RAPID BIOELECTROCHEMICAL METHODS FOR THE DETECTION OF LIVING MICROORGANISMS

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Abstract. Methods suitable for the estimation of microbial biomass are reviewed. Rapid bioelectrochemical techniques for detecting viable bacteria and yeasts were investigated using a fuel cell and poised potential amperometric detectors. *Escherichia coli* in a fuel cell was detected over the range of $10^2 - 10^6$ cells ml$^{-1}$, with an analysis time of approximately 30 min. The three electrode amperometric detector produced a linear calibration curve for *Escherichia coli* in the range $10^6 - 10^7$ cells ml$^{-1}$. The correlation coefficient was 0.999 ($P < 0.001$) and the analysis time < 15 min. The two electrode amperometric system gave linear calibration for $5 	imes 10^6 - 9 	imes 10^7$ *E. coli* cells ml$^{-1}$. The correlation coefficient was 0.999 ($P < 0.001$) and the analysis time < 15 min. Batch growth of several bacteria and a yeast was successfully monitored bioelectrochemically. It was concluded that the two electrode poised potential amperometric method provided rapid, convenient, inexpensive detection of a wide range of microorganisms.

Keywords. Biomass detector; bioelectrochemical detection; microbial fuel cell; amperometric method; biosensor; bioactivity monitor.

INTRODUCTION

In order to control biotechnological processes and to detect microbial contamination efficient detectors of viable cells are required. Generally, the microbial concentrations in contaminated samples are much less than those found in commercial fermenters i.e. less than $10^6$ cells ml$^{-1}$. At present there is no available satisfactory biomass probe for either concentration range. However, current research has yielded devices that go a considerable way towards achieving the goal of an effective biomass detector (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1 Properties of an Ideal Biomass Probe</th>
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</thead>
<tbody>
<tr>
<td>1) Accuracy</td>
</tr>
<tr>
<td>2) Reproducibility of response</td>
</tr>
<tr>
<td>3) Rapid response</td>
</tr>
<tr>
<td>4) Sensitivity (resolution of $10^7$ viable</td>
</tr>
<tr>
<td>cells ml$^{-1}$)</td>
</tr>
<tr>
<td>5) Large linear range</td>
</tr>
<tr>
<td>6) On-line capability</td>
</tr>
<tr>
<td>7) Autoclavability</td>
</tr>
<tr>
<td>8) Low cost</td>
</tr>
<tr>
<td>9) Ease of operation</td>
</tr>
<tr>
<td>10) Durability</td>
</tr>
<tr>
<td>11) Biological inertness</td>
</tr>
<tr>
<td>12) Harmless to the biomass</td>
</tr>
<tr>
<td>13) No added reagents</td>
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<tr>
<td>14) No sample pre-treatment</td>
</tr>
<tr>
<td>15) Operation in clear or opaque samples</td>
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<td>16) Differentiation between viable and non-viable biomass</td>
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</table>

A recent review of biomass detection (Harris and Kell, 1985) discusses most of the current methods. Table 2 lists some of the analytical parameters of the non-bioelectrochemical biomass detection methods. Acoustic resonance densitometry (ARD) and the piezoelectric membrane methods are described in detail because of their promising potential. Tables 3 and 4 list selected analytical parameters of the bioelectrochemical biomass detection methods. Following these tables some applications of the techniques are described. The following definition of a bioelectrochemical method was used for technique classification: a method in which the analytical signal is generated by the biomass producing or consuming electrical charge, this change being detected at electrodes.

NON-BIOELECTROCHEMICAL METHODS OF BIOMASS DETECTION

See Table 2.

Piezoelectric Membrane

A novel method for total biomass detection was reported by Inhibori, Harube and Suzuki (1981). In this procedure an ultrasound signal was generated and directed through the microbial sample onto a piezoelectric membrane. This membrane provided a measure of the intensity of the ultrasonic signal. Both yeast and bacterial cells attenuated this signal and could therefore be detected at concentrations above $10^6$ cells ml$^{-1}$.

