

## Evaluating what is measured by BDOC and AOC tests ?

Que mesurent les tests de CODB et de COA ?

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### RÉSUMÉ

Un des objectifs de cette recherche est d'examiner les différences entre les résultats obtenus par les tests de dosage des matières organiques biodégradables (MOB). L'autre objectif est de déterminer comment les résultats peuvent correspondre à la valeur vraie de la MOB. L'étude a été menée en employant un modèle mathématique qui tient compte des principes cinétiques et stoechiométriques.

Le tableau 1 présente les exemples des équations de bilan de masse qui entrent dans le modèle. Celui-ci permet de suivre la croissance de la biomasse, la dégradation du substrat (MOB), le carbone organique dissous (COD), ainsi que la production et la dégradation des produits microbiens solubles (PMS). Les PMS, qui possèdent des poids moléculaires allant de moyens à élevés, sont produits durant le métabolisme normal des cellules (RITTMANN *et al.*, 1987). Les PMS peuvent être divisés en deux groupes de produits associés : les PAU qui sont le résultat direct de l'utilisation du substrat et les PAB qui sont produits proportionnellement à la biomasse (PAB).

Certaines hypothèses sont à la base des équations du bilan massique. La biomasse n'est constituée que d'hétérotrophes. La MOB est modélisée en tenant compte de substrats facilement et difficilement dégradables. Chaque substrat se distingue par sa valeur K inscrite au tableau 3. La densité de biomasse en début de test est de 1 mg/l (2400 UFC/ml), sauf quand la densité est modifiée dans le modèle. Pour les besoins de la modélisation, les valeurs de MOB, de CODB et de biomasse ont été converties en demande chimique en oxygène (DCO). Les facteurs de conversion utilisés sont : 1,42 mg de MOB exprimée en DCO/mg de MOB exprimée en solides volatils dissous,  $4,16 \times 10^{-7}$  mg DCO/cellule et 2,67 mg acétate exprimé en DCO/mg de C-acétate.

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Un ensemble de courbes typiques pour le modèle est présenté aux figures 1 et 2. La figure 1 montre les résultats obtenus pour un substrat facilement dégradable, tandis que la figure 2 présente ceux obtenus pour un substrat difficilement dégradable. Dans les deux cas, la biomasse s'accroît graduellement pour atteindre un maximum, puis rediminue. Les vitesses et intensités de réaction dépendent toutefois beaucoup des cinétiques de dégradation de la MOB. Les deux figures traduisent l'accumulation continue des PMS, qui représentent des proportions respectives de 43 % et 30 % de la MOB d'origine pour les substrats facilement et difficilement dégradables. L'accumulation des PMS est importante, car la courbe de décroissance du COD est le résultat net de la MOB consommée moins les PMS accumulés. Ceci implique que le changement dans le niveau de COD, qui représente le paramètre de contrôle pour les tests CODB, n'égale pas la MOB vraie. Le CODB mesuré ne représenterait plutôt que 50 à 60 % de la MOB d'origine.

La figure 3 montre la relation qui existe entre le CODB et la MOB pour les deux types de substrats. Le CODB n'est pas égal à la MOB, ce qui est démontré par l'écart observé par rapport à la droite d'équivalence de pente 1. Cette différence est due à deux phénomènes : l'accumulation des PMS dépend de la MOB, tandis que l'écart entre les deux types de substrat est le résultat des courbes s'approchant de  $S_{\min}$  sur l'axe de la MOB, lorsque le CODB tend vers zéro. Ce résultat est significatif, car des études ont démontré que la MOB dans les eaux brutes contient surtout des substrats difficilement dégradables (LECHEVALLIER *et al.*, 1991). Ainsi, faire l'hypothèse que le CODB soit égal à la MOB pour les substrats difficilement assimilables se traduirait par une importante sous-estimation de la MOB dans l'échantillon.

La figure 4 montre la relation observée entre la biomasse maximum, employée avec les tests COA (carbone organique assimilable), et la vraie MOB pour les deux substrats. Cette figure présente aussi l'étalon de calibration proposé par van der Kooij *et al.* (1982), qui convertit le nombre de cellules en C-acétate ( $4,1 \times 10^6$  cellules par mg C-acétate). Ni le substrat facilement utilisable ni le substrat difficilement utilisable, ne s'approche de la courbe de calibration. Ces écarts sont causés par la variation du premier ordre en ordre zéro de l'équation de Monod et aussi parce que les courbes approchent le  $S_{\min}$  où la croissance des cellules est presque nulle. Lorsque la MOB dans l'échantillon est principalement constituée d'un substrat difficilement dégradable, l'usage d'un étalon d'acétate produit une forte sous-estimation de la MOB vraie.

La figure 5 montre la relation directe entre le CODB et le COA pour les deux types de substrats. L'augmentation du rapport CODB/COA avec la diminution de la MOB s'explique par le fait que la biomasse tend vers une croissance zéro lorsque la MOB s'approche de  $S_{\min}$ . Cette figure démontre clairement qu'il existe une différence fondamentale entre les mesures des tests CODB et COA, lorsque la MOB tend vers  $S_{\min}$ . Toutefois, le rapport CODB/COA est presque unitaire dans le cas du substrat facilement dégradable, quand la MOB se situe à l'intérieur des limites de détection pour le dosage du CODB (environ 100 mg/l à la figure 5). Ainsi, il est possible d'obtenir le même résultat avec les deux types de tests.

