# Sociobiology of biodegradation and the role of predatory protozoa in biodegrading communities

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Predatory protozoa are known to enhance biodegradation by bacteria in a variety of systems including rumen. This is apparently counterintuitive since many protozoa do not themselves produce extracellular degradative enzymes and prey upon bacterial degraders. We propose a mechanism of protozoal enhancement of bacterial biodegradation based on the sociobiology of biodegradation. Since extracellular enzyme production by degraders involves a cost to the bacterial cell, cheaters that do not make the enzyme will have a selective advantage. In the presence of cheaters, degraders that physically attach to water-insoluble substrate will have a selective advantage over free-floating degraders. On the other hand, cheaters will benefit by being free floaters since they consume the solubilized products of extracellular enzymes. Predatory ciliated protozoa are more likely to consume free-floating cheaters. Thus, due to protozoan predation a control is exerted on the cheater population. We illustrate the dynamics of such a system with the help of a computer simulation model. Available data on rumen and other biodegradation systems involving protozoa are compatible with the assumptions and predictions of the model.

[Modak T, Pradhan S and Watve M 2007 Sociobiology of biodegradation and the role of predatory protozoa in biodegrading communities; *J. Biosci.* **32** 775–780]

## 1. Introduction

A variety of protozoa are associated with different biodegrading microbial communities. Some of these protozoa have been shown to produce extracellular enzymes and hence play a direct role in biodegradation (Jouany and Martin 1997). However, a large number of protozoal species play only a predatory role and mainly consume bacteria (Jouany and Ushida 1999). It has been shown reproducibly that predatory protozoa enhance the efficiency of bacterial biodegradation (Biagini *et al* 1998; Jouany 1996; Jouany *et al* 1998). Inoculation of protozoa into defaunated rumen markedly stimulated bacterial cellulases and bacterial xylanolytic activity (Jouany and Martin 1997). Refaunation increased the fibrolytic activity by 4–8-fold. This enhancement of biodegradation was due to the presence of the non-cellulolytic genus *Entodinium* spp. in the defaunated rumen. The predatory soil flagellate *Heteromita globosa* stimulated toluene biodegradation by a *Pseudomonas* sp. in a model food chain (Mattison and Harayama 2001).

The mechanisms of enhanced biodegradation in the presence of protozoa are not clearly known but speculations have been made. Protozoa are suggested to stabilize the pH of the rumen and decrease the redox potential of rumen digesta (Jouany and Ushida 1999). Better pH regulation has been demonstrated in the presence of protozoa. However, it is not clear whether the enhancement of biodegradation is a cause or an effect of pH control. No mechanism by which protozoa could regulate pH has been suggested. Other explanations such as metabolic synergy between protozoa and adherent bacteria, physical contribution of protozoa to the disorganization of the fibre structure and protozoal facilitation of the attachment of bacteria on plant fibres (Jouany and Ushida 1999) are equally unsubstantiated. We suggest as well as model a mechanism for predator enhancement of bacterial biodegradation, which is based on the sociobiological aspects of biodegradation.

Sociobiology deals with the evolutionary origins and stability of altruism and cooperative behaviour between

**Keywords.** Biodegradation; ciliates; rumen; sociobiology

two or more individuals (Hamilton 1964; Wilson 1975). Since clones of bacteria have genetic relatedness close to 1, according to the kin selection hypothesis, high levels of sociality are expected in bacteria. However, there might be other reasons why cooperation need not be common among bacteria (Watve *et al* 2004). Well-studied examples of cooperation in microorganisms include fruiting body formation in myxobacteria (Velicer *et al* 2000) and slime moulds (Bonner 1982). It has been shown empirically as well as theoretically that such cooperative societies are often invaded by cheaters that need not contribute to the cooperative act but can benefit from it (Buss 1982; Watve and Matapurkar 1997). Social behaviour in bacteria is much more common than these classical examples. Simple acts such as the production of extracellular molecules can be interpreted as social behaviours.

