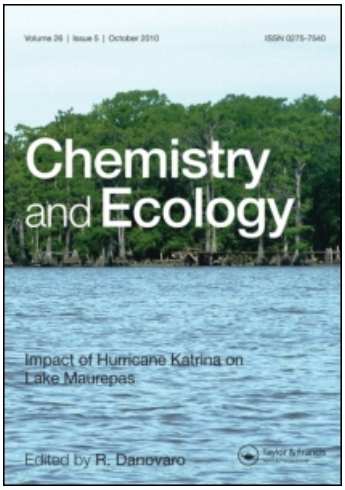

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RATE OF CHANGE OF BOD FOR MILK IN NATURAL SEA WATER

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As part of a project to determine the fate of contaminated milk in the marine environment (Elliott *et al*., 2001), the Biochemical Oxygen Demand (BOD) of unprocessed natural milk in sea water was followed in standard BOD bottles and in an open system. The best estimate for the BOD was ~170,000 $mg \cdot l^{-1}$ although measurable effects could still be seen with dilutions up to one in a million. If incubated in the light, simulating summer conditions, the algae produced oxygen and offset some of the loss through bacterial degradation thereby reducing any slump in oxygen. There was also a "nutrient effect" where the presence of milk increased the oxygen production at low concentrations in sea water. In an open system akin to a slick being dispersed, the change in BOD was exponential after an initial activation period. The length of this activation period was greater in more concentrated mixtures of milk and sea water. These results have been utilised in a model to determine the best strategy for disposing of contaminated milk (Elliott *et al*., 2001).

Keywords: Milk; BOD; Degradation; Seawater; Disposal

INTRODUCTION

Milk is a complex mixture of organic and inorganic compounds. Fresh whole milk is about 88 percent water with the remainder made up of solids that contain fat, protein, and lactose. Fat makes up approximately 3 percent of whole milk and most of the calorific value comes from this fat. The protein in milk is in the form of casein and whey. Lactose, the principal carbohydrate, is a disaccharide sugar ($C_{12}H_{22}O_{11}$), which produces galactose and glucose upon hydrolysis. Milk is also rich in minerals (calcium, phosphorus, zinc and iron) and vitamins (riboflavin, niacin, folic acid, thiamin (members of the B-complex family of vitamins) and vitamin A. However, cows milk is low in vitamins D and C.

In the event of an incident (such as a radiation incident like Windscale in 1957) where large volumes of milk need to be disposed of, discharge into the sea initially appears attractive. However, care is needed so as not to exceed an appropriate Biochemical Oxygen Demand (BOD). Modelling work (*e.g.* Helton *et al*., 1999) has indicated the likely pathways

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and important radionuclides in the transfer of dose after a nuclear power station accident and the effect on disposal routes.

BOD can be simply described as the quantity of oxygen required to completely biodegrade the organic matter present in a volume of waste material giving units of $mg \cdot l^{-1}$. However, it takes time for biological processes to work and the oxygen is utilised over an extended period. Often five days are chosen (BOD_5) but this is arbitrary and in the case of wastes with high BOD, under-estimates the total amount of oxygen required. Sea water contains approximately $7.6\,mg\,O_2\,l^{-1}$, thus unless sufficient volumes of water are provided or the oxygen is resupplied, the water will become anoxic and no further reduction in oxygen can be seen. Therefore, it is usual to dilute wastes into the water until there is still oxygen present even after the incubation period. A blank is also usually incubated for the same period and the change in oxygen is used to correct for the "waste effect" independent of the water effect. The BOD is then calculated from the dilution and the corrected amount of oxygen utilised in the biodegradation of that dilution of waste material. A full treatment of the BOD concept and its role in waste disposal can be found in Clark (1997).

Estimates of BOD for milk range from 100,000 to 140,000 $mg \cdot l^{-1}$ (Porteous, 1996; Holmes, *pers. comm.*) but no information is available on the rate of change of BOD in sea water. In order to determine the feasibility of this disposal route, which could require continuous discharges into the same body of water, the rate of change of BOD of oxygen consumption/production in various dilutions in sea water was investigated. As a basis for experimental investigation a historical BOD limit of $30\,mg \cdot l^{-1}$ was chosen in order to define a level around which an experimental dilution would be set.