Le modèle permet aussi d'examiner l'effet des concentrations en biomasse initiale pour une [MOB] fixée. Pour un substrat facilement dégradable, qui est entièrement consommé en présence d'un faible inoculum, la modélisation montre que le CODB et la biomasse maximum ne sont pas affectés. Cependant, le résultat diffère pour un substrat difficilement dégradable qui n'est pas entièrement consommé avec un inoculum de faible densité. Tel que présenté à la figure 6, le CODB et la biomasse maximum augmentent fortement avec la densité de l'inoculum. Cet effet est dû à la faible croissance de la biomasse qui survient en présence d'un inoculum de faible densité ; la biomasse maximum et le COD minimum sont atteints après 30 jours. Avec un inoculum important, la biodégradation survient plus rapidement et le CODB maximum est atteint avant 30 jours.

Mots clés : COA, CODB, biodégradation, matière organique, test biologique, croissance bactérienne, eau potable.

## SUMMARY

Batch type biodegradable organic material (BOM) tests are modeled using basic kinetic and stoichiometric principles. The modeling results reveal that for biodegradable dissolved organic carbon (BDOC) tests, the change in dissolved organic carbon (DOC) is not equal to BOM. The formation of soluble microbial products (SMP) and the degradation kinetics of the BOM must be considered to estimate the true BOM from BDOC results. For assimilable organic carbon (AOC) tests, using a calibration standard based on an easy to degrade substrate, such as acetate, does not necessarily give an accurate indication of the true BOM. The kinetics of BOM degradation must be estimated before an AOC test can be used to interpret the true BOM in a sample. The inoculum density can also influence the results of AOC and BDOC tests. When the BOM is hard to degrade, using a low density test can underestimate the amount of BDOC in a sample.

*Key-words* : AOC, BDOC, biodegradation, organic matter, bioassay, bacterial growth, drinking water.

## INTRODUCTION

Growth of bacteria can cause major problems in water distribution systems. Besides causing violations of drinking water standards such as coliform counts (RICE *et al.*, 1991), bacteria can form taste and odor compounds, decrease hydraulic capacity, and enhance pipe corrosion (RITTMANN and HUCK, 1989). Many studies, including VAN DER KOOIJ (1992) and LECHEVALLIER *et al.* (1991), demonstrate that growth is directly proportional to the amount of biodegradable organic material (BOM) in the post-treatment water. Therefore, accurately measuring the low concentrations of BOM that exist in water supplies is a key to assessing bacterial growth potential of drinking water.

The need to minimize BOM in treated water is especially important now that regulations are placing strict limits on the concentrations of halogenated organic by-products (RITTMANN and HUCK, 1989). To prevent growth in water containing excessive BOM will require chlorine residuals far in excess of standards allowed for disinfection by-products. Only if the amount of BOM entering a distribution system is known will it be possible to balance between removing BOM during treatment and optimizing the chlorine residual to minimize halogenated organic formation while assuring the control of bacterial growth. Even with the use of biological treatment methods like those currently used in Europe, an accurate measurement of BOM is essential to monitor system performance and assure control of bacterial growth.

During the 1980s and 90s, numerous BOM measurement techniques were developed and are summarized by HUCK (1990). Most involve batch incubations, and they can be divided into three main classes:

1. Tests of the assimilable organic carbon (AOC) type measure BOM as the increase in biomass as assayed by plate counts, ATP, or other biomass-specific parameter. The BOM is typically determined by multiplying the increase in biomass by a calibration factor established by biomass growth on

known amounts of a simple substrate, such as acetate. The biomass inoculum density is very small; thus, these tests require a long incubation period of 5 to 30 days, but can detect small BOM concentrations, typically to 10  $\mu\text{g C/l}$ . The original version of this test was developed by VAN DER KOOIJ (1982).

2. Tests of the biodegradable dissolved organic carbon (BDOC) type equate BOM to the decrease in the dissolved organic carbon (DOC). The biomass inoculum density is very small, thus requiring a long incubation period. An example of this test was developed by SERVAIS *et al.* (1987). BDOC type tests typically have a minimum detection limit of 100-200  $\mu\text{g/l}$ , which may be a serious limitation, since problematical growth has been correlated with BOM levels as low as 10-50  $\mu\text{g/l}$  in studies by VAN DER KOOIJ (1992) and RICE *et al.* (1991).

3. High inoculum-density tests of the BDOC type use a large inoculum in order to decrease the incubation time. An example of this test was developed by JORET *et al.* (1991). This reduces the incubation time to 1-7 days, but the effect on test accuracy is not known.

The most prevalent problem with these tests is the inconsistencies in the values obtained. These variations exist for several reasons: variation in laboratory techniques, use of natural versus pure cultures, the use of different substrates to calibrate AOC type tests, or differences that result from the fundamental principles that govern bacterial growth. The purpose of this research is to examine if fundamental differences exist between what BOM tests measure and how these measurements relate to the true amount of BOM. The research accomplishes these goals by applying a mathematical model encompassing the basic kinetic and stoichiometric principles involved in bacterial growth during BOM tests.

