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# Bioreactor Sensors Based on Nucleic Acid Hybridization Reactions

## Scientific Note

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**Index Entries:** Biosensor; hybridization kinetics; gene probes; affinity chromatography; DNA immobilization.

## INTRODUCTION

Mixed culture microbial ecosystems are important in engineered biological waste treatment systems. For many xenobiotic hazardous materials there are only a small number of organisms that possess the necessary genes that code for biodegradation enzymes for a particular organic compound. In bacteria, these genes are often found on extrachromosomal DNA called plasmids. Variations in ecological community structure influenced by environmental conditions or stresses can strongly influence the critical subpopulation and the resulting potential for expressing activity (*1–4*). Blackburn et al. (*1,5*) have found that small subpopulations of $10^7$ degraders/mL (less than 0.2% of the living cells

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present) have a dramatic influence on the biodegradation of polynuclear aromatics in relation to stripping. For effective environmental control of stripping in a biological treatment system it may be necessary to monitor and control these small subpopulations.

The current method used to determine subpopulation concentrations is known as colony hybridization (*6–10*). This method involves the detection of DNA sequences specific to a given gene(s) or trait. Although this is a standard method that has been used in research for more than a decade, it requires the culturing of viable cells on solid media. The process can take up to several weeks for the cells to form colonies, rendering it impractical for many biological waste treatment system control applications.

Specific biological molecules can be detected using a technique known as affinity chromatography (*11–13*). This type of chromatography utilizes highly specific biosorbents as the stationary phase and greatly facilitates the isolation of large molecular weight biological substances by exploiting the specific functional properties of the macromolecules (*13*).

A DNA molecule consists of two nucleic acid polymers held together in a double helix by hydrogen bonding. Under proper conditions the hydrogen bonds can be broken and two single strands of DNA can be produced. This single stranded DNA under permissive conditions can reassociate to form the original double helix macromolecule. When this process is performed with DNA from different sources, it is called hybridization and can be very specific in nature (*6,14–16*).

A biosensor for rapid (several hours) detection of important subpopulations can be developed by utilizing affinity chromatography and DNA hybridization kinetics. Single-stranded probe DNA can be immobilized on a support material to form an affinity chromatography adsorption bed. The performance of this adsorption bed can then be quantified by the method of Michaels (*17*). The input to the sensor would be the presence of a genotype, the output could be either visual, photometric, radiation, or an indirect signal.

## MATERIALS AND METHODS

### *DNA*

Plasmid DNA is isolated from *E. coli* carrying the pBR322 plasmid (4.3 kb) or *Pseudomonas putida* carrying the NAH7 plasmid (83 kb) using the lysozyme-SDS procedure of Anderson and McKay (*18*). The experimental procedure is outlined in flowsheet form in Fig. 1. Purification of the plasmid DNA is accomplished by ultracentrifugation at 40,000 rpm for 48 h in a cesium chloride-ethidium bromide gradient. The isolated closed circular DNA is linearized and reduced in size with a restrictive endonuclease *Eco RI* and then suspended in a 0.12*M* sodium phosphate solution (*19*).

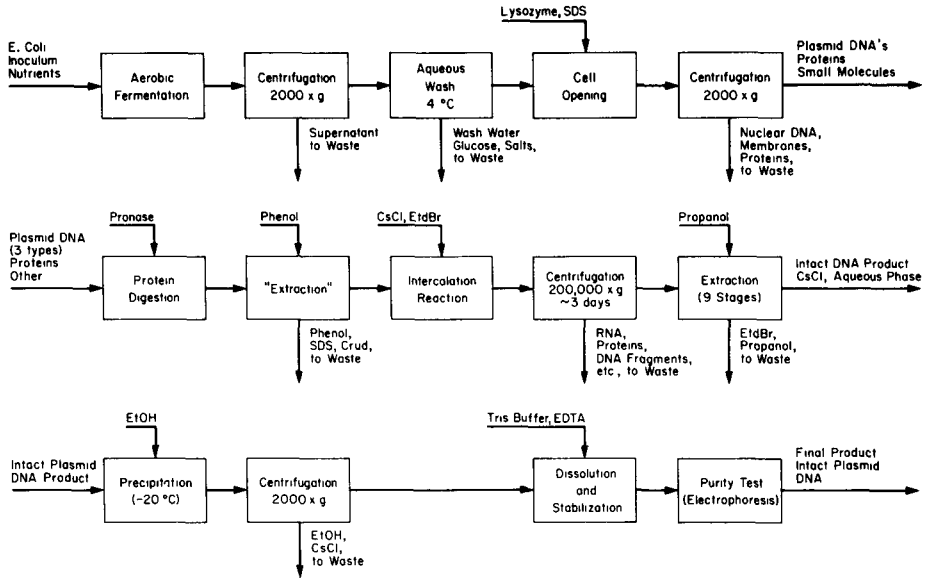


Fig. 1. Flowsheet for the production of DNA.

