



**Estimation of Biodegradation Kinetics for
Envirotemp[®] FR3[™] Dielectric Coolant in
Pond Water**

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1. Introduction

Cooper Industries have developed a dielectric fluid, Envirotemp[®] FR3[™] (FR3), for use in new and retrofitted electrical transformers. This product is an edible seed oil-based fluid, which contains food grade additives. According to the manufacturer's specifications, it is readily biodegradable and the standard acute aquatic toxicity and oral LD50 tests have proven the product to be non-toxic (1). However, there are additional concerns regarding spills of even non-toxic vegetable oil based products into aquatic surface waters, the hazard of oils due to non-solubility in water and lower specific gravity than water (2). These two characteristics will cause the oils to cover the aquatic surfaces in thin films which can lead to potential coating of animals, respiration interference, and restrictions in the absorption of oxygen from the air into the water. In addition, there is the potential for such a spill to further reduce the oxygen content in the water due to their relatively rapid biological oxidation demand (BOD) (3).

We have seen statements that claim just one tablespoon of vegetable oil in a one acre pond of 3 foot average depth would deplete oxygen from typical pre-spill values.

Unfortunately, neither real world or even laboratory produced data on the rate and degree of actual oxygen depletion in a surface waters due to spills of vegetable oil are not readily available. With the encouragement of the US EPA SPCC office, the sponsor of this study contracted the University of Cincinnati to obtain data on actual rate and degree of oxygen depletion in pond water in a controlled laboratory process reflecting real world conditions in a practical way. For this reason, the goal of this study was to evaluate the impact of a

1,000 gallon spill of FR3 in a 100-acre pond on the concentration of dissolved oxygen (DO) in the water column.

2. Experimental

In order to determine the rate and extent of the biodegradation due to a spill of FR3, a respirometry test was designed considering two variables, water depth (2 and 8 in.) and mixing level (zero, low, and high). The combination of depth and mixing resulted in three scenarios, namely, experiment A (water depth 8 inches/no mixing), experiment B (water depth 8 inches/low mixing), and experiment C (water depth 2 inches/high mixing).

The water (40 L) used in the test was collected from an urban pond located at Burnet Woods (Cincinnati, OH). Aliquots were analyzed for nutrients (nitrogen and phosphorous) on a Dionex DX 500 Ion Chromatograph with a CD 25 Conductivity meter. Ammonia-nitrogen concentration was determined using a gas-sensing ion selective electrode (ISE) connected to a 720A Orion pH/ISE meter (Orion Research Inc., Boston, MA). Total and soluble chemical oxygen demands (CODs) were measured using prepared low range digestion vials (Hach, Loveland, CO). Finally, total organic carbon (TOC) and inorganic carbon (IC) were analyzed on a TOC-V CSH total organic carbon analyzer (Shimadzu, Wood Dale, IL). A pH value of 8.0 was found in the pond water. Results of the pond water analyses are shown in Table 1.

Parameter	Concentration (mg/L)
Nitrate	ND
Nitrite	ND
Phosphate	ND
Ammonia	ND
Total COD	45.3
Soluble COD	35.3
TOC	38.1
IC	20.7

Table 1. Chemical analysis pond water. COD, chemical oxygen demand; TOC, total organic carbon; IC, inorganic carbon; ND, not detected.

The experiments were conducted, for 30 days at 20 °C, in four computerized respirometers (N-Con Systems, Crawford, GA) with a total capacity for 48 flasks. The oxygen uptake by each flask was measured continuously and recorded hourly by the computer system. The oxygen consumed was measured and replenished through a calibrated valve in response to pressure drop within the headspace of the vial resulting from oxygen utilization. CO₂ produced as a result of biodegradation is removed from the headspace in a KOH trap. The O₂ demand of the un-metabolized FR3 was calculated as the difference between the product O₂ demand and the cumulative O₂ utilized at each time point (collected hourly) throughout the experiment. As mentioned above, each flask was equipped with a trap containing 0.1 N potassium hydroxide (KOH) to remove the evolved CO₂ from the system. The amount of CO₂ produced within the system was calculated at discrete points based on the relative drop in pH of the KOH solution. The

KOH solution in the traps was replaced when the solution pH dropped below approximately 10.5, as indicated by the pH indicator dye Alizarin Red.

Experiments A and B were performed in 70 mm i.d. × 326 mm tall flasks, with a 1 L volume of water in the column. Experiment C was carried out in 70 mm i.d. × 176 mm tall flasks. In this case, the volume of the water was 250 mL. Each experiment consisted of 10 replicates (pond water and FR3), two controls (pond water), and two blanks (distilled water and FR3). The volume of FR3 added to samples and blanks was calculated by Equation 1 to simulate a spill of 1,000 gallons (3785.4 L) in a 100 acre (404,685.6 m²) water body. Since respirometry flasks had a surface area of 0.0038485 m², the spiked volume of FR3 was 36 μL.

$$V(\text{FR3}) = 3785.4 \text{ L} \times \frac{0.00384885 \text{ m}^2}{404,685.6 \text{ m}^2} \times \frac{10^6 \mu\text{L}}{1\text{L}} = 36 \mu\text{L} \quad [1]$$

In regard to the oxygen depletion within the aqueous phase, DO was measured, under anoxic conditions, in duplicated samples by means of a 550A dissolved oxygen meter (YSI, Yellow Springs, OH). For each experiment, two samples were sacrificed at time zero and successive sampling events took place according to the oxygen uptake.

3. Results and Discussion

On July 9, the FR3 biodegradation test was started. After a seven-day run, no oxygen uptake was measured under the three scenarios, indicating that no degradation was occurring. It was speculated that such a phenomenon could be due to the lack of nutrients

found in the water pond. For this reason, the test was repeated, but this time 6 mg N-NO₃ and 1.2 mg P-PO₄ were added to all samples and controls. Results for average cumulative oxygen uptake with time are showed in Figures 1 and 2. The three curves revealed Monod-type degradation profiles. Lag phases were found in all cases primarily due to time needed for biomass acclimatization and the low density of biomass present in the pond that can biodegrade the oil. For both mixed scenarios, B and C, this period lasted for approximately two days, 42 and 50 hours, respectively. On the other hand, the samples with no mixing (experiment A) exhibited a delay of 84 h.

