The Effect of Containment Measures
Applied to the Exhaust Gas of a Fermenter.

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Abstract.

Many industrial fermentations have the potential to release micro-organisms either accidentally or incidentally during the course of their operation. The agitation and aeration of microbial broths can produce foams and aerosols that must be contained to meet biosafety regulations. The Turbosep foam separator, which is widely used, is a static device designed to separate the foams emerging from highly aerated fermentation processes. The foam is recycled to the fermenter, allowing the exhaust gases to pass out to the local environment often through a High Efficiency Particulate Air (HEPA) filter.

The influence of the operating parameters of fermentation on the release of microorganisms into the exhaust gas was investigated. The release of process cells was monitored using a cyclone-quantitative polymerase chain reaction methodology. It was found that microbial release rates increased with increasing agitation and aeration rates. However, micro-organisms were released into the exhaust gas in only very low numbers. At an agitation rate of 500 rpm and an aeration rate of 1.5 L min\(^{-1}\), approximately 1.5 \(\times 10^5\) \textit{E.coli} cells were released per minute from a 2L fermenter. Based on this result it can be estimated that 5 \(\times 10^7\) cells will be released over the course of a 5.5 hour fermentation. This is equivalent to the loss of 3.3 µL of broth at harvest.

The installation of the Turbosep onto a fermenter was found to reduce the microbial burden of the exhaust gas by approximately 6 orders of magnitude whilst recirculating foam. In the absence of foam the microbial burden of the exhaust gas was reduced by approximately 4 orders of magnitude. Exhaust gas containment can, in light of the data presented here, utilise alternative methods of prefiltering to minimise the challenge to the HEPA filter. However as this challenge is minimal, containment systems should be designed to reduce the accidental release rather than the incidental.

A novel control strategy for the addition of anti-foam into a fermenter fitted with a Turbosep has been developed. Differential pressure measurements across the Turbosep were linked to the provision and regulation of antifoam addition. Using the Turbosep in conjunction with the differential pressure control strategy allowed the addition of antifoam to be totally automated and dependent on the requirements of the fermentation.
at any time. The Turbosep was determined to reduce the antifoam requirements of a 6000 L *E.coli* fermentation by approximately 66 %, leading to a 16 % increase in productivity without changing any of the process operating parameters.

Computational fluid dynamics (CFD) has been used to model the performance of the Turbosep in terms of particle collection efficiency. Performance curves were produced with approximately the same shape and $d_{50}$ as those obtained by experiment. Furthermore the predicted pressure drops were in excellent agreement with the measured data. The CFD model was able to predict the salient features of the Turbosep flow field, thus providing a better understanding of the fluid dynamics of the device. The CFD model also provided a reliable and relatively inexpensive method of redesigning the Turbosep to increase performance. The predicted particle collection efficiency of the new design was at least 30 % higher than the original design.
Microbial Risk Assessment Statement.

A risk assessment for the micro-organisms used during the course of this study was completed according to the regulations of the University of London. A copy of the risk assessment can be obtained from the pilot plant manager at the Advanced Centre for Biological Engineering, University College London.

The micro-organisms, *E.coli* RV308 pHKY531 and *S. cerevisiae* S150Δhsp82 and all experiments were deliberately chosen so that any microbial release would be no greater than Good Manufacturing Practice.
for Pen
Acknowledgements.

I would like to thank Professor Mike Turner for his helpful supervision of this project. In particular, I am indebted to him for his careful reading of this manuscript and thoughtful suggestions and improvements. I would also like to thank Dr Eli Keshvaraz-Moore for her advice in the early stages of this work and for her willingness to discuss the progress at all times. I would also like to thank David Ridaelgh at Domnick Hunter for his technical assistance.

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For their moral support, I would also like to thank my friends and colleagues the ACBE, with whom I've shared the joys, despair and beers that are an integral part of any PhD.
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<tr>
<td>ACBE</td>
<td>Advanced Centre for Biochemical Engineering</td>
</tr>
<tr>
<td>ACDP</td>
<td>Advisory Committee on Dangerous Pathogens</td>
</tr>
<tr>
<td>ACGM</td>
<td>Advisory Committee on Genetic Manipulation</td>
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<tr>
<td>AEA</td>
<td>Atomic Energy Agency</td>
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<tr>
<td>ASM</td>
<td>Algebraic Stress Model</td>
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<tr>
<td>CFD</td>
<td>Computational Fluid Dynamics</td>
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<tr>
<td>CCCT</td>
<td>Curvature Compensated Convection Transport</td>
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<tr>
<td>CPU</td>
<td>Central Processing Unit</td>
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<tr>
<td>CV</td>
<td>Control Volume</td>
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<tr>
<td>DNA</td>
<td>Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>DOT</td>
<td>Dissolved Oxygen Tension</td>
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<tr>
<td>EC</td>
<td>European Community</td>
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<tr>
<td>GILSP</td>
<td>Good Industrial Large Scale Practice</td>
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<tr>
<td>GMMOs</td>
<td>Genetically Manipulated Micro-Organism</td>
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<tr>
<td>HASAWA</td>
<td>Health and Safety at Work Act</td>
</tr>
<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Filter</td>
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<tr>
<td>HSE</td>
<td>Health and Safety Executive</td>
</tr>
<tr>
<td>HSW</td>
<td>Health and Safety at Work</td>
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<tr>
<td>OECD</td>
<td>Organisation for Economic co-operation and Development</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Pe</td>
<td>Peclet Number</td>
</tr>
<tr>
<td>PID</td>
<td>Pipe and Instrumentation Diagram</td>
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<tr>
<td>PISO</td>
<td>Pressure Implicit with Splitting of Operators</td>
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<tr>
<td>QPCR</td>
<td>Quantitative Polymerase Chain Reaction</td>
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<tr>
<td>QUICK</td>
<td>Quadratic Upstream Interpolation for Convective Kinetics</td>
</tr>
<tr>
<td>RAM</td>
<td>Random Access Memory</td>
</tr>
<tr>
<td>rDNA</td>
<td>Recombinant Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse Osmosis</td>
</tr>
<tr>
<td>RSM</td>
<td>Reynolds Stress Model</td>
</tr>
<tr>
<td>SIMPLE</td>
<td>Semi-Implicit Method for Pressure Linked Equations</td>
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<tr>
<td>SIMPLEC</td>
<td>Semi-Implicit Method for Pressure Linked Equations-Consistent</td>
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<td>SIMPLER</td>
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<td>TRS</td>
<td>Thiosulphate Ringers Solution</td>
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<tr>
<td>VVM</td>
<td>Volume for Volume per Minute</td>
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Nomenclature.

Symbols.

A \quad \text{Area (m}^2\text{).}
C \quad \text{Computational position.}
\text{d}_{50} \quad 50\% \text{ cut off diameter.}
E \quad \text{Node to the east of the general node.}
F \quad \text{Force acting on particle (N).}
g \quad \text{Acceleration due to gravity (ms}^{-1}\text{).}
k \quad \text{Turbulent kinetic energy (m}^2\text{s}^{-2}\text{).}
K \quad \text{Thermal conductivity (Wm}^{-1}\text{K}^{-1}\text{).}
M \quad \text{Mass (kg).}
n \quad \text{Number of iterations.}
N \quad \text{Node to the north of the general node.}
p \quad \text{Pressure (Nm}^{-2}\text{).}
P \quad \text{General node.}
q \quad \text{Heat flux (Wm}^{-2}\text{).}
Q \quad \text{Volumetric sampling rate (m}^3\text{s}^{-1}\text{).}
Re \quad \text{Reynolds Number}
S \quad \text{Node to the south of the general node.}
S_0 \quad \text{Source term.}
t \quad \text{Time (s).}
T \quad \text{Temperature (K).}
u \quad \text{Velocity component in the Cartesian x-direction.}
v \quad \text{Velocity component in the Cartesian y-direction.}
V \quad \text{Volume (m}^3\text{).}
w \quad \text{Velocity component in the Cartesian z-direction.}
W \quad \text{Node to the west of the general node.}
x \quad \text{Distance in the Cartesian x-direction (m).}
y \quad \text{Distance in the Cartesian y-direction (m).}
z \quad \text{Distance in the Cartesian z-direction (m).}

\text{Greek Symbols.}

\alpha \quad \text{Under-relaxation factor.}
\beta \quad \text{Curvature constant.}
\Gamma \quad \text{Effective diffusivity (m}^2\text{s}^{-1}\text{).}
\varepsilon \quad \text{Rate of dissipation of turbulent kinetic energy (m}^2\text{s}^{-3}\text{).}
\nu_t \quad \text{Eddy viscosity (kgm}^{-1}\text{s}^{-1}\text{).}
\xi \quad \text{Computational velocity (ms}^{-1}\text{).}
\rho \quad \text{Density (kgm}^{-3}\text{).}
\Theta \quad \text{General property.}
\tau \quad \text{Viscous stress (kgm}^{-1}\text{s}^{-2}\text{).}
Superscripts.

\( g \)  gas
\( p \)  particle
\( * \)  Guessed.
\( ^\prime \)  Correction.
\( \Lambda \)  Instantaneous.
\( - \)  Mean.
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The majority of large-scale bioprocesses are recognised as being safe (WHO, 1984). However, some processes may use a pathogenic organism or one that has been genetically modified, and if hazardous to man or to the environment will by law require containment (HSE, 1992). Current UK and EC biosafety guidelines suggest that processes should, depending on the level of containment required, “minimise” or “prevent” release of the process organisms. However, these terms are qualitative in nature and thus difficult to interpret into a mechanical engineering design. Industry has reacted to these guidelines by over-engineering containment measures which inevitably results in more complex processes and higher plant capital costs.

When designing for containment purposes it is important to realise that a process cannot be completely contained (Turner, 1989). There is always the chance of a failure in a system that could result in a release. The cost of containing a bioprocess rises with the level of containment required and so does the complexity of the system. It has been argued that a highly contained system due to its complexity is more likely to release micro-organisms due to operator error than a simpler less contained one.

It is apparent that there is the need for a programme to quantitatively assess the release of micro-organisms from a bioprocess, both incidental during normal operation and accidental due to mechanical failure or operator error. When there is data available on the scale of incidental and accidental release then performance criteria could be written more confidently and as a consequence contained bioprocesses designed with reference to a measurable set of performance parameters.

The aims of this project were to quantify the incidental release of bioaerosols into the fermenter exhaust gas and also to correlate the internal design of the Turbosep exhaust gas containment system with the degree of containment provided. In this way a quantitative assessment of total microbial release could be determined which would allow for the redesign or optimisation of exhaust gas containment measures. Furthermore, as the primary role of the Turbosep is as a foam separator, the development of a system where a high efficiency of foam separation is combined with
an effective removal of microbial cells would result in a more cost-effective containment of the exhaust gas from a fermenter.

1.1 Biosafety Regulations and Guidelines.

This section will summarise the current UK biosafety legislation. It will explain the chronological development of the legislation, highlight some of its weaknesses and explain why a more quantitative approach is required.

1.1.2 The Development of UK Biosafety Legislation.

Current UK legislation concerning the use of genetically modified micro-organisms (GMMOs) and their effects on both workers and the environment are made under the power of the Health and Safety at Work Act (HSW, 1974). This act came into existence as a direct result of recommendations made in the Robens Report (1972). The report provided a turning point for safety legislation in the UK as it was critical of the retrospective approach to safety regulations. At the time of publication there were no safety regulations that encompassed all places of work. The report proposed the development of a unified approach to health and safety at work and made two recommendations; the first was that a comprehensive health and safety at work act (HSW, 1974) should be passed, which should be supported by a combination of regulatory and non-statutory codes and standards. The second was that an administrative body should be formed to make arrangements and provide a mechanism for linking regulations and non-statutory codes at work. Today the Health and Safety Commission (HSC) and Health and Safety Executive (HSE) are responsible for the safety of all employees within the UK.

The Health and Safety at Work Act (1974) places a duty on the employer:

"...requiring a provision and maintenance of a working environment for the employees that is as far as is reasonably practicable, safe without risks to health and adequate as regards facilities and arrangements for the welfare at work."
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"...carry out or arrange for the carrying out of any necessary research with a view to the discovery and, so far as is reasonably practicable, the elimination or minimisation of any risks to health and safety to which the design or article may give rise."

The phrase “so far is reasonably practical” qualifies these duties such that the employer must balance the risk involved in carrying out the work against the cost of avoiding the risk.

The first attempt to regulate the use of recombinant DNA technology was made at the Gordon Research Conference on Nucleic Acids held in the United States in 1973 (Singer and Soll, 1973). This pivotal conference on molecular genetics recognised that it was possible to form hybrid DNA and transfer it between organisms. Although no hazard had been identified there was a request for the establishment of a study committee to consider the risks posed by this new technology. This conference led to the formation of the Committee on Recombinant DNA Molecules, set up by the US National Academy of Science, which called for a voluntary embargo on a number of DNA manipulations with a perceived hazard (Berg et al, 1974). Among the prohibited experiments were those that would introduce plasmids carrying antibiotic resistance into strains not known to carry these traits. This therefore represented the first broad classification of rDNA experiments according to the perceived hazard of the activity (Turner, 1989).

The following year at the Asilomar Conference on Recombinant DNA molecules (Berg et al, 1975) the first steps to create formal safety guidelines for genetic engineering were made. It was concluded that the potential risks of rDNA technology should be dealt with by incorporating containment as an essential feature of experimental design and matching the level of containment used with the estimated risk (Berg et al, 1975). The conference was also notable in that it stated that the most significant contribution to limiting the spread of rDNA was the use of biological barriers. These barriers are of two types: fastidious bacterial hosts that would be unable to survive outside of the laboratory environment and fastidious vectors which would be non-transmissible, except in well specified host organisms. Physical containment was suggested to provide an additional factor of safety. With regard to large-scale operations, it was noted that such
experiments were inherently more hazardous than small-scale work. Consequently an arbitrary upper limit of 10 L was suggested for laboratory scale experiments.

As a consequence of two small outbreaks of smallpox, derived from clinical laboratories in the 1970s (Schofield, 1992), the Advisory Committee on Dangerous Pathogens (ACDP) was formed. The ACDP defined a system of classification of naturally occurring organism into four different hazard groups based on their pathogenicity. Corresponding levels of containment to minimise infection when handling these organisms were also defined.

In the UK, regulations on the use of GMMOs were first made in 1978; the Health and Safety (Genetic Manipulation) Regulations. These were based on a voluntary code of conduct and backed by a series of guidance notes. Then in 1984 the Advisory Committee on Genetic Manipulation (ACGM) was formed. The ACGM advises the HSC, HSE and other government departments on the human health and environmental aspects of the contained use of GMMOs. This came into fruition in 1988 when the ACGM published a protocol for assessing the hazard posed by GMMOs (ACGM, 1988). The potential hazard associated with an experiment was derived from three component factors, access, expression and damage. Each of these factors was scored on the Brenner scale from $10^{-2}$ (low risk) to 1 (high risk), and the product of the three used to define the category of containment required.