## METHODS

The model described here was designed to capture all of the phenomena that can affect bacterial growth in a BOM testing environment. The model tracks the growth of biomass, the degradation of substrate (BOM), the amount of dissolved organic carbon (DOC), and the production and degradation of soluble microbial products (SMP).

SMP are molecules of moderate to high molecular weight that are produced during normal cell metabolism (NAMKUNG and RITTMANN, 1986). SMP can be a dominant part of the dissolved organic carbon, and they are distinctively different from the original substrate, the true BOM. SMP can be subdivided into two categories: utilization associated products (UAP), which are produced directly as a result of substrate utilization, and biomass associated products (BAP), which are produced in proportion to biomass (RITTMANN *et al.* 1987).

Table 1 shows the mass-balance equations that comprise the model. Table 2 defines the variables and units used in the equations. Features and assumptions of the mass-balance equations are as follows:

- Only heterotrophs are present.
- Oxygen is never limiting.
- Growth of bacteria is represented by a Monod type growth expression (equation 1). As discussed by RITTMANN *et al.* (1987), growth of biomass can result from the utilization of BOM substrate and both types of SMP (UAP and BAP), as shown by the  $R_1$  term (equation 2). Biomass decay can occur by endogenous respiration of biomass ( $b_h$ ) and by the formation of BAP ( $k_2$ ).
- Substrate (BOM) utilization is represented by a Monod type expression (equation 3). For this study, the BOM was subdivided into a relatively easy to degrade substrate (S1) and a hard to degrade substrate (S2). They are distinguished by varying the value of the kinetic parameter K.
- Separate SMP mass balances for the UAP (equation 4) and BAP (equation 5) show positive terms for production and negative terms for degradation. As developed by NOGUERA (1991), a Monod type expression is used for the formation of UAP and the degradation of UAP and BAP. BAP production is proportional to the concentration of active biomass.
- A non-degradable portion of the initial DOC is allowed and called SX.
- Dissolved organic carbon (equation 6) is the summation of the substrate (BOM), the non-degradable portion of the original DOC (SX), and both forms of SMP (UAP and BAP).

**Tableau 1** Équations de bilan de masse pour une espèce bactérienne et un substrat.

**Table 1** Mass balance equations for one-species and one-substrate.

Biomass:	
$V \frac{dX_h}{dt} = Y \left( q_m \left[ \frac{S}{K+S} \right] \right) X_h V + Y_p R_1 X_h V - b_h X_h V - k_2 X_h V$	(1)
$R_1 = q_{uap} \left[ \frac{UAP}{K_{uap} + UAP} \right] + q_{bap} \left[ \frac{BAP}{K_{bap} + BAP} \right]$	(2)
Substrate (BOM):	
$V \frac{dS}{dt} = -q_m \left[ \frac{S}{K+S} \right] X_h V$	(3)
Soluble Microbial Products:	
$V \frac{dUAP}{dt} = k_1 \left( q_m \left[ \frac{S}{K+S} \right] \right) X_h V - q_{uap} \left[ \frac{UAP}{K_{uap} + UAP} \right] X_h V$	(4)
$V \frac{dBAP}{dt} = k_2 X_h V - q_{bap} \left[ \frac{BAP}{K_{bap} + BAP} \right] X_h V$	(5)
Dissolved Organic Carbon:	
$DOC = S + SX + UAP + BAP$	(6)

Tableau 2 Notations utilisées pour les equations de bilan de masse.

Table 2 Notations used for mass-balance equations.

$b_h$	=	biomass endogenous-decay coefficient [ $d^{-1}$ ]
BAP	=	biomass associated products [ $\mu g \text{ COD}_{\text{bap}}/l$ ]
$k_1$	=	UAP formation rate constant [ $\mu g \text{ COD}_{\text{uap}}/\mu g \text{ COD}_s^{-1}$ ]
$k_2$	=	BAP formation rate constant [ $\mu g \text{ COD}_{\text{bap}}/\mu g \text{ COD}_{\text{cell}}^{-1} d^{-1}$ ]
$K_{\text{bap}}$	=	half-maximum-rate concentration for BAP degradation [ $\mu g \text{ COD}_{\text{bap}}/l$ ]
$K$	=	half-maximum-rate concentration for BOM degradation [ $\mu g \text{ COD}_s/l$ ]
$K_{\text{uap}}$	=	half-maximum-rate concentration for UAP degradation [ $\mu g \text{ COD}_{\text{uap}}/l$ ]
$q_{\text{bap}}$	=	maximum specific rate of BAP degradation [ $\mu g \text{ COD}_{\text{bap}}/\mu g \text{ COD}_{\text{cell}}^{-1} d^{-1}$ ]
$q_m$	=	maximum rate of substrate degradation [ $\mu g \text{ COD}_s/\mu g \text{ COD}_{\text{cell}}^{-1} d^{-1}$ ]
$q_{\text{uap}}$	=	maximum specific rate of UAP degradation [ $\mu g \text{ COD}_{\text{uap}}/\mu g \text{ COD}_{\text{cell}}^{-1} d^{-1}$ ]
$R_1$	=	specific SMP consumption rate [ $\mu g \text{ COD}_{\text{smp}}/\mu g \text{ COD}_{\text{cell}}^{-1} d^{-1}$ ]
$S$	=	substrate concentration (BOM) [ $\mu g \text{ COD}_s/l$ ]
SX	=	non-biodegradable dissolved organic concentration [ $\mu g \text{ COD}_s/l$ ]
UAP	=	utilization associated products [ $\mu g \text{ COD}_{\text{uap}}/l$ ]
$V$	=	volume [l]
$X_h$	=	heterotrophic biomass concentration [ $\mu g \text{ COD}_{\text{cell}}/l$ ]
$Y$	=	growth yield associated with substrate [ $\mu g \text{ COD}_{\text{cell}}/\mu g \text{ COD}_s^{-1}$ ]
$Y_p$	=	growth yield associated with SMP degradation [ $\mu g \text{ COD}_{\text{cell}}/\mu g \text{ COD}_{\text{smp}}^{-1}$ ]

