# EXPERIMENTAL SIMULATION OF BIODEGRADATION IN RIVERS OXYGEN, ORGANIC MATTER AND BIOMASS CONCENTRATION CHANGES

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# EXPERIMENTAL SIMULATION OF BIODEGRADATION IN RIVERS

## OXYGEN, ORGANIC MATTER AND BIOMASS CONCENTRATION CHANGES

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**Abstract**—Dissolved oxygen (DO) and biochemical oxygen demand (BOD) concentration changes after an organic matter discharge into a river have been studied in the absence of oxygen transfer. According to these laboratory experiments, biodegradation of various organic compounds (glucose, glutamic acid, starch, ovalbumin and ethanol) in Seine river samples incubated at 15–30°C follow a biphasic behaviour. During a lag-phase of 10–20 h, DO decreases linearly (0.12 ppm $h^{-1}$ at 20°C), whereas BOD is constant. During a subsequent aerobic exponential phase, DO and BOD uptake are proportional and increase exponentially with time (0.13 $h^{-1}$ at 20°C). Using cell ATP as biomass indicator, the latter phase was shown to correspond to a cell division step. A kinetic model was developed for stimulating DO and BOD concentration changes after a waste water discharge at temperatures ranging between 15 and 30°C.

*Key words*—biodegradation, dissolved oxygen (DO), biochemical oxygen demand (BOD), biomass, adenosine triphosphate (ATP)

### INTRODUCTION

Organic waste water discharge in a river results in dissolved oxygen uptake, either directly by chemical oxidation of the reducing pollutants, or by their metabolism by microorganisms, i.e. their biodegradation. Raising the temperature of rivers reduces oxygen solubility and accelerates the kinetics of chemical and microbiological oxygen and polluting load reduction.

Numerous kinetic models of stream water quality have been proposed to describe dissolved oxygen (DO) and biochemical oxygen demand (BOD) variations along a stream. The first, and most widely used model, was proposed by Streeter & Phelps (1925) for the Ohio river. It states that oxygen uptake is equal to BOD uptake, and that both kinetics are first order. Since then, several modifications have been proposed to take into account the effects of dispersion (O'Connor, 1961, 1967), sedimentation and adsorption (Dobbins, 1964), photosynthesis and respiration (O'Connor & Di Toro, 1970), thermal exchanges (Lin *et al.*, 1973a, b, c), benthal demand (Shelton *et al.*, 1978) and nitrogeneous biodegradation (Shelton *et al.*, 1978; Knowles & Wakeford, 1978). Nevertheless, it was frequently observed that first order curves do not fit well with experimental variations of BOD and second order kinetics were proposed for DO and BOD uptakes (Woodward, 1953; Revelle *et al.*, 1965; Young & Clark, 1965; Zanoni, 1967; Edeline & Lambert, 1979). Their success in describing actual river evolution was not generally accepted. Besides these field studies, Flegal & Schroeder (1976) and Rauwel & Thévenot (1976) conducted laboratory experiments without renewal of dissolved oxygen: after organic discharges, both observed lag-phases with no oxygen evolution followed by exponential phases, similar to those encountered in experiments on cellular growth.

In order to understand phenomena occurring in rivers, in the vicinity of organic waste discharge, we followed the concentration variations of DO, pollutant and biomass. These experiments were conducted between 1978 and 1981 in Seine river samples, added with different organic wastes (glucose, ovalbumin, starch, glutamic acid, ethanol) and incubated at different temperatures in closed cells (to avoid reoxygenation).

In contrast to the usual BOD laboratory tests, all river samples received neither additional micro-organism seed nor salt solution. Adenosine triphosphate (ATP) was chosen for evaluation of biomass: indeed it is a fundamental constituent of all living cells, and only the living ones, since it is destroyed very rapidly after cell death. It was shown that the amount of ATP is proportional to the amount of cell organic carbon, which is a usual indicator of biomass (Holm-Hansen, 1970). Furthermore, trace amounts of ATP can be easily determined by bioluminescence, after cell filtra-

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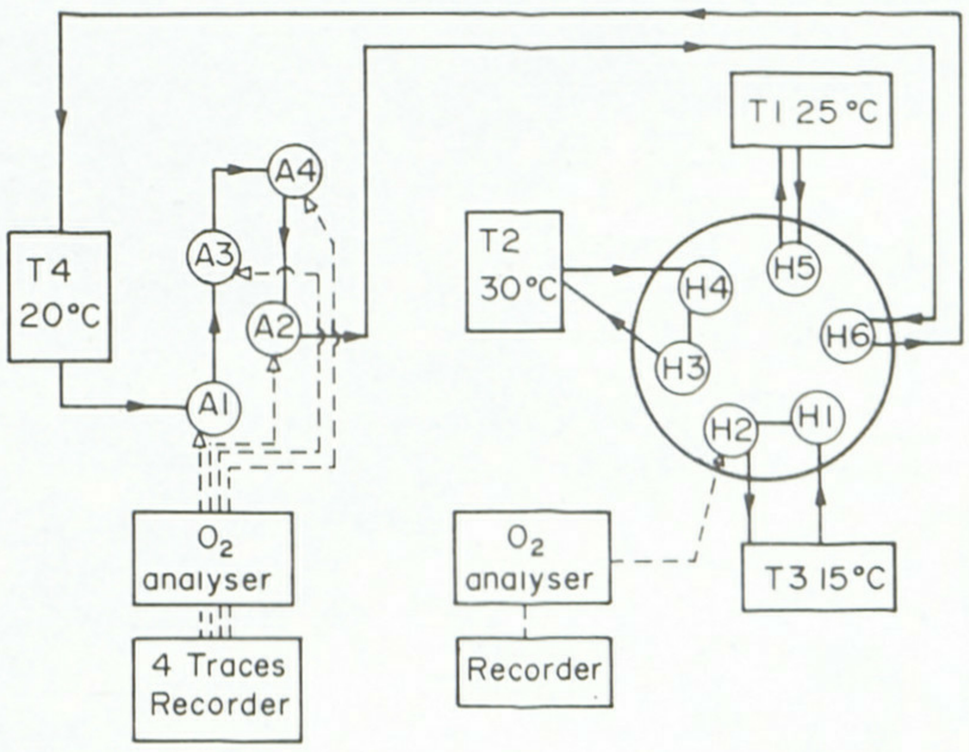