In the years following the Asilomar Conference, the manufacture of proteins with recombinant organisms grew in scale and it became necessary to implement control over these larger scale processes. Much of the guidance on large-scale use is based on the recommendations and conclusions of a major international study by the Organisation for Economic Co-Operation and Development (OECD, 1986). The containment suggested by the OECD ranges from Good Industrial Large Scale Practice (GILSP) through a series of three increasing stringent levels of containment (see Table 1.1). The OECD recognised that GILSP was not a containment category in itself, but adopted it because many organisms used in traditional manufacture were regarded as safe since they had been used for many years and had not caused any problems.
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The OECD guidelines are operational guidelines that attempt to control the scale of release. Incidental release however cannot be prevented so rDNA organisms were required to have built in environmental limitations permitting optimal growth in an industrial setting but limited survival without adverse consequences in the environment. As a result complete physical containment was unnecessary.

The Genetic Modified Organisms (Contained Use) Regulations (HSE, 1992) came into force in 1993, under the powers of the HSW (1974) act to cover both human and environmental risks and implement EC Directive 90/219/EEC (EC, 1990). Contained use refers to any operation involving GMMOs including cultured, stored, used, transported, destroyed or disposed of. This therefore includes waste streams. The regulations stipulate before proceeding with any operation the GMMO concerned is first categorised into one of two hazard groups. Either group I for zero or low risk GMMOs, or group II for GMMOs that could be hazardous to either man or the environment.

The operation being undertaken also has to be categorised, Group A for research or teaching work, or Group B for commercial work. The level of containment required for the operation must also be determined, either B1 (equivalent to GILSP), B2, B3 or B4 (equivalent to OECD large scale categories 1, 2 and 3.) To make classification simpler and more logical, guidance was provided in the ACGM note 7 (ACGM, 1993).

A risk assessment must then be performed which considers both human health and the environment. This includes categorisation of the organism and the operation for notification and the determination of the appropriate measures.
## Table 1.1 OECD Containment Levels (from OECD, 1986).

<table>
<thead>
<tr>
<th>Specification</th>
<th>Containment Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Viable organisms should be handled in a system which physically, separates</td>
<td>1</td>
</tr>
<tr>
<td>the process from the environment</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Exhaust gases from the closed system should be treated as to:</td>
<td>Minimise release</td>
</tr>
<tr>
<td>3. Sample collection, addition of materials to a closed system and transfer</td>
<td>Minimise release</td>
</tr>
<tr>
<td>of viable organisms to another closed system, should be performed so as to:</td>
<td>Inactivated by validated means</td>
</tr>
<tr>
<td>4. Bulk culture fluids should not be removed from the closed system unless the</td>
<td>Inactivated by validated means</td>
</tr>
<tr>
<td>viable organisms have been:</td>
<td>Prevent release</td>
</tr>
<tr>
<td>5. Seals should be designed so as to:</td>
<td>Minimise release</td>
</tr>
<tr>
<td>6. Closed systems should be located within a controlled area.</td>
<td>Optional</td>
</tr>
<tr>
<td>a. Biohazard signs should be posted.</td>
<td>Optional</td>
</tr>
<tr>
<td>b. Access should be restricted to nominated personnel only.</td>
<td>Optional</td>
</tr>
<tr>
<td>c. Personnel should wear protective clothing.</td>
<td>Yes, work clothing</td>
</tr>
<tr>
<td>d. Decontamination and washing facilities should be provided for personnel.</td>
<td>Yes</td>
</tr>
<tr>
<td>e. Personnel should shower before leaving controlled area.</td>
<td>No</td>
</tr>
<tr>
<td>f. Effluent from sinks and showers should be collected and inactivated before</td>
<td>No</td>
</tr>
<tr>
<td>release.</td>
<td>Optional</td>
</tr>
<tr>
<td>g. The controlled area should be adequately ventilated to minimise air</td>
<td>Optional</td>
</tr>
<tr>
<td>contamination.</td>
<td>No</td>
</tr>
<tr>
<td>h. The controlled area should be maintained at an air pressure negative to</td>
<td>No</td>
</tr>
<tr>
<td>atmosphere.</td>
<td>Input air and extract air to the controlled areas should</td>
</tr>
<tr>
<td>i. Input air and extract air to the controlled areas should be HEPA filtered</td>
<td>No</td>
</tr>
<tr>
<td>j. The controlled area should be designed to contain spillage of the entire</td>
<td>No</td>
</tr>
<tr>
<td>contents of the closed system.</td>
<td>Optional</td>
</tr>
<tr>
<td>k. The controlled area should be sealable to permit fumigation</td>
<td>Inactivated by validated means</td>
</tr>
<tr>
<td>7. Effluent treatment before final discharge</td>
<td>24</td>
</tr>
</tbody>
</table>
New regulations governing the contained use of GMMOs came into force in April 1996, which amended the 1992 Contained Use Regulations (HSE, 1992). The amendments implemented EC directive 94/51/EC (EC, 1994) and addressed some of the difficulties that had arisen in the practical application of the 1992 Regulations. For example under the 1992 regulations a person undergoing gene therapy would be classified as a GMMO and therefore subject to containment. A new and simpler system for classifying GMMOs has also been implemented which details more stringent criteria for classifying a GMMO as a group I organism (Table 1.2).

The 1992 regulations remain unchanged with regard to risk assessment, control measures to protect health and the environment and the disclosure of information to the public. The new regulations now address the problem of classifying micro-organisms as either Group I or group II, which was an area of contention under the 1992 guidelines.

### Table 1.2 Criteria for classifying GMMOs into Group I (from 94/51/EC).

<table>
<thead>
<tr>
<th>Criteria to fulfil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. The recipient or parental micro-organism is unlikely to cause disease to humans or plants.</td>
</tr>
<tr>
<td>2. The nature of the vector and the insert is such that they do not endow the genetically modified micro-organism with the phenotype likely to cause disease to humans, animals or plants, or likely to cause adverse effects in the environment.</td>
</tr>
<tr>
<td>3. The genetically modified micro-organism is unlikely to cause disease in humans, animals or plants and is unlikely to have adverse effects on the environment.</td>
</tr>
</tbody>
</table>

With these amendments the EC and UK governments are taking the right steps to simplify essential biosafety legislation. However the guidelines are drafted in very general terms and thus difficult to interpret. Qualitative terms such as “minimise” and “prevent” release are inadequately defined and are difficult to translate into mechanical engineering design terms. As a result no universal design standards have been implemented. Industry has reacted to the legislation by over-engineering containment measures, which inevitably results in more complex processes and higher plant cost.
Pennman (1989) estimated that the basic bioreactor cost would increase by 30% for the increase in the applied containment level for B3 and B4 containment classes. This may result in a process becoming uneconomical. Moreover, containment is normally applied to a process to prevent the release of micro-organisms, but any change to the process itself has the potential to introduce new modes of failure and risks (Dowell and Hendershot, 1997). It has been argued that a highly contained process due to its increase complexity could produce greater incidents of operator error, which could result in a more serious accident occurring than if a simpler less contained process was used.

A report by the Select Committee on Science and Technology in 1993 expressed concern that the current biosafety regulations would lead to a lack of competitiveness in the UK biotechnology industry. The difficulties experienced by the industry has been attributed to the attempt to apply the same containment legislation to all sectors of the industry, where such legislation is only applicable to a small area of the total industry. Additionally, the administrative burden placed on the industry by existing legislation is high. Although the current legislation has attempted to simplify essential legislative criteria, the reporting systems in place are still complex, in need of harmonisation and neglect the industries view (Dickson, 1996).

Ideally, the biosafety legislation should reflect the degree of process containment intended i.e. that no detectable release will occur when high-risk organisms are used and that some release is tolerable with low risk organisms. Kirsop (1993) stated that assigning numerical values to situations where some release is acceptable is extremely difficult, especially as little quantitative data is available for existing processes.

1.1.3 Current Regulations on the Contained Use of GMMOs.

The current regulations governing the contained use of GMMOs came into force in May 2000 (HSE, 2000), and amend the 1996 Contained Use Regulations (EC, 1994). The key features of these amendments are summarised below.
• A new procedure for risk assessment that will promote a common approach throughout Europe.

• A simplified method of notification linked to the actual risk of the activity or process.

• The removal of the requirement to classify organisms and activities separately (into group I/II and Type A/B respectively).

• The improved and extended specification of containment and control measures which will be applicable to all activities involving GMMOs (not all measures are stated explicitly in the current regulations). Details of these specifications can be found in the CEN Standards (2001).

However, the basis of the current legislation remains unchanged where the owner performs a microbial risk assessment and the process containment measures are then selected accordingly.
1.2 The Containment of Bioprocesses.

The following section will describe the types of bioprocess containment possible and the design considerations required for a contained process. It will then discuss the implications of the current biosafety regulations on the design of exhaust gas containment systems.

1.2.1 Bioprocess Containment Design.

There are two broad categories of process containment. Primary containment should provide for the protection of personnel and the immediate vicinity of the process from exposure to process materials (Turner, 1989) and is achieved through good equipment design. Secondary containment should provide for the protection of personnel and the environment external to the facility from exposure to process materials (Turner, 1989) and is achieved through good building design and good manufacturing practice (Tubito, 1991).

Bioprocesses can be defined as either open or closed. A closed system is one that has no connections to the external environment as opposed to an open system that allows material to enter the process from or exit to the external environment. A closed system is difficult if not impossible to achieve in practice (Turner, 1989) due to the complexity of containment measures both to install and operate. It follows that when designing for containment it is important to realise that a process cannot be completely contained.

1.2.2 Types of Bioprocess Release.

Accidents are always unexpected but it is essential that the risk of accidental failure is assessed and measures are implemented to prevent the event from occurring. In the majority of unit operations the statistical chance of unit failure is far less significant than the probability of an accident occurring. The probability of an accident resulting in a release can be analysed by using a statistical method such as fault tree analysis (Jefferis and Schlager, 1986).
Principally there are two types of release, incidental and accidental. The incidental release of micro-organisms from a process results from less than perfect containment. The accidental release of micro-organisms into the environment could be the result of a simpler operator error or a catastrophic failure of a unit operation. As has already been stated containment cannot be absolute so it follows that a bioprocess will release micro-organisms into the environment due to its operation. Therefore the safety precautions applied to the process needs to balance the two types of risk. This is difficult to achieve in the bioprocess industry, as there is no reliable method of quantitatively assessing process safety. The effect of this is often to introduce an expensive additional layer of protection to ensure a fail-safe system of combined protection in order to comply with the necessary level of containment. An example of this might be the use of two HEPA filters on an exhaust gas line and no attention to the release produced when operating a sampling port.

The type of release from a process will be dependent upon the nature of the material breaking the containment and the manner in which the containment is breached. Leaver and Hambleton (1992) stated that the bioreactor was the most likely point in a bioprocess at which significant amounts of biological material could be released. Ashcroft and Pomeroy (1983) carried out experiments to simulate accidents that might occur during fermenter operation and included; fermenter operation in the absence of an exhaust gas filter and fermenter operation without the addition of any antifoam to simulate a failure in the antifoam supply. The absence of an exhaust gas filter resulted in gross contamination of surfaces up to 1m away from the vessel. Failure of the antifoam supply resulted in the culture medium being forced through the exhaust gas filter and contaminating the surrounding area. Settle plates showed widespread contamination of the floor with up to 40 particles per cm². The authors found that most of the culture fluid expelled was disseminated into large droplets that were deposited onto surfaces. Although spillages and leaks can cause gross contamination of equipment surfaces and buildings, uncontrolled release beyond the production area can be limited by appropriate building design and the use of effective decontamination procedures. However, whether it is continuous low levels of incidental release or an occasional high level of accidental release that poses the most significant hazard to health and the environment is an important question. Only when sufficient data that enables accurate quantification of the
incidental release from a process is available, can the relative contributions to overall release from incidental and accidental sources be assessed. It will then be possible to determine the most important potential mode of release and to design bioprocess containment accordingly.

1.2.3 The Incidental Release of Bioaerosols into the Fermenter Exhaust Gas.

Most studies have been focused on the release of micro-organisms in aerosols, since, in this state the released organisms may pose a threat to health and the environment and can not be easily detected or contained (Hambleton et al, 1992).

Aerosols are metastable suspensions of particles in gases and generally occur in dilute multiphase flows, with mass fractions below $10^{-3}$ and particulate volumes below $10^{-6}$. The generic term, aerosol was first coined near the end of the First World War and was used to describe clouds of microscopic and sub-microscopic particles in air. Aerosols are produced when a force is exerted onto a liquid. If sufficient force is exerted small droplet aerosols are formed which can provide an effective mode of transport for a wide range of minute particles of dust, minerals, trace elements and micro-organisms attached to the surface of or incorporated into the droplet.

The properties of aerosols that are important in terms of biosafety are the concentration of hazardous material and the particle size distribution. In terms of micro-organisms their concentration in the culture broth will be at their greatest towards the end of a fermentation, this will be reflected in the composition of the aerosol produced. For the majority of bio-aerosols, aerodynamic diameters are generally greater than 2 μm (Upton et al, 1994). However, the size distribution of the aerosol is dependent on the manner in which the aerosol was produced and the nature of the liquid from which it was derived (Szewczyk et al, 1991). Pilancinski et al (1990) described the process of aerosol droplet formation. The air bubble generated in the bulk of the liquid travels upwards to the surface where it forms a hemispherical film cap above the liquid surface. The bubble stays on the surface due to equilibrium between the buoyancy and surface tension forces. As the liquid from the bubble drains due to gravity the film becomes thinner and weaker until the bubble bursts. Fragments of the broken film are released as a large
number of film droplets. As the liquid depression is filled by the surrounding liquid, a jet is created at its centre. Disintegration of this jet releases a few large jet droplets which if large enough could potentially carry process micro-organisms. Hage and Wessels (1980) quantified the number of bacteria contained in varying sizes of aerosol droplets. The authors estimated that an aerosol containing $2 \times 10^9$ cells mL$^{-1}$ would produce droplets of approximately 5 μm radius that would contain 1 bacterial cell. However larger droplets of 100 μm radius would contain approximately 8000 bacterial cells. The authors suggested that the larger particle of 100 μm would sediment about 100 times faster than the smaller 5μm particle. However, since the terminal settling velocity is proportional to $d^2$ the 100 μm radius particle should theoretically sediment 400 times faster than the 5 μm radius particle.

Winkler (1987) investigated the number of contaminated particles in the exhaust gas and reported that in the fermenter headspace there are about $10^6$ contaminated particles per m$^3$ of gas. Since each contaminated particle contains at least one viable organism, then this describes only the minimum number present. Additionally it has been reported that a large portion of viable cells are not culturable (Colwell et al, 1985) and since this report relied on the measurement of the culturable cells, which in the aerosolised state is likely to be very low (Neef et al, 1995), then the reported numbers are likely to be an underestimate of the total number of cells present in the exhaust gas. Neef et al (1995) reported that less than 1% of cells collected by filtration from an aerosol were able to grow as mini-colonies, compared to 90% culturability of cells filtered from the suspension used to produce the aerosol.