The modeling runs presented in the results of this study used the following standard conditions, which illustrate all the key points. First, the biomass was composed of only one species, modeled as *Pseudomonas fluorescens* type cells for the purposes of converting cell numbers to biomass COD (chemical oxygen demand). Second, the substrate was either easy-to-degrade or hard-to-degrade; they were never present together. Finally, the inoculum density was 1  $\mu g/l$  (2400 CFU/ml), except for when the effect of varying the inoculum density was modeled.

## PARAMETER VALUES AND CONVERSIONS

Kinetic and stoichiometric parameter values used in the model are given in Table 3. Most of these values are representative of what is found in the literature, such as LAWRENCE and MCCARTY (1970) and FURUMAI and RITTMANN (1992). However, values for the formation and degradation of SMP applicable to the highly oligotrophic conditions that exist in drinking water are not available. Reasonable estimates were made from existing literature values taken from NOGUERA (1991) and TINGLEY (1991). These studies indicate that the degradation kinetics of SMP is many times slower than in the original simple substrates. One result of this relationship is that the model is not highly sensitive to SMP parameter values.

**Tableau 3** Valeurs des paramètres cinétiques.**Table 3** Kinetic parameter values.

	Variable	Value
$b_h$	$[d^{-1}]$	0.1
$k_1$	$[\mu g \text{ COD}_{uap}/\mu g \text{ COD}_s^{-1}]$	0.1
$k_2$	$[\mu g \text{ COD}_{bap}/\mu g \text{ COD}_{cell}^{-1} d^{-1}]$	0.1
$K(S1)$	$[\mu g \text{ COD}_s/l]$	1000
$K(S2)$	$[\mu g \text{ COD}_s/l]$	7500
$K_{bap}$	$[\mu g \text{ COD}_{bap}/l]$	20,000
$K_{uap}$	$[\mu g \text{ COD}_{uap}/l]$	30,000
$q_m$	$[\mu g \text{ COD}_s/\mu g \text{ COD}_{cell}^{-1} d^{-1}]$	10.0
$q_{bap}$	$[\mu g \text{ COD}_{bap}/\mu g \text{ COD}_{cell}^{-1} d^{-1}]$	2.0
$q_{uap}$	$[\mu g \text{ COD}_{uap}/\mu g \text{ COD}_{cell}^{-1} d^{-1}]$	13.0
$Y$	$[\mu g \text{ COD}_{cell}/\mu g \text{ COD}_s^{-1}]$	0.6
$Y_p$	$[\mu g \text{ COD}_{cell}/\mu g \text{ COD}_s^{-1}]$	0.6

The results of BOM tests in experimental studies are given as BDOC, acetate C, or biomass. Since organic carbon can have an oxidation state from +3 to -4, direct comparisons of these values cannot be made. For the purposes of this model, values of BOM, BDOC, and biomass were converted to chemical oxygen demand (COD). The conversion factors used are as follows: 1.42  $\mu g$  BOM as COD/ $\mu g$  BOM as VSS,  $4.16 \times 10^{-7}$   $\mu g$  COD/cell, and 2.67  $\mu g$  acetate as COD/ $\mu g$  acetate C. Appendix A shows the derivation of these conversions.

## RESULTS AND DISCUSSION

A typical set of output curves for the model is shown in Figures 1 and 2. Figure 1 shows the results of modeling growth on the easy-to-degrade substrate, while Figure 2 depicts growth on the hard-to-degrade substrate. In both cases, the biomass slowly rises to a peak value, then slowly declines. The location of the peak is highly dependent on the ease of degradation; it occurs much sooner for the easy-to-degrade substrate. This biomass peak is critical, because it is used to assess BOM by AOC type tests.

Degradation of the original BOM also is shown in both figures. For the easy-to-degrade substrate, the most rapid degradation occurs during the first 7 days, and the BOM is fully removed by 25 days. For the hard-to-degrade substrate, the degradation is much slower, and degradation is not complete even after 30 days.