Fig. 1. Experimental device for DO uptake kinetic studies. T1–T4: thermostats; A1–A4: cells used for continuous DO determinations; H1–H6: cells for discontinuous DO determinations (Hexacel).

tion and extraction: when mixed with luciferine and luciferase, ATP extracts yield light emission in which the intensity maximum is proportional to the ATP amount, the lower detection limit being as low as $10^{-12}$ mol (Tobin *et al.*, 1978; Apoteker, 1981).

## MATERIALS AND METHODS

### *Instrumentation*

*Dissolved oxygen.* Pollution load dependence of oxygen uptake was studied in 4 cells of 280 ml thermostated at $20.0 \pm 0.02$°C and containing 4 Beckman 76 835 oxygen electrodes (Fig. 1). The latter were connected via an automatic switch to a process Beckman 778 oxygen analyser and to a four-traces Honeywell Y 153 recorder: the usual sensititivity was 0.4–0.6 ppm $cm^{-1}$. This group of instruments was generously provided by P. Marcellin, Laboratoire des Organes Végétaux aprés Récolte (Meudon, France).

Temperature dependence of oxygen concentration changes was studied at 15, 20, 25 and $30 \pm 0.2$°C in a 6 cells device developed by Rauwel & Thévenot (1976). These cells were successively in contact with a Beckman 39 550 oxygen electrode fixed on a glass disk which was rotated with respect to the cells. The electrode was connected to a Beckman Fieldlab analyser and to a Sefram graphispot recorder; the usual sensitivity was 0.5 ppm $cm^{-1}$.

The 10 previously mentioned cells were temperature controlled by 2 Haake F J thermostats (25 and 30°C), a Papst NB.DS 283 ultrathermostat (20°C) cooled by a Lauda ultrakryomat TK 30 D cryostat and a Colora WK5 cryothermostat (15°C) (Fig. 1).

BOD and batch-type oxygen concentration determinations were carried out in 90 ml vessels placed at $20.0 \pm 0.2$°C in a BTL water bath. Oxygen concentration was measured with a Beckman 39 550 oxygen electrode connected to a Beckman Fieldlab analyser and to a Sefram graphispot recorder; the usual sensitivity was 0.5 ppm $cm^{-1}$.

*Glucose sensor.* The glucose sensor was a differential device including a glucose electrode, consisting of a platinum disk covered by a $\beta$-D-glucose oxidase collagen membrane, and a compensating electrode mounted with a non-enzymatic collagen membrane (Sternberg *et al.*, 1979; Thévenot *et al.*, 1979). Current outputs of both electrodes were subtracted and differentiated against time giving steady-state and dynamic responses. For this study both electrodes were mounted with flow-through caps, the circulating solution being either 0.2 M acetate buffer, 0.1 M KCl, pH 5.6 or a mixture of buffer and samples (1:1). Circulation and dilution were effected with a two-channel Gilson polystaltic pump.

Electronics were supplied by Soléa-Tacussel and consisted of a Deltapol two channels current amplifier, a PRG 5 or a PRGE potentiostat and a Dérivol derivating amplifier using a time-base of 1 s. The recorder was a three traces Linear 395.

The temperature of all solutions was carefully thermostated at $30.0 \pm 0.1$°C using a Huber ministat cryothermostat.

*ATP determinations.* River samples were filtered through 47 mm dia membranes presenting pore size of 1.2 $\mu$m. As soon as the filtration was complete, membrane filters were cut in stripes and immersed in 5 ml dimethylsulfoxide (DMSO) for ATP extraction. One ml of the suspension was mixed with 5 ml $10^{-2}$ M morpholinopropane sulphonate (MOPS) buffer and frozen at −20°C for storage.

ATP was determined by bioluminescence with a Jobin-Yvon PICO-ATP photometer generously provided by Direction des Etudes et Recherches d'Electricité de France (Chatou, France). Intensity of the light emission was followed on a galvanometric Sefram graphispot or a potentiometric EPL 2 Soléa-Tacussel recorder: its maximum value was determined on the recording and not with the electronic device provided by the instrument.

### *Procedure*

For each set of experiments, about 8 l. of Seine river water were taken above Paris, near the Choisy bridge, and filtered on porous glass (Sovirel, grade 3, pore size 20–40 $\mu$m) for homogenization. This river sample was then saturated with a mixture of air and nitrogen in order to fix oxygen concentration to a value lower than saturation at the highest experimental temperatures, i.e. 7.7 mg $l^{-1}$. Under such conditions, no supersaturation phenomena or bubble formation was observed within the cells, even at 30°C. Initial concentrations of added pollutant (glucose, ovalbumin, starch, glutamic acid, ethanol) were obtained by adding directly into the different cells, already filled with Seine water, small volumes of standard solutions. Before each experiment, oxygen electrodes were calibrated by controlling their output current when placed in stirred distilled water thermostated at 20°C and saturated by air bubbling. Their zero was checked by immersion either in sulphite solution or in distilled water where nitrogen was bubbling. 35 small vessels were simultaneously filled either with BOD dilution water or with solutions identical to those present in the larger cells, in order to monitor DO, glucose (when the latter was added as the model pollutant) and biomass evolutions. They were all incubated at 20°C. Oxygen concentration was continuously recorded in the 10 cells during 10–100 h and, from time to time, some of the small vessels were opened and checked independently till all oxygen concentrations decreased below 1 mg $l^{-1}$.