## *Support Material*

Three support materials are under consideration, cellulose, nitrocellulose, and nylon 6,6. Nylon-6,6 (Rainin Instruments, Woburn, MA) with a particle size distribution of 20–32 μm is the preferred material. This material is nonwetting, has a simple geometric shape (spherical), the DNA binding method is simple, and the quantity of bound DNA can be easily controlled. Cellulose (alpha-cellulose, 99.5% purity, Sigma Chemical, St. Louis, MO) is more difficult to bind and will present problems with expansion in the adsorption bed caused by water adsorption. It remains under consideration because the binding method may offer some advantages. Nitrocellulose has the same advantages and disadvantages as cellulose.

## *Binding Methods*

Covalent binding of DNA to cellulose can be accomplished using the method of Moss et al. (*20*), utilizing the bifunctional oxisane 1,4-butanediol diglycidyl ether to activate the cellulose and subsequently link DNA to the cellulose or by using the method of Noyes and Stark (*21*). DNA can be linked to nitrocellulose by the method described by Bresser and Gillespi (*22*). For nitrocellulose binding, the DNA is added to a 12.2*M* sodium iodide solution and incubated in a boiling water bath for 10 min to denature the DNA. Fine nitrocellulose powder is added to the solution, dried and baked for 2 h at 80°C. DNA can be thermally bound to nylon-6,6. Fine nylon-6,6 beads are added to a 0.12*M* sodium phosphate solution at pH 5.5, containing DNA. A denaturing solution composed of

1.5*M* sodium chloride and 0.5*M* sodium hydroxide is added and the mixture is allowed to stand for 5 min. After the solution is neutralized with 3 M sodium acetate to pH 6.5, the nylon 6,6 beads are air dried. The beads are then baked at 80°C for 1 h in a conventional air-circulating oven to complete the attachment.

### *ANALYTIC METHODS*

Both plasmid DNA hybridization kinetics in free solution and adsorption bed breakthrough curves are determined using a spectrophotometer (Beckman DV-7). Initially, DNA hybridization reactions occurring within the biosensor will be detected by radioactively labeling the sample DNA with $^{32}P$ by nick translation as specified by the supplier (Bethesda Research Laboratory, Gaithersburg, MD). The labeled DNA is purified by a Sephadex G-50 column chromatograph. The amount of radioactivity is correlated to the amount of bound DNA. Colorimetric detection of hybridization will employ the method of Haas and Fleming (*23*) or Foster et al. (*24*).

#### *Colony Hybridization*

Colony hybridization (*6,8,9*) is the normal procedure used for the detection of specific genotypes. The general procedure for colony hybridization is summarized by Sayler et al. (*8*). Bacterial colonies are transferred to a hybridization membrane (nylon or nitrocellulose), the cells are alkaline-lysed and the denatured DNA is thermally fixed in the single-stranded form to the hybridization membrane. Following a prehybridization treatment to eliminate nonspecific binding sites, the $^{32}P$ labeled (single-stranded) probe DNA is added and, under permissive conditions, allowed to hybridize to homologous target sequences. A low salt (10 m*M* NaCl) wash buffer is used to disrupt hybrids of greater than 5% bp mismatch (95% stringency), and the positive colonies are detected by autoradiography.

## CONCEPTUAL DESIGN OF A BIOSENSOR

Figure 2 presents a conceptual design of the biosensor. A known quantity of the specific DNA (either pBR332 or NAH7), in single stranded form, is immobilized on nylon 6,6 beads with a diameter of 20–32 μm. The remaining active sites on the nylon beads are saturated with salmon sperm DNA. The biosensor is then formed by uniformally packing the nylon 6,6 beads in a borosilicate glass column with a volume of about 1.0 mL and a column length of approximately 20.0 cm. The DNA is extracted from a sample taken from a biological system, and then put in single stranded form and injected into the biosensor.

