000	49,89	0,491	161,66	1,591	391,66	3,855	0,00	0,000
26/05/2005	L4	F	29,7	29,8	27,4	27,6	17,6	63,77	0,628	49,45	0,487	290,88	2,863	1130,31	11,125	53,95	0,531
26/05/2005	L4	F	29,7	29,8	27,4	27,6	17,6	61,66	0,607	27,80	0,274	300,15	2,954	1043,05	10,266	46,80	0,461
26/05/2005	L14	F	33,8	34,2	33	33,2	15	49,11	0,483	29,77	0,293	243,73	2,399	329,27	3,241	0,00	0,000
26/05/2005	L14	F	33,8	34,2	33	33,2	15	60,79	0,598	38,91	0,383	300,10	2,954	370,50	3,647	0,00	0,000
26/05/2005	L15	F	33,7	34,1	32,1	32,3	16,3	46,87	0,461	33,38	0,329	201,38	1,982	247,71	2,438	0,00	0,000
26/05/2005	L15	F	33,7	34,1	32,1	32,3	16,3	56,55	0,557	44,73	0,440	246,54	2,427	253,97	2,500	0,00	0,000
26/05/2005	L16	F	33,3	34,8	33,3	33,5	11,6	36,29	0,714	15,26	0,300	82,70	1,628	2442,48	48,080	58,23	1,146
26/05/2005	E21	F	27,8	27,6	25	25,2	17,5	75,08	0,739	62,20	0,612	252,42	2,484	1244,80	12,252	131,46	1,294

26/05/2005	E21	F	27,8	27,6	25	25,2	17,5	75,08	0,739	62,20	0,612	252,42	2,484	1244,80	12,252	131,46	1,294
26/05/2005		NF			17,7	17,9	17,4	109,11	1,074	54,64	0,538	134,77	1,327	401,42	3,951	115,07	1,133
26/05/2005		NF			17,7	17,9	17,4	84,47	0,831	64,92	0,639	177,66	1,749	396,51	3,903	104,73	1,031
26/05/2005	L1	NF	26,0	26,9	25	25,2	17,7	77,56	0,763	37,12	0,365	170,33	1,676	702,19	6,911	99,53	0,980
26/05/2005	L1	NF	26,0	26,9	25	25,2	17,7	76,50	0,753	32,94	0,324	220,83	2,174	837,60	8,244	94,57	0,931
26/05/2005	L4	NF	29,7	29,8	27,4	27,6	17,6	0,00	0,000	34,10	0,336	92,97	0,915	446,52	4,395	0,00	0,000
26/05/2005	L4	NF	29,7	29,8	27,4	27,6	17,6	80,44	0,792	39,47	0,388	250,63	2,467	1005,36	9,895	74,16	0,730
26/05/2005	L6	NF	31,5	31,7	28,9	29,1	17,4	0,00	0,000	50,56	0,498	171,87	1,692	956,41	9,413	25,88	0,255
26/05/2005	L6	NF	31,5	31,7	28,9	29,1	17,4	52,72	0,519	31,10	0,306	203,16	2,000	1165,48	11,471	37,52	0,369
26/05/2005	L15	NF	33,7	34,1	32,1	32,3	16,3	63,68	0,627	7,55	0,074	214,63	2,112	243,90	2,401	0,00	0,000
26/05/2005	L15	NF	33,7	34,1	32,1	32,3	16,3	49,65	0,489	29,70	0,292	185,53	1,826	208,10	2,048	0,00	0,000
26/05/2005	L16	NF	33,3	34,8	33,3	33,5	11,6	226,86	2,233	12,17	0,120	47,32	0,466	3029,34	29,816	188,48	1,855
26/05/2005	L16	NF	33,3	34,8	33,3	33,5	11,6	3,46	0,068	47,97	0,944	52,75	1,038	1428,06	28,111	0,00	0,000
26/05/2005	E19	NF	30,0	30,2	29,3	29,5	16,8	522,74	5,145	107,43	1,057	45,63	0,449	862,43	8,489	187,35	1,844
26/05/2005	E19	NF	30,0	30,2	29,3	29,5	16,8	0,00	0,000	0,00	0,000	20,81	0,205	0,00	0,000	0,00	0,000
26/05/2005	E20	NF	29,0	29,4	29,6	29,8	17	88,20	0,868	36,76	0,362	104,43	1,028	1055,23	10,386	106,49	1,048