In the batch experiments where glucose was the pollutant added to Seine river samples, DO, glucose and ATP were successively determined in the same sample incubated 16–24 h at 20°C. Calibration of the oxygen electrode, of the glucose sensor and of the photometer were respectively checked before each set of determinations.

For ATP monitoring, the extracts were immediately frozen and kept at −20°C to be all determined the same day: indeed, luciferine–luciferase reacting solutions were prepared from Lumac reaction mixture just before ATP determinations and completely used within a few hours. The photometer and enzymatic responses were checked each hour with standard ATP solutions and the standardizations were performed by adding known amounts of ATP in the extracts.

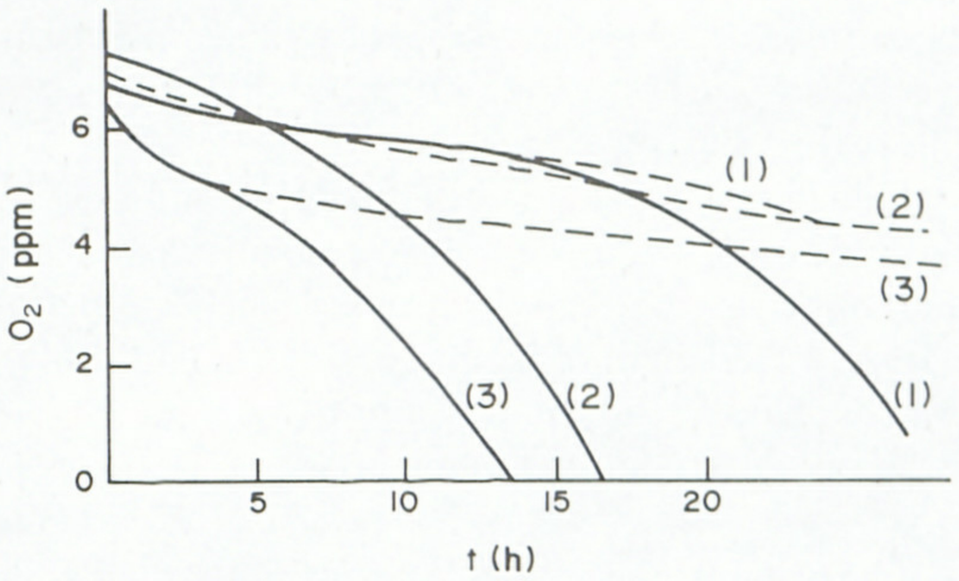


Fig. 2. DO concentration changes in Seine water samples either crude (– – –) or added with 25 mg $l^{-1}$ glucose (——) and incubated at 20°C. Seine water sampled on (1) 6 May, (2) 9 June and (3) 22 July, 1981.

In order to improve precision in DO uptake kinetics, experimental curves were fitted by polynomes of degree 4, estimated by a least-square regression using Chebyshev polynomes with discrete time intervals and a Hewlett–Packard 97 calculator: differences between calculated values and experimental data were usually smaller than 0.05 mg $l^{-1}$. Curves obtained with the Beckman 76 365 electrodes (with large cathode) were also corrected from their own consumption using blank experiments in distilled water (Thévenot *et al.*, 1979).

## EXPERIMENTAL RESULTS

### *Oxygen evolution*

Figure 2 presents 3 typical recordings of DO concentration changes in Seine samples, either crude (dashed lines) or containing initially 25 mg $l^{-1}$ glucose (solid lines). These experiments were performed with Seine Water sampled respectively on 6 May, 9 June and 22 July 1980, i.e. with different micro-organisms seeding. These curves show that addition of glucose does not affect DO uptake during a first period of several hours. After this lag-phase, addition of glucose accelerates DO uptake. We can also notice on Fig. 2 the great importance of microorganism seed in river samples used, since all oxygen has disappeared within about 13, 16 and more than 25 h for experiments 3, 2 and 1 respectively. The lag-phase is also different according to the river sample and varies from 4 to 15 h in this set of experiments. The mean value obtained in experiments performed at 20°C and with 10 mg $l^{-1}$ added $BOD_5$ equals 15 h (Table 3). Such a biphasic behaviour of oxygen uptake was observed in all experiments where a biodegradable organic pollutant had been added to Seine river samples. Semi-logarithmic plots of oxygen uptake after lag-phase demonstrate that it is exponential, the mean value of constant $k_1$ being equal to 0.13 $h^{-1}$ at 20°C (Table 3).

### *Glucose evolution*

When the pollutant added to Seine was glucose, we monitored its concentration changes during biodegradation experiments (Fig. 3). During the first hours after glucose discharge, DO uptake of samples added