It is essential for the bacterial population to produce extracellular enzymes such as cellulases, xylanases and amylases for the degradation of water-insoluble substrates. The enzymatic degradation results in soluble products that can then be easily taken up by the cell. However, since the degradation process is extracellular, the cell has no control over it. As a result, the degradation products can also benefit a cell that does not produce the extracellular enzyme. In a fluid environment a 'cheater' cell that does not pay the cost of making the extracellular enzyme can share the benefit almost equally with a degrader cell. In effect, the cost–benefit ratio would be more favourable for the cheater cell which would get selected over the degrader. This is likely to result in a collapse of the biodegradation system.

As a counterstrategy, degraders should attempt to protect biodegradation products in such a way that their benefit is greater than that of cheaters. This can be achieved by means such as physically attaching to the substrate particle or physically surrounding the particle. Attachment will help them take up greater amounts of degraded products from the particle being degraded before the products leak out into the fluid medium. On the other hand, it will be more advantageous for cheaters to remain mobile or free floating as they depend upon the uptake of solubilized product and attachment will reduce the effective surface area available for absorption. Ciliated protozoa are more likely to prey on free-floating bacteria owing to their method of feeding. This would exert a control on the cheater population. Orpin and Letcher (1984) found that defaunation increased the concentration of liquid-associated bacteria while it had no effect on solid-adherent bacteria. This indicates that protozoa chiefly prey on free bacteria. There is some evidence that the population of bacteria fixed on particles is higher in faunated than in defaunated rumen (Jouany and Ushida 1999).

We demonstrate with a computer simulation model that predatory protozoa can effectively keep cheaters in control and therefore stabilize the system while enhancing degradation. In the absence of predators the system collapses due to invasion of the biodegrading community by cheaters.

## 2. The model

The model assumes a chemostat system with an inflow rate D at which a complex substrate (such as cellulose fibre) is supplied continuously from a reservoir at a constant concentration *fr*. The degrading organisms act upon the added fibre converting a part of it to a soluble form while a part is removed from the system in the overflow. The model assumes two types of degraders—the ones that attach to the substrate ($X_1$) most of the time and the ones that are always freely suspended in the medium ($X_2$). Attachment facilitates better absorption of the degraded substrate so that a fraction $n$ of the degraded substrate is taken up by the cell producing the extracellular enzymes. The rest, i.e. (1–$n$) is dispersed in the medium and can be taken up by any cell. On the other hand, for an attached cell the surface area in contact with the substrate will not be available for transport of soluble nutrients. Therefore, the rate of uptake of free-floating nutrients by the attached forms is $u$ times ($u$ <1) the free-floating forms.

Cheaters, i.e. organisms that do not invest in enzyme production but consume the degradation products, may also have alternative strategies to remain in the attached ($X_3$) or unattached ($X_4$) form.

The growth of the populations $X_1$, $X_2$, $X_3$ and $X_4$ can be represented as

$$\frac{dX_1}{dt} = r_1(X_1) - DX_1$$

$$\frac{dX_2}{dt} = r_2(X_2) - DX_2$$

$$\frac{dX_3}{dt} = r_3(X_3) - DX_3$$

$$\frac{dX_4}{dt} = r_4(X_4) - DX_4.$$

The growth rates of $X_1$, $X_2$, $X_3$ and $X_4$ depend upon the substrate available to each one. Accordingly

$$r_1 = \frac{R\max(Su + fmn)}{Km + Su + fmn} - Ce - Ca$$

$$r_2 = \frac{R\max S}{Km + S} - Ce$$

$$r_3 = \frac{R\max Su}{Km + Su} - Ca$$

$$r_4 = \frac{R\max S}{Km + S}.$$

Here, the $S$-dependence is assumed to follow the well known functional form used by Monod (1942; *see* Smith,

*http://math.la.asu.edu/~halsmith/bacteriagrow.pdf*) in his model of the chemostat. $S$ is the standing concentration of soluble substrate. $f$ is the standing concentration of the total fibre available for degradation. $u$ is the fraction of soluble substrate taken up by the attached organisms after considering the loss of surface area due to attachment. $m$ is the total amount of substrate degraded by the degraders $X_1$ and $X_2$. $n$ is the fraction of degraded fibre directly absorbed by $X_1$ owing to an advantage of attachment. $Ca$ is the cost of attachment. $Ce$ is the cost of producing extracellular enzymes for degradation.