Ferris (1995) showed that the release of micro-organisms into the fermenter exhaust gas could be collected by the use of a sampling cyclone. In these experiments it was shown that E.coli cells could be detected, but the quantity of cells collected was not known precisely as the enumeration method used (microscope cell counting) was not sensitive enough. Noble et al (1997) extended the work of Ferris by monitoring the release of cells into the fermenter exhaust gas from a 2L fermenter using a sampling cyclone - quantitative polymerase chain reaction (QPCR) methodology. It was found that over the course of a 5.5 hour period, $3 \times 10^7$ process cells were released, this number corresponding to less than 2μL equivalent of fermentation broth at harvest.
Other studies on aerosols produced by fermentation have concentrated on aerosol particle diameter rather than cell concentration in the aerosol. Pilancinski et al (1990) showed that the aerosol size distribution in the fermenter headspace, measured using an aerodynamic particle sizer was influenced by several factors such as the air flow rate, agitation rate and the rheological properties of the broth. The number of particles released was shown to increase with both agitation and aeration rate, with a significant fraction of the measured particles (30-40%) exceeding 2μm in diameter, large enough to carry biological material from the broth. This work was extended by Szewczky et al (1991) who measured the aerosol size distribution in the fermenter headspace and the effect of cell growth on the change in aerosol properties. The authors observed a decrease in particle concentration with increasing bacterial growth; this change being more pronounced in the size range above 2μm. The aerosol size range was found to be practically independent to air flow rate and agitation rate for sizes less than 2μm. However, for particles larger than 2μm the concentration was found to increase with both agitation rate and air flow rate. Ferris (1995) postulated that as the cell density increases it is more likely that clumping of cells in the broth will occur. Larger clumps of cells may not be lifted into the aerosol due to their size distribution not being compatible with the particle size distribution of the aerosol formed or due to the increased settling velocity. This may account for the fall in number observed by Szewczky et al (1991).

Huang et al (1994) attempted to monitor aerosol generation as an on-line method for biomass monitoring in an E.coli fermentation. Since there were very few particles of greater than 1μm diameter detected, the aerosol size distribution in the size range 0.1 - 1μm was measured and compared with the bacterial growth rate and rheological properties of the broth. It was found that the exhaust gas number concentration increased during the growth phase and subsequently decreased after bacterial cell concentration had reached a stable level. Metabolic changes in the composition of the fermentation medium subsequently caused the surface tension to decrease, leading to a greater forming tendency, which in turn caused greater aerosol release.

Due to the different methods of microbial capture and enumeration employed in each of these studies, direct comparisons between observed release rates is difficult. However,
the data presented does provide a good first approximation to quantify the hazards posed by the incidental release of bioaerosols.

1.2.4 The Containment of Fermenter Exhaust Gases.

The general interpretation of the biosafety legislation is that incidental release of category B2 or higher organisms should be strictly controlled. The most operationally secure method to contain an exhaust gas is to filter it through an absolute rated, sterilising grade HEPA filter. A HEPA filter is rated by its efficiency in removing particles greater than 0.3 μm and typically has a rating of 99.97% (DIN Standard). Under ideal conditions HEPA filters have been shown to be 99.9999% efficient in capturing bacterial spores (Harstad, 1969) and 99.9999% efficient in capturing viruses (Thorne and Burrows, 1960), thus providing an effective barrier between the process and the environment.

HEPA filters are generally constructed from a single pleated sheet of filter paper, commonly fabricated from a synthetic fibrous material. Particulate matter is separated from the bulk flow by impacting upon these fibres or upon previously captured particles. Once attached the particulate matter will not be re-entrained. There are several capture mechanisms. As air flows around a fibre, larger particles with sufficient momentum will leave the streamline flow and impact on a fibre. Smaller particles move around randomly as they are constantly bombarded by other particles. This random motion causes them to come into contact with the fibres where they are retained. Other particles are captured as they pass a fibre tangentially.

The precise quality and composition of the exhaust gas depends largely on the process from which it is emitted. However certain general qualities can be attributed as follows, the gas may contain foams or entrained aerosols and liquid. It may be fully saturated with water vapour, have a high bioburden and be warm or hot. The efficiency of a HEPA filter is greatly affected by the air velocities across it and also by the amount of filtering medium it contains (Cadwell and Whyte, 1991). Additionally, the filters can become blinded if they come into contact with quantities of foam or liquid, resulting in containment being compromised and a reduced gas flow capacity, effectively shutting
down the fermenter. The primary requirement for an exhaust gas containment system is therefore to condition the gas stream so that continuous and effective filtration can be assured. A typical approach for conditioning the exhaust gas stream is shown schematically in Figure 1.1. After passing through the condenser and foam catch pot the exhaust gas will be free from entrained foam, liquids and larger aerodynamic diameter aerosol particles but will still have a relative humidity of approximately 100%. To prevent condensate forming in the pipe work upstream of the terminal filter, the gas stream is heated by 10 - 15 °C.

Figure 1.1 A schematic representation of a typical exhaust gas containment system.

Other methods employed for conditioning the exhaust gas included treatment with a hydrophobic pre-filter, or the use of a mechanical separator such as a cyclone.
1.3 Cyclone Design and Application.

The following section will describe the use of cyclones for the separation of microorganisms from a gas stream. It will discuss the difficulties in optimising the design of cyclones for this specific duty and will summarise the theoretical studies that have attempted to model the performance of cyclones in terms of particle collection efficiency.

1.3.1 Cyclone Design.

Equipment can be broadly described as a cyclone if it derives centrifugal separational ability through the use of a pump-induced tangential velocity in a stationary vessel. Furthermore the tangential velocity can be partly or wholly induced by some kind of impeller mechanism. Cyclones have long been used in industry and technology as simple de-dusting devices, for the removal of particulates from contaminated gas streams (Lapple, 1950; Stairmand, 1952). They have also been used to collect size-selected samples of airborne dusts (Davies, 1952) and airborne micro-organisms (Errington and Powell, 1969). Due to the widespread use of cyclones it is important to try and understand their particle collection characteristics.

Cyclones generally consist of two sections, an upper cylindrical section and a lower conical section. The gas flow is introduced tangentially into the upper section and is constrained to take a downward spiral motion. A fraction of this flow, referred to as the underflow, leaves the cyclone through a duct at the apex of the conical section, while the remained reverses direction and swirls upwards in a central air core to exit from the vortex finder (see Figure 1.2).

Separation of the particles is due to the centrifugal force caused by the spinning gas stream. This force throws the particles outwards and onto the cyclone wall where they are collected.

A second important flow feature is short circuit flow. A fraction of the flow forms a thin stream that follows the top of the cyclone and the outer wall of the vortex finder, before
turning rapidly through 180 ° at the tip of the vortex finder and leaving the cyclone through the overflow. The practical implication is that particles following this stream are more likely to escape than be captured.

The flow field inside a cyclone is very complex. In addition to the interaction of the particles with the fluid (also called the carrier phase), the fluid swirls and re-circulates along the length of the cyclone. The cause of this swirl and its effect on particle separation can be described by examining the individual velocity components of the flow.

The tangential velocity component of the flow, illustrated schematically in Figure 1.3, has been shown by Kelsall (1952) to increase as the diameter decreases.
The radial velocity component is generated because not all the fluid can enter the underflow but some must be discharged through the vortex finder. The radial velocity varies from a maximum at the cyclone wall to zero at the air core (see Figure 1.4). A force balance is generated between the centrifugal force and the force generated by the inward radial flow. Fine particles have a small centrifugal force and are carried inwards whereas large particles penetrate against the flow to the wall. At the cyclone wall the radial velocity component is at its greatest while the tangential component is fairly moderate. Thus only the larger diameter particles will tend to stay at the wall. At smaller radii from the core, the radial velocity component is reduced and the centrifugal forces increase. Thus smaller particles will attain a balance of forces and lose their radial velocity component.
The axial velocity component is responsible for the particle discharge from a cyclone, it does not take part in the force balance. The axial velocity is greatest at the wall diminishing towards the air core. There exists a locus of zero vertical velocity (see Figure 1.5). Inside this locus the direction of the vertical velocity is upwards and all particles in this zone will go to the vortex finder. Those particles that are balanced by acting forces to stay at this point form the size that has an equal opportunity of passing to either the underflow or overflow (see Section 1.3.3.2)
1.3.2 Optimisation of Cyclone Design.

Cyclone collection efficiency is defined as the fraction of the particles of a given size that are retained by the cyclone. There are eight common dimensions used to characterise cyclone collection efficiency, often expressed as a ratio to the cyclone body diameter. However from the literature it is clear that there is still a great deal of uncertainty over the best way to optimise the design of the cyclone. This problem is compounded further due to variations in the feed rate and feed composition from process to process.
The effect of cyclone diameter on efficiency has been reported by Kim and Lee (1990). The authors recognised that the smaller the diameter of the cyclone the greater the collection efficiency. This is paid for by an increased pressure drop at the same flow rate or by the need to accept a smaller flow rate at the same pressure requirement. An increase in the overall length of the cyclone has been shown to give an increase in both capacity and efficiency for a given pressure drop (Rietema, 1961).

To allow an opportunity for re-entrainment of the particles in the short circuit flow it is usual to remove the overflow stream by means of a vortex finder (Svarovsky, 1979). Re-entrainment then occurs as particles flow down the outside wall. Increase in the length of the vortex finder therefore allows more time for this re-entrainment and increases the efficiency of separation of the course particles. However, the majority of the fine particles reach the overflow in the return stream from the apex of the cone. An increase in the vortex finder length therefore allows less time for their re-entrainment (Bradley, 1965) and consequently causes a decrease in efficiency. An optimum length therefore exists, dependent on feed size and distribution, and cut point in relation to this size distribution.

The principal design variables that control cyclone performance are the three aperture sizes; feed, overflow and underflow. The feed and overflow sizes control the size of separation and the pressure drop, the underflow size controls the flow ratio. However it has now been recognised that there is probably no such thing as an optimum diameter for each aperture applicable to all duties and to all sizes of cyclones (Bradley, 1965)

1.3.3 Models of Cyclone Behaviour.

As many different types of cyclone have been constructed it is important to characterise the design when attempting to model it effectively. Standard cyclone designs exist but there is no reasoning to prove that they are optimal. This is where the real value of modelling appears, as a good model could predict changes in dimensions and operation that should improve performance.
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Dirgo and Leith (1985a) related that there is general agreement between different investigators that the operating parameters of the system should be used to predict performance, and most theories account for the effects of particle size, particle density, gas velocity and viscosity. They also noted that there is less agreement relating to the effects of the dimensions and geometry of the cyclone. All cyclone theories attempt to create a force balance between the outward centrifugal force and the inward drag force. Leith (1979) outlined three general approaches to predict the collection efficiency of a cyclone as outlined below.

1.3.3.1 Critical Diameter, Timed Flight Models.

The timed flight models assume that particles enter the cyclone at a certain radius from the cyclone axis. Particles must then travel outwards from this point to the cyclone wall where they are collected. The critical diameter is that particle size that travels exactly this distance during its residence time in the cyclone. This theory was originally proposed by Lapple (1950) and was used as a starting point for more widely used models.

1.3.3.2 Critical Diameter, Static Particle Models.

The static particle models involve determining the particle diameter for which the centrifugal force is exactly balanced by the drag force. These ‘static’ particles possess a
critical diameter that, in theory should rotate indefinitely around the edge of the cyclone core. The models assume that the critically sized particle will have a collection efficiency of 50%, and hence the critical particle diameter is referred to as the $d_{50}$. The static particle approach predicts a sharp increase in cyclone efficiency from zero for particles smaller than $d_{50}$ to unity for larger particles. The sharp discrete separation is never seen in practice due to variations in radial and tangential gas velocities over the height of the cyclone. This theory was first developed by Stairmand in 1950 and later refined by Barth (1956). According to the theory the $d_{50}$ can be calculated for any cyclone and operating conditions if the tangential velocity at the core and the core length are known.

1.3.3.3 Fractional Efficiency Models.

The fractional efficiency models assume that the gas flow is highly turbulent and mixes any uncollected particles in any plane perpendicular to the cyclone axis. These particles are assumed to remain inside the cyclone for as long as it takes the air supporting them to penetrate the cyclone. This allows the entire fractional efficiency curve to be determined without resorting to a generalised curve based on a critical particle diameter. This theory was first proposed by Leith and Licht (1972). However the theory does suffer from a simplified handling of the gas mixing which was later refined by Dietz (1981).

1.3.4 Experimental Performance of Cyclones.

Dirgo and Leith (1985b) carried out an experimental investigation using a number of 'pilot plant' cyclones to measure the collection efficiency and pressure drop over a range of sampling rates. The experimentally derived collection efficiency curves were compared with data generated from a number of cyclone efficiency theories. Their modified form of Barth's (1956) theory produced the best correlation with the experimental data compared to competing models. Although this model may predict efficiency significantly better than other methods there was still a great deal of scatter about the results.
Computational fluid dynamics has also been used to examine the correlation between models and experiments (Griffiths and Boysan, 1992; Griffiths and Boysan, 1996). The authors reported that CFD predicted particle collection efficiency for a range of small sampling cyclones had the same shape and $d_{50}$ and approximately the same gradients as those obtained by experiment. Of the empirical models considered in this study, only the Barth (1956) theory was able to match the experimentally derived data. The authors concluded that CFD was a reliable method for modelling the performance of cyclones.

What is clear from the models proposed is that each works best under certain conditions specific to the cyclone and the operating conditions used. With particles in the size range 1 to 10 μm discrepancies among theories and variation between results are the greatest. Particles of this size are more likely to be affected by small variations in the internal gas dynamics than larger particles, therefore attempting to model then is more difficult.

An empirical approach to cyclone design has been adopted by some designers (Swift, 1986), but most engineers tend to develop the model first and then test it experimentally with reference to other existing models. A number of papers have focused on the separation of particles in cyclones and the best ways to design experiments and collect data (Burkholz, 1985; Kim and Lee, 1990).

Moore and McFarland (1993) recommended that a designer should start with the cyclone dimensions as laid down by Stairmand (1951) as most information is available on this design. However when analysing the performance of a particular cyclone it is important to note the scale of the experiment and the type of aerosol used. It has been shown that the smaller the cyclone diameter, the greater the collection efficiency (Kim and Lee, 1990). Large variations exist in experiments carried out using homogenous aerosols compared to heterodisperse aerosols. The heterodisperse experiments cannot be accurately replicated and as a result make drawing conclusions between models difficult.
1.4 Fermentation Foam and its Control.

Blinding of the exhaust gas filter by contact with liquid or foam is one of the major causes of accidental fermentation failure (Leaver and Hambleton, 1992) and increases the probability of microbial release. The following section describes the factors affecting foam formation during a fermentation, the adverse effects of the foam presence and the control strategies commonly employed to limit excessive foam build up.

1.4.1 Fermentation Foam and its Consequence.

Foam, a dispersion of gas in liquid with a density approaching that of the gas is an agglomeration of gas bubbles separated from each other by a thin liquid film or lamella (Bikerman et al, 1953). Foams are produced whenever a solution containing a surfactant is aerated bubbles rise towards the surface forming two distinct regions, the top layer where the bubbles are densely packed is described as the foam, whereas the liquid fraction beneath is described as the froth. Foam is also formed in anaerobic fermentations due to the agglomeration of gas bubbles generated during cellular metabolism (Solomans, 1967). In many of these processes foaming occurs to such an extent that the process is either severely impaired or even completely arrested.