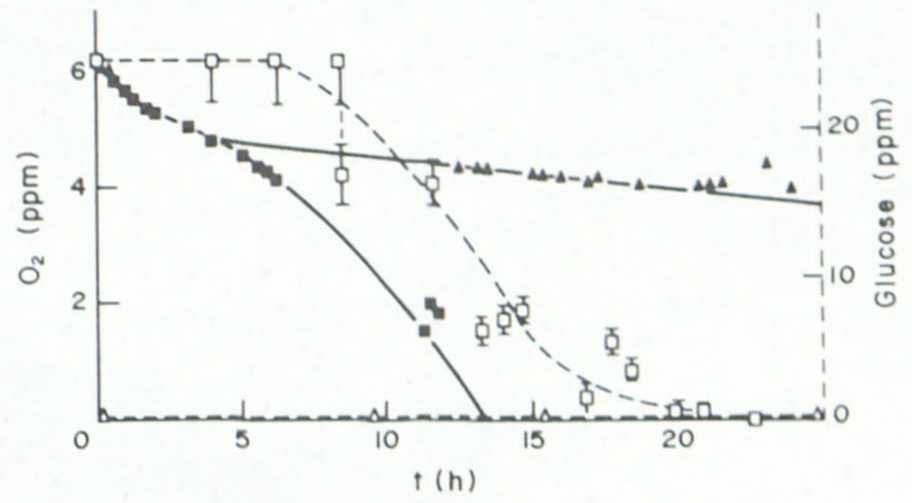


Fig. 3. DO (——) and glucose (– – –) concentration changes in Seine water samples either crude (▲△) or added with 25 mg $l^{-1}$ glucose (■□) and incubated at 20°C.

with glucose is equal to that of crude samples and there is no glucose uptake. Later, when DO uptake becomes exponential, glucose uptake starts, and accelerates rapidly, until there is no more DO in the sample. Under anaerobic conditions, glucose uptake becomes much slower.

Thus, the lag-phase observed for DO uptake corresponds also to a lag-phase for the pollutant uptake. In order to determine the actual stoichiometry of DO uptake during glucose biodegradation, we plotted the number of moles of glucose vs oxygen uptake after lag-phase (Fig. 4). Despite the differences between experiments, we observed an apparent stoichiometry of about 1 mol oxygen $mol^{-1}$glucose, independent of initial glucose concentration and of incubation time after lag-phase.

This value is much lower than the expected figure for complete metabolism of glucose, i.e. 6 mol oxygen $mol^{-1}$ $glucose^{-1}$ (Lehninger, 1970) and lower than the values obtained for glucose $BOD_5$ determinations, i.e. 2.8–4.4 mol oxygen $mol^{-1}$ $glucose^{-1}$ (Bond & Straub, 1973).

### *Biomass evolution*

Being aware of microbiological seed modification during filtration and/or gas bubbling, we monitored

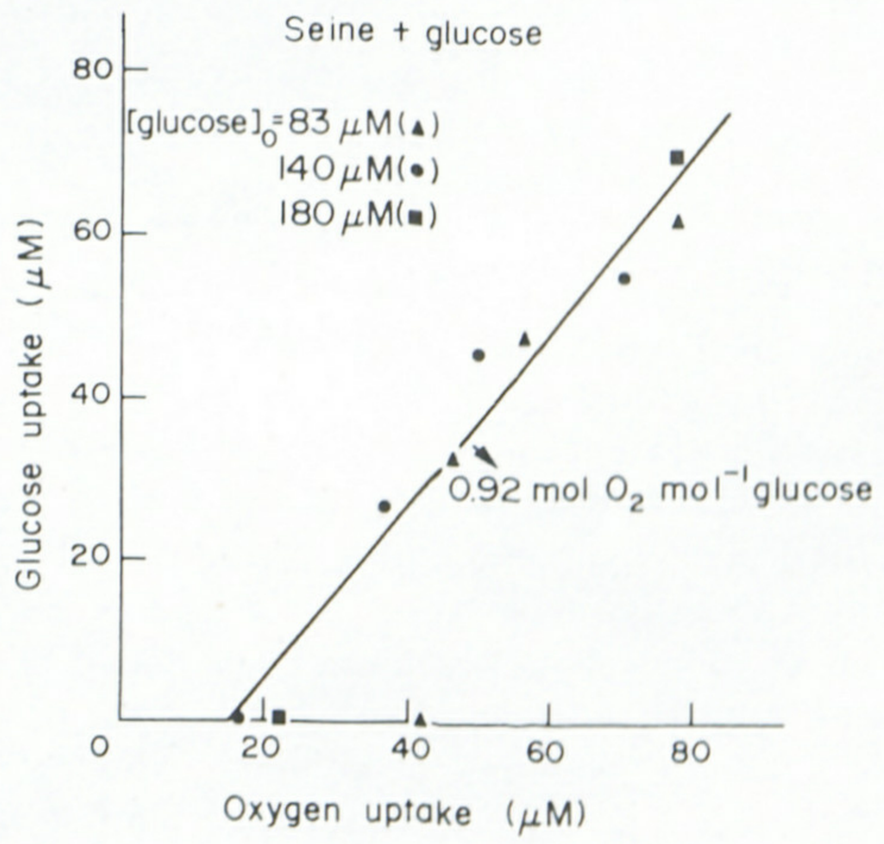


Fig. 4. Glucose biodegradation in Seine water. Stoichiometry between glucose and DO uptake at 20°C.