The upper limit of detection was $10^6$ *Saccharomyces cerevisiae* cells ml$^{-1}$ and $10^5$ cells ml$^{-1}$ for *Mailiotus antarcticus* or a *Klebsiella* species. In addition to providing real time analysis the detection cell could be autoclaved. However, after autoclaving several times, the epoxy resin attaching the piezoelectric membrane in position cracked necessitating replacement.

A dilution step would be required for monitoring industrial bacterial fermentations and some yeast fermentations. In addition since there is no discrimination between biomass and non-biomass particles serious errors would occur if the fermentation broth contained a suspension of substrate material. However, this method promises inexpensive real-time analysis of total fermenter...
The specific gravity can be calculated. The specific weight per ml of suspended solids is then obtained by subtracting the specific gravity of the culture medium (Clarke and co-workers, 1985; Krakty and Leopold, 1968). This device is extremely robust and autoclavable. Analysis is rapid but not in real time, as the specific gravity of the growth medium containing no particulate matter has to be measured. The linear range of this method $10^7 - 10^9$ cells ml$^{-1}$ is sufficient to monitor fermentations and downstream processing (Blake-Coloeman and colleagues, 1984; Blake-Coloeman and Clarke, 1988b, 1984c. In addition, the price of the ARD instrumentation could be quite low.

Table 2. Analytical Parameters of Non-bioelectrochemical Biomass Detection Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection Limit (cells ml$^{-1}$)</th>
<th>Analysis Time (h)</th>
<th>Sample Pre-Treatment</th>
<th>Source of Analytical Signal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioluminescence</td>
<td>$10^6$</td>
<td>0.2</td>
<td>ATP Extraction</td>
<td>Viable Cells</td>
<td>Chappelle (1978)</td>
</tr>
<tr>
<td>Radiometry</td>
<td>1</td>
<td>8-16</td>
<td>Incubation</td>
<td>Viable Cells</td>
<td>Waters (1972)</td>
</tr>
<tr>
<td>Direct Microscopic Counts</td>
<td>$5 \times 10^5$</td>
<td>1</td>
<td>Staining</td>
<td>Viable Cells</td>
<td>Pettipher (1951)</td>
</tr>
<tr>
<td>Epifluorescence</td>
<td>$5 \times 10^5$</td>
<td>0.5</td>
<td>Staining</td>
<td>Viable Cells</td>
<td>Pettipher (1950)</td>
</tr>
<tr>
<td>Viable Counts</td>
<td>10</td>
<td>24-72</td>
<td>Incubation</td>
<td>Viable Cells</td>
<td>Postgate (1969)</td>
</tr>
<tr>
<td>Dry Weights</td>
<td>0.02 mg ml$^{-1}$</td>
<td>8</td>
<td>Drying</td>
<td>Suspended solids</td>
<td>Neufeld (1962)</td>
</tr>
<tr>
<td>Nephelometry</td>
<td>$2 \times 10^7$</td>
<td>Real time</td>
<td>None</td>
<td>Suspended solids</td>
<td>Mallette (1969)</td>
</tr>
<tr>
<td>Turbidimetry</td>
<td>$2 \times 10^6$</td>
<td>Real time</td>
<td>None</td>
<td>Suspended solids</td>
<td>Mallette (1969)</td>
</tr>
<tr>
<td>Fluorimetry</td>
<td>$0.4 \mu g ml^{-1}$</td>
<td>Real time</td>
<td>None</td>
<td>Fluorophores</td>
<td>Zabriakie (1972)</td>
</tr>
<tr>
<td>Microcolormetry</td>
<td>$10^6$</td>
<td>2</td>
<td>Incubation</td>
<td>Viable Cells</td>
<td>Reezer (1972)</td>
</tr>
<tr>
<td>Filtration</td>
<td>-</td>
<td>0.5</td>
<td>Filtration</td>
<td>Solid Particles</td>
<td>Westas (1983)</td>
</tr>
<tr>
<td>Electrical Counting</td>
<td>-</td>
<td>0.1</td>
<td>None</td>
<td>Suspended solids</td>
<td>Dow (1979)</td>
</tr>
<tr>
<td>Flexoelectric Membrane</td>
<td>$10^6$</td>
<td>Real time</td>
<td>None</td>
<td>Suspended solids</td>
<td>Ishimori (1981)</td>
</tr>
<tr>
<td>Acoustic Resonance</td>
<td>$1.0 \times 10^8$</td>
<td>Real time</td>
<td>None</td>
<td>Suspended solids</td>
<td>Clarke (1985)</td>
</tr>
</tbody>
</table>