The amount of fibre degraded by the degrader population and converted into soluble substrate is $mf(X_1+X_2)$. Therefore, the change in standing concentration of fibre in the system is given by

$$\frac{df}{dt} = Dfr - mf(X_1 + X_2) - Df\,.$$

The rate of change of soluble substrate concentration is decided by the rate of solubilization by the degraders, rate of uptake by all the four populations and the fraction lost in the overflow.

$$\frac{dS}{dt} = [mf((1-n)X_1 + X_2)] - y[r_1(X_1) + r_2(X_2) + r_3(X_3) + r_4(X_4)] - DS$$

where, $y$ is the substrate utilization constant which is assumed to be identical for all populations.

If predators were introduced into this system then their presence would alter the growth equations of the populations. The rate of predation of the unattached population (*Pu*) is assumed to be higher than that of the attached population (*Pa*).

The growth of the predator population can be written as

$$\frac{dX_5}{dt} = r_5X_5Pa(X_1 + X_3) + r_5X_5Pu(X_2 + X_4) - DX_5\,,$$

where $r_5$ is the rate of conversion of prey consumed into predator biomass.

The altered growth equations of the degraders and cheaters are

$$\frac{dX_1}{dt} = r_1(X_1) - DX_1 - PaX_5,$$

$$\frac{dX_2}{dt} = r_2(X_2) - DX_2 - PuX_5,$$

$$\frac{dX_3}{dt} = r_3(X_3) - DX_3 - PaX_5,$$

$$\frac{dX_4}{dt} = r_4(X_4) - DX_4 - PuX_5.$$

Using the five types of populations, the concentration of fibre and solubilized substrate as interdependent variables, simulations were run for 5000 to 50,000 generations. Simulations were continued until a stable steady state or clear extinction of populations was achieved.

## 3. Results and discussion

In the model system, survival of microbial populations critically depended on the degraders as the water-insoluble fibre was the only available substrate which the cheaters were unable to degrade. However, as part of the fibre was solubilized, cheaters could grow. In the absence of predators, attached degraders had an all-time advantage over free-floating degraders since they could utilize a larger share of the degraded fibre. On the other hand, free-floating cheaters had an all-time advantage over attached cheaters since both depended on the solubilized substrate and the attached forms lost some of the absorbing surface area due to attachment. In the absence of predators a typical result of the simulation was that the cheaters outnumbered the degraders since they

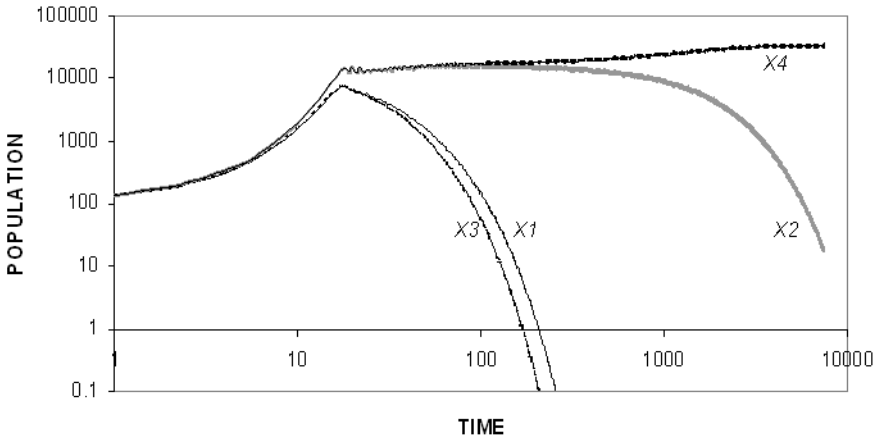



**Figure 1.** Typical dynamics of degraders and cheaters in the absence of predators. Both time and population are represented on a log scale for clarity. Free-floating cheaters drive degraders to extinction. After complete extinction of degraders the entire system collapses and all species become extinct (not shown). Other parameters for the simulation were *D=0.13, Rmax=0.5, Km=20, Ce=0.001, Ca=0, u=0.4, y=0.005, m=0.05, n=0.6, Pa=0.0005, Pu=0.0009.*