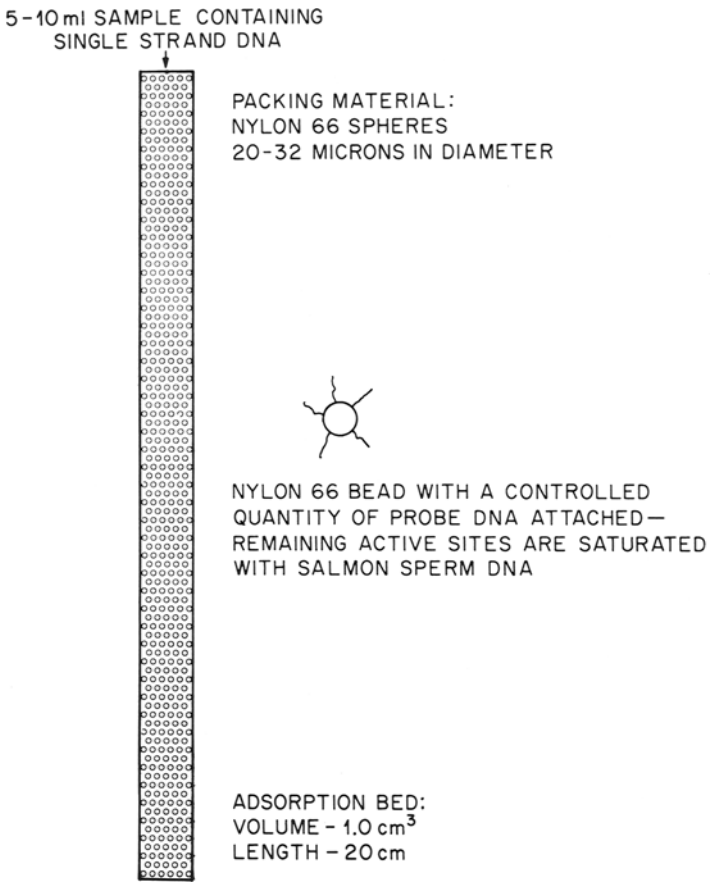


Fig. 2. Conceptual design of a biosensor.

The process can be analyzed as a simple adsorption problem with mass transfer limitations imposed by hybridization kinetics, steric hindrance, and flow rate. Figure 3 depicts the process as it evolves with time in the biosensor (assuming a very large sample has been injected). There will be three regions in the adsorption bed at any time, a region in which all of the probe DNA has been saturated, i.e., completely hybridized with the incoming sample DNA, a mass transfer zone in which the probe DNA is partially hybridized, and a region where all of the probe DNA is in the single stranded form.

Knowing the concentration of probe DNA on the packing material, the packing density, and the cross-sectional area of the bed, the amount of probe DNA/mm of bed length can be calculated. If a square wave front is obtained (zero mass transfer zone length), the saturation zone length could be measured using any of several detection methods. This length could then be converted into the concentration of cells in the sample of the particular subpopulation that was desired (*see* Fig. 4).

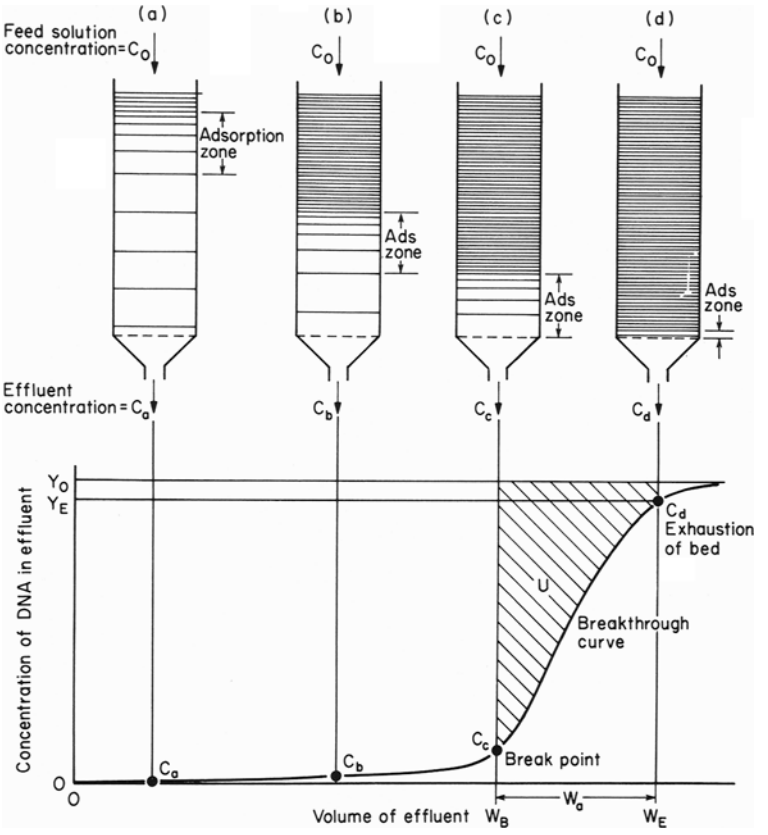


Fig. 3. Typical breakthrough curve.