Table 3. Examples of Impedimetric Detection of Microbial Contamination

<table>
<thead>
<tr>
<th>Initial Concentration (cells ml$^{-1}$)</th>
<th>Sample</th>
<th>Detection time (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^6$</td>
<td>cerebrospinal fluid</td>
<td>5.8</td>
<td>Kahn and colleagues (1976)</td>
</tr>
<tr>
<td>$10^5$</td>
<td>raw milk</td>
<td>8.9</td>
<td>Gran and Luetscke (1982)</td>
</tr>
<tr>
<td>$10^5$</td>
<td>frozen vegetables</td>
<td>5.0</td>
<td>Hardy and colleagues (1977)</td>
</tr>
<tr>
<td>$10^5$</td>
<td>urine</td>
<td>2.5</td>
<td>Zafari and Martin (1977)</td>
</tr>
<tr>
<td>$10^6$</td>
<td>blood</td>
<td>6.8</td>
<td>Hadley (1976)</td>
</tr>
<tr>
<td>$10^6$</td>
<td>blood</td>
<td>8.5</td>
<td>Kahn and colleagues (1976)</td>
</tr>
<tr>
<td>$10^6$ to $10^7$</td>
<td>sewage effluent</td>
<td>1.5</td>
<td>Munos and Silverman (1979)</td>
</tr>
</tbody>
</table>

All experiments carried out using a Bactometer 32 instrument.

Table 4. Analytical Parameters of some Bioelectrochemical Biomass Detection Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Detection range (cells ml$^{-1}$)</th>
<th>Analysis Time (h)</th>
<th>Sample Pre-Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potentiometric</td>
<td>$1 \times 10^7$ to $1 \times 10^6$</td>
<td>7.5 to 1.0</td>
<td>Incubation</td>
<td>Wilkins (1978)</td>
</tr>
<tr>
<td>Fuel Cell</td>
<td>$10^7$ to $10^9$</td>
<td>0.2</td>
<td>None</td>
<td>Matsunaga (1979)</td>
</tr>
<tr>
<td>Fuel Cell</td>
<td>$6 \times 10^6$ to $4 \times 10^9$</td>
<td>0.5</td>
<td>None</td>
<td>Turner (1982)</td>
</tr>
<tr>
<td>Amperometric</td>
<td>$3 \times 10^6$ to $2\times 10^6$</td>
<td>0.3</td>
<td>Preconcentration</td>
<td>Maitshigawa (1982)</td>
</tr>
<tr>
<td>Amperometric</td>
<td>$7 \times 10^6$ to $4\times 10^6$</td>
<td>0.2</td>
<td>Preconcentration</td>
<td>Matsunaga (1981)</td>
</tr>
<tr>
<td>Amperometric</td>
<td>$6 \times 10^6$ to $6\times 10^5$</td>
<td>0.2</td>
<td>None</td>
<td>Ramsay (1983)</td>
</tr>
<tr>
<td>Amperometric</td>
<td>$3 \times 10^5$ to $2\times 10^5$</td>
<td>0.1</td>
<td>None</td>
<td>Matsunaga (1984)</td>
</tr>
</tbody>
</table>

All experiments carried out using a Bactometer 32 instrument.
Impedimetric Methods of Biomass Detection

This technique uses the change in electrical conductivity that results from microbial metabolism. To achieve reproducible results the composition of the growth medium and its temperature have to be carefully controlled. Instrumentation costs are high but many samples can be assayed simultaneously. Typically the analyte sample is placed in sterile microelectrodes for several hours until the impedance changes by a pre-set amount. The most successful applications have occurred where large numbers of samples containing quite low levels of microbial contamination need to be analysed. Hence the analysis of foodstuffs and clinical samples is often carried out impedimetrically (see Table 3).