The formation of foam in submerged culture is affected by the hydrodynamic conditions that in turn are affected by the introduction of gas, the nature and composition of the medium, the presence of growing cells and the physical conditions of the fermentation (Duitschaever et al, 1988). The problems created by foaming in submerged culture fall into two categories, those in which the foam appears within the bioreactor and those in which foam escapes if some form of containment is not applied. When foaming occurs, the effective volume of the liquid in the reactor increases. This may lead to loss of culture liquid and micro-organisms through the air exhaust line and seepage into bearings and attachments causing sterility and containment problems (Bryant, 1970). This problem is further compounded by the fact that foam formation is often autocatalytic, the foam being able to create conditions in the culture that promotes lysis of some cells which in turn leads to the generation of more foam (Bryant, 1970).
1.4.2 Strategies for Controlling Foam Build-Up.

To avoid excessive foam build-up, anti-foam agents or mechanical foam destroyer are used. In some cases a combination of both chemical and mechanical methods are used for more effective control of the foam.

1.4.2.1 Chemical Methods of Foam Control.

Chemical anti-foam agents are surface-active substances that decrease the surface elasticity of liquids and prevent stable foam formation (Solomans, 1969). The foam breaks as a result of the tendency to attain the equilibrium between the surface elasticity of the liquid and the surface-active substance (Vardar-Sukan, 1992). Consequently an agent capable of destroying foam in one case may well act as a foam stabiliser in another. Thus the issue of foam control in bioprocesses is more complex than one of simple foam suppression.

The use of anti-foam agents is well established. They can be classified as oils, waxes, aliphatic acids or esters, alcohols, sulphates, sulfonates, polyglycols and siloxanes (Prins and Vant Riet, 1987). Examples of some of the members of these groups that are recommended and employed in fermentations are listed in Table 1.3. The mode of action of a chemical anti-foam agent varies with the type of compound and foam and the nature of the substance causing the foam formation. Ross (1950) described two mechanisms to account for foam destruction by an anti-foam agent.

1. The antifoam is dispersed into very small droplets that penetrate into the foam lamella and form a duplex film. This film spreads on the lamella. The foam bursts because of the strain caused by the extension of the duplex film.

2. The antifoam penetrates into the lamella and forms a mixed monolayer on the lamella that has less cohesion than the lamella-stabilising, surface-active agent film in the absence of the antifoam. This causes the lamella to burst.
Many of the commercial antifoams require the presence of carriers to assist in the suppression of the foam (Solomans, 1967). Additionally, a mixture of more than one antifoam is sometimes required for effective foam control.

Table 1.3 Antifoam agents commonly used in fermentations.

<table>
<thead>
<tr>
<th>CHEMICAL ANTIFOAM AGENT</th>
<th>EFFECTIVENESS.</th>
<th>REFERENCE.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceride oils and fatty acids</td>
<td>Stable to heat sterilisation. Effective on own but more effective with paraffin oil as carrier.</td>
<td>Solomons, 1967</td>
</tr>
<tr>
<td>Natural Oils (castor oil, corn oil, cotton seed, linseed oil, olive oil, poppy seed oil, sesame oil and sunflower oil)</td>
<td>Effectiveness varies greatly with the type of fermentation broth used.</td>
<td>Varder-Sukan, 1988</td>
</tr>
<tr>
<td>Liquid paraffin and silica hydrophobised with trimethylsilane</td>
<td>Effective general antifoam.</td>
<td>Garrett, 1994</td>
</tr>
<tr>
<td>Aqueous emulsion with 10% silicon oil</td>
<td>Effective general antifoam. Effect on OTR + Kla significant at start but effect gradually decreases.</td>
<td>Koch, 1995</td>
</tr>
<tr>
<td>Silicon oil and polypropylene glycol</td>
<td>Effective general antifoams. Less effect on OTR + Kla than silicon oil emulsion.</td>
<td>Koch, 1995</td>
</tr>
</tbody>
</table>

1.4.2.2 Adverse Effects of Chemical Antifoam.

Antifoam agents have been reported to change the physical properties of the culture broth, resulting in the deterioration of the operational characteristics of the fermentation, and a marked decline in fermenter performance (Kawase and Moo-Young, 1987). The presence of antifoam agents in the system has also been shown to increase the difficulty in extraction and purification of the product (Evans and Hall, 1971). Enzyme systems of the fermenting micro organism may be damaged by some oils, causing rates of sugar utilisation to decrease and production of desired metabolites such as antibiotics to be inhibited (Moller et al, 1992)
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Most antifoam agents also have a profound effect on the oxygen transfer rates (OTR) either lowering or increasing the rates (Koch, 1995). Evans and Hall (1971) found that some antifoam agents lowered the OTR as much as 12-50% which resulted in a waste of expensive sterile air and energy input to the agitator. In such cases the strategy is often to switch to another antifoam agent, however Philips et al (1960) noted that the addition of antifoam in smaller doses affected the OTR to a lesser extent. In contrast, about a 10-fold increase in OTR was reported with a silicon compound antifoam (Philips et al, 1960).

1.4.2.3 Mechanical Methods of Foam Control.

The basis of mechanical foam breaking is to subject the foam lamella to shear stress (Zlokarnik, 1986). The mechanical methods overcome many of the disadvantages associated with chemical antifoam agents however they may not be totally effective alone and linked to their high running costs, complicated designs and the possible damage to the micro organism their use is limited.

Despite their many shortcomings, mechanical foam breakers are indispensable in some fermentations where the most efficient antifoam is not able to cope with the large amounts of foam produced. For example the cultivation of Pseudomonas sp Rsan ver for the production of a biosurfactant is impossible to carry out equipped using a chemical antifoam alone due to excessive foam formation (Guerra-Santos et al, 1983), however a mechanical foam breaker proved successful in the cultivation of this strain.

Comprehensive reviews on the use of mechanical foam separators/breakers have been published by Viestures et al, (1982) and Ghildyal et al, (1988) and are summarised in Table 1.4.
Table 1.4 Mechanical foam breakers employed in fermentations.

<table>
<thead>
<tr>
<th>TYPE</th>
<th>MECHANISM OF ACTION</th>
<th>USAGE</th>
<th>EFFECTIVENESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal foam</td>
<td>Condenser directs steam onto the surface of the foam. Elevated temperatures cause</td>
<td>Rarely used in the fermentation industry due to the heat sensitivity of</td>
<td>Cost intensive and unsuitable with heat sensitive materials</td>
</tr>
<tr>
<td>breaker</td>
<td>bubble expansion, evaporation of the moisture content, a decrease in surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>viscosity and a thermal degradation of foam producing material.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrical foam</td>
<td>Electric current passed through the foam. Disintegration of foam based on the</td>
<td>Rarely used in the fermentation industry as the efficiency of the</td>
<td>Information on the influence of the electric field is lacking</td>
</tr>
<tr>
<td>breaker</td>
<td>appearance of forces which act differently on liquid and gas</td>
<td>method in uncertain</td>
<td></td>
</tr>
<tr>
<td>Sonic foam</td>
<td>Ultrasonic vibrations passed through the foam. Disintegration based on a combination</td>
<td>Limited use in the fermentation industry</td>
<td>A 6-11 kc/second pulse is reported to be able to control foam</td>
</tr>
<tr>
<td>breaker</td>
<td>of acoustic pressure, turbulence produced by the sonic weaves, a higher internal</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pressure in the foam bubbles compared to the surrounding particles and a vacuum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>caused by sonic energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impact spray</td>
<td>Liquid or air is sprayed on to the surface of the foam. High pressure jet</td>
<td>Limited to large scale foaming in sewage effluent treatment plants</td>
<td>Effective foam control but cost intensive</td>
</tr>
<tr>
<td>defoamer</td>
<td>generates compression and shear forces which disrupt the foam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYPE</td>
<td>MECHANISM OF ACTION</td>
<td>USAGE</td>
<td>EFFECTIVENESS</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Nozzle foam breakers</td>
<td>Foam is rapidly accelerated through a nozzle which results in almost complete separation into liquid and gas. Liquid is recycled to the fermenter by a positive displacement pump, air is expelled to the atmosphere</td>
<td>Limited use due to the high running costs for pumping gas</td>
<td>Simple device, almost complete destruction but unsuitable for particulate broths</td>
</tr>
<tr>
<td>Centrifugal foam breaker</td>
<td>Number of designs. Simplest form consists of a device rotating within a housing. Foam broken by centrifugal forces into liquid and gas. The liquid is recycled to the fermenter, air is expelled to atmosphere.</td>
<td>Limited use in the fermentation industry</td>
<td>Simple device, but only partial destruction of the foam</td>
</tr>
<tr>
<td>Vacuum foam breaker</td>
<td>Number of designs. Vacuum results in a large pressure drop which ruptures the inter bubble film</td>
<td>Rarely used in the fermentation industry due to high capital costs</td>
<td>Very efficient method</td>
</tr>
<tr>
<td>Cyclone type defoamers</td>
<td>Foam flows through the inlet pipe into the cyclone, the gas is forced to the centre of the cyclone by centrifugal forces and exits through the top of the cyclone, the liquid is recycled to the fermenter</td>
<td>Extensive use in the separation of solids from a gas stream.</td>
<td>Efficiency improved by the use of twin cyclones, addition of baffles and a truncated cone inside</td>
</tr>
<tr>
<td>Fundafoam</td>
<td>Device mounted on a flange within the fermenter vessel. Consists of a rotating perforated hollow shaft, conical disc with radial veins</td>
<td>Use limited to heavily foaming processes</td>
<td>Elimination of the need for any antifoam addition. A 50% increase in the productivity of antibiotic and citric acid fermentations is reported</td>
</tr>
<tr>
<td>MFARD (Mechanical foam breaking rotating disc)</td>
<td>Broth is removed from the fermenter and pumped through a rotating disk from which it is atomised. Utilises the impact action between the atomised liquid particles and the foam from the aerated liquid</td>
<td>Use limited to heavily foaming processes.</td>
<td>Good foam breaking but also applicable to power requirements</td>
</tr>
</tbody>
</table>
1.5 The Turbosep Exhaust Gas Containment System.

The Turbosep mechanical foam separator is a cyclone type device that connects directly to the exhaust gas line of the fermenter. The exhaust gas stream passes through the Turbosep where foams, aerosols and liquids are separated from the air. The liquids are re-cycled back into the fermenter while the separated gas exit upwards through a central vortex finder. The general design of the turbosep is shown in Figure 1.6, with detailed drawing presented in Appendix A.

![Figure 1.6 General design of the Turbosep foam separator.](image)
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There are nine different sizes of Turbosep, operating over an air flow range of 150 L/min$^1$ to 140K L/min$^1$. The principal of operation for each size of Turbosep is identical and is described below, where each number corresponds to a position in Figure 1.6.

1. The exhaust gas containing entrained cells, foam and liquid enters the Turbosep.
2. The airflow separates around the central vortex finder and is directed through static angled vanes that impart a swirling motion to the flow.
3. The flow is directed onto the impingement plate. This causes the flow to change direction and a phase separation of the liquid/foam and gas phases occurs. The separated liquid droplets coalesce into larger droplets as the liquid drains over the slope of the impingement plate and down to the base of the Turbosep.
4. The gas spirals down between the vortex finder and the inside of the outer wall. The axial velocities generated ensure that the separated foam does not re-entrain into the clean air.
5. The vortex arrester halts the swirl of the gas stream and creates a quiescent zone. This serves to prevent re-entrainment of the separated material and allows it to drain freely down the return pipe back into the fermenter.
6. The gas stream reverses direction and exits the Turbosep through the inside of the vortex finder.

1.5.1 Fermentation Foam Control Using the Turbosep.

The Turbosep has been reported to provide an effective separation of entrained foam, liquids and aerosols from the exhaust gas. However, under gross foam out conditions it is possible to exceed the capacity of the Turbosep causing it to overflow. By positioning an expansion tank in the liquid return pipe between the Turbosep and fermenter (see Figure 1.7), any rise in foam or liquids can be detected. A standard foam probe inserted into the expansion tank detects such a build up and uses a conventional anti-foam dosing system directly into the return pipe. The anti-foam is then transported into the fermenter where it acts to prevent further foam build up.
Figure 1.7. A schematic representation of the Turbosep foam control system. The degree of exhaust gas containment provided by the Turbosep was determined by measuring the cell release rates both upstream (position 1 and 2) and downstream (position 3).

Foam separation in the Turbosep is thought to be due to the centrifugal force caused by the spinning gas stream (Ridaelgh, personnel communication). The similar nature of operation between the Turbosep and a cyclone has lead to some speculation as to whether the Turbosep would be able to remove small particles from the exhaust gas stream and will be investigated further within this thesis.

This chapter lists the materials and methods used during the course of this project. Equipment and consumable suppliers can be found in Appendix B.

2.1 Microbial Methods.

2.1.1 Details of the Micro-Organisms Used.

Two micro-organisms were used during the course of this project (see Table 2.1). *E. coli* (RV308 pHKY531) was constructed by Eli Lilly and Company (Indianapolis, USA), where the host (RV308) is a derivative of the thiamine deficient *E. coli* K-12. The vector is a rop' plasmid that expresses met-asp-ovine somatotropin. It also contains genes for tetracycline resistance and the cI857 temperature sensitive repressor that controls BST expression. *S. cerevisiae* (S150Δhsp82) was genetically modified by the insertion of a KANMXR cassette into the genome, which is used as a target for a Quantitative Polymerase Chain Reaction assay (see Section 2.2).

Table 2.1 Details of micro-organisms used.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Characteristics</th>
<th>Strain prepared by</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> RV308</td>
<td>Tc^r, met-asp-bovine</td>
<td>Dr W. Cook^a</td>
<td>Cockshott, 1993</td>
</tr>
<tr>
<td>pHKY531</td>
<td>somatotrophin</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>-</td>
<td>Dr M. Noble^b</td>
<td>Wach <em>et al</em>, 1994</td>
</tr>
<tr>
<td>S150Δhsp82</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Km^r; kanamycin resistant; Tc^r: tetracycline resistant; ^aEli Lilly & Co. Ltd., Speke Operations, Liverpool, UK; ^bPA Consulting, Melbourne, Cambridge, UK.

2.1.2 Culture and Maintenance of Micro-Organisms.

2.1.2.1 Culture on Solid Media.

*E. coli* RV308 pHKY531 was grown at 28 °C on 28 gL^-1 Lauria agar (Sigma), which was supplemented with 10 μg mL^-1 filter sterilised tetracycline (Sigma) after
autoclaving. *S. cerevisiae* S150Δhsp82 was grown at 28 °C on sterile YPD agar, containing per litre; 10 g yeast extract (Oxoid), 20 g peptone (Oxoid), 20 g glucose (Oxoid) and 20 g agar (Sigma).

### 2.1.2.2 Culture in Liquid Media (Shake Flasks).

*E. coli* strain RV308 pHKY531 was grown at 28 °C in Lauria broth (Sigma) containing per litre; 10g bacto tryptone, 5 g yeast extract and 10 g NaCl with the addition after autoclaving of 2μg mL⁻¹ filter sterilised tetracycline. Seed cultures were grown by inoculation of a 5 mL volume of the sterile culture medium in a sterile universal tube with a single colony. The universal tube was then loosely capped and placed on an orbital shaker (New Brunswick) at 200 rpm. After approximately 4 hours, or sooner if turbidity was noted, 0.5 mL of the seed culture was aseptically transferred to a 250 mL shake flask containing 50 ml of the sterile medium. The culture was grown overnight on an orbital shaker at 200 rpm.