From Figure 1, it can be deduced that cumulative oxygen uptake for experiments A and B was similar, though slightly faster for the mixed samples. After 30 days, oxygen uptake values at the plateau were alike and close to 70 mg/L. Nevertheless, the curve for experiment A presented higher standard deviation values, because of the non-homogenized matrix. Experiment C (Figure 2) yielded a much faster FR3 metabolization.

In order to compare the results of the three scenarios, oxygen uptake data were normalized to the spill surface area, which was constant for all samples, and plotted in Figure 3. Although the three experiments achieved equivalent plateau levels, when collated with A and B, experiment C is the best-case scenario, since the combination of a smaller volume (250 mL) and high mix level led to a quicker removal of FR3 fluid. On the other hand, FR3 fluid removal was slower for the 1-liter samples, higher biomass amount notwithstanding. So the effect of mixing is critical to achieve a homogeneous

matrix, where biomass and FR3 fluid are well-dispersed and continuously stirred, which increases the contact area for enzymatic activity and facilitates the degradation process.

Degradation rates can be calculated using Equation 2, which is the standard expression used to define the BOD for wastewater (4).

$$O_t = O_m (1 - e^{-kt}) \quad [2]$$

Where O_t = oxygen uptake at time t

k = first-order reaction rate constant

O_m = ultimate oxygen uptake

t = time

Equation 2 can be linearized, as follows:

$$1 - \frac{O_t}{O_m} = e^{-kt} \quad [3]$$

$$\text{Ln} \left(1 - \frac{O_t}{O_m} \right) = -kt \quad [4]$$

The representation of time versus $\text{Ln} (1 - O_t/O_m)$ yields a straight line whose slope is the reaction rate constant. In the present case, O_t and t were monitored by the respirometers and O_m is the final oxygen uptake value at the plateau (Figures 1 and 2). Figure 4 shows the plots for the three experiments. After acclimation, the three curves had a linear trend, which coincided with the exponential growth of biomass. Once the substrate concentration became limited, the linear trend ends when the stationary growth phase (plateau) is reached.

The linearization results are given in Table 2. From these data, it can be estimated that the first-order reaction rate constants for experiments A, B, and C were 0.0033 h^{-1} (0.079 d^{-1}), 0.0039 h^{-1} (0.094 d^{-1}), and 0.0077 h^{-1} (0.18 d^{-1}), respectively.

Experiment	Slope	S_D^*	R^2	S_D^*
A (1 L no mixing)	-0.0033	7.58×10^{-6}	0.998	0.043
B (1 L mixing)	-0.0039	1.32×10^{-5}	0.995	0.075
C (250 mL mixing)	-0.0077	3.15×10^{-5}	0.996	0.073

Table 2. Statistical parameters for linear fits. S_D^* , standard deviation.

In the biodegradation experiments, the carbon dioxide production is related to the mineralization of the carbon source (5). Figures 5 and 6 show the CO_2 release for experiments A, B, and C. From Figure 5 (1-liter samples), it can be concluded that the effect of mixing resulted in a higher production rate and, therefore a faster degradation, although the final amount was similar after 30 days. In order to compare the three scenarios, measured CO_2 production was related to surface area in Figure 6. As stated for the oxygen consumption, final CO_2 production resulted in the same value for the three experiments at the end of the run. However, lower volume (250 mL) and higher mixing, which implies better homogenization and no transfer limitations, led to a higher production rate and faster mineralization.

The second goal of the present study was to determine how the biodegradation would affect the dissolved oxygen in the water column. Hence, samples were sacrificed at different times during the respirometry and DO was measured along the water column. The results are given in Figures 8, 9 and 10.

According to Figures 8 and 9, the impact of depth on DO was not significant. In fact, for any sampling event, the variation of DO along the 8-inches water column was negligible. When compared with time zero values, oxygen depletion was only found in experiment A (Figure 8). Dissolved oxygen dropped from 4 mg/L to 3 mg/L during degradation, this reduction being attributed to stagnation. Figure 10 shows that over experiment C, DO was always close to the initial value of 5 mg/L.

4. Conclusions

For the simulated conditions of a 1,000-gallon spill in a water body with a surface area of 100 acre, Envirotemp FR3 was found to be readily biodegradable. After 30 days, FR3 mineralization was completed under the three studied scenarios. The highest degradation rate was achieved in experiment C, where a high mixing level and a small volume led to a well-dispersed mix of FR3 in water. This situation enhanced enzymatic activity on FR3 and resulted in faster removal. Experiments A and B showed similar degradation rates, though FR3 removal was slower than in experiment C. This phenomenon may be due to a

lower FR3 dispersion in water, which reduces the contact between biomass, nutrient and substrate.

When it comes to dissolved oxygen concentration within the aqueous phase, no relevant depletion was found attributable to the biodegradation process. In spite of the stagnant conditions, no dramatic drop in DO was measured during experiment A. Stirred systems (experiments B and C) had DO levels above 4 mg/L during the degradation process. This phenomenon is due to the break of the FR3 film on top of the water column, which permits faster oxygen exchange between both gas and liquid phases. In addition, no differences in DO values with water depth were observed. Since there was no significant depletion of dissolved oxygen noted, there should be no adverse effect on aquatic life, even under stagnant conditions

5. References

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- (3) Groenewold, J. C.; Pico, F. R.; Watson, K. S. Comparison of BOD relationships for typical edible and petroleum oils *J. Water Pollut. Control Fed.* **1982**, *54*, 398-405.
- (4) Metcalf & Eddy, I. *Wastewater Engineering. Treatment and reuse*; McGraw-Hill: New York, 2003.
- (5) OECD "OECD guideline for testing of chemicals: ready biodegradability", Organization for Economic Co-operation and Development, 1992.