Microbial contamination in frozen vegetables was investigated by Hardy and co-workers (1977). Extracts containing 10^9 viable cells per ml were analysed in 5h. This was far more rapid than the conventional viable count method which took several days and there was good agreement. Martinez and colleagues (1980) established that pasteurised milk with a detection time of 13.5h or more had a shelf life of at least 12 days. Samples with a shorter detection time supplied within 11 days. This impedimetric method was considerably faster than the viable count assay which took 7 days.

Several studies have been carried out on impedimetric detection of microbial contamination in urine (Cady and colleagues, 1974; Specter and co-workers, 1976; Safari and Martin, 1977). Samples containing 10^7 cells ml^-1 were assayed in approximately 3h. This compared favourably with the 4th conventionally required and there was good correlation with the standard method. Hadly (1976) reported that positive blood samples, i.e. those containing 10^6 cells ml^-1 or more, were detected in 6-8h and there was good agreement with the plate count. Kahn and colleagues (1976) assayed samples of cerebrospinal fluid to detect bacterial meningitis. They found that the threshold level of this condition, 10^3 cells ml^-1 was detected in 5.8h. This was far more rapid than the plate count method.

Potentiometric Methods of Biomass Detection

Table 4 summarises potentiometric and amperometric methods of biomass detection. The basis of potentiometric analysis is the measurement of the potential developed at an electrode with respect to a reference electrode. No external biasing potential is applied. Wilkins (Wilkins, 1974; Wilkins and colleagues, 1976) measured the potential developed at a Pt electrode with respect to a saturated calomel electrode (SCE) by Enterobacter. A linear relationship was obtained between the initial viable microbial concentration and the time required to reach a potential of 30mV. Escherichia coli, with an initial concentration of 1 x 10^7 cells ml^-1 was detected in 1h. One cell ml^-1 could be detected in 7.5h. In later work, Wilkins (1976) replaced the SCE with a Pt electrode. Results for E. coli were similar to those obtained previously. The method was also shown to be applicable to a wide range of bacteria. At a concentration of 1 x 10^6 cells ml^-1 the range of detection times for the microorganisms used was 1.5-3.4h; at 1 cell ml^-1, the range was 8.4-16.9h.

The potentiometric method is simple to operate, has low capital cost and detects a wide range of viable bacteria with a large dynamic range. However, the lengthy analysis time is a disadvantage.

Fuel Cell

Matsunaga, Karube and Suzuki (1979) reported the use of a non-mediated fuel cell to detect microbial cells (mediator is a small redox compound used to transfer electrons from biological material to an electrode or vice-versa). Two fuel cells were used with only the anode of each in contact with the analyte sample. To provide a background signal the anode of one of the fuel cells was covered with dialysis membrane, hence excluding the microorganisms but allowing the oxidation of any interfering chemical species. Thus the analytic signal was the difference between the electrical currents from the two fuel cells. Although not a sensitive method the batch growth of Saccharomyces cerevisiae was successfully followed with the biomass probe as-fabry, Lactobacillus fermentum gave a linear calibration curve in the range 9 x 10^6 - 3 x 10^10 cells ml^-1. Only viable cells produced a response.

Turner and co-workers (1982) increased the analytical sensitivity of the fuel cell method by using the mediator phenazine ethosulphate (PES) to detect E. coli. Analysis time was approximately 30 min and the limit of detection 4 x 10^6 cells ml^-1.