The most revealing aspect of these figures is contained in the SMP curves. Both show continuous accumulation of SMP, reaching a value that is 43% of

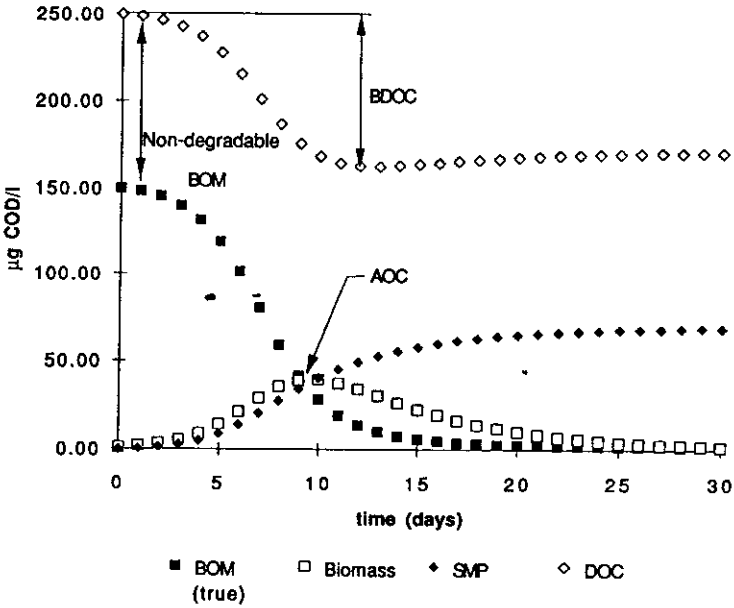


Figure 1 Résultat typique pour 150 µg/l de substrat facilement utilisable.  
Example BOM model output curve for 150 µg/L of the easy-to-degrade BOM.

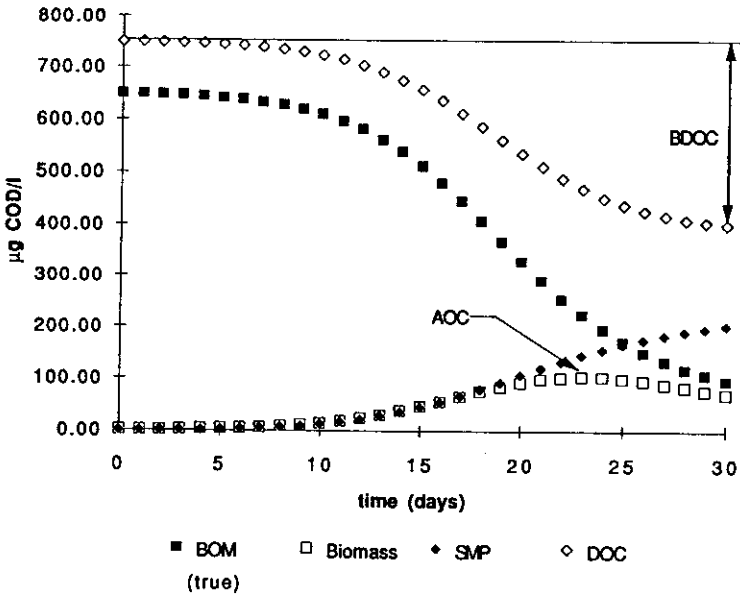


Figure 2 Résultat typique pour 650 µg/l de substrat facilement utilisable.  
Example BOM model output curve for 650 µg/L of the hard-to-degrade BOM.



the original BOM level for the easy-to-degrade substrate and 30% for the hard-to-degrade substrate. The build-up of SMP is significant, because the decline shown in the DOC curve is the net result of the BOM degraded minus the amount of SMP accumulated. This means that the change in the DOC level, the assay for the BDOC tests, is not equal to the true BOM; instead the measured BDOC is only about 50-60% of the original BOM.

### Comparing BDOC and AOC to BOM

The model was used to simulate the range of BOM typically found in drinking water in order to compare the measurable quantities – the amount of biomass and the change in DOC – with the true BOM. Figure 3 shows the relationship between BDOC and BOM for an easy-to-degrade substrate (S1) and a hard-to-degrade substrate (S2). The BDOC is not equal to BOM, as evidenced by the deviation from the 1:1 ratio line in the figure. This deviation, which depends on the true BOM and on the degradation kinetics of the substrate, is caused by two phenomena. First, the amount of SMP produced is a function of the BOM. A higher amount of BOM directly increases the accumulation of UAP, and it indirectly increases the amount of BAP because biomass accumulation is proportional to the BOM. Second, the difference between the easy-to-degrade and hard-to-degrade substrate is a result of the curves approaching  $S_{\min}$  on the BOM axis, where BDOC approaches zero.  $S_{\min}$  is defined as the minimum amount of substrate required to maintain a positive growth rate and is defined mathematically for this model by equation 7:

$$S_{\min} = K \left( \frac{b_h + k_2 - Y_p R_1}{Y_{q_m} - (b_h + k_2 - Y_p R_1)} \right) \quad (7)$$

$S_{\min}$  is 34.5  $\mu\text{g/l}$  for the easy-to-degrade substrate and 259  $\mu\text{g/l}$  for the hard-to-degrade substrate.