MATERIALS AND METHODS

Determination of Dissolved Oxygen Concentration

Measurements of the oxygen content of sea water, and sea water mixed with milk are based on the Winkler titration according to the protocol of Strickland and Parsons (1972). The principle of the method is the precipitation of manganous hydroxide which is subsequently oxidised by free oxygen to form a tetravalent manganese compound. This compound oxidises iodide ions to iodine which is determined by titration with sodium thiosulphate.

Experimental Set-up

This study has been carried out in two main steps: the first step is the study of milk degradation in isolated conditions, and the second one is the study of milk degradation in an open system, (super)saturated with oxygen. In both systems, the temperature was maintained at $12\,^{\circ}C$ under atmospheric pressure. The traditional method of calculating BOD for any waste material involves incubation in the dark so only the bacterial processes are measured. However, in the real environment, light can penetrate the surface waters so that algae can photosynthesise and offset the reduction in oxygen caused by bacterial growth. Once the milk and sea water are in the sealed bottle, there can be no resupply of oxygen from the outside. In surface waters there can be diffusion of atmospheric oxygen into the system to continue the bacterial degradation. Therefore, in the second experimental setup, the sea water

was incubated in an open system with air added through an air stone. This allowed the bacteria to degrade the milk without reducing the oxygen in the waters. After pre-selected times, aliquots were drawn off and incubated in the traditional (closed bottle) way to calculate the amount of oxygen needed to degrade what was left.

The milk used in this study was produced by Friesian cows from a local herd. Milk was collected each time it was needed in order to use fresh milk, straight from the cow without pasteurisation. Sea water was collected on the day of the experiment directly from the Menai Strait. In general, pre-calibrated 250 ml BOD bottles were used for all incubations. The equipment was composed of a chiller (GRANT FC25G) and pump (GRANT FH 16-D) which controlled the temperature and a tank where the bottles were incubated. An external temperature probe connected to the pump was used to control the temperature in the tank.

Milk in sea water in a closed system

This study was conducted in two different light régimes, "winter", (low light, short period) and "summer" (natural summer daylight period). The "winter" light conditions were created by partially covering the tank with an opaque top.

Seven different dilutions of milk in sea water were chosen to achieve a logarithmic series. The dilution series was 1 in 3,333; 10,000; 33,333; 100,000; 333,333; 1,000,000 and sea water only (infinite).

For each dilution, seven different incubation times were chosen: 0, 1, 3, 10, 30, 100, and 300 hours. These should represent the appropriate timescales for the initial spreading and dilution of the milk in sea water.

A second series included samples containing higher concentrations of milk in order to determine the point where oxygen "generation" by algal photosynthesis was balanced by oxygen consumption by bacteria. The dilution series was 1 in 100; 300; 1,000 and 3,000.

Milk in sea water in an open system

Two dilutions of milk in sea water were selected for this experiment; 1 in 3,000 and 1 in 10,000. These two solutions were placed in 5 litre beakers and agitated gently with air input. This maintained the oxygen concentration at or above the saturation point. In order to avoid evaporation due to the stirring, the beakers were covered with an opaque top with a free passage for the air-flow. Thus, the light illumination was low and similar to the "winter" light condition in the first experiment.

Samples of the milk–sea water mixtures were removed from the beakers after 0, 1, 3, 10, 30, 100, 300 hours and then placed in sealed BOD bottles wrapped in aluminium foil for a further 100 hours incubation. Sub-samples of the stock solution were analysed at the same time to determine the oxygen content prior to BOD analysis.

RESULTS

The results of the winter and summer incubations are presented graphically. In summary, oxygen content of the BOD bottles ranged from 0 to $8.8\,\mathrm{mg}\cdot\mathrm{l}^{-1}$ with one standard error