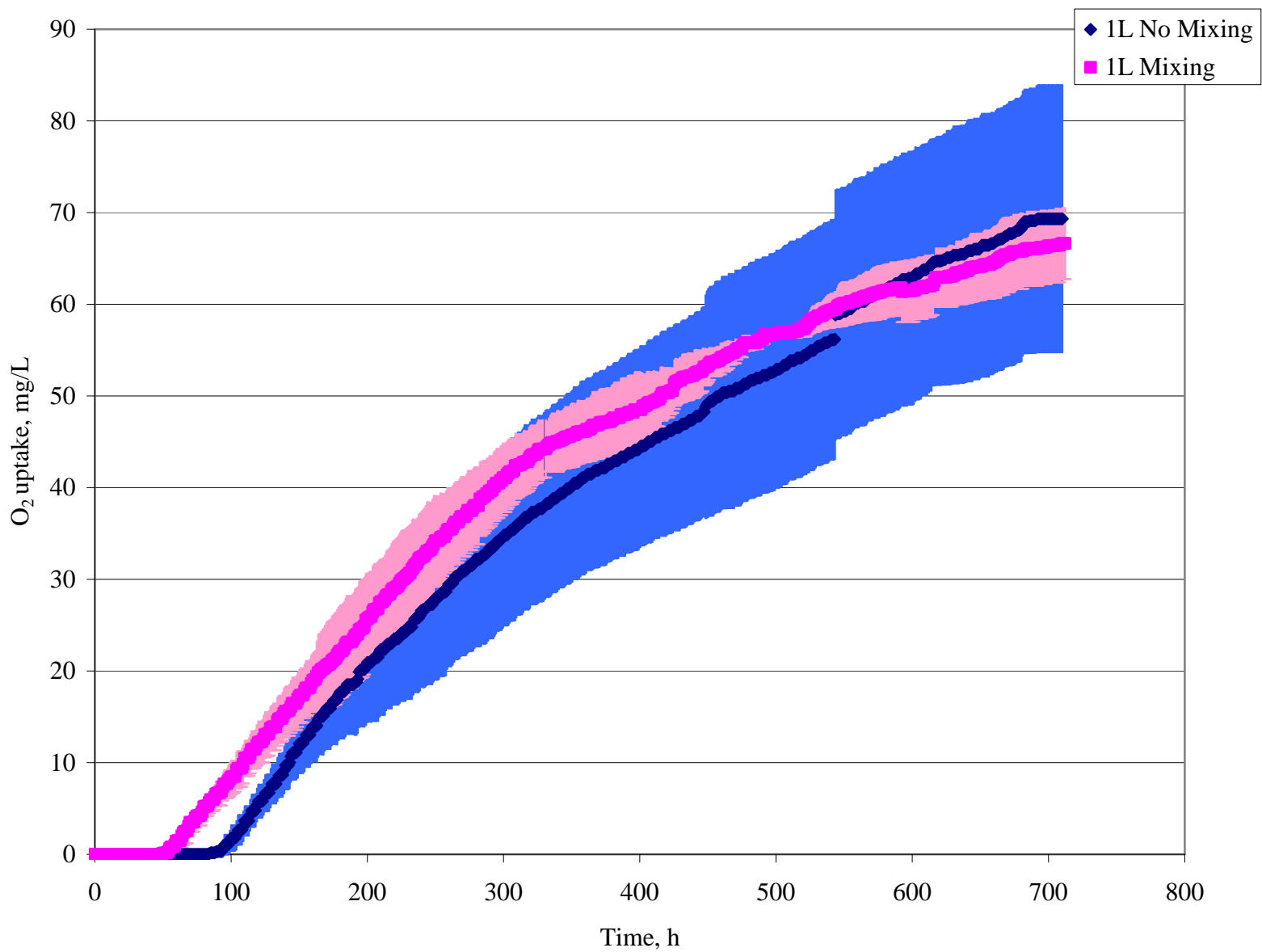


Figure 1. Average cumulative oxygen uptake for experiment A, and B.

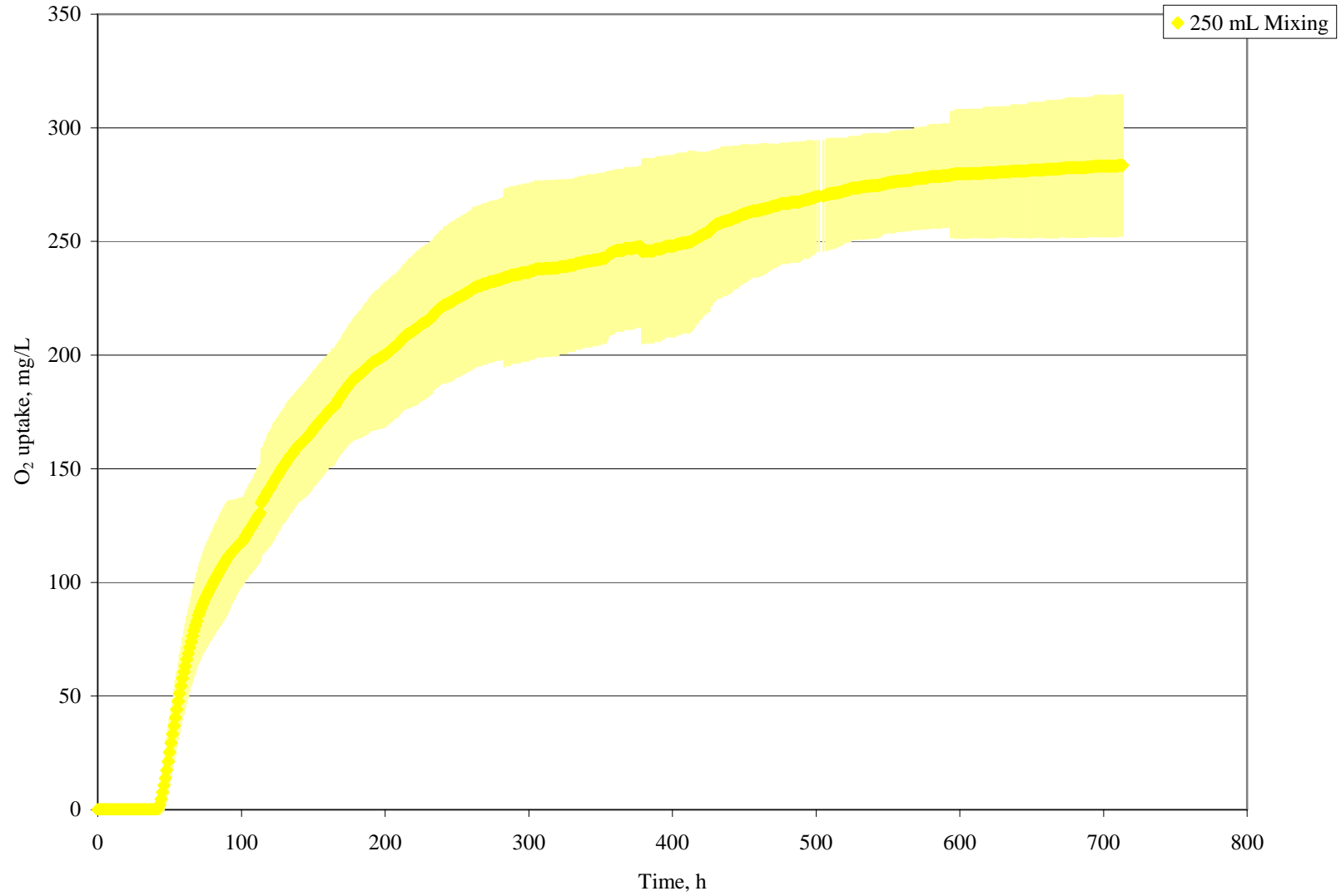


Figure 2. Average cumulative oxygen uptake for experiment C.