26/05/2005		NF			27,9	28,1	17,4	75,60	0,744	45,59	0,449	136,17	1,340	1159,69	11,414	121,07	1,192
26/05/2005	E21	NF	27,8	27,6	25	25,2	17,5	83,92	0,826	54,30	0,534	173,75	1,710	1057,31	10,407	127,87	1,259
26/05/2005	E21	NF	27,8	27,6	25	25,2	17,5	79,83	0,786	61,35	0,604	228,95	2,253	1163,44	11,451	135,95	1,338
29/05/2005	L6	F	31,5	31,7	28,9	29,1	17,4	47,64	0,469	34,12	0,336	219,58	2,161	1188,73	11,700	39,03	0,384
29/05/2005	E21	F	27,8	27,6	25	25,2	17,5	99,23	0,977	71,92	0,708	531,54	5,232	1457,36	14,344	120,67	1,188
29/05/2005	E23	NF	24,1	24,3	23,9	24,1	17,9	19,39	0,191	73,79	0,726	126,48	1,245	727,13	7,157	92,88	0,914
30/05/2005	L6	F	31,5	31,7	28,9	29,1	17,4	38,82	0,382	45,44	0,447	277,34	2,730	1568,92	15,442	52,55	0,517
30/05/2005	L7	F	33,5	33,8	31,2	31,4	16,5	0,00	0,000	23,39	0,230	26,80	0,264	847,66	8,343	0,00	0,000
30/05/2005	L7	F	33,5	33,8	31,2	31,4	16,5	0,00	0,000	5,00	0,049	0,00	0,000	249,56	2,456	0,00	0,000
30/05/2005	L5	F	32,6	32,9	30,8	31,0	17,3	84,76	0,834	59,88	0,589	219,56	2,161	1181,22	11,626	35,69	0,351
30/05/2005	L5	F	32,6	32,9	30,8	31,0	17,3	54,85	0,540	78,59	0,774	251,90	2,479	1458,30	14,353	55,46	0,546

30/05/2005	E23	F	24,1	24,3	23,9	24,1	17,9	67,73	0,667	79,39	0,781	237,78	2,340	1158,86	11,406	105,82	1,041
30/05/2005	E23	F	24,1	24,3	23,9	24,1	17,9	81,30	0,800	69,20	0,681	220,30	2,168	1078,59	10,616	120,38	1,185
30/05/2005	E24	F	22,1	22,1	22,8	23,0	17,4	100,03	0,985	57,56	0,567	971,13	9,558	2461,52	24,228	157,87	1,554
30/05/2005	E24	F	22,1	22,1	22,8	23,0	17,4	94,83	0,933	19,21	0,189	134,75	1,326	643,15	6,330	103,29	1,017
30/05/2005		F			20,8	21,0	17,8	82,85	0,815	71,53	0,704	226,35	2,228	966,64	9,514	112,88	1,111
30/05/2005		F			20,8	21,0	17,8	67,59	0,665	43,26	0,426	184,30	1,814	897,20	8,831	121,43	1,195
30/05/2005	E26	F	18,0	18,1	18,5	18,7	17	92,98	0,915	65,67	0,646	188,04	1,851	820,12	8,072	121,42	1,195
30/05/2005	E28	F	14,0	13,9	13,3	13,5	16	72,86	0,717	52,20	0,514	192,12	1,891	819,41	8,065	107,43	1,057
30/05/2005	L7	NF	33,5	33,8	31,2	31,4	16,5	42,90	0,352	14,39	0,118	107,41	0,881	2864,50	23,495	0,00	0,000
30/05/2005	L7	NF	33,5	33,8	31,2	31,4	16,5	0,00	0,000	20,64	0,203	0,00	0,000	452,09	4,450	0,00	0,000
30/05/2005	L5	NF	32,6	32,9	30,8	31,0	17,3	102,42	1,008	18,92	0,186	134,22	1,321	938,12	9,233	0,00	0,000
30/05/2005	L5	NF	32,6	32,9	30,8	31,0	17,3	74,52	0,733	52,72	0,519	178,45	1,756	1056,55	10,399	0,00	0,000
30/05/2005	L3	NF	33,2	33,5	31,1	31,3	16,4	19,60	0,193	37,84	0,372	175,02	1,723	1916,98	18,868	15,26	0,150
30/05/2005	E23	NF	24,1	24,3	23,9	24,1	17,9	85,69	0,843	78,61	0,774	218,77	2,153	1207,80	11,888	104,99	1,033