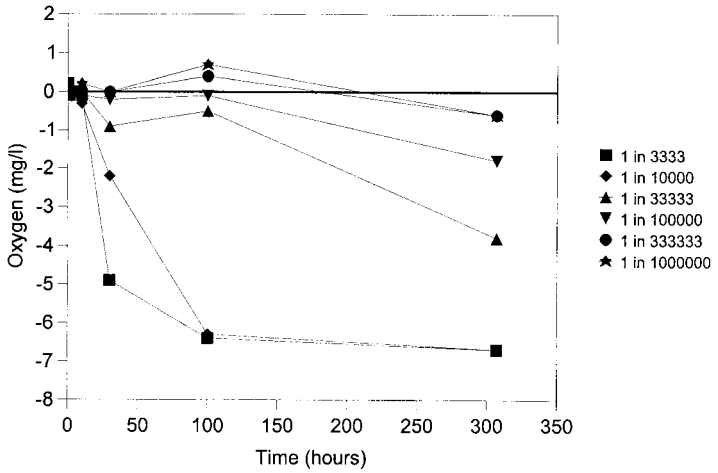

FIGURE 1 The effect of milk on the oxygen content of sealed bottles (see text for explanation). Experiments were conducted in the winter light régime at 12 °C.

being ~5% based on three replicate analyses of three bottles. The effect of the milk on the oxygen concentration with time for the winter condition can be seen graphically in Figure 1. As expected, the concentration of oxygen in the sealed BOD bottles decreases with time and with increasing milk added. The "no milk" blank is removed from each value to indicate the magnitude of the milk effect. The same data for the summer illumination experiment can be seen in Figure 2. When concentrations of milk are low, more oxygen is produced compared with the "no milk" blank. This can be clearly seen in Figure 2. With the inclusion of the extra, more concentrated series, it is possible to see the point at which there is no change in the oxygen content even though milk has been added. This is shown graphically in Figure 3.

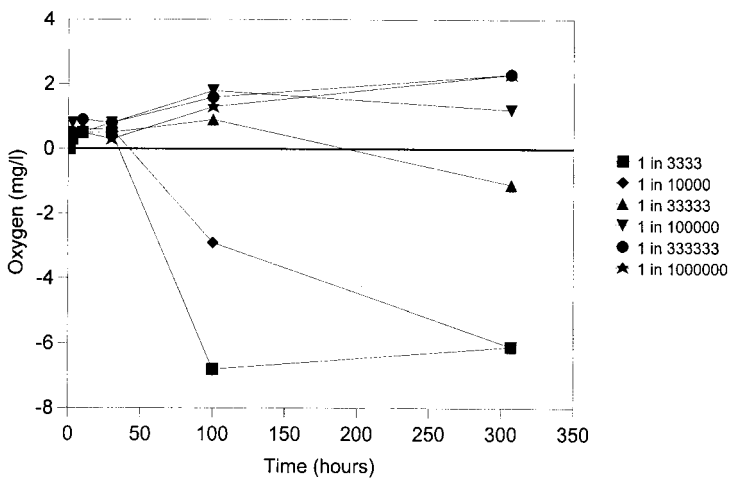

FIGURE 2 The effect of milk on the oxygen content of sealed bottles. Experiments were conducted in the summer light régime at 12 °C.

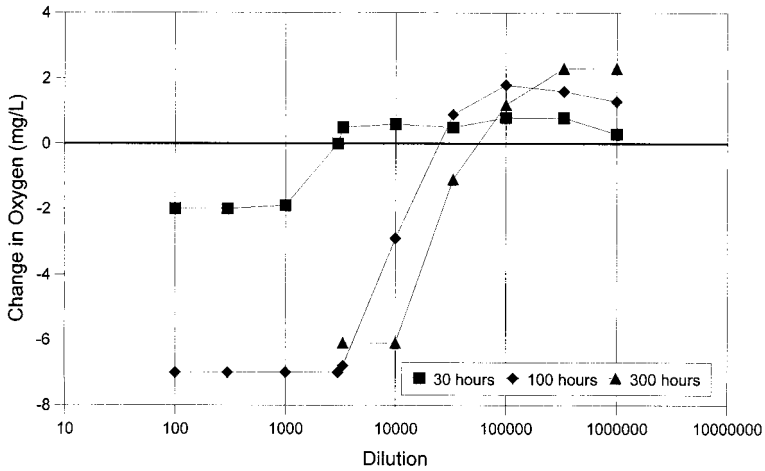

FIGURE 3 The change in oxygen content as a function of both time and dilution in the summer conditions. Positive values indicate the production of oxygen within the sealed system.