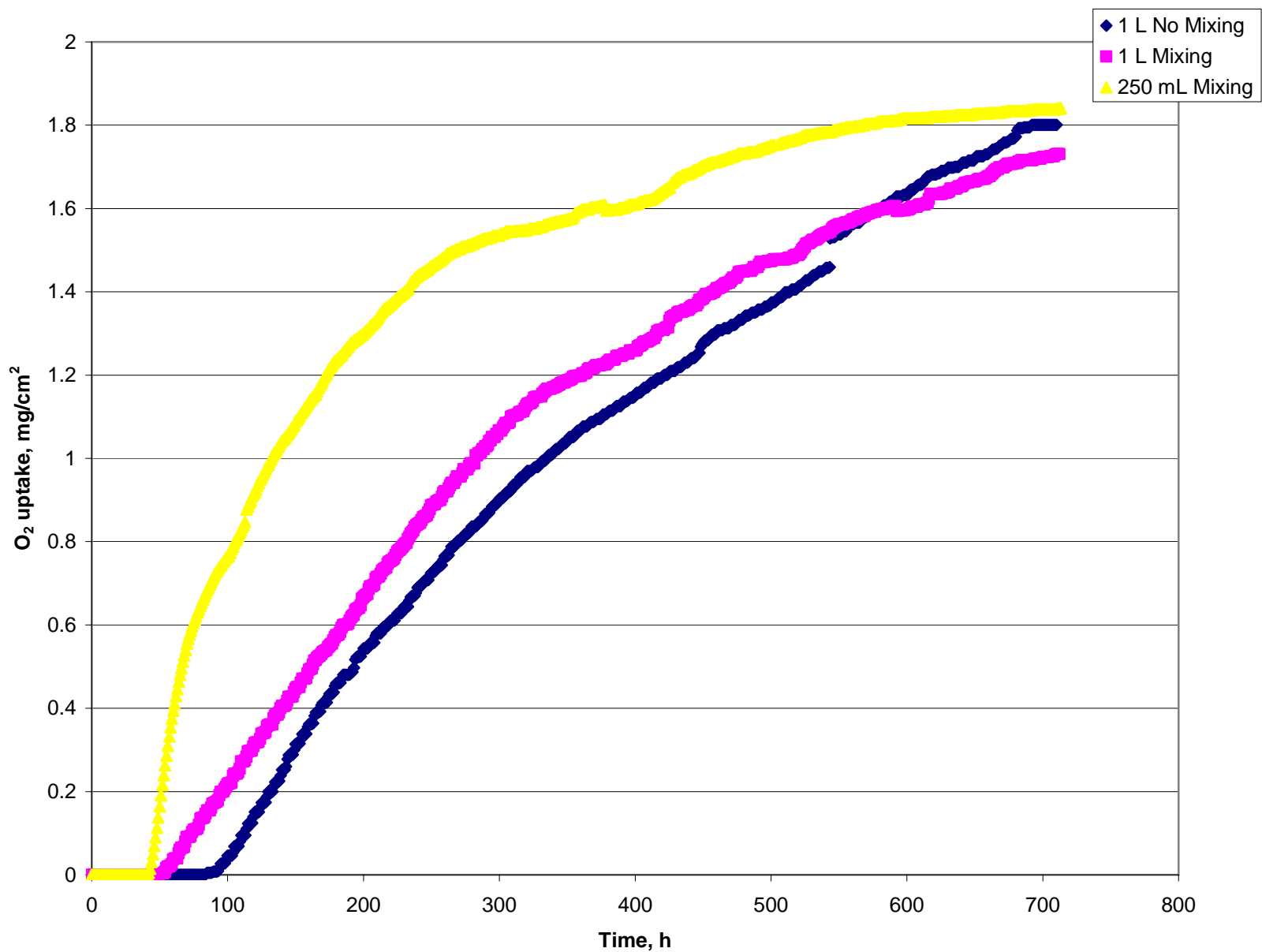


Figure 3. Oxygen uptake per surface area experiments A, B, and C.