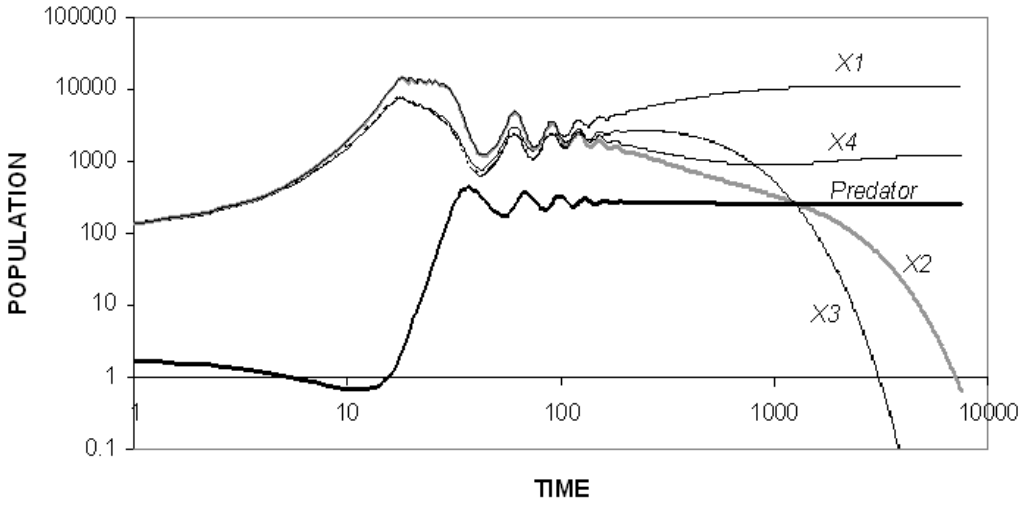



**Figure 2.** Typical dynamics of degraders and cheaters in the presence of predators. The cheater population is kept in control by the predator resulting in coexistence. Other parameters are the same as in figure 1.

A. Without predator

| n → u ↓ | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 0.95 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | | | | | | | | | | |
| 0.3 | | | | | | | | | | |
| 0.5 | | | | | | | | | | |
| 0.7 | | | | | | | | | | |
| 0.9 | | | | | | | | | | |
| 0.99 | | | | | | | | | | |

B. With predator

| n → u ↓ | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 | 0.95 |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.1 | | | | | | | | | | |
| 0.3 | | | | | | | | | | |
| 0.5 | | | | | | | | | | |
| 0.7 | | | | | | | | | | |
| 0.9 | | | | | | | | | | |
| 0.99 | | | | | | | | | | |

**Figure 3.** Parameter areas of stability of biodegradation (shown by the grey area) when the dilution rate is high. D = 0.13 and the other parameters are the same as in figure 1.

saved the cost of making the extracellular enzyme. This resulted in gradual extinction of the degraders leading to a collapse of the system (figure 1). Biodegradation was stable only when both *u* and *n* were large. When *u* was close to 1 the disadvantage for the attached forms was negligible, so even a small advantage of differential pick-up of the degradation product '*n*' was sufficient for the survival of the attached degraders. At low and moderate values of *u* and *n* cheaters proliferated and ultimately drove the system to extinction.

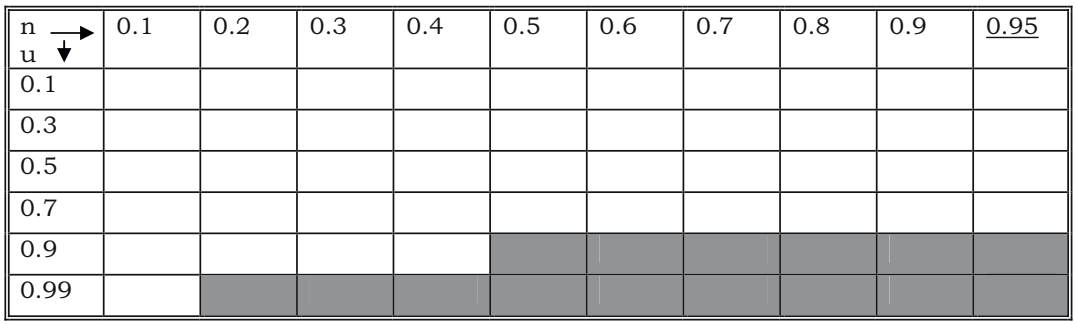
Although the model treats *u* and *n* as two independent parameters, a negative correlation is expected between these two. Both the parameters are related to the cell surface attached to the substrate in such a way that *n* increases with the fraction of surface attached and *u* decreases with it. It is unlikely that both *u* and *n* will be simultaneously large. Therefore, in the absence of predators, stability of the system is doubtful.