For larger scale shake flask studies, a 2 mL sample of the overnight cell culture was aseptically transferred to a 2 L baffled shake flask containing 500 mL of the sterile medium. The culture was grown overnight on an orbital shaker at 200 rpm.

*S. cerevisiae* S150Δhsp82 was grown at 28 °C in YPD broth, containing per litre; 10 g yeast extract (Oxoid), 20 g peptone (Oxoid) and 20 g glucose (Oxoid). The seed time was extended to 8 hours, otherwise the same procedure as for *E. coli* was used.

### 2.1.2.3 Culture in Liquid Media (Fermentations).

#### 2.1.2.3.1 *E.coli* RV308 pHKY531.

For 2 L scale fermentation studies the medium used was a modified version of T-broth (Tartof and Hobbs, 1987) made up according to Table 2.2. Essentially this is a 2/3 x T-broth, the reduction in nutrient content allowing a more controlled fermentation (Noble *et al*, 1997).
A 2 L fermenter (IncelTech (UK) Ltd.) was inoculated from a shake flask with 500 mL of an overnight cell culture, this represents 33% of the final working volume of the fermenter. A large seed volume allows the cell culture to reach stationary phase more rapidly and results in a reduced fermentation time. This was necessary for the timing of subsequent experiments (see Chapter 5).

Table 2.2 Composition of modified T-broth.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (gL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>2.3 g L⁻¹</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>3.8 g L⁻¹</td>
</tr>
<tr>
<td>Bactotryptone</td>
<td>8 g L⁻¹</td>
</tr>
<tr>
<td>Yeast Extract</td>
<td>16 g L⁻¹</td>
</tr>
<tr>
<td>Glycerol</td>
<td>3 mL L⁻¹</td>
</tr>
<tr>
<td>Polypropylene glycol (PPG, antifoam, BDH)</td>
<td>0.2 mL L⁻¹</td>
</tr>
<tr>
<td>Kanamycin*</td>
<td>20 mg L⁻¹</td>
</tr>
</tbody>
</table>

* Filter sterilised and added after autoclaving.

For 75 L and 450 L scale fermentation studies the defined medium of Cockshott (1996) was used, made up according to Table 2.3. This medium supports growth to medium cell densities and is more cost effective for pilot/large scale fermentations.

The 75 L (Chemap) fermenter was inoculated from a shake flask with 500 mL of an overnight cell culture, this represents 1% of the final working volume of the fermenter. After approximately 12 hours or at the onset of stationary phase, whichever occurred first the entire contents of the 75 L vessel was used to seed the 450 L (Chemap) fermenter. This represents a 16% inoculum. The fermentation time was approximately 6 hours.

At the onset of stationary phase the contents of the 450 L vessel were harvested. Solid-liquid separation was achieved semi-continuously in a Carr P5 Powerfuge centrifuge at 15000 g and a liquid feed rate of 60 L hour⁻¹. The recovered cell paste was resuspended in 50% w/v glycerol and stored at -70 °C.
Table 2.3 Composition of Cockshott medium.

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (g L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>0.33</td>
</tr>
<tr>
<td>(NH₂)Fe(SO₄)₆.6H₂O</td>
<td>0.60</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.0032</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.00033</td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>0.00057</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.98</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>1.32</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.05</td>
</tr>
<tr>
<td>K₂SO₄</td>
<td>6.62</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>0.33</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>0.66</td>
</tr>
<tr>
<td>Thiamine hydrochloride</td>
<td>0.0066</td>
</tr>
<tr>
<td>Tetracycline hydrochloride*</td>
<td>0.002</td>
</tr>
<tr>
<td>Glucose (batch)</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* Added after sterilisation as a filter sterilised solution.

At each scale the temperature of the fermentation was maintained at 28 °C. The pH was maintained at 7 and controlled by the addition of 0.5 M NaOH. No acid additions were made to prevent the pH from rising. The aeration rate in all experiments was 1 v.v.m (i.e. 1.5 L min⁻¹ in the 2 L vessel, 50 L min⁻¹ in the 75 L vessel and 300 L min⁻¹ in the 450 L vessel). The dissolved oxygen tension (DOT) was maintained above 20 % by the automatic variation of the stirrer speed. In each fermentation pH and DOT were logged and the exit gas was analysed by mass spectrometry (Prima 600, Fisons Scientific Instruments) to determine the oxygen uptake rate and the carbon evolution rate. A real time data acquisition system (RTDAS) was used.

The dimensions of the vessels used are detailed in Figures 2.1, 2.2 and 2.3. Each vessel contained four baffles and was agitated using standard configuration 6 blade Rushton Turbines.
2.1.2.3.2 *S. cerevisiae* S150Δhsp82.

For 2 L scale fermentation studies YPD broth was used, made up as described in section 2.1.2.2. This is a complex medium that supports growth to medium cell densities.

The fermenter was inoculated from a shake flask with 500 mL of an overnight cell culture, this represents 33% of the final working volume of the fermenter. The same process variables and unit operations were used as for *E. coli* RV308 pHKY531 fermentation (see Section 2.1.2.3.1).

2.1.2.4 Maintenance of Micro-Organisms.

Stock cultures of *E. coli* were maintained in 20 % w/v glycerol solution at -70 °C. Glycerol stocks were prepared by pipetting 3 mL of a sterile 20 % glycerol solution onto an agar plate on which the culture had grown without contamination. Using a sterile inoculation loop, colonies were lifted off the agar into suspension. Finally, using a sterile pipette tip, the suspension of the micro-organisms in glycerol solution was returned to the sterile glass bijoux, which was then stored at -70 °C.

Stock cultures of *S. cerevisiae* were stored on slopes at 4 °C. Slopes were prepared using 50 gL⁻¹ malt extract agar.

Working cultures of all micro-organisms were maintained on agar at 4 °C. Cultures were re-plated every two weeks. Stock and working cultures were stored in triplicate.
Figure 2.1 Geometry of the 2 L IncelTech Fermenter (all dimensions in mm).
Figure 2.2 Geometry of the 75 L Chemap Fermenter (all dimensions in mm).
Figure 2.3 Geometry of the 450 L Chemap Fermenter (all dimensions in mm).
2.1.3. Optical Density (OD\textsubscript{600}) Measurement.

Optical density of broth cultures was measured at 600 nm using a Beckman DU-64 spectrophotometer (Beckman Instruments). Cultures were diluted in reverse osmosis (RO) water to ensure that the OD\textsubscript{600} reading was in the range 0.05-0.3 absorbance units. Sterile broth diluted in RO water was used as the blank.

2.1.4 Cell Enumeration.

2.1.4.1 Microscopic Cell Counting.

The concentration of \textit{E.coli} cells in a sample was determined using a Helberg bacteria counting chamber (Weber Scientific International Ltd). A small drop of sample was pipetted onto the counting chamber and a cover slip was slid into place. The presence of 'Newtons rings' around the sample chamber was used to confirm an adequate seal. The chamber was viewed under phase contrast and a x 400 magnification (x 40 objective, x 10 eyepiece, Nikon Optiphot Microscope). The number of cells in the whole grid was counted and recorded. In order to convert the number of cells counted per grid to number of cells per mL a multiplication factor of $7.8 \times 10^4$ was used, giving a limit of detection of $1.25 \times 10^6$ cells mL$^{-1}$.

The concentration of \textit{S.cerevisiae} cells in a sample was determined using a haemocytometer with improved Neubauer ratings (Weber Scientific). The ‘slide’ was viewed at x100 magnification (x10 objective, x10 eyepiece) using normal phase illumination. The number of cells in the whole grid was counted and recorded. In order to convert the number of cells counted per grid to number of cells per mL a multiplication factor of $1 \times 10^4$ was used, giving a limit of detection of $2.5 \times 10^6$ cells mL$^{-1}$.
2.1.4.2 Viable Plate Counts.

The number of colony forming units (CFU) in a sample of *E. coli* RV308 pHKY531 was determined by the use of spread plates. A 0.1 mL volume of a cell suspension was pipetted onto the surface of a nutrient agar plate supplemented with 20 μL filter sterilised tetracycline.

The suspension was spread over the agar surface using a sterile glass rod. Plates were thoroughly dried before use to prevent spreading of colonies. The number of colonies was counted after 1-2 days incubation at 28 °C.

2.1.4.3 Determination of Plasmid Stability.

The proportion of CFU in a sample of *E. coli* RV308 pHKY531 that expresses the plasmid of interest was determined by comparing the number of CFU mL⁻¹ using nutrient agar as the plate count medium (Nₐ), with the CFU mL⁻¹ using nutrient agar supplemented with tetracycline (N₉). Plasmid stability was calculated as follows:

\[
\text{Plasmid stability (\%)} = \frac{N_t}{N_a} \times 100
\]
2.2 Quantitative Polymerase Chain Reaction (QPCR) Assay.

The method of QPCR used was based on the use of a competitive standard in the PCR (Janson, 1995). Further details relating to QPCR and an example calculation to quantify the amount of target DNA in a sample can be found in Appendix C.

The PCR technique amplifies in vitro, and is illustrated in Figure 2.4. PCR involves amplification of the two oligonucleotide primers that flank the DNA sequence to be studied. These primers hybridise to opposite flanks of the target sequence and are orientated such that DNA synthesis proceeds across the region between the primers, effectively doubling the amount of that DNA segment. Since the extension products are also complementary and are capable of binding primers, the cycle can be repeated after a denaturation step. Repeated cycles of denaturation, priming and extension result in a rapid exponential accumulation of the specific target fragment.

![Figure 2.4 The polymerase chain reaction (PCR).](image)

To quantify the number of target fragments within a sample an internal standard and a standard sample of plasmid DNA, of known concentration, are included in the reaction. Since the internal standard is a fragment of DNA it is amplified with an efficiency that is proportional to the amplification of the target fragment.
The PCR products are separated by agarose gel electrophoresis, run alongside molecular weight markers. Quantification is based upon the comparison of the peak area ratios of the standard and the samples.

QPCR has a number of advantages over traditional plating methods. It is selective allowing the target organism to be distinguished from other strains present in the environment, even closely related ones. The technique is also highly sensitive, in theory a single organism can be detected. However, the sensitivity of the technique is also one of its drawbacks since samples can be contaminated by product carry-over from previous runs. This problem is exaggerated by the subsequent amplification of the contaminant DNA. Recommendations are reported elsewhere on the best ways to avoid carry-over of PCR products (Kwos and Higuchi, 1989; Sarkar and Sommer, 1990; Kitchin and Bootman, 1993).

2.2.1 PCR Sample Preparation.

Samples prepared in sterile thiosulphate ringers solution (TRS) were used directly in the PCR assay. Alternatively samples were stored at -20 °C until required and then thawed at room temperature before analysis. All PCR assays were carried out in triplicate in a Class II microbiological safety cabinet to prevent contamination of the reactions. This safety cabinet was not used for any other operations involving the target strain.

The stock reagents used in the PCR are shown in Table 2.4 and were prepared in advance using sterile consumables. Details of the primers used are shown in Table 2.5. The internal standard used in each reaction was appropriate to the concentration of the target plasmid in the sample and is described in more detail in Appendix C.
Table 2.4 Stock reagents used in the PCR assay.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stock Concentration</th>
<th>Supplier</th>
<th>Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfu polymerase</td>
<td>5 units μL^{-1}</td>
<td>Stratagene</td>
<td></td>
</tr>
<tr>
<td>Pfu polymerase buffer</td>
<td>200 mM Tris-HCl pH 8.4, 500 mM KCl</td>
<td>Stratagene</td>
<td></td>
</tr>
<tr>
<td>dNTP mix (dATP, dTTP, dCTP, dGTP)</td>
<td>1.25 mM each</td>
<td>Pharmacia</td>
<td>SROW</td>
</tr>
<tr>
<td>Primers</td>
<td>20 μM</td>
<td>Pharmacia</td>
<td>SROW</td>
</tr>
</tbody>
</table>

Table 2.5 PCR primers used in this study.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Primer</th>
<th>Sequence 5'-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> RV308 pHKY531</td>
<td>P1</td>
<td>ATG GAT TTT CCG GCT ATG TCT</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>GTA CGT CTC CGT CTT ATG CAG</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> S150Δhsp82</td>
<td>KANMXR</td>
<td>AAG ACT GTC AAG GAG GGT</td>
</tr>
<tr>
<td></td>
<td>hsp-5' *</td>
<td></td>
</tr>
</tbody>
</table>

* Contact Peter Piper, Department of Biochemistry and Molecular Biology, UCL, London for details.

2.2.1.1 *E. coli* RV308 pHKY531 PCR Protocol.

The PCR was carried out in 600 μL reaction tubes containing 25 μL reaction volume made up of 0.5 μL Pfu polymerase, 2.5 μL of 10x Pfu polymerase buffer, 4 μL of dNTP solution, 2.5 μL of each primer (P1 and P2), 1 μL of internal standard (IS(B)), 2 μL SROW and 10 μL of sample. The components were overlaid with 25 μL of light mineral oil (Sigma) to prevent evaporation before commencing the reaction.

PCR was carried out by placing the reaction tubes into a Hybaid Omnigene temperature cycler which was programmed for 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, 55 °C for 1 minute, 72 °C for 1 minute and finally a single step of 72 °C for 10 minutes.
2.2.1.2 *S. cerevisiae* S150Δhsp82 PCR Protocol.

Prior to the PCR reaction step, samples were subjected to an enzymatic lysis to disrupt the cell membrane; 20 μL of a 5x lysis buffer containing 3M sorbitol (Sigma), 0.5 M sodium phosphate (Sigma), pH 7.4 and 1600 units mL⁻¹ Lyticase (Sigma) were added to 80 μL of the sample and vortexed briefly before incubation at 37 °C for 30 minutes. After incubation the sample was spun at top speed in a benchtop centrifuge for 5 minutes, to pellet the 'spheroplasts.' A 90 μL volume of the supernatant was withdrawn and discarded followed by the addition of 90 μL of SROW. The sample was then vortexed for 30 seconds before being used in the PCR assay.

The PCR was carried out in 600 μL reaction tubes containing 25 μL reaction volume made up of 0.5 μL *Pfu* polymerase, 2.5 μL of 10x *Pfu* polymerase buffer, 4 μL of dNTP solution, 2.5 μL of each primer (hsp-5' and KANMXR), 1 μL of internal standard (IS(Y)), 2 μL SROW and 10 μL of sample. The components were overlaid with 25 μL of light mineral oil (Sigma) to prevent evaporation before commencing the reaction.

PCR was carried out by placing the reaction tubes into a Hybaid Omnigene temperature cycler that was programmed for 92 °C for 5 minutes, followed by 35 cycles of 92 °C for 30 seconds, 56 °C for 1 minute, 72 °C for 1 minute and finally a single step of 72 °C for 10 minutes.