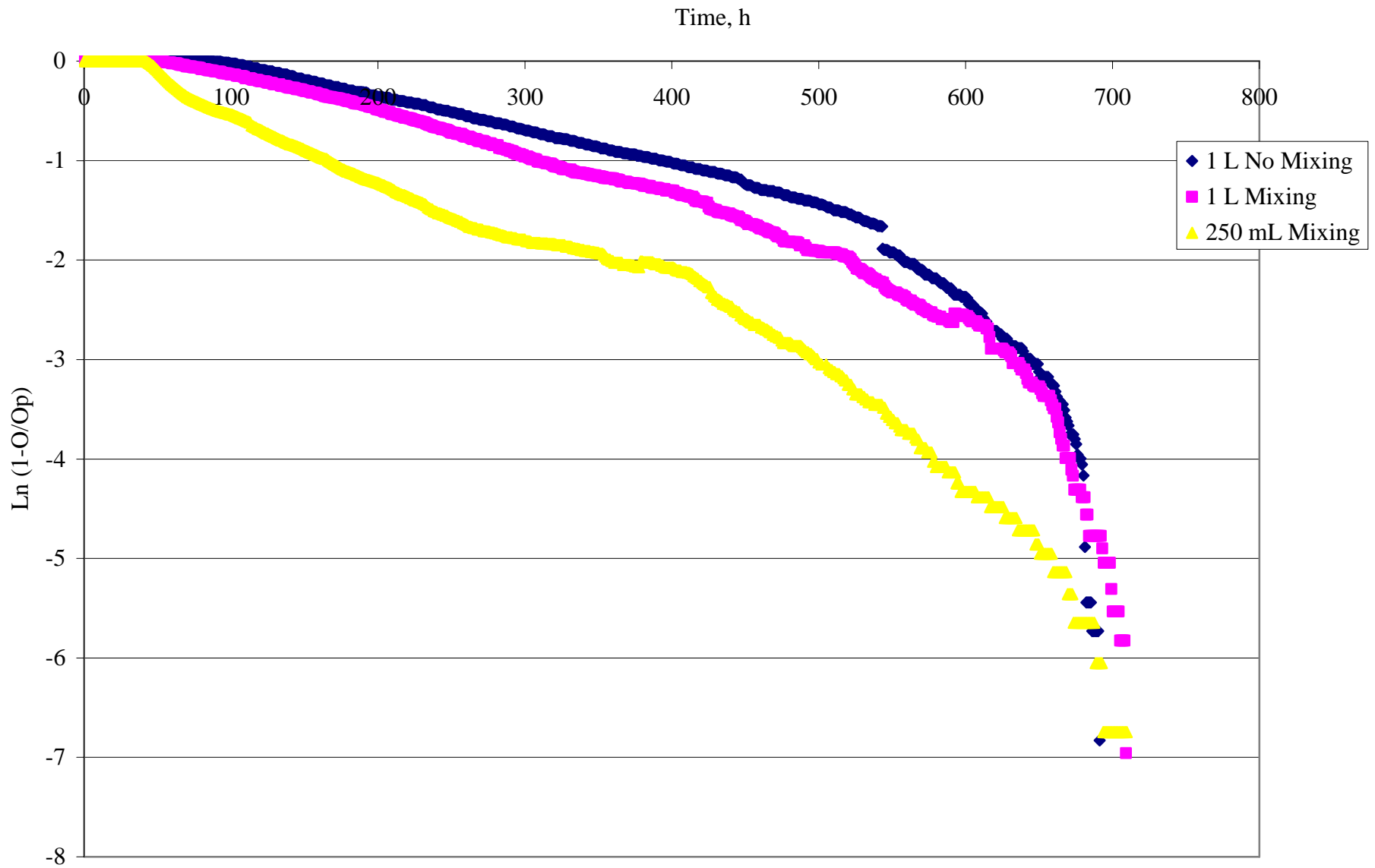


Figure 4. Linearized BOD data for experiments A, B, and C.

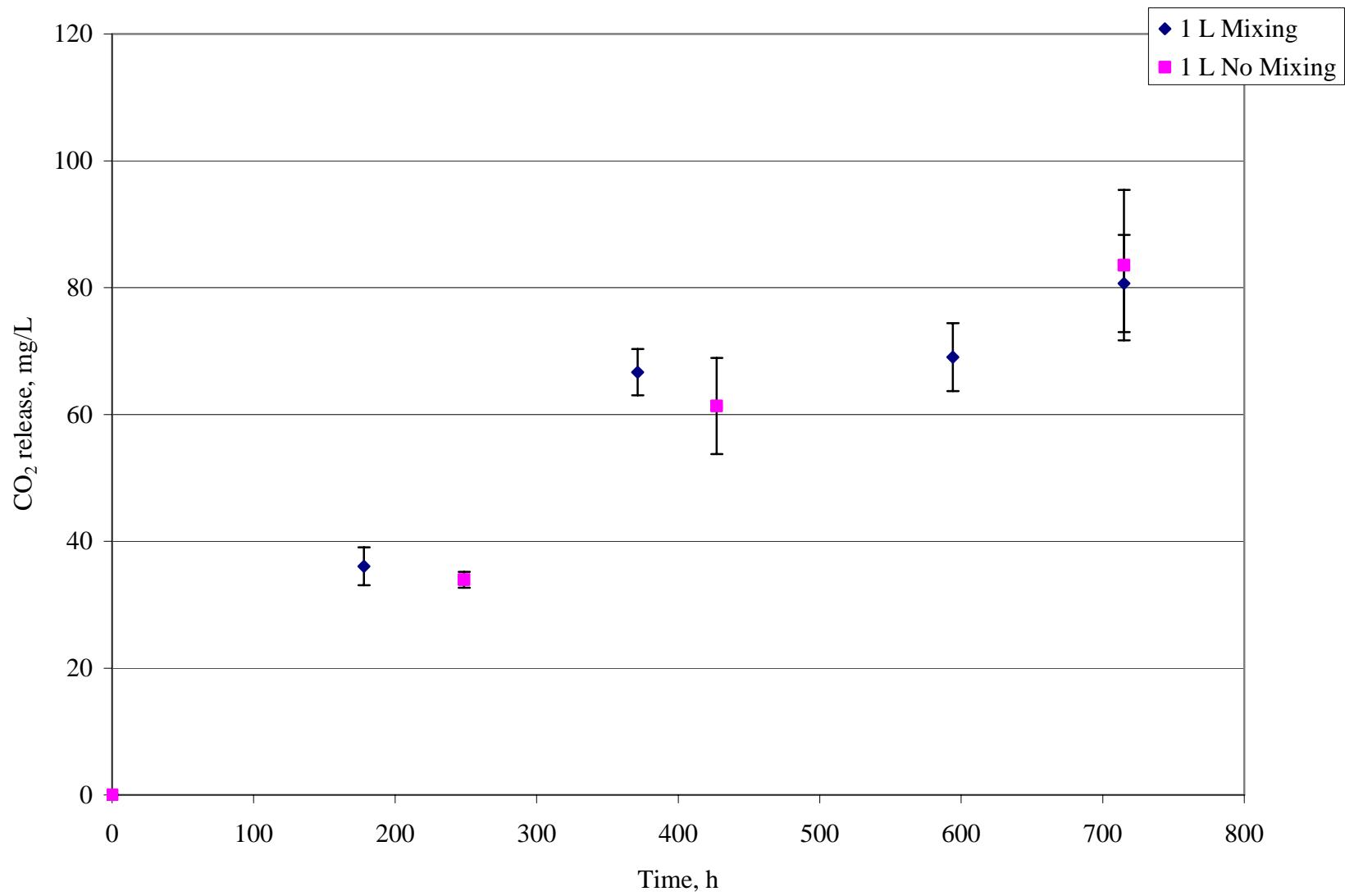


Figure 5. Carbon dioxide released experiments A and B.