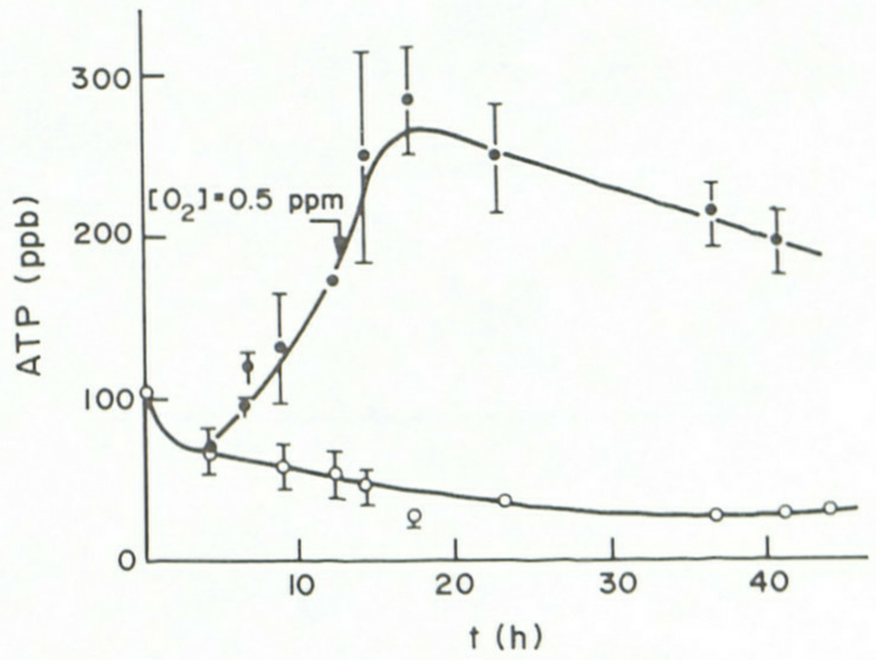


Fig. 5. Evolution of ATP concentration in Seine water samples either crude (○) or added with 25 mg $l^{-1}$ glucose (●) and incubated at 20°C.

ATP evolution during the different steps of river sample conditioning. We observed that the rough glass frit filtration yielded a very important reduction of cell ATP, which became, just after filtration, about 10% of its initial value. Later, during the gas saturation period, the amount of ATP increased, reaching 30–100% of the initial value after 20–30 h. So, the seed used in these biodegradation experiments was not equilibrated, since biomass was increasing, but taking into account the cell ATP level, it was not very different from the microbiological seed of the initial river sample.

During the actual biodegradation experiments, we followed simultaneously concentration changes of biomass in Seine river samples with and without glucose (Fig. 5). We observed that the amount of ATP in crude Seine samples slowly decreased until equilibrium was reached about 30–40 h after the vessel had been filled. For Seine samples added with glucose, ATP begins to slowly decrease like the crude Seine sample, then in increases very rapidly and reaches a maximum a little later when all DO has disappeared. During this phase of rapid growth, the increase of ATP seems proportional to the oxygen deficit and hence exponential. This cell ATP variation gives evidence of cell division during the active part of biodegradation, i.e. after lag-phase.

*Temperature and organic waste dependence*

We have studied the effects of organic discharge and temperature on the two phases of DO uptake during biodegradation. When lower than 25 mg $l^{-1}$, the pollution load (expressed in $BOD_5$) seems to have no or little influence on the length of lag-phase and on the exponential phase kinetic constant $k_1$ (Fig. 6). Nevertheless, the importance of the seed present in the river sample is noticeable. In about half of the experiments that we undertook, we observed no significant effect of polluting load on lag-phase (Fig. 6a). In the other half, an increase of the polluting load slightly decreased the lag-phase (−0.15 to −1.4 h per $BOD_5$ ppm), as shown on Fig. 6(c). This effect does not seem to be related to the nature of the pollutant, since it was observed in about half of the experiments with each pollutant. Concerning $k_1$, the effect of polluting load was never significant (Fig. 6b and d).

On the other hand, we observed in all experiments that an increase of temperature between 15 and 30°C shortened the length of lag-phase (Fig. 7a and c). The average value of this temperature dependence of lag-phase, at 20°C and for 10 ppm added $BOD_5$ was about −1.1 h per °C or −7.8% per °C (Tables 1 and 3). The exponential phase kinetic constant $k_1$ increases with temperature. This temperature dependence seems linear between 15 and 30°C (Fig. 7b and d), the slope ranging between 0.009 and 0.049 $h^{-1}$ per °C according to river samples and organic pollutant. Consequently, Arrhenius plots present different slopes at different temperatures. At 20°C, the activation energy of $k_1$ ranged between −8.7 and −26 kcal. Temperature coefficient $\theta$ is usually referred to the Streeter and Phelps kinetic model:

$$k_1^T = k_1^{20} \times \theta^{(T-20)}$$

where $T$ is expressed in °C. At 20°C, $\theta$ was found to be in the 1.05–1.14 range with a mean of 1.097 which corresponds to a temperature dependence of $k_1$ = 9.7% per °C.

*Comparison of biodegradation of different organic species*

We conducted experiments with different added polutants: glucose, glutamic acid, starch, ovalbumin