These data for the summer conditions indicate that there is no net loss of oxygen up to the 3,333 dilution after 30 hours, 30,000 after 100 hours and 70,000 after 300 hours. At each of these times, more dilute systems had more oxygen present due to the photosynthesis of naturally occurring algae. In winter conditions (Fig. 4), there is no obvious positive benefit in terms of oxygen production. Therefore, the negative effects occur at much lower dilutions than in the summer condition (c.f. Fig. 3).

Calculations of the BOD were made by multiplying the oxygen utilisation in each bottle by its dilution. The results span a wide range of values which are dilution and time dependent. This highlights the difficulty in using the BOD_5 value to describe the behaviour of milk in sea water. The oxygen concentrations in an open sea water system after incubation with milk can be seen in Table I. The final concentrations of oxygen after incubation in sealed BOD bottles for 100 hours after sampling can also be seen in Table I.

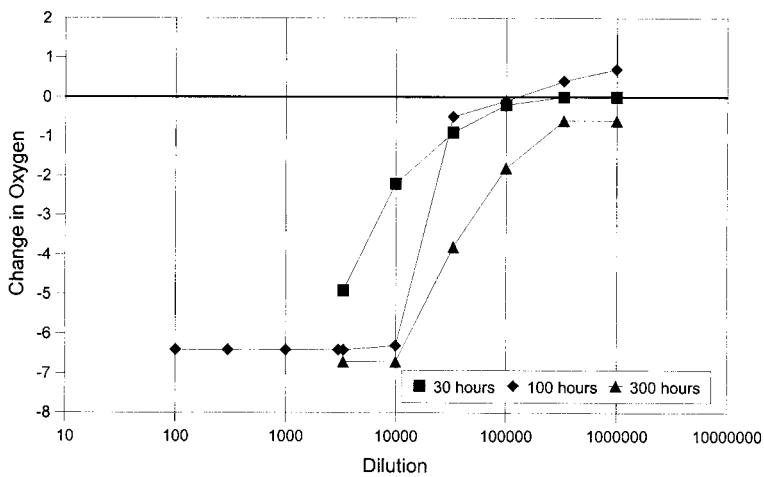

FIGURE 4 The change in oxygen content as a function of both time and dilution in the winter conditions. Positive values indicate the production of oxygen within the sealed system.

TABLE I BOD calculations for milk in an open sea water system followed by incubation for 100 hours in sealed BOD bottles at 12 °C under winter light conditions

Incubation time (h)	*Dilution*	*Concentration $O_2 \pm$ s.e. at sampling ($mg \cdot l^{-1}$)*	*Concentration $O_2 \pm$ s.e. ($mg \cdot l^{-1}$)*	*BOD ($mg \cdot l^{-1}$)*	*BOD of undiluted milk ($mg \cdot l^{-1}$)*
0	3,000	9.7 ± 0.4	0.3 ± 0.2	9.4	28,200
1	3,000	9.3 ± 0.4	0.2 ± 0.2	9.1	27,300
3	3,000	9.8 ± 0.4	0.2 ± 0.2	9.6	28,800
10	3,000	9.3 ± 0.4	0.2 ± 0.2	9.1	27,300
30	3,000	9.3 ± 0.4	0	≥ 9.3	$\geq 27,900$
100	3,000	9.4 ± 0.4	0.2 ± 0.2	9.2	27,600
332	3,000	8.8 ± 0.4	7.7 ± 0.2	1.1	3,300
0	10,000	10.2 ± 0.4	0.2 ± 0.2	10.0	100,000
1	10,000	9.8 ± 0.4	2.6 ± 0.2	9.2	72,000
3	10,000	9.7 ± 0.4	0.5 ± 0.2	7.2	92,000
10	10,000	9.3 ± 0.4	2.6 ± 0.2	6.7	67,000
30	10,000	8.9 ± 0.4	2.5 ± 0.2	6.4	64,000
100	10,000	9.4 ± 0.4	5.9 ± 0.4	3.5	35,000
332	10,000	8.6 ± 0.4	8.1 ± 0.4	0.5	5,000

The oxygen content in the 5 litre beakers at the time of BOD sampling indicated that the water remained supersaturated with oxygen throughout the entire experiment with little difference between the two beakers. The reduction in oxygen content after a further 100 hours incubation in the sealed BOD bottles can be seen in Figure 5. The 1 in 3,000 dilution BOD bottles contained little or no oxygen until the open incubation was 300+ hours. In contrast, the 1 in 10,000 dilution contained low concentrations of oxygen from 3 hours onwards.

