Plasmid Transfer for Enhancing Degradation Capabilities

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The kinetics of plasmid conjugation for the TOL and RP4 plasmids depend strongly on the donor cells’ specific growth rate and substrate concentration, both of which determine the cells’ energy availability. Although transfer rates can be large when energy availability is high, normal biological processes have low energy availability. Therefore, we propose and evaluate preliminarily a simple scheme to create a small zone of high energy availability. — Environ Health Perspect 103(Suppl 5):113–115 (1995)

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One of the pressing goals for environmental engineers is to increase the rates of biodegradation of hazardous organic chemicals in processes for treating wastewaters, drinking waters, groundwaters, and soils. Because discharges of hazardous chemicals in effluent waters, off-gases, and wasted sludges or soils are increasingly restricted by law, the only alternative for controlling hazardous organic chemicals is biodegradation, which can destroy the contaminant molecules. When the degree of biodegradation is not sufficient with the normal means of biological treatment, more advanced strategies are needed to enhance the biodegradation rates.

This report summarizes research aimed at enhancing biodegradation rates by directly controlling the microorganisms’ genetic capability through horizontal gene transfer. The application of this research involves using plasmid conjugation to extend or augment the biodegradative capacities of bacteria that are well suited to function in treatment systems. The particular scientific goal of the research is to define the kinetics for conjugative plasmid transfer by bacteria relevant to biological treatment.

**Plasmid and Horizontal Transfer**

Horizontal transfer of genetic information is a naturally occurring phenomenon involving plasmids, which are covalently closed circular strands of DNA that exist and replicate autonomously from the host chromosome (1). Being accessory and mobile, plasmids can be introduced into bacteria indigenous to a treatment process without requiring a chromosomal change. Thus, the genetic elements responsible for making the indigenous species ecologically fit for a treatment system can be maintained while new elements needed for specific degradation reactions can be added via the plasmid. Furthermore, since replication of plasmid DNA is not dependent on cell division, the genetic information can be amplified and can proliferate throughout the microbial population without the need for creating a growth rate advantage for any added or indigenous species. Because the selective pressures inherent to treatment processes (e.g., low substrate concentrations of diverse substrates, slow specific growth rates, and aggregation) are not going to be alleviated, proliferation of critical, new genetic information to ecologically fit, indigenous bacteria is an extremely promising strategy for enhancing process performance for degradation. This is especially true when the target contaminants are present in concentrations too low (e.g., much less than 1 mg/l) to exert any significant selective pressure themselves.

The mechanisms affecting plasmid transfer and stability are illustrated schematically in Figure 1. Three types of cells exist:

- donor cells, signified by D, contain the plasmid;
- recipient cells, signified by R, do not contain the plasmid; they are chromosomally distinct from donor cells; and
- transconjugant cells, noted by T, are recipient cells that have gained the plasmid. Thus, they are chromosomally R, but also contain the plasmid.

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**Figure 1.** Schematic summary of the plasmid-transfer phenomena, the type of cells (D, R, and T), and the kinetic expressions for each phenomenon (kt1DR for DR transfer, kT2R for TR transfer, and -bpT for plasmid loss).
Figure 1 also illustrates the three plasmid-transfer processes:

- **DR transfer** is a conjugation event in which D and R cells come into contact, the plasmid DNA is replicated, and the replicated plasmid is partitioned into both cells, creating D and T cells. Note that conjugation increases the number of plasmids and plasmid-containing cells, even though the total number of cells is the same;
- **TR transfer** is a similar conjugation event, except that the plasmid is donated by a transconjugant and the result is two T cells; and
- **Plasmid loss from the transconjugant cell** can occur through segregation (the improper partitioning of plasmid DNA during cell division), restriction of the plasmid, or unfaithful replication during cell division (2,3).

Each transfer process occurs at a rate that is dependent on the plasmid, the bacterial species, and conditions within the system. Figure 1 indicates the rate expressions we employed for each process (4). Although we investigated all three transfer processes (5–7), we report here only on DR transfer, for which the rate expression is

\[ r_{DS} = k_{DS} DR \]

in which \( r_{DS} \) is the rate of formation of transconjugants by DR transfer (Mₙ L⁻³ T⁻¹), \( D \) is the concentration of donor cells (M₀ L⁻³), \( R \) is the concentration of recipient cells (M₀ L⁻³), \( k_{DS} \) is the DR-transfer coefficient (M₀ M₀⁻¹ L⁻¹ T⁻¹).

Trends for TR transfer were parallel to those for DR transfer (5–7), while loss rates were small for the systems studied (5,6). In the remainder of this report, we present experimental results for the value of \( k_{DS} \) and what affected its value.

### Transfer of the TOL Plasmid

Our initial work (6,7) investigated the transfer kinetics for the TOL plasmid, which codes for the degradation pathway of toluene. The donor was *Pseudomonas putida* PAWI (TOL), and a restriction-deficient recipient, *Pseudomonas aeruginosa* PAO 1162, was employed. Transconjugants were assayed routinely by their ability to grow on toluic acid, and hybridization of plasmid DNA with oligonucleotide probes was used periodically to confirm the presence of the TOL plasmid (5).

