

## Oxygen utilization rate in sea water during decomposition of pellet fish food

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*Vessels with 20 liters nonfiltered sea water and pellet concentrations of 10,20,40,80,160 mg dm<sup>-3</sup> were incubated in the dark at 17°C with strong aeration during 26 days. Every two days samples of the water were taken in the WINKLER's bottles. Oxygen concentrations were measured by WINKLER method two days after incubation.*

*Initial velocity of oxygen consumption at decomposition of pellet fish food in the sea water increases with increasing pellet concentration. It can be mathematically described by equation of rectangular hyperbola ( $v_o$  (mgO<sub>2</sub>dm<sup>-3</sup>day<sup>-1</sup>) = 4.2C<sub>F</sub>/(31.2+C<sub>F</sub>), C<sub>F</sub> in mg dm<sup>-3</sup>). Apparent MICHAELIS-MENTEN constant indicates that initial rate of oxygen consumption changes a little with increasing pellet concentration above 60 mg dm<sup>-3</sup>. At low pellet concentration initial rate of oxygen consumption could be probably expressed simpler with kinetic reaction of first order.*

### INTRODUCTION

The most critical factor for fish rearing is the concentration of oxygen. Oxygen consumption in rearing units, tank or cage type, occurs in two ways: by fish respiration and decomposition of organic substance. In commercial systems oxygen consumption depends on heterotrophic and nitrification organisms (WICKINS and HELM, 1981). Uneaten pellets are sources of carbon and nitrogen for autotrophic and heterotrophic organisms in the sea water. At decomposition of organic matter oxygen consumption is 2.67 mgO<sub>2</sub>/mgC and 4.57 mgO<sub>2</sub>/mgN (RICHARDS, 1965).

Substrate oxidation by microorganisms and oxygen consumption in natural and waste water, is usually mathematically described by the kinetic reaction of the first order (O'CON-

NOR, 1967; BRAUN and BERTHOUEX, 1970; METCALF and EDDY, 1979). However, the growth of microorganisms and nutrient removal demand more complex kinetic proposed by MONOD (BRAUN and BERTHOUEX, 1970). However, the MONOD equations of microorganisms have found application in the modeling of aerobic and anaerobic biological waste treatment processes with their mixed cultures and complex nutrient compositions (BRAUN and BERTHOUEX, 1970; METCALF and EDDY, 1979). Pellets fish food is for microorganisms just such a complex nutrient mixture.

Knowledge of oxygen consumption at decomposition of pellet fish food is necessary for a correct estimation of fish number in rearing tanks, and for the prediction of possible consequences in aquatoria surrounding rearing cages.

## MODEL EQUATIONS OF OXYGEN CONSUMPTION

As the concentrations of substrates and other ligands are precisely present at zero time, analysis of the initial reaction velocity benefits from a minimum of errors along the concentration scale (WONG, 1975). The series of utilized oxygen measurements are obtained at successive times, and the series of points  $(y_1, t_1), (y_2, t_2), \dots, (y_k, t_k)$  fit an extrapolating formula e.g. power series in time

$$y(t) = a_0 + a_1 t + a_2 t^2 + a_3 t^3 + \dots \quad (1)$$

Initial velocity of oxygen consumption is obtained by time derivation of equation (1) and put time  $t=0$ :

$$(dy/dt)_{t=0} = a_1 \quad (2)$$

According to the kinetic of the first order, consumption of oxygen is (METCALF and EDDY, 1979):

$$\frac{dy}{dt} = k(S_0 - y) \quad (3)$$

where:

- $y$  = utilized oxygen ( $\text{mg dm}^{-3}$ )
- $t$  = time (day)
- $k$  = first order reaction constant ( $\text{day}^{-1}$ )
- $S_0$  = initial substrate concentration ( $\text{mg dm}^{-3}$ ).

When the interaction between the growth rate of microorganisms and rate of substrate utilization is included, then the oxygen consumption rate is (BRAUN and BERTHOUEX, 1970; METCALF and EDDY, 1979):

$$\frac{dy}{dt} = \frac{\mu_{\max} X (S_0 - y)}{Y(K_m + S_0 - y)} \quad (4)$$

where:

- $y$  = utilized oxygen ( $\text{mg dm}^{-3}$ )
- $t$  = time (day)
- $S_0$  = initial substrate concentration ( $\text{mg dm}^{-3}$ )

$\mu_{\max}$  = microorganisms maximum growth rate ( $\text{day}^{-1}$ )

$X$  = microorganisms concentration at time  $t$  ( $\text{mg dm}^{-3}$ )

$Y$  = yield factor

$K_m$  = apparent MICHAELIS-MENTEN constant ( $\text{mg dm}^{-3}$ ).