Nishikawa and co-workers (1980) used filter pre-concentration as well as a mediator (4,6-dichlorophenol indophenol) to increase analytical sensitivity. Linear calibration curves over the range 10^9 - 10^10 cells ml^-1 were obtained from the four bacterial species tested. The analytical sensitivity of the method differed between species. However, when the viable counts in two water samples were determined, using E. coli calibration curve, good correlation was obtained. Analysis time was about 20 min and only viable cells gave a response.

Amperometric Methods

These methods are based on measuring the current produced on reaction of the analyte species at a working electrode. They can be classified into two or three electrode systems. Three electrode systems consist of a working electrode poised at a pre-set potential, a reference electrode and a counter electrode which completes the electrical circuit. In two electrode systems the counter and reference electrodes are combined. This system is simpler to operate but the potential at the working electrode is not maintained as accurately as in the three electrode system (Sawyer, 1974; Turner, 1985).

Matsunaga and colleagues (1980) reported the detection of Bacillus subtilis in a non-mediated three electrode system. Two sets of electrodes were used, one with the working electrode covered in dialysis tubing to provide a background signal. Since no mediator was used the detection limit was high approximately 3 x 10^9 cells ml^-1. However, the absence of a soluble mediator and the stability of the system to autoclaving, allowed the successful in-situ monitoring of batch growth. Response time was less than 3 min and only viable cells responded. Ramsay and co-workers (1983) reported a three electrode method in which a mediator (PES) was used. A linear calibration curve over the range 6 x 10^9 - 6 x 10^10 cells ml^-1 was obtained for Escherichia coli with an analysis time less than 15 min. Only viable cells gave a response.

Recently Matsunaga and Hamada (1984) have reported a cyclic voltammetric method for the detection of yeast and bacterial species. Linear calibration was obtained over the range 3x10^6 - 2x10^9 cells of a Saccharomyces cerevisiae ml^-1. It was shown that
modification of a graphite electrode, with \(4,4\)-bipyridine, enhanced the current from Escherichia
col on the electrode. The electrode and the
to detection limit was decreased
by 25%. If the \(4,4\)-bipyridine
layer immobilized on the electrode and the
electrodes were stable to sterilisation this probe
could theoretically be used in a fermentor for
online analysis.

Matsumaga and colleagues (1981) reported a two
electrode biomass sensor based on a Clark oxygen
electrode with a filter pre-concentration step to
increase analytical sensitivity. The analysis time
was approximately 10 min. An immobilized bacteria
yeasts were detected at low concentrations. The
limit of detection for the yeasts was approximately
10² cells ml⁻¹ and for the bacteria 10⁶ cells ml⁻¹.
Both the calibration curves were obtained over a one
decade concentration range.

Both the fuel cell and the amperometric methods
have in common rapid response, applicability to
both bacteria and yeasts, low cost, medium sensi-
tivity and ease of operation. For these reasons it is
likely that these methods will be further
developed.

Possible Developments Leading to Practical Biomass
Detectors

All the biomass estimation methods reviewed have
gone disadvantages. However, acoustic resonance
densitometry, the piezoelectric membrane method and
the amperometric methods merit further attention.
In particular in AER the successful develop-
ment of a real time measure of the specific gravity
of the cell free culture is necessary for real time
continuous monitoring of fermenter biomass. The
piezoelectric membrane method requires the develop-
ment of a membrane attachment system that is stable
at repeated autoclaving. The amperometric methods
need to be made more sensitive and if possible
based on an immobilized mediator. An immobilized
mediator could permit on-line analysis of fermenter
biomass.