The result shown in Figure 3 is important because studies, including that of LECHEVALLIER *et al.* (1991), show that BOM in water supplies mostly contains hard-to-degrade substrates. For example, for a BDOC near a TOC detection limit of 100  $\mu\text{g C/l}$ , Figure 3 shows that the true BOM is 500  $\mu\text{g COD/l}$ . Thus, assuming that BDOC is equal to BOM for hard-to-degrade substrates would result in a large underestimate of BOM in the sample.

Figure 4 shows the relationship between maximum biomass ( $X_{\max}$ ) used in AOC tests and true BOM for both substrates. Also shown in the figure is the calibration standard derived by VAN DER KOOIJ, VISSER, and HIJNEN (1982) to convert cells to AOC in the form of acetate carbon, which is  $4.1 \times 10^6$  cells per  $\mu\text{g}$  acetate C. Figure 4 shows that neither of the substrates fits the calibration standard well. Using the calibration standard to translate  $X_{\max}$  to AOC always gives an underestimate of the true BOM. This underestimate is a direct result of the calibration being derived by growing cells on low concentrations of acetate, that is very easy to degrade. The fundamental principle behind the deviation from the calibration standard is mainly due to the modeling curves approaching the  $S_{\min}$  value on the BOM axis, where cell growth approaches

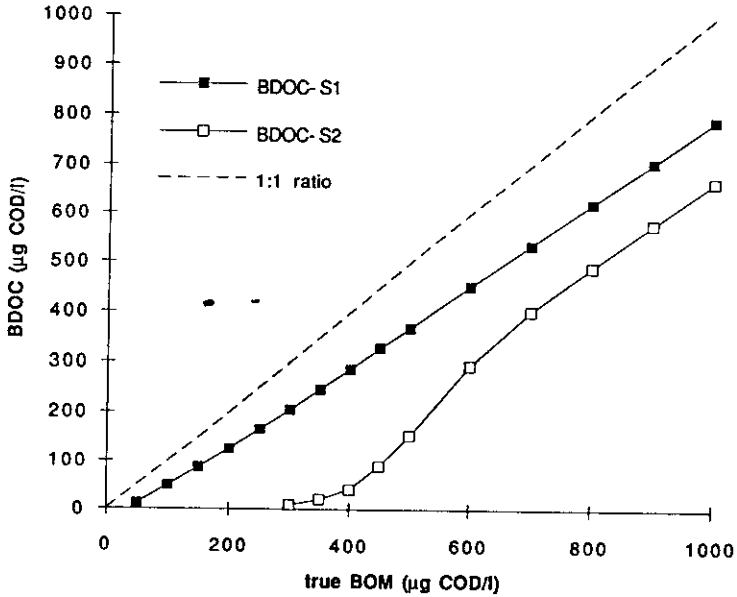


Figure 3 Relation entre le CODB et la MOB pour un substrat facilement utilisable : (S1) et pour un substrat difficilement utilisable (S2).

*The relationship between BDOC and BOM for an easy-to-degrade substrate (S1) and a hard-to-degrade substrate (S2).*

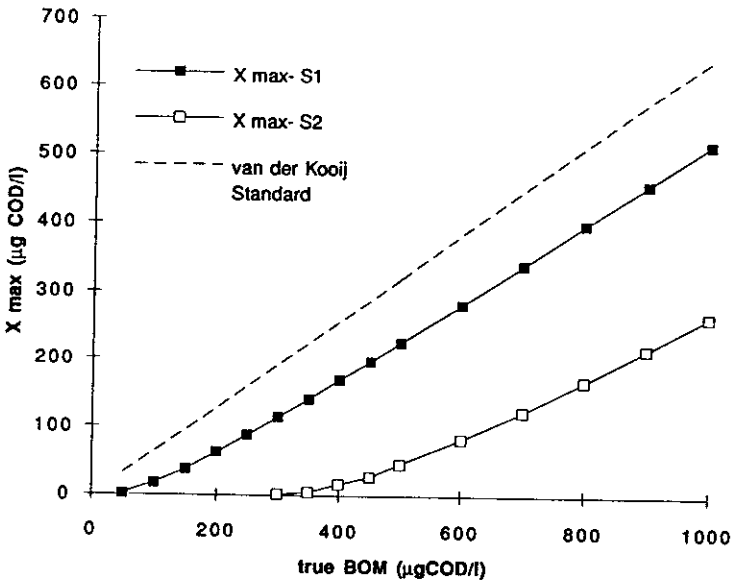


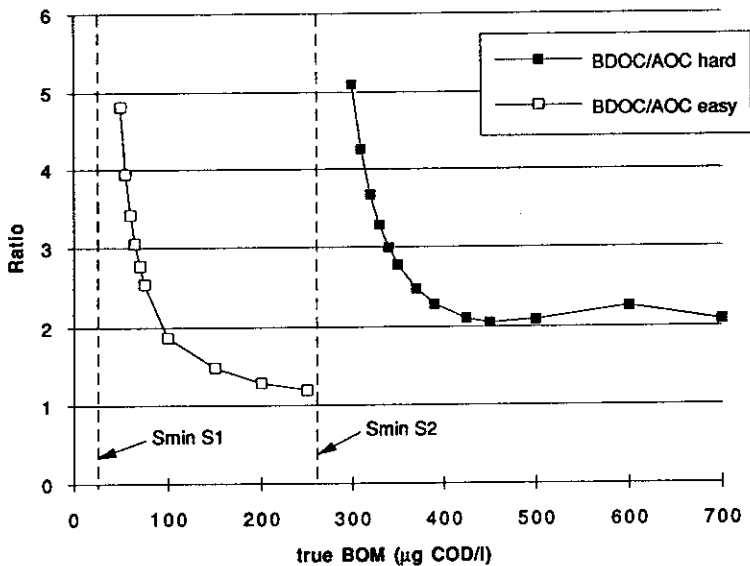
Figure 4 Relation entre la biomasse maximum (Xmax) et la MOB pour un substrat facilement utilisable (S1) et pour un substrat difficilement utilisable (S2).