When  $t=0$  then quantity of utilized oxygen is equal zero to  $(y(t)=0)$ , and from equation (3) is:

$$(dy/dt)_{t=0} = a_1 = kS_0 \quad (5)$$

and from (4):

$$\left. \frac{dy}{dt} \right|_{t=0} = a_1 = \frac{\mu_{\max} X (S_0 - y)}{Y(K_m + S_0 - y)} = \frac{AS_0}{K_m + S_0} \quad (6)$$

where  $A = \mu_{\max} X_0 / Y$ .

## MATERIALS AND METHODS

Twenty liters of non-filtered sea water and ground commercial pellet (Trouvit 2B; moist 9.8%, total protein 46%, cellulose 3.3%, fats 8.5%, carbohydrates 12%) were put in plastic containers in concentrations of 10, 20, 40, 80 and 160  $\text{mg dm}^{-3}$ . The experiment began in October when the sea water temperature was about 17°C. Containers were put in the dark thermostatic chamber at 17±0.5°C with powerful aeration providing sufficient oxygen concentration (100% saturation) and water mixing. The samples of each pellet concentration were taken in two 120 ml WINKLER bottles in successive time intervals. Oxygen concentration in one bottle was measured immediately ( $\text{DO}_t$ ), and in the other one after determined incubation time ( $\text{DO}_{t+\text{dt}}$ ). Utilized oxygen for incubation time interval is the difference between two concentrations of dissolved oxygen ( $y_i = \text{DO}_t - \text{DO}_{t+\text{dt}}$ ). Cumulative values of utilized oxygen, from beginning to any determined time ( $t_k = \sum_{i=1}^k (dt)_i$ ) of experiment, give total utilized

oxygen ( $ty_k = \sum_{i=1}^k y_i$ ). Total experimental

time was 26 days. The analysis of dissolved oxygen was made by WINKLER method (STRICKLAND and PARSONS, 1972).

## RESULTS

In the first two days the oxygen consumption was  $1.68 \text{ mg dm}^{-3}$  in pellet concentration of  $10 \text{ mg dm}^{-3}$ , and  $7.81 \text{ mg dm}^{-3}$  in high pellet concentration of  $160 \text{ mg dm}^{-3}$  (Fig. 1).

The results of utilized oxygen in function of time agree with polynome of the third order. The values of coefficients are shown in Table 1.

Value of coefficient  $a_1$  is initial velocity of oxygen consumption ( $dy/dt=a_1$  at  $t=0$ ) in units  $\text{mgO}_2 \text{ dm}^{-3} \text{ day}^{-1}$ . When the pellet concentra-

tion is  $10 \text{ mg dm}^{-3}$  then the initial velocity of oxygen consumption is  $0.669 \text{ mgO}_2 \text{ dm}^{-3} \text{ day}^{-1}$ , while in concentrations of  $160 \text{ mg dm}^{-3}$  it is  $3.674 \text{ mgO}_2 \text{ dm}^{-3} \text{ day}^{-1}$ .

The oxygen consumption initial velocity in relation to pellet concentration is shown in the Fig. 2. The nonlinear model (equation 6) fits the initial velocity of the oxygen consumption better than the linear model (equation 5) (Fig. 2).

Equation for the linear model of the oxygen consumption initial velocity dependence on the pellet concentration in the sea water is:

$$\left. \frac{dy}{dt} \right|_{t=0} = v_o = 0.016C_F + 1.326$$

$$R^2=0.775 \quad (7)$$

Table 1. Coefficients of polynomial equation (1) ( $a_1$ -initial velocity of oxygen consumption;  $R^2$ -coefficient of determination)

Pellet concentration ( $\text{mg dm}^{-3}$ )	$a_0$	$a_1$	$a_2$	$a_3$	$R^2$
10	0.271	0.669	-0.034	0.0007	0.988
20	-0.689	2.065	-0.108	0.0020	0.962
40	0.829	2.388	-0.136	0.0027	0.982
80	1.536	2.733	-0.163	0.0033	0.947
160	1.043	3.674	-0.205	0.0040	0.985