DISCUSSION

The results of the incubations in winter (low light conditions) (Fig. 1) show that the oxygen content of the BOD bottles is still decreasing even after 300 hours incubation. The more con-

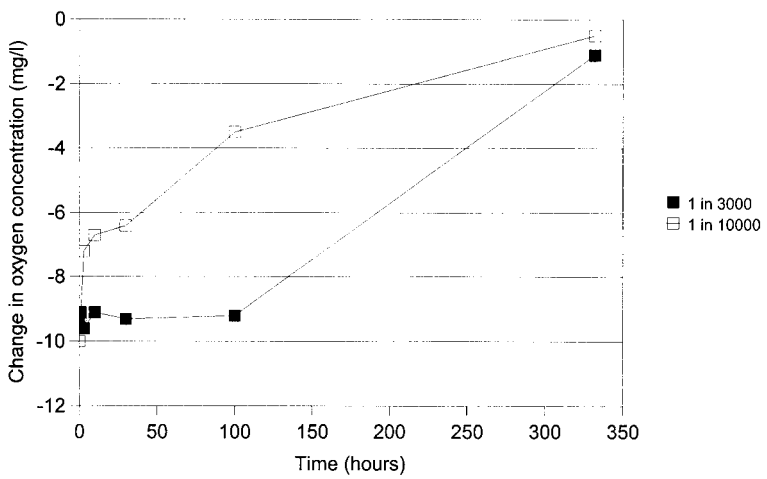

FIGURE 5 The change in oxygen content of the BOD bottles after 100 hours incubation less the oxygen content at the time of sampling.

centrated samples (1 in 3,333 and 1 in 10,000) had reached anoxia within 100 hours and so no further reductions could be measured. Therefore, even at the highest dilutions used here, standard BOD values obtained after 5 days underestimate the total amount of oxygen needed to degrade the milk. There was a reduction in oxygen in the blank due to the degradation of naturally occurring organic matter in the sea water but a small effect could still be seen at a dilution of 1 part milk in 1,000,000 sea water (Fig. 1).

In the summer conditions, the rate of oxygen usage is not as great due to the production of oxygen by naturally occurring algae in the sea water. Indeed, at low dilutions (<1 in 100,000) a beneficial effect could be seen with more oxygen being produced than in the blank. The rate of change in the oxygen in the BOD bottles can be taken from the slope of the linear portion of the lines in Figures 1 and 2 prior to removal of the blank where oxygen was still present. These rates are shown graphically in Figure 6.

A "nutrient effect" can be seen where oxygen is produced above the rate of the blank (plotted nominally at 1E + 7) in the summer conditions. If there was no nutrient effect, the winter and summer lines should be parallel with an offset due to the algal oxygen production. However, there is a dilution dependent difference between the two lines due to the addition of organic and inorganic nutrients contained within the milk to the system. This is different to the milk effect which is simple subtraction of the blank due to usage of oxygen in the sea water by the bacteria in the absence of milk.

The data in Figure 3 indicate the dilution where no net negative effects can be seen due to the addition of the milk in summer conditions. The nutrient effect is again seen with oxygen production in dilute solutions. Up to 30 hours, a dilution of ~1 in 3,000 has no negative effect on the oxygen content of the system. However, this same dilution after 100 hours has utilised all the oxygen in the sealed BOD bottles. Therefore, the "no net effect" dilution moves to the right on the graph (~1 in 30,000 after 100 hours and ~1 in 70,000 after 300 hours). In an environmental system where sea water containing milk is likely to be isolated from the atmosphere, the minimum dilution at which no net effect would be expected should be significantly greater than the values derived from Figure 3 which were based on the estimated BOD of milk alone.