30/05/2005	E24	NF	22,1	22,1	22,8	23,0	17,4	62,76	0,618	32,74	0,322	217,23	2,138	1532,89	15,088	106,93	1,053
30/05/2005	E24	NF	22,1	22,1	22,8	23,0	17,4	127,49	1,255	0,00	0,000	163,92	1,613	758,06	7,461	119,09	1,172
30/05/2005		NF			20,8	21,0	17,8	68,04	0,670	42,74	0,421	209,90	2,066	1017,31	10,013	148,88	1,465
30/05/2005		NF			20,8	21,0	17,8	65,57	0,645	69,21	0,681	255,87	2,518	1067,40	10,506	102,14	1,005
30/05/2005	E25	NF	20,0	20,1	19,7	19,9	17,7	65,67	0,646	67,52	0,665	199,77	1,966	857,38	8,439	131,76	1,297
30/05/2005	E25	NF	20,0	20,1	19,7	19,9	17,7	77,65	0,764	63,24	0,622	240,30	2,365	919,94	9,055	136,66	1,345
30/05/2005	E26	NF	18,0	18,1	18,5	18,7	17	55,06	0,542	54,38	0,535	210,08	2,068	765,60	7,535	133,43	1,313
30/05/2005	E26	NF	18,0	18,1	18,5	18,7	17	93,26	0,918	58,43	0,575	227,80	2,242	939,76	9,250	142,73	1,405
30/05/2005	E26	NF	18,0	18,1	18,5	18,7	17	85,21	0,839	59,29	0,584	188,62	1,857	773,94	7,618	123,22	1,213
30/05/2005	E28	NF	14,0	13,9	13,3	13,5	16	71,23	0,701	57,82	0,569	174,85	1,721	553,74	5,450	133,85	1,317

Annexe 4 - Données concernant la culture phytoplanctonique en photobioréacteur (25/07/06-18/09/06)

		UA moy	nb cell moy (x10^6)	Ag (nM)		H2S moy (nM)(ni=3)		OCS moy (nM)(ni=3)		MeSH moy (nM)(ni=3)		DMS moy (nM)(ni=3)		DMDS moy (nM)(ni=3)	
date	T°C	(ni=3)	(ni =4)	calculé	mesuré	Filtré	Non filtré	Filtré	Non filtré	Filtré	Non filtré	Filtré	Non filtré	Filtré	Non filtré
25/07/06	30,5	0,15	1,79	0	0,00										
26/07/06	30,3	0,13	1,71	0											
27/07/06	29,7	0,12	1,67	0											
28/07/06	29,1	0,16	2,30	0											
29/07/06				0											
30/07/06				0	0,00										
31/07/06	29,2	0,23	4,03	0											
01/08/06	28,1	0,24	5,06	0											
02/08/06	27,9	0,27	4,53	0											
03/08/06	28	0,28	5,53	0											
04/08/06	28,7	0,29		0											
05/08/06				0											
06/08/06				0											
07/08/06	29,2	0,30	5,59	0		0,143	0,124	0,617	1,018	0,000	0,000	4,262	6,440	0,219	0,407
08/08/06	30,6	0,30	5,02	0	0,00	0,386	0,188	0,264	0,196	0,427	0,000	7,312	6,508	0,289	0,349
09/08/06	29,1	0,29	4,50	0		0,234	0,181	0,232	0,235	0,353	0,358	7,538	6,286	0,330	0,361
10/08/06	28,8	0,29	4,60	0		0,177	0,187	0,185	0,166	0,351	0,426	9,928	7,265	0,389	0,436
11/08/06	27,6	0,29	5,45	0		0,187	0,174	0,161	0,157	0,391	0,383	7,473	7,019	0,541	0,484
12/08/06	27,1	0,29		0											
13/08/06	28,1	0,27		0,1	1,38										
14/08/06	28,2	0,29		0,1	1,15										
15/08/06	29,4	0,28		0,1	1,03										
16/08/06	28	0,25	4,45	0,1		0,188	0,211	0,101	0,137	1,747	1,050	9,532	9,343	0,562	0,535