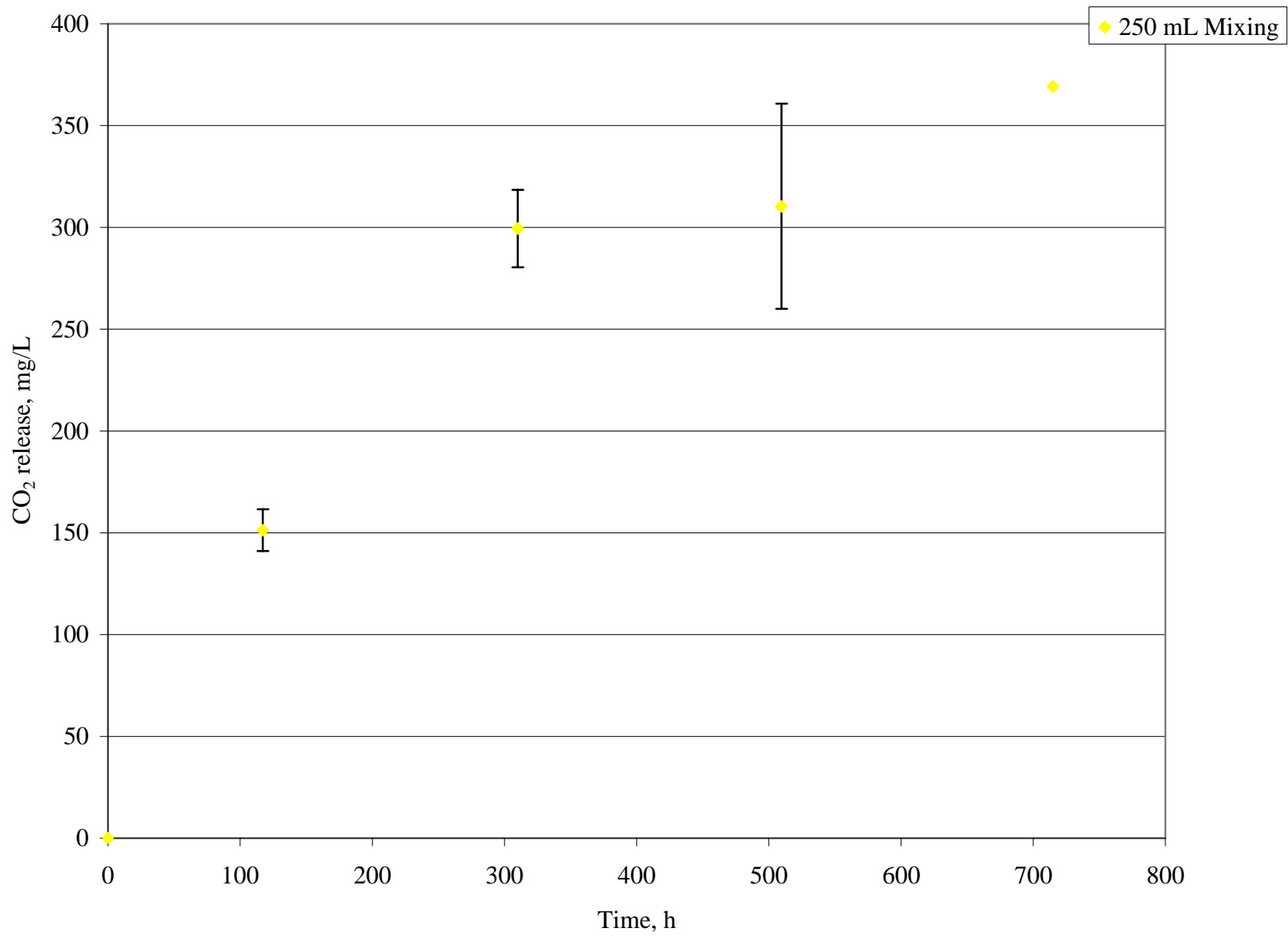


Figure 6. Carbon dioxide released experiment C.