It may not be possible to obtain a zero length mass transfer zone, however, the conditions under which the biosensor is operated, temperature, packing density, concentration of probe DNA on the packing material, sample size, and flow rate, can be controlled to minimize the length of the mass transfer zone. The mass transfer zone can then be calculated by using the mathematical model of Michaels (*17,25*), which assumes a constant pattern zone, i.e., the mass transfer zone does not change shape as it moves through the bed. Using this mathematical model, the mass transfer zone can be converted into an equivalent length of saturated bed, and used to correct the biosensor measurement (*see* Fig. 4).

## PLANNED EXPERIMENTAL WORK

In order to develop the biosensor and quantify its accuracy and response time, a series of experiments are planned. First, the free solution hybridization kinetics must be determined for the two genotypes being evaluated. Preliminary experiments using the NAH7 plasmid indicate

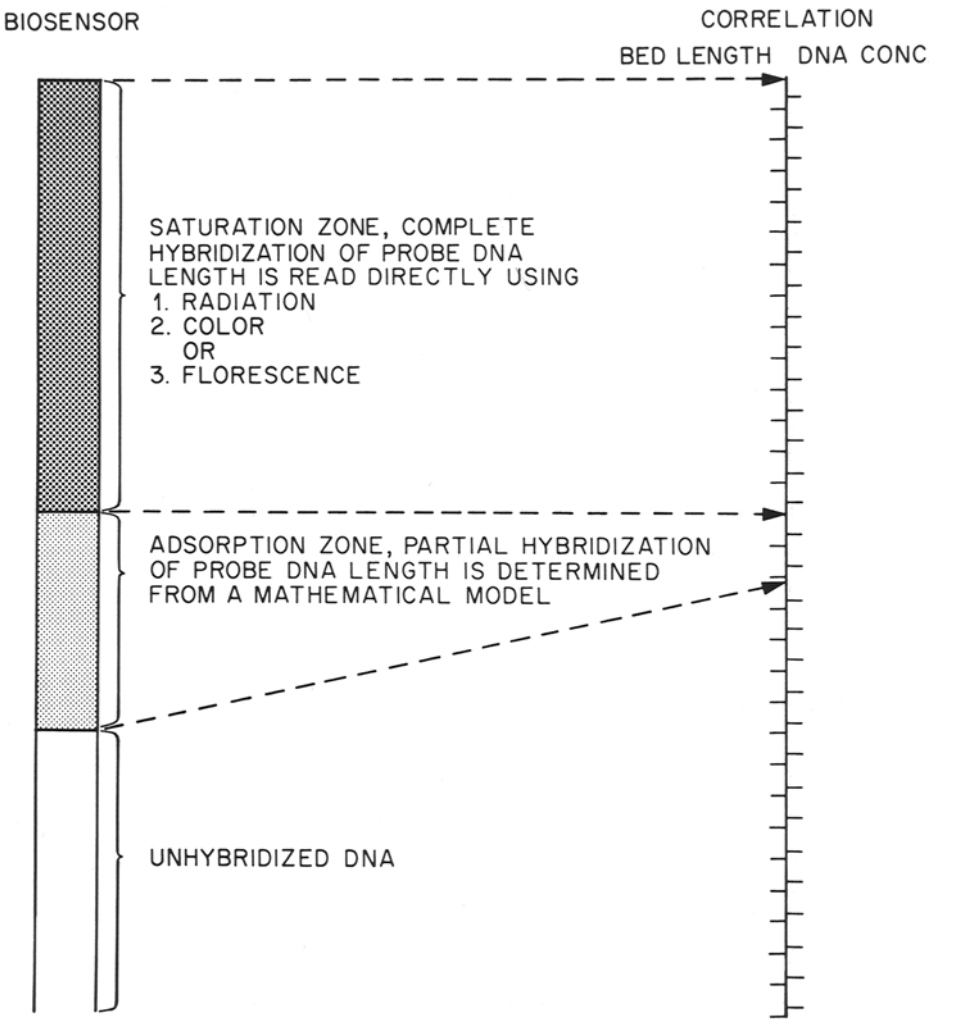


Fig. 4. Biosensor operation using a length of stain detection method.

the hybridization rate is zero at 95°C, and the maximum rate is achieved at approximately 60–65°C. This information is vital for sample preparation and to determine the optimum temperature for operation of the biosensor—the faster the hybridization rate within the sensor, the smaller the mass transfer zone correction.