THE APPLICATION OF AMPEROMETRIC
SYSTEMS TO THE DETECTION OF MICRO-
ORGANISMS

Our early work showed that the current generated
by bacteria in a phenazine ethosulphate mediated
fuel cell was proportional to the bacterial con-
centration (Turner and co-workers, 1980). The
amplitude was oxygenated by bubbling with nitrogen.
This prevented electron loss to oxygen and mediator
to autoclaving. The mediator increased the rate of
cell transfer from the bacterial cells to the
anode. From the anode, the electrons flowed
through a load resistor to the cathode where they
reacted with oxygen and protons to form water. A
potentiometric chart recorder measured the potential
difference across the resistor thus providing a
measure of the current. The mediator concentra-
tion was optimised at 0.2nM and the temperature of the
electrolyte maintained at 30°C. Using reticulated
carbon electrodes Escherichia coli was detected
at concentrations over the range 6 × 10⁷ to 4 × 10⁹
bacteria ml⁻¹. The analysis time was approximately
30 min. When Pt gauze was used in place of reti-
culated carbon the detection limit was decreased
to 4 × 10⁷ cells ml⁻¹. These results were encour-
gaging but the fuel cell was tedious to assemble and
operate. Due to the short working life of the
silver chloride on a silver foil support. A
mediator layer was deposited showing that there w as little o.ata
to repeated autoclaving. The amperometric methods
need to be made more sensitive and if possible
based on an immobilized mediator. An immobilized
mediator could permit on-line analysis of fermenter
biomass.

This required no ion-exchange membrane as there
was only one reaction compartment. Only one
solution had to be prepared and this was sparged
with a single gas, nitrogen. The mediator was
phenazine ethosulphate at an optimum concentration
of 5.8mM. The analysis time (measuring the
initial slope of the analyte peak) was less than
15 min. Linear calibration for Escherichia coli
was obtained from the detection limit 6 × 10⁶
cells ml⁻¹ to 6 × 10⁸ cells ml⁻¹ (see Fig.2). A
correlation coefficient of 0.996 (n = 6, P < 0.001)
was obtained showing that there was little data
scatter.

Development of a Two Electrode Pulsed Potential
Amperometric Biomass Detector

The three electrode system was simplified and made
easier to use by replacing the counter and refer-
ence electrodes with a single electrode. This
consisted of an electrochemically deposited layer
of silver chloride on a silver foil support. A
robust probe was formed by attaching the Pt work-
ing electrode and the reference electrode to
opposite sides of a plastic support (see Fig.3). To
take a sample through a multichannel
system was built, based on the BBC Microcomputer.

Typical experimental conditions were:
Potassium ferricyanide (0.5ml, 250mM)
Ag/AgCl reference electrode (0.5ml, 1.5cm)
Electrolyte de-oxygenated with oxygen free nitrogen
for five minutes and then sparged continuously.
Electrolyte temperature 25°C.
Working electrode poised at 100mV versus Ag/AgCl
reference electrode.

Sodium phosphate buffer (0.55ml, 50mM, pH 7.0)
Potassium chloride (1.000l, 1000mM)
Glucose (0.15ml, 25mM)
Potassium ferricyanide (0.15ml, 250mM)
Microbial cells (0.15ml)

E.coli cells were grown in continuous culture on
defined medium under glucose limitation. The
microbial cells were added after the mediator
oxidation peak had plateaued. The analysis time
when the maximum slope of the analyte peak was
measured was less than 15 min.

Potassium ferricyanide was normally used as the
mediator in place of PBK because it was more
chemically stable. The potassium chloride was
required to stabilise the potential obtained by
the reference electrode.

Two electrode amperometric studies. The relation-
ship between respiration rate and BEC response of
Escherichia coli cells was investigated. Firstly
the respiration rate over a range of substrate
(glucose) concentrations was measured in a Clark
oxygen electrode. The rate increased non-linearly
from zero to zero substrate concentration to a
plateau at 30mM glucose (130nmol, 0.1mg dry
wt⁻¹). In the BEC a similar response was
obtained (see Fig.4). At zero glucose concen-
tration a small peak was obtained (maximum slope
0.034mV s⁻¹). The maximum slope then increased
non-linearly to a plateau (1.64kV s⁻¹) at
30mM. Probably the small peak obtained at zero
concentration was caused by the bacteria
using endogenous reserves. These experiments
demonstrated that for maximum BEC response E.coli
cells needed to be respiring at their max-
imum rate. Having established the saturating glucose
concentration for the BEC response of a high
concentration of E.coli cells (1 × 10⁸ cells ml⁻¹)
the standard glucose concentration was fixed at
10mM.