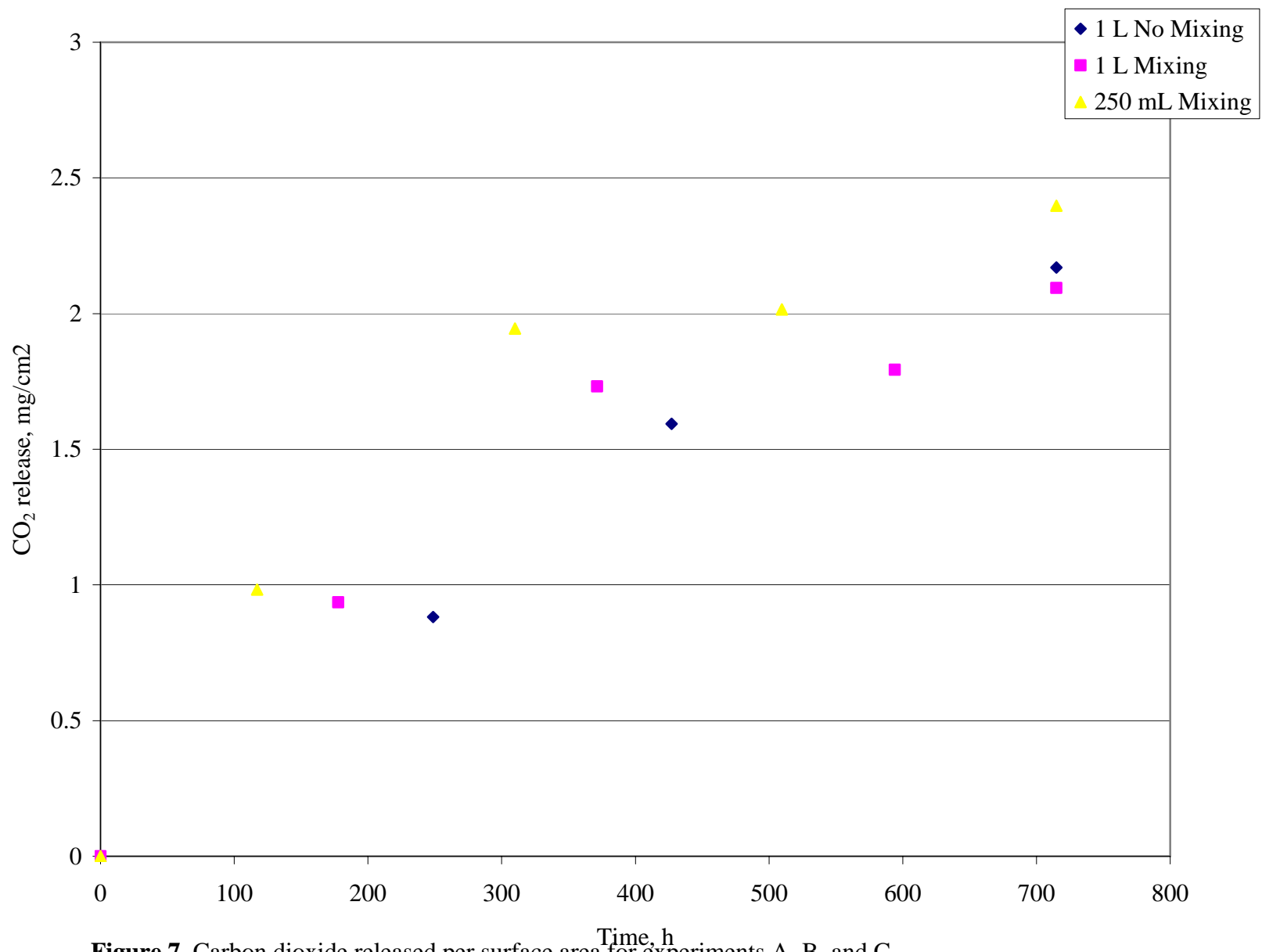


Figure 7. Carbon dioxide released per surface area for experiments A, B, and C.

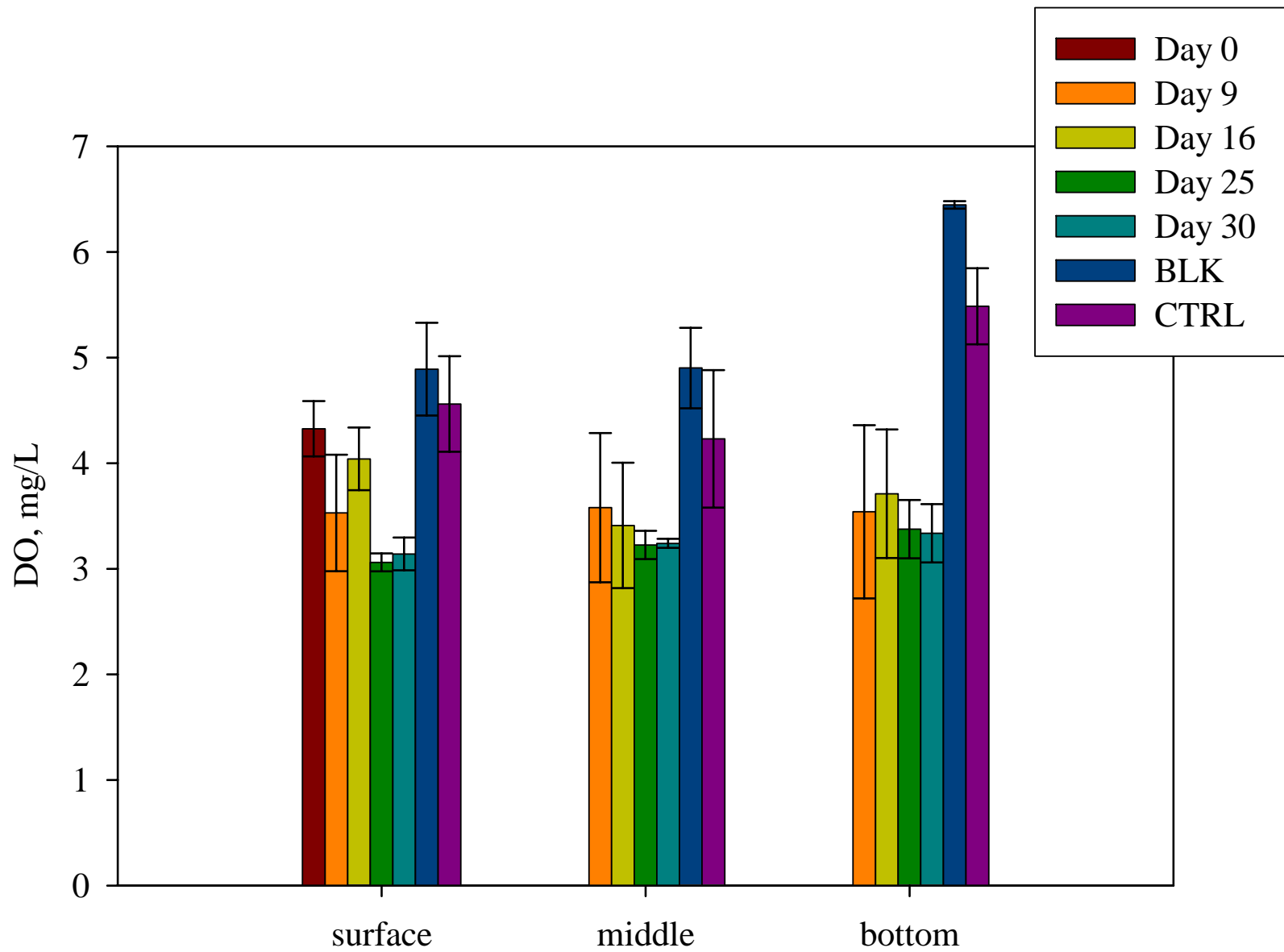


Figure 8. DO and water column depth for experiment A (8 in.-1L no mixing).