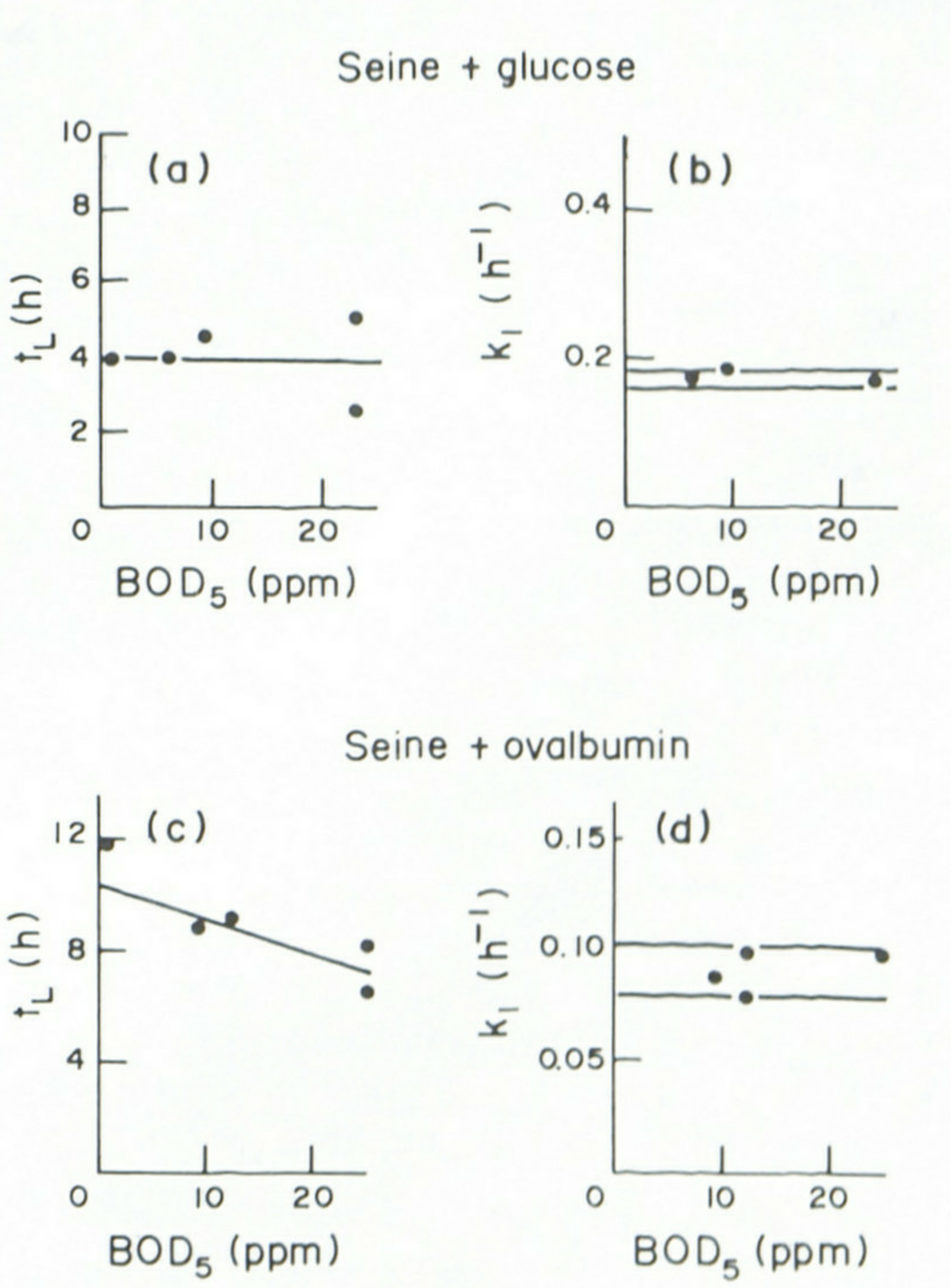


Fig. 6. Organic matter dependance of lag-phase and exponential kinetic constant of biodegradation in Seine river samples incubated at 20°C: (upper diagram) glucose, (lower diagram) ovalbumin.

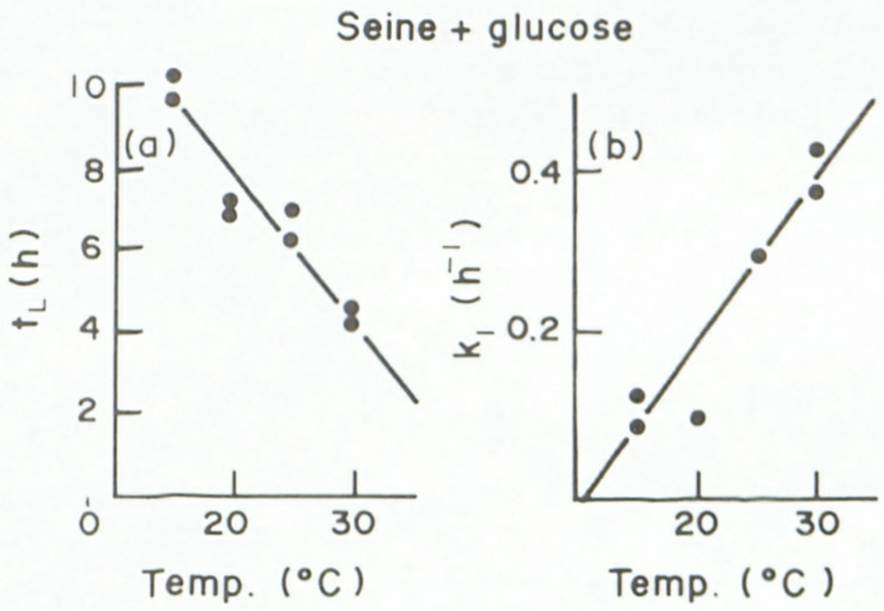


Fig. 7. Temperature dependence of lag-phase and exponential kinetic constant of 25 mg $l^{-1}$ glucose (upper diagram) or ovalbumin (lower diagram) biodegradation in Seine river samples incubated at 20°C.

and ethanol. We observed that DO concentration changes were similar during incubation of these compounds: a lag-phase of 6–20 h was followed by an exponential oxygen uptake whose rate constant ranged between 0.1 and 0.3 $h^{-1}$ (Table 1). Experiments simulating a very large discharge of ethanol gave $t_L$ and $k_1$ values in the same range, i.e. 10 h and 0.22 $h^{-1}$ respectively. We compared more carefully and under similar experimental conditions biodegradation of glucose and ovalbumin; their $BOD_5$ values are very similar, i.e. $0.44 \pm 0.05$ and $0.48 \pm 0.06$ g $O_2$ $g^{-1}$ respectively, but the latter is both carbonaceous and nitrogeneous. We observed that differences between experiments performed with same pollutant were often larger than between those performed with glucose and albumin. Nevertheless, ovalbumin seems to present a somewhat longer lag-phase and a smaller exponential phase kinetic constant than glucose (Figs 6 and 7). Indeed, the mean values for the lag-phase were found to be about 20 and 14 h for ovalbumin and glucose respectively, mean $k_1$ values were about 0.10 and 0.14 $h^{-1}$ respectively (Table 1). Comparison of temperature dependence of lag-phase $t_L$ and exponential phase rate constant $k_1$ showed that they were also smaller when added pollutant was ovalbumin instead of glucose. All these results are consistent with the generally accepted slower biodegradation of nitrogen containing compounds (Velz, 1970; Shübert & Deutsch, 1971).