2.2.2 QPCR Product Analysis.

The analysis of the PCR products was performed by agarose gel electrophoresis as described by Sambrook *et al* (1989). The running buffer used was 0.5 x tris-borate ethylenediaminetetraacetic acid (TBE) with 0.25 μg ml⁻¹ ethidium bromide. Aliquots of 7.5 μL of the PCR product were mixed with 2.5 μL gel loading solution (Sigma) and were run on horizontal 2% agarose (Wide Range/Standard 3:1, Sigma) gels at 80V for 3 hours. This was sufficient to allow separation of the internal standards and the template DNA. The molecular weight markers used were PCR Markers (Sigma).
2.2.3 Gel Documentation and Densitometry.

Gels were documented using a UV transilluminator and gel documentation system with Image store 5000 software (UVP Ltd), which produces a thermal print of the image.

The UVP gel documentation system was also used for densitometry of gels by downloading the gel image to disk, then analysing the data using gelbase software (UVP Ltd). In order to ensure that the downloaded data did not comprise of overloaded images (which would effect the quantification procedure), up to 3 images of each gel were taken and downloaded using different aperture settings on the lens. For maximum sensitivity (i.e. weak bands) a wide aperture was used, but this increases the likelihood of image overload from adjacent intense bands. Since the quantification method used in this study involves measurement of the peak area ratios of one band to another, it is necessary that the intensity of fluorescence emitted by each band is within the linear range of detection. However, if one band, say the internal standard was so weak that it required a wide aperture setting to be visible, and the other band in the same lane, in this case the target product, was very intense, then it would be impossible to accurately measure the ratio of peak areas. This is what limits the dynamic range of detection of the densitometry method. In practice it was found that the ratio of intensities of one band to another within the same lane must be less than twenty, and that of course assumes that neither band is saturating. Peak areas were scored according to these results.

2.2.4 Differentiation of Total and Extracellular Plasmid Concentration.

Extracellular plasmid (free in solution) was measured separately from total plasmid concentration according to the following method: a 0.5 mL aliquot of sample (containing micro-organisms) was micro-filtered in a Costar spin -x- tube (0.22 μm pore size, low DNA binding cellulose acetate membrane) which was centrifuged for 5 minutes at 6500 x g for 5 minutes. A 10 μL aliquot of the filtrate was then used in the PCR and the results compared with those from the unfiltered sample.
2.3 Quantification of Foam Stability.

In order to investigate the foam separational efficiency of the Turbosep it was challenged with different types of foam. The following section describes the method used for characterising some of the physical properties of foam.

A graduated 4.5 litre plastic column 100 mm in diameter with a porous glass aerator was used for the measurement of foaminess. The column was filled with the test solution to a liquid level of 15 cm above the aerator. This corresponds to 2 litres of sample volume. The temperature of the test solution was maintained at 20 °C by an *in-situ* thermostat heater. The liquid layer was aerated with compressed air, the flow rate of which was measured with a rotameter.

Foaming of each test solution was induced by connecting the air supply and was continued for 10 minutes or until the total height (liquid and foam) reached the 4 L level in the apparatus, whichever occurred first. At this point the air supply was cut off and the foam allowed to collapse naturally.

The foaming coefficients were obtained by dividing the final foam height attained by the time period required to reach it, and the time period required to drain it respectively.
2.4 Bioaerosol Sampling.

Many of the experiments performed in this project necessitated the capture and enumeration of bioaerosols. This had to be carried out in a safe and reproducible manner. The following section describes the equipment and protocols used for the capture of bioaerosols.

2.4.1 Aerojet General Cyclone Operation.

The air sampling device used within these studies was an Aerojet General Cyclone (Soham Scientific) (Decker et al, 1969, Upton et al, 1994), a modified version of the Errington-Powell cyclone (Errington and Powell, 1969). The Aerojet General Cyclone was set up as shown in figure 2.5. The principle of operation is that the injection of a recycled scrubbing liquid into the cyclone washes off cells that have been deposited onto the inner walls and collects them in a sample pot. Continuous scrubbing is achieved by inducing a slight suction at the underflow so that the liquid is drawn into the sample pot. This is achieved using a pressure equalisation pipe; a section of flexible tubing connecting the cyclone overflow to the sample pot. Thus the concentration of particles collected increases with time.

When sampling bioaerosol, air was drawn into the cyclone by an air pump (Air Control Installations) at 360 L min⁻¹, and the scrubbing liquid, 80 mL TRS was recirculated at 20 mL min⁻¹ using a peristaltic pump (Watson-Marlow). Sampling was carried out in batch mode for 15 minute periods, at the end of each run the volume of collection liquid remaining was measured by weighing. After collection the sample was analysed using QPCR and/or cells counts.

2.4.1.1 Cyclone Cleaning.

The cyclone and all associated tubing was cleaned after each sample by immersion into a 1% Tego solution (Th. Goldschmidt Ltd.) for 30 minutes. To ensure that all the surfaces were thoroughly wetted the cyclone was inverted half way through the cleaning
period. After the cleaning stage the cyclone and all tubing was rinsed out with tap water and allowed to air dry for 5 minutes.

![Diagram of an Aerojet General Cyclone]

**Figure 2.5** Schematic representation of an Aerojet General Cyclone.

2.4.2 Sampling of Fermenter Exhaust Gases.

Release of process cells into the exhaust gas during the course of a fermentation was measured by removing the exhaust gas filter and connecting the cyclone to the exhaust gas stream. Sampling was carried out as described in Section 2.4.1. In addition to each exhaust gas sample an additional background sample was taken (with the air filter connected) in order to provide information about the levels of background target microorganisms that might be detected in room air. For background samples the cyclone was left unconnected to the exit gas line, and a 10 minute sample of room air taken. After this time, a 1 mL sample was aseptically removed from the pot. On connection to the exhaust gas stream the cyclone was run for a further 15 minutes.
Samples obtained from the cyclone were stored overnight at 4 °C and were assayed by QPCR within 24 hours. The number of cells released was calculated by subtracting the number of cells collected in the background sample from the number of cells collected in the subsequent exhaust-gas cyclone sample. An identical sampling protocol was used to examine the level of exhaust gas containment provided by the Turbosep. The fermenter used in these studies was a 250 litre stainless steel vessel (Sinclair Fabrications, 200 L working volume) agitated by a single 6 blade Rushton turbine impeller. The experimental set up is shown schematically in Figure 2.6. Further details of the 250 L vessel are displayed in Figure 2.7.

**Figure 2.6** Schematic representation of a Turbosep where each number refers to a sampling position.
The Turbosep inlet was connected directly to the exhaust gas pipe while the recycle line was connected onto a second free port on the vessel top plate. The re-cycle line was designed to pass directly into the vessel and open at a level below the liquid surface. Thus any foam re-cycled back into the vessel would not accumulate on the broth surface.
The degree of exhaust gas containment provided by the Turbosep was determined by measuring cell release rates both upstream (position 1 and 2 in figure 2.5) and downstream (position 3 in figure 2.5).

2.4.3 Particle Counting Devices.

The instruments used to count and size liquid and airborne particles within this project are described below.

2.4.3.1 Malvern Instruments 3600Ec Particle Sizer.

The particle size of liquid borne particles was measured using a 3600Ec particle sizer (Malvern Instruments). The instrument uses laser diffraction to measure the size of particles. This is done using the principal of the Fraunhofer theory in measuring and interpreting the angular distribution of light diffracted by the particles. The range of particle sizes that can be quantified is set by the choice of filter, laser focal and beam length. The instrument was set up with a focal length of 63 mm and a beam length of 14.3 mm, with a filter for particles over the size range of 1.2 µm to 188 µm diameter. The instrument was blanked with RO water that had been filtered through a 0.22 µm cellulose acetate filter (Whatman). The sample was then added as fine droplets to prevent the formation of bubbles until the automatic sample indicator displayed optimum. The sample was analysed by the instrument as percentage volume in each of 32 different band sizes. The results were logged to a printer.

2.4.3.2 TSI Incorporated 7450 Laser Particle Counter (LPC).

The particle size of airborne particles was measured using a LPC 7450 (TSI Incorporated) laser particle counter. Similar to the Malvern particle sizer user laser diffraction to measure the size of particles. The system comprises a vacuum pump, LPC and printer. Air is drawn into the LPC at 5 L min$^{-1}$ where it provides a real time count of particles within two size bands, greater than 0.5 µm aerodynamic diameter and greater than 5.0 µm aerodynamic diameter. The instrument was set up to count particles per litre over 1 minute sample periods and the mean results were logged to the printer.
2.5 Computational Fluid Dynamics (CFD).

CFD has been used in this project to provide an understanding of the fluid dynamics of the Turbosep and also to examine the effects of design changes on performance in terms of particle collection efficiency. The Turbosep is shown schematic in Figure 1.6 while detailed drawings are presented in Appendix A.

The CFD analysis was carried out using CFDS-FLOW3D version 4.0 (AEA Technology, Computational Fluid Dynamics Service) which comprised the following:

Pre-processor      CFX MESHBUILD
Processor          CFX F3D
Post-processor     CFX VIEW and CFX VISUALISE

All simulations were run on an IBM RS6000 workstation with 128Mbytes of RAM, under an AIX operating system.

2.5.1 How CFD Codes Work.

The three stages of any CFD analysis are problem definition, simulation and analysis of results and these are performed in the pre-processor, solver and post-processor respectively. The geometry of the problem domain is defined in the pre-processor, i.e., the size and shape of the flow domain and the position of any inlets and outlets. The flow domain is then divided into a grid of control volumes, the solution to the flow problem being defined at a point (or node) within each control volume. The numerical solution to the flow problem is performed by the solver. CFDS-FLOW3D uses a finite volume method to solve the fundamental equations of fluid flow; the Navier-Stokes equations. The numerical algorithm comprises three steps. The first step involves the formal integration of the governing equations over all the (finite) control volumes of the solution domain. These integral equations are then converted into a system of algebraic equivalents by the substitution of a variety of different finite-difference approximations. The final step involves the solution of the algebraic equations by an iterative method.
The results of the simulation can then be displayed in a range of different graphical output forms in the post-processor.

2.5.2 Basic Analysis Assumptions.

Air flow at 293 K was assumed, which was taken to be 3-dimensional, Newtonian, turbulent, isothermal, incompressible and at steady state.
3. CFD Theory and Model Development.

Research on the particle collection characteristics of cyclones has received much attention with some of the earliest research dating back to the 1930’s. However, due to the development of complex fluid flow patterns within the cyclone, the separation process is still not fully understood, and the experimentally developed predictive models are usually only applicable to a group of specific cyclone geometry’s.

CFD has previously been used to model the performance of a range of small sampling cyclones (Griffiths and Boysan, 1996). Performance curves were produced with approximately the same shape and \( d_{50} \) as those obtained by experiment. Furthermore the predicted pressure drops were in excellent agreement with the measured data. Due to the similarities between the cyclone and the Turbosep it was believed that CFD would provide a reliable way of predicting the performance of the Turbosep and potentially provide better correlations with experimentally derived data compared to other semi-empirical methods.

A brief summary of CFD and details of the software used were presented in Section 2.5. Some other aspects of CFD are explained in Appendix D. The current section begins by describing in more detail the theoretical aspects of CFD, and then describes the development of a CFD model for simulating the fluid flow and particle capture in the Turbosep.

3.1 Theoretical Aspects of CFD.

3.1.1 Grid Effects.

3.1.1.1 Grid-Independence.

The solution to a flow problem is defined at nodes inside each control volume. The number of control volumes in a grid governs the accuracy of a CFD solution. In general, the larger the numbers of control volumes, the better the solution accuracy, however the computational cost associated with a large number of control volumes is high. Therefore
a compromise between solution accuracy and computational cost must be reached. To
determine the appropriate number of control volumes, it was necessary to begin with a
course grid and then make it progressively finer until the solution did not change
significantly, i.e. only within a few percent. Further details concerning the numbers of
control volumes required to obtain grid-independence in the Turbosep is given in
Section 3.2.1.2.

3.1.1.2 Grid Refinement.

Optimal grids are often non-uniform; finer in areas where large variation occurs from
point to point and courser in regions with relatively little change. A self-adapting
gridding capability was not incorporated into the CFD code therefore it was down to the
user to define the optimal grid. Such an approach was exploited in this research to
maximise the available computational resources.

3.1.2 Convergence Criteria.

Convergence of a CFD simulation is a necessity to obtain accurate results. Over the time
course of the simulation, the residual was plotted to determine whether the calculation
was proceeding successfully. At the end of each iteration the calculated variables were
substituted into the Navier-Stokes equations (see Appendix D). If the exact solution had
been obtained the individual components would sum to zero. However, as the CFD
calculated solution is only an approximation, the components values will always be
greater than zero. This summed value is described as the residual error and this should
be reduced over the time-course of the simulation. For each simulation the mass source
residual was plotted, which is the sum of the absolute values of the net fluxes into and
out of each control volume in the computational domain. It has dimensions of mass /
time.
3.1.3 **Pressure Velocity Coupling Algorithms.**

The solution of the Navier-Stokes equations presents three particular problems:

- The convective terms of the momentum equations contain non-linear quantities.
- The momentum equations are intricately coupled because every velocity component appears in each momentum equation and in the continuity equation.
- There is no transport equation for pressure yet it appears in all the momentum equations.

If the pressure gradient was known, the process of obtaining discretised equations (see Section 3.1.5) for velocities from the momentum equations would be the same as for any other scalar. However, flow computations calculate the pressure fields as part of the solution, so its gradient is not known beforehand.

For compressible flows, the continuity equation can be used as a transport equation for density and the energy equation for the transport of temperature. The pressure may then be obtained from the density and temperature by using the equation of state:

\[ p = p(\rho, T) \]  

If the flow is incompressible the density is constant and hence by definition is not linked to pressure. In this case, coupling between pressure and velocity introduces a constraint on the solution of the flow field such that if a correct pressure field is applied in the momentum equations, the resulting velocity field should satisfy continuity.

Both the problems associated with the non-linearity in the equations and the pressure-velocity linkage can be resolved by adopting an iterative solution strategy such as the SIMPLE algorithm of Pantankar and Spalding (1972).
3.1.3.1 The SIMPLE Algorithm.

The SIMPLE algorithm (semi-implicit method for pressure linked equations) is essentially a guess and correct procedure for the calculation of pressure. A guessed pressure field, $p^*$ is used to solve the momentum equations to yield the velocity components $u^*$, $v^*$ and $w^*$. The correction $p'$ can be defined as the difference between the correct pressure field, $p$ and the guessed pressure field, $p^*$ so that:

$$p = p^* + p'$$  \hspace{1cm} 3.2$$

Similarly the velocity correlations $u'$, $v'$ and $w'$ can be defined to relate the correct velocities $u$, $v$ and $w$ to the guessed velocities $u^*$, $v^*$ and $w^*$:

$$u = u^* + u'$$ \hspace{1cm} 3.3$$

$$v = v^* + v'$$ \hspace{1cm} 3.4$$

$$w = w^* + w'$$ \hspace{1cm} 3.5$$

These corrections are then used to update the pressure and velocity fields.

3.1.3.2 The SIMPLER Algorithm.

The SIMPLER (SIMPLE Revised) algorithm of Pantankar (1980) is an improved version of SIMPLE. In this algorithm the discretised continuity equation is used to derive a discretised equation for pressure instead of the pressure correction equation of SIMPLE. Thus, the intermediate pressure field is obtained directly without the use of a correction. Velocities are still obtained through the velocity corrections of SIMPLE.