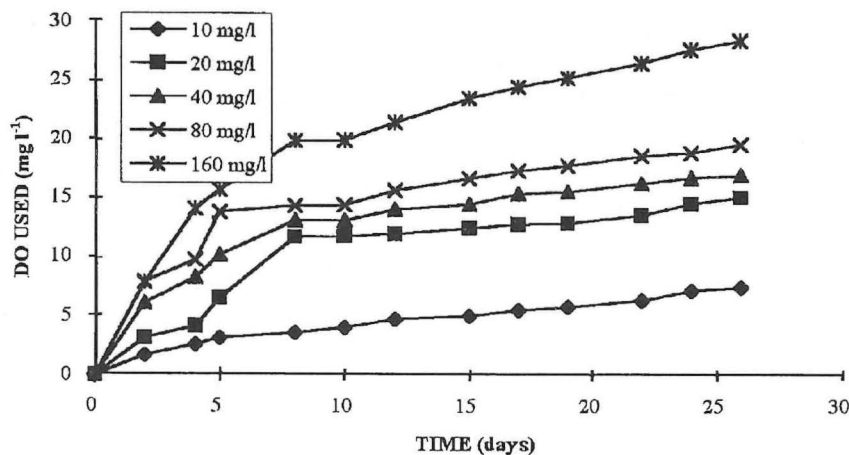


Fig.1. Dissolved oxygen used for different concentrations of the pellet fish food ( $10\text{-}160 \text{ mg l}^{-1}$ ) decomposition

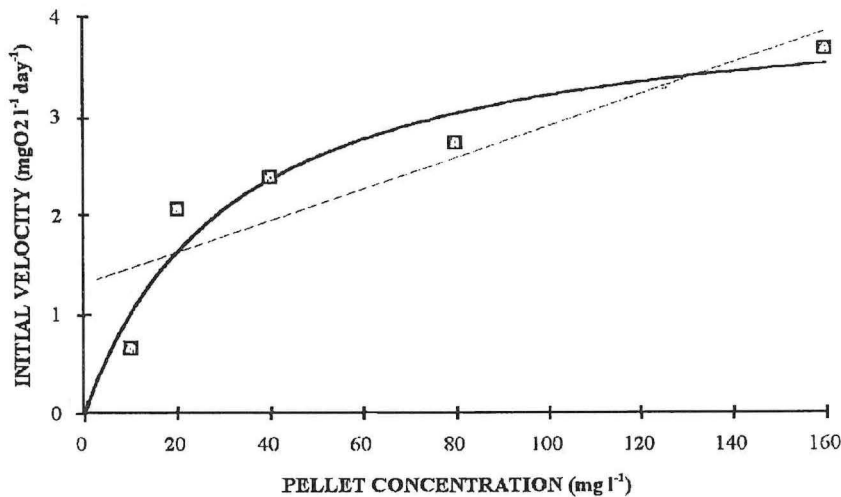


Fig.2. Relationships of the initial rate of oxygen consumption and pellet food concentrations for linear (---) and non-linear (—) models

while for the nonlinear model it is:

$$v_o = \frac{4.21C_f}{31.16 + C_f} \quad R^2=0.914 \quad (8)$$

Coefficient of determination ( $R^2$ ) for the linear model shows that 77.5% variance of oxygen consumption initial velocity depends on pellet concentration, while in the nonlinear model, 91.4% variance depends on it.

## DISCUSSION

The oxygen utilization rate in the seas and oceans is low (order of magnitude  $10^{-4}$  mgO<sub>2</sub> dm<sup>-3</sup>day<sup>-1</sup>) and decreases with water depth (WIRTKI, 1962; TSEITLIN, 1992). In shore waters which are under the influence of urban sewage, the oxygen consumption can be 1 mgO<sub>2</sub> dm<sup>-3</sup>day<sup>-1</sup> (THOMAS *et al.*, 1976).

In absence of aeration in the experimental vessels, dissolved oxygen concentration of 7.5 mgO<sub>2</sub> dm<sup>-3</sup> in vessels with pellet concentration of 80 and 160 mg dm<sup>-3</sup> would be consumed in two days, with pellet concentration of 40 mg dm<sup>-3</sup> in three days, within six days with 20 mg dm<sup>-3</sup>, and to the end of the experimental period in vessels with concentrations of 10 mg dm<sup>-3</sup>.