## KINETIC MODEL OF BIODEGRADATION

Oxygen and pollutant uptakes cannot be described by the Streeter and Phelps kinetic equations which do not take into account the lag-phase observed after organic discharge, and which use first order kinetics instead of exponentially increasing DO and pollutant uptakes. In order to describe our experimental results, we have built a kinetic model which concerns only DO and pollutant uptakes, i.e. $dC/dt$ and $dL/dt$ and does not consider reoxygenation phenomena (Table 2).

### *Lag-phase*

During lag-phase, oxygen uptake rate $k_0$ is independent of organic discharge and roughly constant. Mean value for $k_0$ was found to be 0.12 ppm $h^{-1}$

Table 2. Kinetic equations for organic substances degradation in river samples: concentration changes of dissolved oxygen $C$ and pollution load $L$ during incubation at temperature $T$, without oxygen transfer

(1) *Crude river*

$$\frac{dC}{dt} = k_0 \text{ with } k_0^T = k_0^{20} + \frac{dk_0}{dt}(T - 20)$$

(2) *River discharged with organic waste water*

(a) lag phase: $0 \leqslant t \leqslant t_L$ and $C > 0$

$$\frac{dC}{dt} = k_0 \text{ with } t_L^T = t_L^{20} + \frac{dt_L}{dT}(T - 20)$$

$$L = L_0$$

(b) aerobic exponential phase: $t \geqslant t_L$, $C > 0$ and $L > 0$

$$\frac{dC}{dt} = a\frac{dC}{dt} \text{ with } a \geqslant 1$$

$$k_1^T = k_1^{20} + \frac{dk_1}{dT}(T - 20)$$

Table 1. Biodegradation parameters for different organic substances incubated in Seine river samples at 20°C.

| Wastes | $L_0$ (ppm) | $t_L$ (h) | $dt_L/dL_0$ (h $ppm^{-1}$) | $dt_L/dT$ (h per °C) | $k_1$ ($h^{-1}$) | $dk_1/dL_0$ ($h^{-1}$ $ppm^{-1}$) | $dk_1/dT$ ($h^{-1}$ per °C) |
|---|---|---|---|---|---|---|---|
| Glucose | 0.5–25 | 14 | $0.9 \pm 0.9$ | $-1.2 \pm 0.4$ | 0.14 | $(4 \pm 3)\ 10^{-3}$ | $0.017 \pm 0.003$ |
| Glucose + glutamic acid | 9–36 | 6 | 0 | | 0.29 | | 0.025 |
| Starch | 11–45 | 8 | 0 | | 0.25 | | |
| Ovalbumin | 0.5–25 | 20 | $-0.2 \pm 0.3$ | $-1.0 \pm 0.4$ | 0.10 | $(1 \pm 2)\ 10^{-3}$ | $0.005 \pm 0.006$ |
| Ethanol | 2300–23,000 | 10 | 0 | | 0.22 | | 0.018 |

$L_0$ = initial BOD; $t_L$ = lag-time; $k_1$ = exponential phase rate constant.

Table 3. Numerical values of parameters involved in kinetic equations of Table 2: data obtained during incubation of glucose or ovalbumin in Seine river samples are given at 20°C for 10 ppm added $BOD_5$

| Kinetic parameters (units) | No. experiments | Mean | SD |
|---|---|---|---|
| $k_0^{20}$ (ppm $h^{-1}$) | 31 | 0.12 | 0.07 |
| $dk_0/dT$ (ppm $h^{-1}$) | 4 | 0.004 | 0.002 |
| $T_L^{20,10}$ (h) | 9 | 15 | 6 |
| $dt_L/dT$ (h per °C) | 4 | −1.1 | 0.6 |
| $dt_L/dL_0$ (h $ppm^{-1}$) | 7 | −0.7* | 0.8 |
| $k_1^{20,10}$ ($h^{-1}$) | 7 | 0.13 | 0.04 |
| $dk_1/dT$ ($h^{-1}$ per °C) | 9 | 0.013 | 0.007 |
| $dk_1/dL_0$ ($h^{-1}$ $ppm^{-1}$) | 7 | 0.003* | 0.003 |

*These parameters were neglected in the kinetic equations of Table 2.

at 20°C and it increases linearly with temperature (Table 3).

Lag-phase ends when oxygen uptake of samples added with pollutant becomes more important than those of crude water samples. The length $t_L$ of this lag-phase has a mean value of 15 h at 20°C; it decreases linearly with a temperature increase and is independent of the initial pollutant load $L_0$: indeed $dt_L/dL_0$ values were either not significant or actually negligible.

*Aerobic exponential phase*

At the end of lag-phase, DO and BOD uptake become proportional—but not equal—and both increase exponentially with time till depletion of one of them. The rate constant $k_1$ of this exponential phase presents a mean value of 0.13 $h^{-1}$ at 20°C and seems independent of the initial waste discharge. Temperature dependence of $k_1$ does not follow an Arrhenius relation but can reasonably been assumed to be linear.

Thus, the seven parameters plotted in Table 2, i.e. $k_0^{20}$, $dk_0/dT$, $t_L^{20}$, $dt_L/dT$, $k_1^{20}$, $dk_1/dT$ and $a = dL/dC$ are sufficient for describing DO and BOD concentration changes during aerobic biodegradation of waste discharge in a river (Fig. 8).