3.1.3.3 The SIMPLEC Algorithm.

The SIMPLEC (SIMPLE-Consistent) algorithm of Van Doormal and Raithby (1984) follows the same steps as the SIMPLE algorithm, with the difference that the
momentum equations are manipulated so that the SIMPLEC velocity corrections omit terms that are less significant than those omitted in SIMPLE.

![Diagram](image)

**Figure 3.1** Sequence of operations for the SIMPLE algorithm.

### 3.1.3.4 The PISO Algorithm.

The PISO (Pressure Implicit with Splitting of Operators) of Issa (1986) is a pressure velocity calculation developed originally for the non-iterative computations of unsteady compressible flows. It has, however been adapted successfully for the iterative solution of steady state problems. PISO involves one predictor step and two correction steps and may be seen as an extension of SIMPLE enhanced with a second corrector step.

A comprehensive comparison of SIMPLEC, SIMPLER AND PISO for a variety of flow conditions has been carried out elsewhere (Jang et al, 1986).
3.1.4 Under Relaxation Factors.

Under relaxation had several interlinked purposes in the solution process. Firstly, it reduced the amount by which a variable would change between iterations if the discrete transport equations were solved as they stand. Thus difficulties caused by non-linearity are overcome. Secondly the linear solver was given an easier equation to solve.

The pressure correction equation is susceptible to divergence unless some under relaxation is used during the iterative process. New improved pressures, \( p^{\text{new}} \) were obtained using the following equation:

\[
p^{\text{new}} = p^* + \alpha_p p'
\]

Where \( \alpha_p \) is the pressure under relaxation factor. If \( \alpha_p \) is equal to unity then the guessed pressure, \( p^* \) was corrected by \( p' \). However if the guessed pressure was too far from the final solution, the pressure correction term was often too large for stable computations. If \( \alpha_p \) was chosen to be zero then no correction was applied. A value of \( \alpha_p \) (between 0 and 1) was chosen which was large enough to move the iterative process forward, but small enough to ensure stable computations.

Similarly the velocities were also under relaxed, the iteratively improved velocity components \( u^{\text{new}}, v^{\text{new}} \) and \( w^{\text{new}} \) are obtained from:

\[
\begin{align*}
    u^{\text{new}} &= \alpha_u u + (1 - \alpha_u) u^{(n-1)} \\
    v^{\text{new}} &= \alpha_v v + (1 - \alpha_v) v^{(n-1)} \\
    w^{\text{new}} &= \alpha_w w + (1 - \alpha_w) w^{(n-1)}
\end{align*}
\]

Where \( \alpha_u, \alpha_v \) and \( \alpha_w \) were the u-, v- and w- under relaxation factors, \( u, v \) and \( w \) were the corrected velocity components without under relaxation and \( u^{(n-1)}, v^{(n-1)} \) and \( w^{(n-1)} \) represent the values obtained in the previous iteration.
3.1.5 Discretisation: Finite Difference Scheme.

Discretisation is a technique used to substitute the finite difference approximations into the governing fluid flow equations, thus converting them into their algebraic equivalents that can be solved by the CFD code.

Each transport equation was integrated over each control volume, to obtain the discretised equation that connected the variable at the centre of any control volume with its neighbours. Apart from the continuity equation, all of the equations had the same form. For a general property, \( \theta \), the integral, steady-state convection-diffusion equation is:

\[
\int_A n \cdot (\rho \partial \theta) dA = \int_A (\nabla \cdot r V) \theta dA + \int_{\partial V} S_\theta dV
\]

3.10

Where \( \Gamma \) is the relevant effective diffusivity for the variable \( \theta \).

This equation represents the flux balance over a control volume. The left-hand side gives the net convective flux and the right hand side contains the net diffusive flux and the generation or destruction of the property, \( \theta \), within the control volume (referred to as source terms).

All the terms were discretised in space using a finite differencing treatment. A central differencing scheme (see Table 3.1) worked well for the diffusion and source terms (on the right hand side of equation 3.10). However, the treatment of the convective terms was more crucial to the accuracy of the CFD results. A central differencing scheme could be used for the diffusive term because the diffusion process affects the distribution of a transported quantity in all directions. In contrast, convective processes only exert their influence in the flow direction. When convective forces are more significant than diffusive ones, the central differencing scheme requires a large number of control volumes to obtain physically realistic results. In these cases it was preferable to use an alternative differencing scheme. The general properties of the discretisation schemes are discussed in Section 3.1.5.1 and individual schemes are described in Table 3.1.
3.1.5.1 Properties of Discretisation Schemes.

Three mathematical concepts are useful in determining the success or otherwise of an algorithm.

- **Convergence.**
  Convergence is the property of a numerical method to produce a solution that approaches the exact solution as the grid spacing, control volume, or element size is reduced to zero.

- **Consistency.**
  The numerical scheme must produce systems of algebraic equations that can be demonstrated to be equivalent to the governing equations, as grid spacing tends to zero.

- **Stability.**
  Stability is associated with the damping of errors as the numerical method proceeds. An unstable method will oscillate widely or diverge.

A robust scheme is an accepted alternative to the more rigorous mathematical concepts described above. The essential properties of a robust scheme are conservativeness, boundedness and transportiveness.

- **Conservativeness.**
  Ensures the global conservation of $\theta$ for the whole solution domain in addition to the local conservation of the fluid properties within each control volume that is guaranteed by the finite volume approach. Conservativeness is achieved by representing the flux through a control volume faces in a consistent manner.

- **Boundedness.**
  This is the requirement that successive updates of a variable, $\theta$ leads from the initial guessed distribution to a fully converged solution. If the differencing scheme produces coefficients that satisfy this criterion the resulting matrix of coefficients is diagonally dominant. To ensure diagonal dominance source terms of variable $\theta$, (where $\theta$ is either
being created or destroyed) should always be negative. In the absence of source terms the values of \( \theta \) in a control volume should be bounded by its boundary values. Another essential requirement of boundedness is that all coefficients of the discretised equations should have the same sign. Thus an increase in the value of \( \theta \) in one control volume should result in an increase in \( \theta \) in all neighbouring control volumes.

- **Transportiveness.**

  The dimensionless cell Peclet number, defined as the ratio of the convective mass flux per unit area over the diffusion conductance, is a measure of the relative strengths of convection and diffusion acting on \( \theta \). In the case of pure diffusion (\( Pe = 0 \)) \( \theta \) tends to spread equally in all directions. In the case of pure convection (\( Pe \to \infty \)) \( \theta \) spreads almost exclusively in the direction of the flow. Thus transportiveness can account for the directionality of both convection and diffusion influences on \( \theta \).

### 3.1.5.2 Types of Finite Differencing Scheme.

This section describes the convention for naming nodes and control volume boundaries. To evaluate fluxes at control volume faces, an approximate distribution between nodes was used; the different types of approximation are explained in Table 3.1.

If a node is considered to be at the centre of each control volume, then the boundaries (or faces) of each neighbouring control volume are positioned midway between adjacent nodes. The system of notation used in CFD codes is illustrated in Figure 3.2. A general nodal point is identified by \( P \) and its neighbours to the north, south, east and west are named \( N, S, E \) and \( W \) respectively. The boundaries of the control volume to the north, south, east and west are similarly denoted \( n, s, e \) and \( w \). The distance between the node \( P \) and one of its neighbours e.g. \( W \) is identified by \( \delta x WP \), similarly the other boundaries follow the same pattern.

Each finite differencing scheme will be described by explaining how the value of a property, \( \theta \), at the east and west cell faces is approximated. The properties of each scheme are summarised in Table 3.1.
Figure 3.2 Control volume notation.
Table 3.1 Comparison of Finite Difference Schemes.

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Comments</th>
<th>Value of Variable $\theta$, at E and W faces (when mean flow is in a E to W direction).</th>
<th>Conservative?</th>
<th>Bounded?</th>
<th>Transportive?</th>
<th>Accuracy (Taylor series errors)</th>
</tr>
</thead>
</table>
| Central | Used to represent the diffusion terms of equation 3.10                               | $\theta_w = \frac{1}{2}(\theta_w + \theta_p)$  
$\theta_e = \frac{1}{2}(\theta_e + \theta_p)$ | Yes           | Yes (but only when $Pe < 2$) | Yes               | 2nd                           |
| Upwind  | Takes into account the flow direction when determining the value at a cell face. The convected value of $\theta$, at a cell face is taken to be equal to the value at the upstream node. | $\theta_w = \theta_w$  
$\theta_e = \theta_e$ | Yes           | Yes      | Yes               | 1st                           |
| Hybrid  | Based on a combination of central and upwind differencing schemes. The central differencing scheme is employed at low $Pe$ numbers. The upwind scheme is used at high $Pe$ numbers. | Yes           | Yes      | Yes               | 1st (if $Pe > 2$)  
2nd (if $Pe < 2$) |
Table 3.1 Continued.

|-----------------------|----------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------|-----------|---------------|----------|
| Higher Order Upwind   | Upwind scheme with second order accuracy by extrapolating to the face from two upwind points. This type of scheme is less compact because of the presence of terms such as $\theta_{ww}$ and $\theta_{ee}$. Incorporating more neighbouring points reduces discretisation errors. | $\theta_e = \frac{3}{2} \theta_w - \frac{1}{2} \theta_{ww}$  
$\theta_e = \frac{3}{2} \theta_w - \frac{1}{2} \theta_e$ | Yes           | Yes       | Yes          | 2nd      |
| QUICK                 | Quadratic Upstream Interpolation for Convective Kinetics (Leonard, 1979) scheme uses a three point upstream-weighted quadratic interpolation for cell face values. The face value of $\theta$ is obtained from a quadratic function passing through two bracketing nodes on each side of the cell face and a node on the upstream side. | $\theta_w = \frac{3}{8} \theta_p + \frac{3}{4} \theta_e - \frac{1}{8} \theta_{ww}$  
$\theta_e = \frac{3}{8} \theta_p + \frac{3}{4} \theta_e - \frac{1}{8} \theta_e$ | Yes           | Yes       | Yes          | 3rd      |
| CCCT Modified QUICK   | A modified QUICK (Alderton and Wilkes, 1988) scheme to attempt to alleviate the instabilities associated with QUICK (Leonard, 1979). | $\theta_w = \left(\frac{3}{8} - \beta\right) \theta_p + \left(\frac{3}{4} + 2\beta\right) \theta_e - \left(\frac{1}{8} + \beta\right) \theta_{ww}$  
$\theta_e = \left(\frac{3}{8} - \beta\right) \theta_e + \left(\frac{3}{4} + 2\beta\right) \theta_e - \left(\frac{1}{8} + \beta\right) \theta_e$ | Yes           | Yes       | Yes          | 3rd      |
3.1.6 Turbulent Flow and Turbulence Modelling.

Many if not most flows of engineering significance are turbulent. However the analytical treatment of turbulence is not as well developed as that of laminar flow. Due to the importance of turbulent flow it is essential that it is modelled correctly.

In turbulent flow the fluid and flow variables vary with time such that the instantaneous velocity vector will differ from the averaged velocity vector in both magnitude and direction. As a kind of motion it can be characterised by the following properties.

- Turbulent flows exhibit a random and unsteady variation in both space and time. These fluctuations occur over a wide range of length scales and frequencies.

- The mean flow may be one, two or three-dimensional but the turbulence is always three-dimensional.

- Turbulent flows are strongly diffusive. Turbulence results in the rate of diffusion process to increase to levels far greater than those due to molecular diffusion.

3.1.6.1 Turbulence Modelling.

The Navier-Stokes equations provided a valid description for nearly all practically relevant flows, including turbulent ones. However, it is not currently practical to solve the equations with appropriate boundary conditions using numerical procedures for the three dimensional, non-linear, coupled differential equations. Neither is it practical to utilise a three-dimensional grid as the computational costs would be too excessive.

Since the effect of turbulence upon the mean properties of the flow is generally more relevant than the details of the turbulent motion, there appears to be no need to solve the equations for the instantaneous variables if the averaged values are all that is required.

The decomposition of the instantaneous variable, $\hat{\theta}$, into a mean ($\bar{\theta}$) and fluctuating ($\theta$) components was proposed by Reynolds (1874), thus:
\[ \bar{\theta} = \bar{\theta} + \theta \]  

3.11

The mean component is the long-term average of the instantaneous value.

These definitions are introduced into the instantaneous Navier-Stokes equations and the results averaged. This averaging process introduces some unknown turbulence correlations into the equations, which are known as Reynolds or turbulence stresses. Physically these represent the rate at which momentum is transported by turbulent fluctuations. They have a magnitude many times greater than their laminar counterparts, which accounts for the rigorous mixing associated with turbulent flows. However the Reynolds stresses render the equations insoluble since there are now more unknowns than there are equations.

A turbulence model consists of a set of differential equations and/or algebraic formula that allows for the determination of the Reynolds stresses and hence close the time-averaged equations of fluid motion. The model provides a numerical value for the Reynolds stresses at each point in the flow by adopting one of the approaches described below. The advantages and disadvantages of the different turbulence models are summarised in Table 3.2.

3.1.6.2 Eddy-Viscosity Models.

The eddy-viscosity hypothesis is based on the assumption that the Reynolds stresses are related to the viscous stresses on the mean flow. The eddy-viscosity, \( \nu_t \), is not a function of the fluid, but rather of the flow.

Dimensional analysis of \( \nu_t \) reveals the following relationship that is representative of the large scale of turbulence:

\[ \nu_t \propto \text{velocity scale} \times \text{turbulence length scale} \]  

3.12

The most commonly employed turbulence model, the k-\( \varepsilon \) model (Lauder and Spalding, 1974) utilises the eddy-viscosity hypothesis for turbulence. The eddy-viscosity is
evaluated by the solution of two partial differential transport equations, one for the
turbulent kinetic energy, \( k \) and one for the rate of dissipation of the turbulent kinetic
energy, \( \varepsilon \). The \( \varepsilon \) equation represents a balance between the rate of transport of \( \varepsilon \) by the
mean flow, its rate of diffusion by turbulent fluctuations, its generation by vortex
stretching and its destruction by the action of viscosity.

### 3.1.6.3 Reynolds Stress and Algebraic Stress Turbulence Models.

In the Reynolds stress turbulence model, the Eddy-Viscosity hypothesis is not invoked.
Instead the Reynolds stresses are obtained directly from the solution of differential
transport equations in which they are the dependent variables.

The Algebraic stress model is an economical way of accounting for the anisotropy of the
Reynolds stresses without going to the full length of solving the Reynolds stress
transport equations. In this model the convective and diffusive terms are omitted thus
reducing the Reynolds stresses to a set of algebraic equations.

The advantages and disadvantages of the different turbulence models are described in
Table 3.2.
Table 3.2 Turbulence models utilised by the CFD code.