The effect of steric hindrance on hybridization kinetics must also be evaluated. The probe DNA will be attached to a solid support and will be reacted with sample DNA that is in solution, it is anticipated that this situation will lead to a reduced rate of hybridization as compared with solution hybridization. Experiments must also be conducted on the dynamics of the adsorption bed to determine the breakthrough profile and obtain the operating conditions leading to minimal length of the mass transfer zone. Figure 5 gives a schematic diagram of the experimental ap-

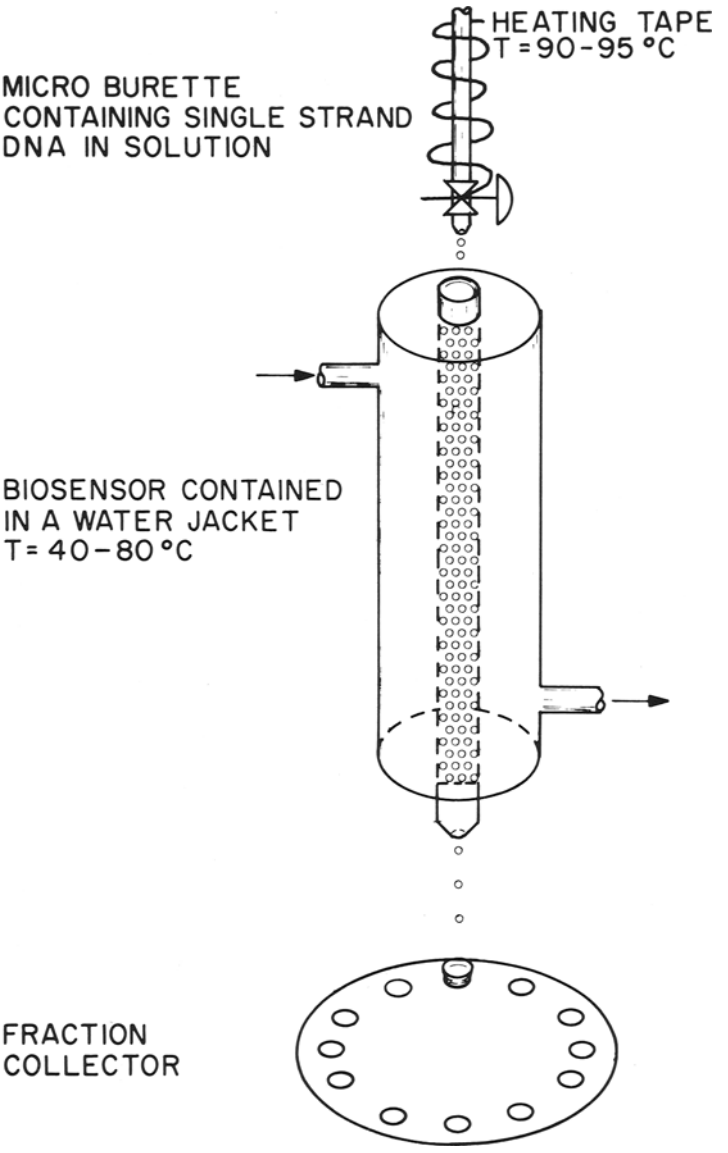


Fig. 5. Experimental measurement of a breakthrough curve.

paratus that will be used to measure the breakthrough profiles in the adsorption bed. The sample DNA will be kept in a microburet made of siliconized borosilicate glass and kept at 90–95°C to prevent the sample DNA from reassociating. A constant flow from the microburet will be maintained to the biosensor, which is kept at about 60–65°C, the temperature at which the hybridization reaction will be at a maximum (based on preliminary experiments). The problem of sample DNA hybridizing in solution within the adsorption bed should be minimal because of the

much higher concentration of probe DNA attached to the nylon-6,6 beads.

Once the optimal flow conditions are determined, the length of the breakthrough curve will be modeled, this model will then be used to make the necessary correction indicated in Fig. 4 to correlate the "length of stain" on the biosensor to a concentration of DNA in the sample. The length of stain (saturated probe DNA) can be determined by radiation, colorimetric, or by using a laser with a fluorescence tag.

The final experiments, to evaluate the method's accuracy, will consist of taking actual reactor samples and analyzing them by both the standard colony hybridization method and with the new biosensor.

## CONCLUSIONS

A new, more rapid method is needed for the determination of critical subpopulations in mixed culture biological systems. Of particular interest are the monitoring and control of systems that are concerned with degrading toxic and hazardous waste materials. The proposed biosensor should lead to specific genotype analyses in mixed culture samples within several hours, as opposed to the days or weeks presently required for colony hybridization.

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