The introduction of predators changed the outcome substantially. Predators mainly kept the free-floating cheaters in control. This generally resulted in a stable coexistence among degrader, cheater and predator (figure 2).

In the presence of predators the parameter area over which biodegradation was stable increased considerably. At a large *u*, small *n* and a large difference between *Pa* and *Pu,* attached cheaters destabilized the system, while at very small values of *u*, free-floating cheaters destabilized the system. Over the rest of the area, there was stable or oscillating coexistence (figures 3 and 4).

Change in dilution rate of the chemostat did not alter the pattern qualitatively but shifted the stability areas. At low dilution rates there was more time available for degradation of fibre. As a result, the standing concentration of fibre was relatively small. As soluble substrate became more important, under low dilution rates advantages conferred by *u* were more important than those conferred by *n*. Therefore, at low dilution rates, the stability area expanded along the *u* axis and contracted along the *n* axis (figures 3 and 4). Over a broad range of *D*, *u* and *n* the predator was required to stabilize the system. The only critical assumption for predator-induced stability was *Pu >Pa.* Under this condition the predator exerted control over the cheater population, thus playing a crucial role in maintaining an equilibrium between degraders and cheaters. In this study, we modelled a classical chemostat which is a fluid environment and we also treated the dynamics of the non-soluble components as if they were soluble. In reality, non-soluble substrates create a structured or socially viscous environment. Such environments are likely to enhance cooperation through increased kin and group selection. Therefore, the dynamics of a real-life system are likely to be even more stable.

A. Without predator




B. With predator

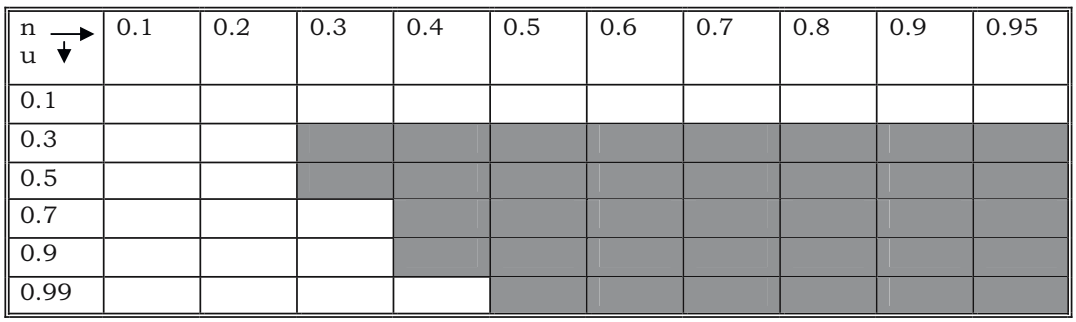



**Figure 4.** Parameter areas of stability of biodegradation (shown by the grey area) when the dilution rate is low. D= 0.07 and the other parameters are the same as in figure1.

The model shows that predatory protozoa can stabilize a biodegradation system by influencing the altruist–cheater dynamics. The finding that protozoa reduce the density of free-floating bacteria and enhance biodegradation (Orpin and Letcher 1984) is in support of the model. It is also possible to test the model by construction of a chemostat system with suitably selected bacterial cultures and a predatory species.

There is another possible mechanism for protozoal enhancement of bacterial biodegradation—enhancement of group selection. If protozoa harbour communities of biodegrading bacterial cells intracytoplasmically or on their surface, there can be competition between these consortia leading to inter-group selection. Currently, empirical data for this are limited and therefore we did not incorporate this possible mechanism in the model. Studies on biodegradation have so far ignored the sociobiological factors. Our model demonstrates that sociobiology could play an important role in the evolution and stability of biodegradation, and opens up a number of possible applications of sociobiology in fields such as pollution control.

## Acknowledgments

We sincerely thank Jean-Pierre Jouany of INRA, France for valuable information and useful interaction at the early stages of concept development.

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*MS received 27 November 2006; accepted 3 April 2007*

*e*Publication: 26 April 2007

Corresponding editor: Vidyanand Nanjundiah