Biochemical oxygen demand in natural and waste waters is divided into the carbonaceous and nitrogen oxygen demand (O'CONNOR, 1967; METCALF and EDDY, 1979; WALTERS, 1981). Oxidation of one mole of ammonium to nitric acid consumes 1.8 mole of dissolved oxygen (GUNDERSEN and MOUNTAIN, 1973). However, oxidation of inorganic nitrogen with the process of nitrification is significantly late in relation to carbonaceous oxygen demand. Von BRAND *et al.* (1973) reported maximum concentrations of ammonium at phytoplankton decomposition between days 8 and 20 but no nitrite occurred by that time. Ammonium oxidation in the sea water is a very slow process with maximum rate of 0.1% per day and half the maximum rate at a substrate concentration of 3.6 mgN dm<sup>-3</sup> (MADELIN, 1990). Therefore, it may be taken that during the experimental period of 26 days nitrification is negligible, so that carbonaceous oxygen demand has been only predominant role on oxygen consumption.

The rate-determining step of bacterial degradation of high molecular weight organic material is probably its exoenzymatic hydrolysis (HALLIBAUGH and AZAM, 1983; LANCELOT and BILLEN, 1985). Heterotrophic population metabolizes amino acids that maintain their C:N ratio (HALLIBAUGH, 1978). The

rate of amino acid oxidation in the sea water is the highest in the late summer and early autumn ( $0.04$  to  $0.1 \text{ day}^{-1}$ ) and the lowest in winter (ANDREWS and WILLIAMS, 1971). The density of proteolytic bacteria in the area where the sea water for the experiment of pellet decomposition was taken from, was highest in autumn, but for other bacterial groups, amilolytic and lipolytic, there is no regular pattern of seasonal distribution (KRSTULOVIĆ and ŠOLIĆ, 1988). Kinetic parameters in equations (3) and (4) depend on the composition of bacterial groups and their initial concentrations. Decomposition of natural organic material can be viewed as several different classes, with first-order decay constant differing from one to another by an order of magnitude (LANCLOT and BILLEN, 1985). The equation (4) is

approaching equation (3) when  $K_m \gg S_o$ . However, kinetic of the first-order reaction is valid only when  $\mu_{max}X/(YK_m) = \text{constant}$  (BRAUN and BERTHOUEX, 1970). This expression implies that changes in organism concentration  $X$  are negligible during biochemical oxygen demand progression, or that the ratio is constant. Magnitude of apparent MICHAELIS-MENTEN constant in this experiment with this type of pellet fish food is  $K_m = 31.16 \text{ mg dm}^{-3}$ . Probably, when concentration of pellet fish food in the sea water is lower than  $30 \text{ mg dm}^{-3}$ , than the kinetic of the first order reaction can be applied. On the other hand, the pellet concentrations over  $50\text{-}60 \text{ mg dm}^{-3}$  ( $\approx 2K_m$ ) have a little influence on further increase of the oxygen consumption initial velocity.

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## Brzina potrošnje kisika u morskoj vodi pri razgradnji peletirane hrane za ribe

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### SAŽETAK

Poznavanje brzine potrošnje kisika za vrijeme razgradnje nepojedene peletirane hrane u morskoj vodi, važno je za upravljanje uvjetima bazenskog i kaveznog načina uzgoja ribe.

Posude s 20 litara nefiltrirane morske vode i s koncentracijama peleta od 10, 20, 40, 80, 160 mg dm<sup>-3</sup> inkubirane su u mraku uz snažnu aeraciju pri 17°C 26 dana. Svaka dva dana uzeta je voda u WINKLER-ove boce koje su inkubirane daljnja dva dana, nakon čega je WINKLER-ovom metodom određena koncentracija kisika.

Početna brzina potrošnje kisika ( $v_0$ ) pri razgradnji peletirane hrane za ribe u morskoj vodi raste povećanjem koncentracije peleta ( $C_F$ ) i matematički se može prikazati jednadžbom istostrane hiperbole ( $v_0$  (mgO<sub>2</sub>dm<sup>-3</sup>dan<sup>-1</sup>) = 4.2C<sub>F</sub>/(31.2+C<sub>F</sub>), C<sub>F</sub> u mg dm<sup>-3</sup>). Visoka prividna MICHAELIS-MENTEN konstanta pokazuje da se povećanjem koncentracije peleta iznad 60 mg dm<sup>-3</sup> početna brzina potrošnje kisika malo mijenja. Pri koncentracijama peleta u morskoj vodi nižim od 60 mg dm<sup>-3</sup> (<2K<sub>m</sub>), početna brzina potrošnje kisika se tada može opisati jednostavnijim modelom kinetike reakcije prvog reda.