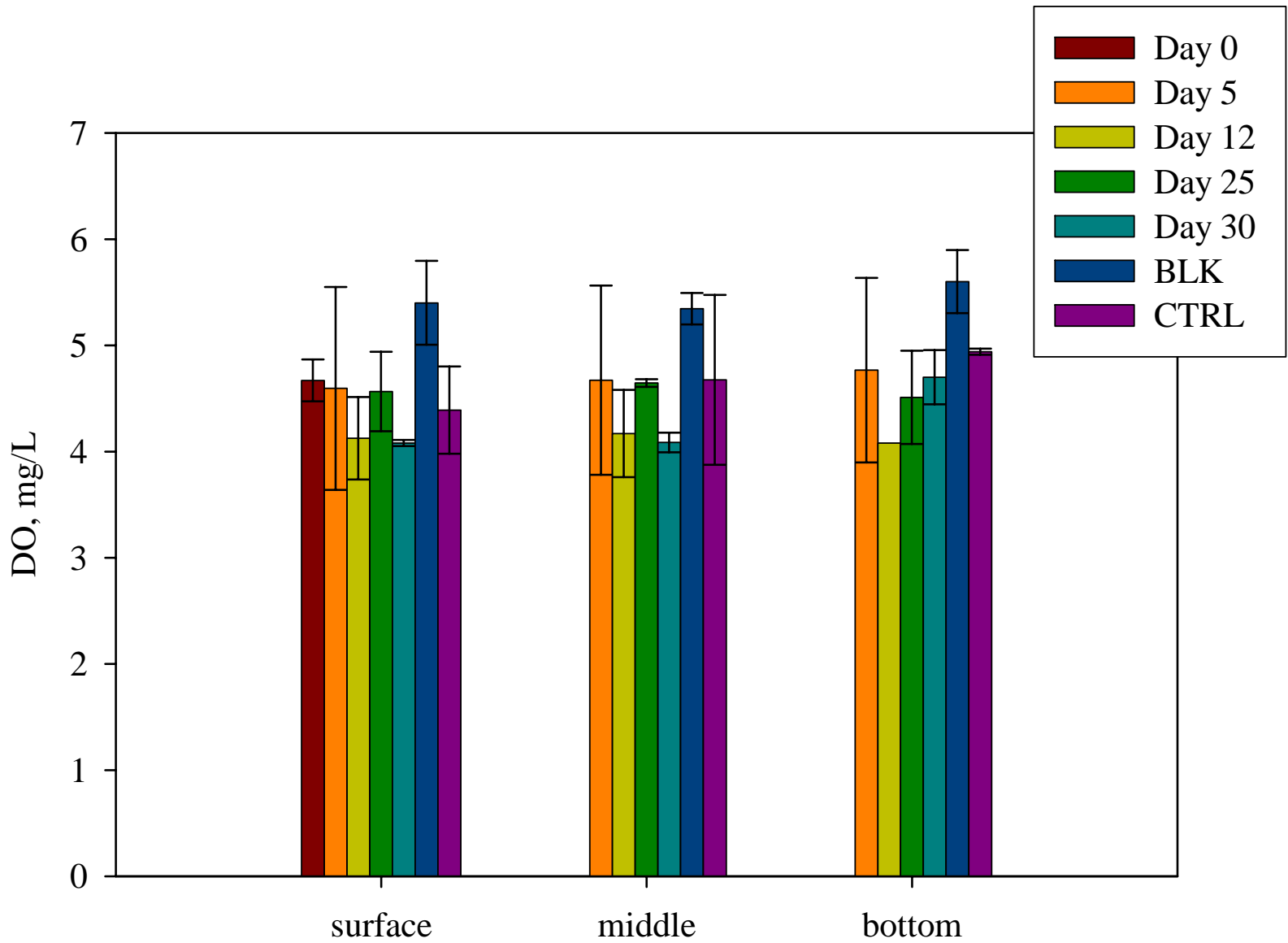


Figure 9. DO and water column depth for experiment B (8 in.-1 L mixing).

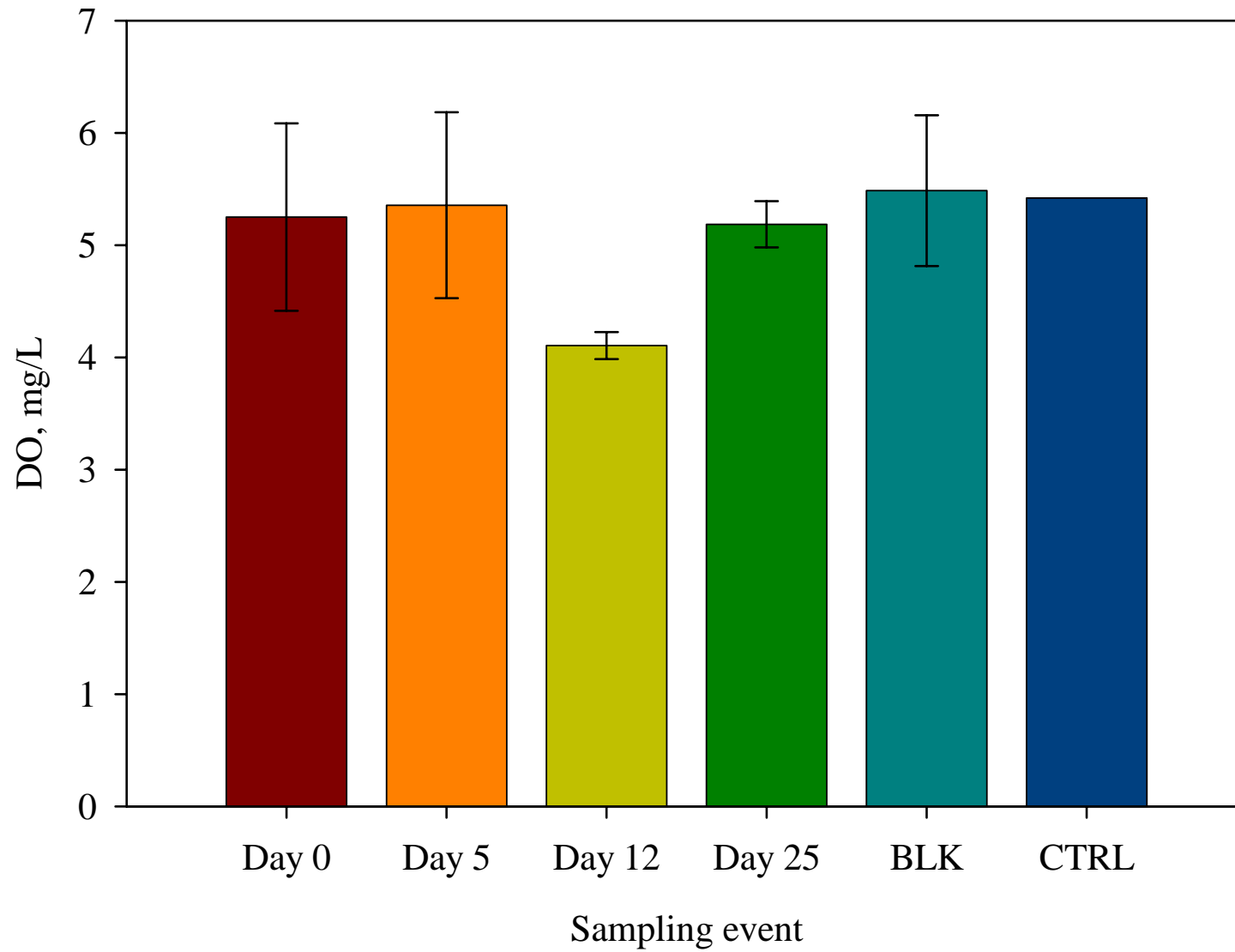


Figure 10. DO for experiment C (2 in.-250 mL mixing).