*The relationship between maximum biomass (X max) and BOM for an easy-to-degrade substrate (S1) and a hard-to-degrade substrate (S2).*

zero. This effect increases for hard-to-degrade substrates, which have higher  $S_{\min}$  values. When BOM in water supplies is mostly hard to degrade, using the acetate calibration standard gives a large underestimate of the true BOM in the sample.

### Comparing BDOC to AOC

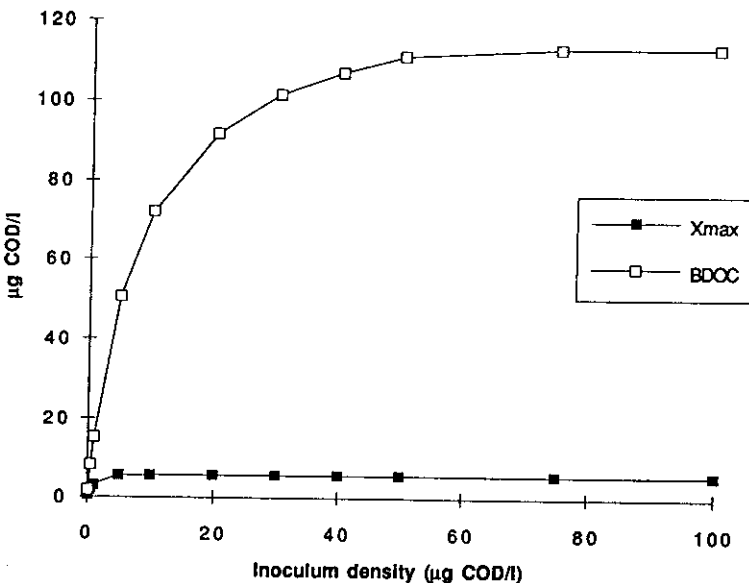
Figure 5 shows the direct relationship between BDOC and AOC for both substrates. AOC is the maximum biomass multiplied by the van der Kooij acetate calibration standard. The BDOC/AOC ratios depend strongly on the degradation kinetics of the substrate and the amount of BOM. The increase in the ratios as BOM decreases is a result of the biomass approaching zero growth as BOM approaches  $S_{\min}$  for each substrate. This figure clearly shows that there is a fundamental difference between what is measured by BDOC and AOC type tests as BOM approaches  $S_{\min}$ . These results could partially explain the observed large differences in the values obtained from these tests. However, for the easy-to-degrade substrate and within the detection limits of the equipment used in BDOC tests (above  $100 \mu\text{g C/l}$ ), the ratio of BDOC/AOC in Figure 5 is about 1/1. A similar analysis for the hard-to-degrade substrate reveals a steady ratio of 2/1 above a true BOM of  $400 \mu\text{g COD/l}$ . Ratios of BDOC/AOC experimental data are typically much higher than these results indicate. Thus, other causes must cause the experimental variations, such as the variations due to the use of pure versus indigenous cultures.



**Figure 5** Proportion CODB:COA pour un substrat facilement utilisable (S1) et pour un substrat difficilement utilisable (S2).  
*Ratio of BDOC:AOC for an easy-to-degrade substrate (S1) and a hard-to-degrade substrate (S2).*

### The effect of inoculum density on BDOC and AOC

The goal of increasing the inoculum density of a BOM test is to decrease the time required to complete the test. Typically, high inoculum tests are BDOC tests, since the change in biomass used by AOC tests would be insignificant compared to the statistical variation of the inoculum biomass. The model was used to examine the effect that increasing inoculum density has on maximum biomass and BDOC for a fixed amount of BOM. For an easy-to-degrade substrate that completely degrades with a low-inoculum density, the results of this modeling show no change in the amount of BDOC and maximum biomass. However, this is not the case for a hard-to-degrade substrate that does not completely degrade with a low-inoculum density. As shown in Figure 6, for the case of 350  $\mu\text{g/l}$  of the hard-to-degrade substrate, the amount of BDOC increased dramatically by increasing the biomass density. This is due to the slow formation of significant biomass from low inoculum densities to degrade the BOM within a reasonable time, shifting the minimum DOC values well beyond 30 days. With a high inoculum, biodegradation occurs more quickly, and the maximum BDOC is achieved within 30 days.



**Figure 6** Effet de la taille de l'inoculum sur le CODB et  $X_{\text{max}}$  quand la MOB n'est pas utilisée complètement avec un faible inoculum. La MOB est de 350  $\mu\text{g/l}$  en substrat difficilement utilisable.