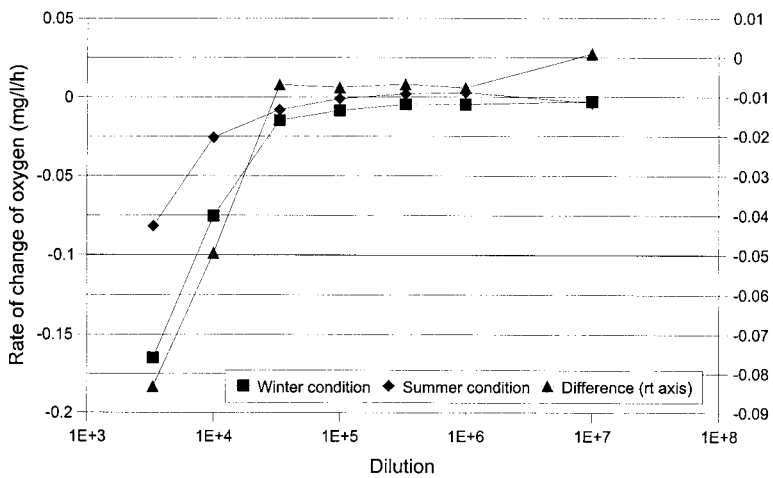

FIGURE 6 The rate of change in oxygen content of the BOD bottles with dilution under the winter and summer conditions. The difference between the two lines is plotted on the 2nd Y-axis.

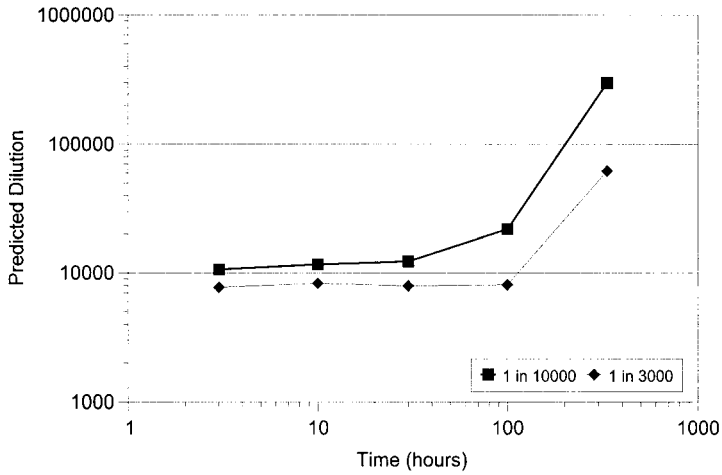

FIGURE 7 The predicted dilution of the milk based on the rate of change of oxygen concentration.

In winter conditions, the effect is more pronounced due to the lack of oxygen production by algae. In turbid waters, this is also likely to be the case even in summer. No net effect values in these conditions are less than 1 in 1,000,000 (Fig. 4) for 300 hours. This and all the higher concentrations still caused a depletion of at least some of the oxygen.

In an open system where the water body is completely saturated with oxygen, it is possible to calculate the rate of change in the system based on the oxygen concentration after 100 hours applied to Figure 1. When these slopes are applied to Figure 5, it is possible to predict the dilution at the time of sampling (Fig. 7). Incubation in the open system increases the rate of change in the dilution exponentially due to increased degradation after initial "activation" of the bacteria. This period of activation is longer for the more concentrated solution (1 in 3,000) compared with the dilute system. However, between 100 and 300 hours, the rates of change in dilution increase.

An estimate of the BOD for milk can be made from these data. For 300 hours, BODs for the three dilutions from 1 in 33,333 to 1 in 333,333 all suggest a value around 170,000 mg of oxygen per litre of milk. This is marginally greater than the previous estimates (see Introduction) but can be accounted for by the increased time of incubation. If the milk/sea water system had been left longer than 300 hours, it is possible that even greater values may be measured.

CONCLUSIONS

The degradation of milk in sea water is not a simple process as the results demonstrate. The application of a simple BOD value does not highlight the time dependent effects nor the effects of nutrients seen in summer conditions. The main conclusions are:

- The BOD for 300 hours incubation is ~170,000 $mg \cdot l^{-1}$.
- In the summer conditions with light present, algae naturally present in the water are able to photosynthesise and produce oxygen which goes some way towards reducing the oxygen debt caused by the bacterial degradation of the milk. In the winter (no light) condition, this effect is not present and so the milk effect can be seen in more dilute systems.

- The minimum dilution that has no effect is time and season dependent and is approximately 1 in 1,000,000 under winter conditions after 300 hours.
- As expected, in an open system, such as the surface or well-mixed waters, the degradation of milk is more rapid than in a closed system. The rate of change in predicted dilution based on the oxygen consumption suggests it is best described by an exponential function up to at least 300 hours.

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