The kinetic experiments with TOL were conducted in the same manner as for RP4. As with RP4, we found that the donor's antecedent growth rate and substrate concentration during the kinetic experiment affected \( k_{DS} \) dramatically. BF Smet's (5) quantified the two effects with Equation 2:

\[ k_{DS} = 0.021 \exp\left(0.55 \mu \right) \]

in which \( k_{DS} \) has the units g₉⁻¹ l (g₀ g₀ day⁻¹); \( \mu \) is the donor's antecedent specific growth rate (day⁻¹); and \( S \) is the donor's substrate concentration (g/l) during the kinetic test. Equation 2 shows that increases in \( \mu \) and \( S \) increase \( k_{DS} \), and the effect is very dramatic for large values of \( \mu \), greater than about 3/day. Near the cell's maximum specific growth rate, \( k_{DS} \) approaches 2.5 g₉⁻¹ l (g₀ g₀ day⁻¹), a very large value. On the other hand, low specific growth rates and low substrate concentrations reduce \( k_{DS} \). When \( \mu = 0.2/day \) and \( S = 2.5 \) mg/l, \( k_{DS} \) drops to 0.001 g₉⁻¹ l (g₀ g₀ day⁻¹), a modest value.

### Application to Treatment Processes

Although \( k_{DS} \) values can be quite large, the conditions normally found in biological treatment processes are quite the opposite of the conditions giving rise to high transfer rates. In order to achieve treatment goals and process stability, specific growth rates are very low (usually 0.2/day or smaller) and substrate concentrations small (typically only a few milligrams per liter). Thus, the normal conditions are much closer to those giving \( k_{DS} = 0.001 \) g₉⁻¹ l (g₀ g₀ day⁻¹) than those giving 2.5 g₉⁻¹ l (g₀ g₀ day⁻¹).

Table 1 summarizes \( k_{DS} \) values for a range of typical steady-state process conditions (5). The \( k_{DS} \) values probably are too small to sustain the plasmid in a significant fraction of the population (4,5) without prohibitively high costs for adding the donor cells.

| Time of donor harvesting, min | \( k_{DS} \) g₉⁻¹ l (g₀ g₀ day⁻¹) | Growth phase* |
|------------------------------|---------------------------------|---------------|
| 500                          | 0.03                            | end of early exponential |
| 520                          | 0.012                           | mid-exponential |
| 550                          | 0.004                           | mid-exponential |
| 570                          | 0.0006                          | late exponential |
| 580                          | 0.0002                          | late exponential |

*The exponential phase was from approximately 250 min until 600 min.

### Table 2. Typical values of specific growth rate, substrate concentration, and \( k_{DS} \)

| \( \mu \), day⁻¹ | \( S \), mg/l | \( k_{DS} \), g₉⁻¹ l (g₀ g₀ day⁻¹) |
|-----------------|-------------|-----------------------------------|
| 1.0             | 0.3         | 6.3 \times 10^{-4}                |
| 0.2             | 0.06        | 2.0 \times 10^{-4}                |
| 0.05            | 0.03        | 1.3 \times 10^{-4}                |

*Parameters for generating these results taken from Smets (5).
obtained by creating zones of high $S$ and $\mu$ within a treatment process that has low $\mu$ and $S$ values overall. This concept is shown schematically in Figure 2, which contrasts the normal format of the activated-sludge process with a modified process that creates a high-$S$ zone in reactor 2.

The modified process adds a small preliminary tank (denoted reactor 2) that receives the influent substrate and maintains a high substrate concentration. Table 3 shows that the substrate concentration in reactor 2 can be significantly augmented when reactor 2 is small enough, but the effluent substrate concentration remains low because of the long contact time in reactor 1.

The trends shown in Table 3 suggest that plasmid-transfer rates can be increased by creating a high-$S$ zone. However, the practical impacts have not been assessed theoretically or experimentally. Further work should address whether the faster plasmid-transfer rates in the small high-$S$ zone are significant enough to offset the low $S$ and low $\mu$ conditions in reactor 1.

**Summary**

Our research has demonstrated that plasmid transfer occurs at relatively fast rates among bacterial strains relevant to biological treatment. Most importantly, the rate coefficients for conjugation are not constants, but depend strongly on the donor cells' specific growth rate and substrate concentration, both of which affect the cells' energy availability. The relationship for how $k_{tr}$ increases for increasing $\mu$ and $S$ was quantified for the transfer of the TOL plasmid between two *Pseudomonas* strains (Equation 2) and the phenomena were qualitatively the same for transfer of RP4 from *Rhodobacter capsulatus* to *Pseudomonas* sp.

Although transfer rates can be high when $\mu$ or $S$ is large, normal biological processes have low values of $\mu$ and $S$. Therefore, we propose that zones of high $S$ or $\mu$ be created within processes whose overall $\mu$ and $S$ values remain low. One simple scheme—a modification to the activated-sludge process—was presented as an example.

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