*Effect of inoculum density on BDOC and  $X_{\text{max}}$  for a BOM which does not fully degrade at low inoculum densities. The BOM is 350  $\mu\text{g/L}$  of the hard-to-degrade substrate.*

## CONCLUSIONS

Modeling what occurs in batch BOM tests using basic kinetic and stoichiometric principles reveals fundamental issues to consider when interpreting what is measured by these tests. For BDOC tests, the change in DOC is not equal to the amount of BOM in the sample. The formation of soluble microbial products (SMP) and the degradation kinetics must be considered to estimate the true BOM in a sample. For AOC tests, using a calibration standard based on an easy-to-degrade substrate, such as acetate, does not always give a good estimate of BOM, because the kinetics of degradation must be considered. Also, varying the inoculum density can influence the results of AOC and BDOC tests. Using a low-density inoculum for a hard-to-degrade substrate can seriously underestimate the amount of BOM in a sample.

One consistent finding is that knowledge of the degradation kinetics of the BOM is very useful for interpreting the results of BOM tests. The same conclusion will apply to any modeling effort directed toward distribution system growth. Thus, further study should be directed toward means to estimate such kinetic parameters as  $q_m$  and  $K$  from AOC and BDOC data.

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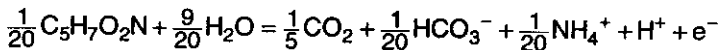
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## APPENDIX A: CONVERSION FACTORS

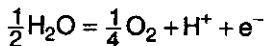
For the purposes of this model, values of BOM, BDOC, and biomass were converted to chemical oxygen demand (COD). COD is the theoretical grams of  $O_2$  used in a redox reaction in which all electrons contained in the carbon are removed and transferred to  $O_2$ . The conversion factors used are as follows: 1.42  $\mu\text{g}$  BOM as COD/ $\mu\text{g}$  BOM as VSS,  $4.16 \times 10^{-7}$   $\mu\text{g}$  COD/cell, and 2.67  $\mu\text{g}$  acetate as COD/ $\mu\text{g}$  acetate C.

The following assumptions are used in these calculations: Organic compounds that make up the BOM in natural waters are mostly the remnants of natural biomass. Natural biomass can be assumed to have the chemical equation of  $C_5H_7O_2N$ , which has a molecular weight of 113 g/mole and an oxidation state of zero for C. The following half reactions are used in formulating these conversions:

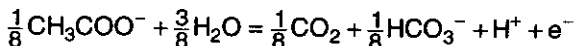
1. Reaction for bacterial cell synthesis with ammonia as the nitrogen source (and BOM decay):



2. Reaction for oxygen as an electron acceptor:



3. Reaction for acetate as an electron donor:



**BOM as VSS to COD:**

$$\left( \frac{\frac{1}{4} \text{ mole } O_2}{1 e^- \text{ eq.}} \right) \left( \frac{32 \text{ g } O_2}{\text{mole } O_2} \right) = 8 \text{ g } O_2 / e^- \text{ eq.}$$

$$\left( \frac{\frac{1}{20} \text{ mole } C_5H_7O_2N}{1 e^- \text{ eq.}} \right) \left( \frac{113 \text{ g } C_5H_7O_2N}{\text{mole}} \right) = 5.65 \text{ g } C_5H_7O_2N / e^- \text{ eq.}$$

$$\left( \frac{8 \text{ g } O_2}{1 e^- \text{ eq.}} \right) \left( \frac{1 e^- \text{ eq.}}{5.65 \text{ g } C_5H_7O_2N} \right) = 1.42 \text{ g } O_2 / \text{g } C_5H_7O_2N$$

### Cells to COD:

For the cell-to-COD conversion, Kaplan (1993) measured *P. fluorescens* cells to have a carbon content of  $1.56 \times 10^{-7} \mu\text{g C/cell}$ , thus:

$$\left( \frac{8 \text{ g } O_2}{1 e^- \text{ eq.}} \right) \left( \frac{(4-0) e^- \text{ eq.}}{\text{mole C}} \right) = 32 \text{ g } O_2 / \text{mole C}$$

$$\left( \frac{32 \mu\text{g } O_2}{\mu\text{mole C}} \right) \left( \frac{1.56 \times 10^{-7} \mu\text{g C}}{\text{cell}} \right) \left( \frac{\mu\text{mole C}}{12 \mu\text{g C}} \right) = 4.16 \times 10^{-7} \mu\text{g } O_2 / \text{cell}$$

### Acetate to COD:

Acetate C has an oxidation state of zero, thus:

$$\left( \frac{8 \text{ g } O_2}{1 e^- \text{ eq.}} \right) \left( \frac{(4-0) e^- \text{ eq.}}{\text{mole C}} \right) = 32 \text{ g } O_2 / \text{mole C}$$

$$\left( \frac{32 \text{ g } O_2}{\text{mole C}} \right) \left( \frac{2 \text{ mole C}}{\text{mole acetate}} \right) \left( \frac{\text{mole acetate}}{24 \text{ g acetate C}} \right) = 2.67 \text{ g } O_2 / \text{g acetate C}$$