17/08/06	27,9	0,26	4,55	0,1		0,146	0,198	0,047	0,063	0,475	1,034	9,170	9,995	0,537	0,509
18/08/06	27,8	0,26	4,57	0,1		0,192	0,165	0,078	0,063	0,705	0,752	13,445	9,569	0,539	0,505
19/08/06				0,1		0,168	0,188	0,048	0,058	0,780	0,693	10,540	9,660	0,529	0,518
20/08/06				0,1											
21/08/06	27,9	0,26	4,53	0,5	1,15										
22/08/06		0,29		0,5		0,332	0,659	0,157	0,461	2,488	2,022	13,515	14,716	0,510	0,512

23/08/06				0,5	1,61	0,418	0,428	0,269	0,270	0,657	0,237	10,781	12,936	0,430	0,472
24/08/06	27,4	0,37	6,72	0,5	1,38	0,503	0,475	0,229	0,236	1,139	0,348	14,110	11,287	0,490	0,409
25/08/06	28,3	0,35	6,00	0,5											
26/08/06				0,5											
27/08/06				0,5											
28/08/06	27,9	0,27	4,98	0,5	1,15	0,278	0,417	0,238	0,166	1,303	0,801	11,099	9,904	0,497	0,436
29/08/06	27,5	0,26		1	1,38	0,352	0,464	0,259	0,219	1,525	0,339	10,790	8,303	0,506	0,333
30/08/06				1	0,92	0,304	0,529	0,195	0,197	0,665	0,317	11,297	10,147	0,396	0,378
31/08/06				1	0,92	0,305	0,483	0,260	0,154	0,641	0,220	10,505	9,437	0,451	0,396
01/09/06				1		0,302	0,448	0,296	0,200	0,528	0,233	10,039	9,815	0,364	0,358
02/09/06				1											
03/09/06				1	0,92										
04/09/06		0,31	5,54	5	5,05	0,247	0,286	0,270	0,154	0,733	0,250	8,472	9,094	0,519	0,492
05/09/06		0,30	6,00	5	2,75	0,215	0,293	0,188	0,157	0,662	0,226	10,060	9,192	0,508	0,460
06/09/06	29,6	0,30	5,03	5	1,38	0,214	0,164	0,295	0,175	0,863	0,188	10,058	8,909	0,544	0,409
07/09/06		0,23	4,50	5	0,92	0,266	0,185	0,231	0,178	0,608	0,292	8,362	7,785	0,658	0,698
08/09/06	28,8	0,22	4,25	5	0,92	0,281	0,215	0,227	0,186	0,656	0,458	8,938	6,109	0,722	0,697
09/09/06	28,7	0,21	4,19	5	1,15	0,239	0,225	0,219	0,194	0,455	0,456	7,857	5,776	0,582	0,724
10/09/06	28,9	0,21		5	9,40	0,227	0,212	0,241	0,179	0,408	0,453	7,406	5,456	0,562	0,689
11/09/06	28,8	0,19	3,88	10	7,34	0,183	0,210	0,189	0,171	0,396	0,471	6,805	5,485	0,464	0,765
12/09/06		0,19		10	3,90	0,202	0,205	0,210	0,169	0,460	0,485	7,285	5,394	0,466	0,761
13/09/06	28,5	0,19	3,85	10	2,75	0,181	0,205	0,191	0,168	0,398	0,491	6,456	5,460	0,407	0,762
14/09/06	28,6	0,17	3,74	10	1,83	0,243	0,200	0,248	0,168	0,518	0,485	8,333	5,426	0,538	0,749
15/09/06	28,1	0,17	3,72	10	1,12	0,187	0,204	0,189	0,165	0,374	0,475	6,155	5,348	0,404	0,756
16/09/06	27,6	0,17	3,66	10	1,10	0,180	0,204	0,189	0,167	0,356	0,484	6,226	5,355	0,391	0,731
17/09/06	27,4	0,16	3,61	10	1,08	0,179	0,203	0,194	0,167	0,365	0,486	6,249	5,410	0,404	0,708
18/09/06	27,9	0,16	3,57	10	1,06	0,182	0,204	0,196	0,168	0,372	0,487	6,275	5,394	0,367	0,722