*Oxygen transfer*

As in usual Streeter & Phelps (1925) kinetic models, oxygen transfer can be taken into account with a linear relation:

$$dC/dt = K_2 (C_s - C)$$

where $C_s$ is the saturated oxygen concentration at temperature $T$ and $K_2$ a function of temperature and river speed and depth (O'Connor & Dobbins, 1958; Churchill *et al.*, 1964; Owens *et al.*, 1964; Langbein & Durum, 1967; Davidson & Bradshaw, 1967; Isaacs & Gavoy, 1968).

An alternative Log deficit form, i.e.

$$\mathrm{Log}\,(C_s - C) = \mathrm{Log}\,(C_s - C_0) - K_2 t$$

and an exponential form, i.e.

$$C = C_s - (C_s - C_0)\exp(-K_2 t)$$

have recently been developed and discussed by Howe (1977) and Brown & Stenstrom (1980).

## CONCLUSION

The experiments presented in this paper simulate a waste water discharge into a river and aerobic biodegradation, i.e. DO and BOD concentration changes, occurring after discharge. Although oxygen transfer has been avoided in such batch experiments, results differ significantly from usually accepted biodegradation models:

(1) an approx. 15 h lag-phase occurs after waste water discharge into river sample; DO uptake is slow and BOD constant during this phase;

(2) an aerobic exponential phase follows; DO and BOD uptake become proportional;

(3) an increase of biomass—estimated by cell ATP—occurs during the aerobic exponential phase.

Such a biphasic behaviour has been observed with various polluants (glucose, glutamic acid, starch, ovalbumin and ethanol) and various Seine river samples. It is similar to the results of BOD experiments with glutamic acid (Flegal & Schroeder, 1976) and may be interpreted as a cell division step following a lag-phase necessary for microorganisms to adapt themselves to their new environment and metabolites. Cell division time had a mean value of 5.3 h at 20°C during the exponential period. It is possible that waste waters discharged into streams are not sterile and will

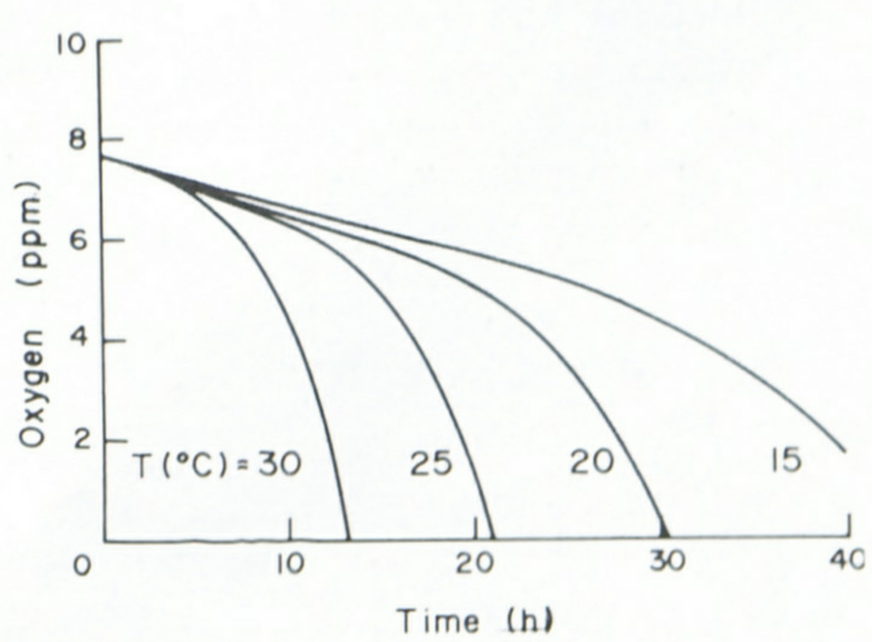


Fig. 8. DO concentration changes after a waste water discharge at 15, 20, 25 and 30°C. Simulated curves according to equations of Table 2 and parameters values of Table 3.

contain an active population of microorganisms adapted to and able to utilize the organic substrates present. Nevertheless, this population will only be stabilized on the stream bed and on aquatic plants of the discharge site vicinity. Its role will be important for biodegradation only if the stream is shallow and if the dilution factor of waste waters is not too high. Such a situation is not likely to occur in the main part of river Seine, Rhine or Thames for example.

The Streeter & Phelps kinetic model, which uses first order kinetics, should only be valid of (a) DO was constant and (b) wastes were directly oxidized by a first order reaction and without contribution of any microorganisms. It is obvious that if microorganisms play a prominent role in waste degradation, cell division and death will occur and first order kinetics should not represent correctly the actual biodegradation reactions. If this first order kinetic model has known such a success in water quality studies, we think that it is related to at least three deficiencies of field experiments:

(1) the almost unceasing waste discharge along rivers, averaging and integrating lag and cell division phases;

(2) the large time and space intervals used both for kinetic constants evaluation and for DO and BOD calculations; a day if not a week interval is usual; river quality determination or calculations are frequently performed over very large distances (several hundreds of km);

(3) the uncertainty in evaluating waste discharge and even DO or BOD, as well as river speed, flow and depth.

Field experiments are now rapidly changing. Field data are improving, both in frequency and quality. Calculations are using smaller space and time intervals to take into account day/night variations and river modifications. Through increase of sewage treatment plants, domestic and industrial wastes are becoming more concentrated and more constant. For all these reasons, we think that stream water quality surveys which use first order kinetics are going to face serious difficulties and that lag-phases and biomass variations should be taken into account. The kinetic model presented in this paper, at half way between empirical and physico-chemical models, should be tested along a stream and in the vicinity of a sewage plant or of a sequence of sewage plants, in order to demonstrate the occurrence of such biphasic biodegradation reactions in polluted rivers.

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