BEC : Bioelectrochemical Cell.
Calibration of E.coli BEC response. The calibration standards were prepared by diluting fresh fermenter samples. Three replicate analyses were carried out for each calibration standard. A linear calibration curve was obtained from the detection limit 5 x 10⁻⁵ to 9 x 10⁻⁹ cells ml⁻¹ (see Fig. 5). The upper limit of detection was determined by the current density that the reference could conduct before the reference potential began to drift. This occurred at approximately 100 nA. There was little data scatter (correlation coefficient of 0.999, n=8, P<0.001).

The equipment and running costs of all three bioelectrochemical methods were low, response was rapid, and analytical sensitivity was quite good. Also the lower detection limits were very similar for all three techniques. In practice the method chosen would be the two electrode amperometric system as it is the simplest to use. In addition, the microbial concentration was sufficient to generate enough electrical current to destabilise this system then the three electrode system could be used.

Detection of Bacteria Naturally Occurring in Milk by the Two Electrode Amperometric Method

There is considerable commercial interest in rapidly detecting and quantifying microorganisms contaminating milk. One of the bacterial species that routinely occurs in pasteurised milk is E.coli. To date there is little data scatter (correlation coefficient of 0.999, n=8, P<0.001). The organism used was E.coli 0157:H7. The bacterial isolate in nutrient broth was followed (30°C, 150 rpm on orbital shaker). During growth the optical density, BEC response, viable count and respiration rate were measured. Fig.6 shows the variation of maximum slope and the viable count during the batch growth. There is close correlation between the maximum slope and the viable count. Similarly there was good agreement between the maximum peak height and viable count.

Range of Microorganisms Detected in BEC

In order to assess the applicability of this technique to microorganisms other than E.coli a range of organisms has been tested in the BEC. These organisms were chosen because they occur in one or more of the following matrices: lubricating oil, milk or cooling tower water. From the many microbial contaminants found in lubricating oil, Paracoccus, Alcaligenes, Bacillus, Pseudomonas aeruginosa, Alkaligenes faecalis, Nocardia NCIB 8863 and Achromobacter album were chosen as they occur in one or more of the following matrices: lubricating oil, milk or cooling tower water. From the many microbial contaminants found in lubricating oil, Paracoccus, Alcaligenes, Bacillus, Pseudomonas aeruginosa, Alkaligenes faecalis, Nocardia NCIB 8863 and Achromobacter album were chosen as they occur in one or more of the following matrices: lubricating oil, milk or cooling tower water.

CONCLUSION

The results in this paper have shown that both the polied potential and fuel cell bioelectrochemical techniques provide simple, rapid, inexpensive and moderately sensitive means of detecting viable biomass. Such techniques could be cost-effective in detecting microbial contamination in samples such as foodstuffs, industrial waste waters and clinical samples. It could also be useful in monitoring the viable biomass concentrations in fermenters.

REFERENCES


Fig. 1. Three electrode poised potential amperometric system
1. Potentiostat; 2. chart recorder; 3. load resistance; 4. air inlet; 5. water bath; 6. counter electrode; 7. magnetic stirring bar; 8. working electrode: 9. glass capillary; and 10. saturated-calomel reference electrode.


Fig. 2. Variation of maximum slope of the analyte peak with concentration of Escherichia coli in the three electrode amperometric cell
Fig. 3. Two electrode poised potential amperometric biomass detector.

Glucose concentration in bioelectrochemical cell (µM)

Fig. 4. Maximum slope versus glucose concentration for *Escherichia coli* cells.

Experimental conditions as in text.
*Escherichia coli* concentration $4 \times 10^7$ cells ml$^{-1}$

Fig. 5. Maximum slope versus *Escherichia coli* bioelectrochemical cell concentration: two electrode amperometric system.

Experimental conditions as in text.

Fig. 6. Log viable count and log maximum slope versus growth of *Milk Isolate No. 3* in batch culture. Experimental conditions as in text.

- : log maximum slope; : log viable count