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>k-( \varepsilon )</td>
<td>• Simplest, most computationally efficient model.</td>
<td>• Assumes Reynolds stress isotropy that results in poor performance in swirling flows.</td>
</tr>
<tr>
<td></td>
<td>• Only initial and boundary conditions need to be supplied.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Widely validated for a number of industrially relevant flows.</td>
<td></td>
</tr>
<tr>
<td>Reynolds Stress</td>
<td>• The most general of all turbulence models.</td>
<td>• Very large computing cost (seven extra PDEs).</td>
</tr>
<tr>
<td>Stress (RSM)</td>
<td>• Only initial and boundary conditions need to be supplied.</td>
<td>• Not as widely validated as the k-( \varepsilon ) model.</td>
</tr>
<tr>
<td></td>
<td>• Accurate calculations of mean flow properties and all Reynolds stresses</td>
<td>• Performs just as poorly in some flows owing to identical problems with the ( \varepsilon )-equation modelling (e.g. unconfined recirculating flows).</td>
</tr>
<tr>
<td></td>
<td>for both simple and complex flows.</td>
<td></td>
</tr>
<tr>
<td>Algebraic Stress</td>
<td>• Cheap method to account for Reynolds stress isotropy.</td>
<td>• Only slightly more computationally expensive than the k-( \varepsilon ) model.</td>
</tr>
<tr>
<td>Stress (ASM)</td>
<td>• Combines the generality of the RSM with the computational economy of the</td>
<td>• Not widely validated</td>
</tr>
<tr>
<td></td>
<td>k-( \varepsilon ) model.</td>
<td>• Severely restricted in flows where convection and diffusion terms are significant.</td>
</tr>
<tr>
<td></td>
<td>• If convection and diffusion are negligible the ASM performs as well as</td>
<td></td>
</tr>
<tr>
<td></td>
<td>the ASM.</td>
<td></td>
</tr>
</tbody>
</table>
3.2 The Application of CFD in Modelling the Performance of the Turbosep.

3.2.1 Problem Definition and Simulation Details.

The Turbosep has previously been described in Section 1.5 with detailed drawings presented in Appendix A. The primary purpose of this study was to examine the effectiveness of CFD in modelling and assessing the performance of the Turbosep. The CFD model was used to determining the following:

- To predict the pressure drop across the Turbosep
- To simulate the flow and capture of bioaerosols within the Turbosep using different inlet boundary conditions.

3.2.1.1 Specification of Initial Boundary Conditions.

3.2.1.1.1 Inlet Boundary Conditions.

A mass flow boundary defines a flow into or out of a system. In CFX F3D these boundary conditions can be defined in two ways:

- A fixed boundary condition. The magnitude and direction of flow defined initially does not change over the course of the simulation.

- A variable boundary condition. The flow magnitude and direction change as the simulation progresses, depending upon the flow solution throughout the domain.

The Turbosep unit used (the smallest sized in the range) had a rated air flow range of 100 - 500 Lmin\(^{-1}\), therefore, by specifying an inlet flow rate and assuming both a linear velocity distribution across the inlet plane and steady state, the inlet velocity could be calculated and defined as a fixed boundary condition. As the velocities at the underflow and overflow ducts were not known they were defined as variable boundaries with the velocity being calculated as part of the solution.
3.2.1.2 Wall Boundary Conditions.

Immediately adjacent to each solid surface is a boundary layer comprising of a thin viscous sub-layer and a buffer layer. To calculate the flow fields in these regions without employing excessively fine grids, a no slip velocity boundary condition was used. The normal component of the velocity was set to zero at the turbulent boundary layer allowing the discretised momentum equations at the adjacent cells to be evaluated without modification. For all other scalar variables the logarithmic law of the wall was implemented.

3.2.1.2 Grid Independence and Convergence Behaviour.

The computational domain of the Turbosep was discretised in space using a full three-dimensional Cartesian co-ordinate system and a non-orthogonal grid structure. Figure 3.3 shows the effect of the number of control volumes on the predicted axial velocity profile measured across a horizontal plane perpendicular to the end of the vortex finder. It can be seen that grid independence was achieved when 80000 control volumes were used. However, due to sharp velocity fluctuations as the flow passes over the vanes and impingement plate (see Figure 3.6), the grid was further refined in these regions to increase solution accuracy. This resulted in the number of control volumes increasing to 91000. A two-dimensional representation of the computational grid is shown in Figure 3.4.

3.2.1.3 The Effect of the Turbulence Model and Finite Differencing Scheme on the Accuracy of the CFD Solution.

Due to turbulent flow in the Turbosep, the key to success in CFD lies with the accurate description of the turbulent behaviour. A number of turbulence models are available (described in Table 3.2) ranging from the industry standard k-ε model to the more complex Reynolds Stress model. However, the k-ε model has shown to be inadequate for the calculations of flows with swirl (Boysan et al, 1982) because it leads to excessive levels of turbulent viscosity and unrealistic tangential velocity distributions.
Figure 3.3 The effect of number of control volumes on the CFD predicted axial air velocities across the radius of the Turbosep at a simulated air flow rate of 500 Lmin⁻¹. The vortex finder is positioned at a radius of 4 mm.

The Reynolds Stress model performs much better than the k-ε model in swirling flows, but it has the disadvantage of being computationally expensive. Turbulence models based on statistical rather than continuum mechanics such as the RNG k-ε model (Yakhot and Smith, 1992) have been reported to have the mathematical simplicity of the k-ε model whilst having the accuracy of the Reynolds Stress model. The main difference between the k-ε model and the standard one is in the calculation of the turbulent viscosity, which in the case of the RNG k-ε model uses an ordinary differential equation, which includes the effect of rotation.

To select the most suitable turbulence model for describing the turbulent behaviour in the Turbosep a series of comparative simulations were performed using both the standard and RNG k-ε models. Due to the high computational demand of the Reynolds Stress model and the limited computer power available, the model wasn’t utilised during the course of the project, the increased computational time required to achieve a converged solution being prohibitively long.
Figure 3.4 A two-dimensional representation of the computational grid employed. The grid was composed of 91000 control volumes.

Figure 3.5 shows a comparison of the experimentally measured and CFD predicted pressure drops across the Turbosep. The RNG k-ε model was found to provide an excellent correlation with the experimental data. The standard k-ε model performed less well, the false predictions being attributed to the generation of anisotropic Reynolds stresses caused by the swirling motion of the fluid (see Table 3.2). Moreover, the sudden deviation of the k-ε model from the experimental data at a flow rate of 500 L.min\(^{-1}\) can be explained by the development of increased swirl at the vanes resulting in a greater level of anisotropy throughout the computational domain.
The finite differencing scheme has been reported to have an effect upon the accuracy of a CFD simulation, although the more accurate schemes tend to be less robust or slower. Indeed when the unmodified QUICK and CCCT schemes were used the solution diverged. However as grid independence had been achieved the hybrid differencing scheme would be expected to have only a minimal effect on solution accuracy. This is evident in Figure 3.5 where the hybrid scheme used in combination with the RNG k-ε turbulence model was able to accurately predict the pressure drop across the Turbosep. Based on the previous discussions, all further CFD simulations used a computational grid composed of 91000 control volumes, the RNG k-ε turbulence model, a hybrid treatment for convection and the default SIMPLEC algorithm (Van Doormal and Raithby, 1984) to couple pressure and velocity. A high level of under relaxation (see Section 3.1.4) was also required for the models to converge adequately.
3.2.1.4 CFD Predictions of the Air Flow in the Turbosep.

Figure 3.6 shows a vector plot of the flow field generated from the CFD model at a simulated air flow rate of 500 L.min⁻¹, the maximum rated flow capacity of the Turbosep used (for clarity the azimuthal components of velocity have been suppressed). The flow field is also presented as a velocity contour plot in figure 3.7. It can be seen that the flow forms descending and ascending streams which flow parallel to one another before leaving the Turbosep through the vortex finder. This behaviour is expected and is due to the fact that swirling flows have a tendency to resist radial motion.
After entering the Turbosep, the main body of the flow passes through the vanes which impart a swirling motion. The flow then streamlines along the outer wall towards the bottom of the Turbosep. The acceleration of the flow as it passes along the outer wall of the vortex finder is due to area reduction. Upon reaching the vortex arrester, this stream changes direction and moves upward towards the vortex finder.

![Figure 3.7 Velocity contour plot of the flow field generated from the CFD model at a simulated air flow rate of 500 Lmin⁻¹.](image)

**Figure 3.7** Velocity contour plot of the flow field generated from the CFD model at a simulated air flow rate of 500 Lmin⁻¹.
Figure 3.8 shows the pressure field generated from the CFD model at a simulated air flow rate of 500 Lmin⁻¹. It appears that the pressure loss within the Turbosep is principally due to the constriction of the flow as it passes over the vanes and impingement plate and streams along the outer wall of the vortex finder.

Figure 3.8 Pressure contour plot of the flow field generated from the CFD model at a simulated air flow rate of 500 Lmin⁻¹.
Boysan *et al.* (1982) discussed how the prediction of the correct shape and quantitative features of the velocity field is crucial in terms of defining both collection efficiency curves and pressure drops, and is highly sensitive to the turbulence model used. The tangential velocity profile measured across a horizontal plane perpendicular to the end of the vortex finder (see Figure 3.9) exhibits a typical combined vortex structure (Griffiths and Boysan, 1996) i.e. the tangential velocity increases with increasing radius from the core and reaches a maximum approximately at the vortex finder radius, and decreases thereafter with increasing radius. The axial velocity profile has previously been shown in Figure 3.3.

![Tangential velocity profile](image)

**Figure 3.9** The CFD predicted tangential velocity profile across the radius of the Turbosep at a simulated air flow rate of 500 Lmin⁻¹. The vortex finder is positioned at a radius of 4 mm.
3.2.2 Particle Trajectories and Collection Efficiency.

Having obtained the fluid flow solution for the Turbosep, the trajectories of different sized particles was modelled to simulate the flow and capture of bioaerosols in the Turbosep.

3.2.2.1 Numerical Approach to Particle Transport.

Particle transport was carried out using a discrete trajectory Langrangian approach. The total flow of particles was modelled by tracking a small number of particles individually through the continuum phase. It was assumed in the simulation that the volumetric ratio of the concentration of the dispersed particles to that of the air was small and the particles were relatively far apart. Therefore the impact of the continuum phase due to the presence of the particles can be neglected but also can particle-particle interactions.

For ease of tracking the particle and where it crosses control volume boundaries, the tracking was carried out in three-dimensional computational space. The equations for position have the form:

\[ C = \frac{d\xi}{dt} \] 3.13

Where \( C \) is the computational velocity, \( \xi \) is the computational position and \( t \) is time.

The computational velocity and position can be related back to the velocity and position in physical space.

The particle trajectory equations for a range of particle sizes injected from different locations along the inlet plane was calculated to obtain cut sizes that corresponded to different operating conditions. One such calculation for a given particle diameter gives the fractional collection efficiency for that size. If these calculations are repeated for different sizes, it is possible to construct an entire collection efficiency curve and determine the \( d_{50} \).
3.2.2.2 Particle Tracking Boundary Conditions.

The initial boundary conditions used as a basis for predicting the Lagrangian transport of particles can have a substantial impact upon the subsequent trajectories calculated by the CFD simulation. This section describes the selection of the initial conditions, such as particle size distribution, density and the coefficient of restitution.

*E. coli* is considered to have an average diameter of 1 μm while *S. cerevisiae* has an average diameter of 5 μm. However, when organisms are released in the form of an aerosol, particle coalescence will occur and composites will form with salts in solution. The average diameter of the aerosolised micro-organism will also vary according to the concentration of organisms within the cell suspension (Bennett and Norris, 1989; Hambleton et al, 1992). The only readily available information concerning the size of aerosolised micro-organisms is provided by Ferris (1995), although even this data has its limitations since the sample measured was only a fraction of that released. Based on the results of Ferris, the size distribution of aerosolised micro-organisms has been taken to be:

*E. coli* 1-10 μm diameter, equally distributed  
*S. cerevisiae* 2-10 μm diameter, normally distributed, average 5 μm.

An analogous problem to that of defining particle size is that of particle density. This will vary according to the level of amalgamation between the organism and the salts in the original suspension. Agutter (1997) showed that the difference in density had only a minimal effect upon the CFD predicted trajectories in a biological containment cabinet. Although gravitational forces have a greater effect upon the more dense particles, this effect is slight and does not influence the paths taken by the organisms. Based on the results of Ferris, the density of *E. coli* was assumed to be 1400 kg/m³.

The coefficient of restitution defines the behaviour of a particle when it hits a surface. It is the ratio of its rebound velocity normal to the surface compared to the same component of its incoming velocity. If the value is zero the particles will stick to the surface but as the value increases to the maximum value of 1 then the particles will tend
to rebound with increasing momentum. A micro-organisms will tend to stick to walls, however the extent to which it does so is unknown. The situation is further complicated due to the recirculating flow patterns observed in the inlet section of the Turbosep. These high velocity secondary flows could re-entrain particles adhered to a wall back into the continuum fluid. A number of authors have investigated the deposition and re-entrainment of particles on surfaces (Dahneke, 1975; Paw and Braaten, 1995; Dunn et al, 1995 and Reynolds, 1999). However, these studies were all based on synthetic particles and it has been acknowledged that the coefficient of restitution is different for each material. Silica glass has been shown to have a coefficient of restitution of 0.95 (Paw and Braaten, 1995). Agutter (1997) used CFD to simulate the flow and deposition of E.coli cells onto the walls of a biological containment cabinet. The author stated that the coefficient of restitution was a function of both the aerosol released and possibly the material out of which the cabinet was constructed. Furthermore, in order to obtain a good correlation with experimental data the author had to give half the micro-organisms a coefficient of restitution of 0 and half a coefficient of restitution of either 0.1 or 0.2.

A limitation of the CFD software is its ability to accurately model both the movement of particles through a turbulent boundary layer and the entrainment of particles onto a surface and due to the experimental difficulties in measuring the interaction of a microscopic particle with a surface, particularly at high impact velocities the coefficient of restitution will be determined on a trial and error basis.

3.2.2.3 CFD Predicted Particle Trajectories.

CFD simulations were performed using 100 particles with an identical size distribution. Collection statistics were obtained by assuming that particles had an identical coefficient of restitution, which was varied between 0 and 0.3. The results were compared to an experimentally constructed collection efficiency curve for dust particles (data supplied by Domnick Hunter, see Appendix E for further details). A simulated air flow rate of 500 Lmin$^{-1}$ was defined at the fixed boundary patches.

The calculated trajectories for 2, 5, 7.5 and 10 $\mu$m diameter particles in the Turbosep are shown in Figure 3.10. The different results reflect the effect of changing the coefficient
of restitution. In this instance, the curve derived with a coefficient of 0.1 provides a good correlation in terms of shape and \( d_{50} \) with the experimental collection efficiency curve. The CFD predicted \( d_{50} \) was determined to be 4.5 \( \mu \text{m} \).

Figure 3.10 Comparison of the experimental and CFD predicted collection efficiency in the Turbosep at a simulated air flow rate of 500 Lmin\(^{-1}\), (▲) Domnick Hunter experimental data, (♦) predicted with a coefficient of restitution of 0.0 (■) predicted with coefficient of restitution of 0.1, (●) predicted with coefficient of restitution of 0.2 and (▼) predicted with coefficient of restitution of 0.3.

Figure 3.11 shows the trajectories for 10 \( \mu \text{m} \) diameter particles injected from 100 locations along the inlet duct at a simulated air flow rate of 500 Lmin\(^{-1}\). In a ‘traditional’ cyclone, particle separation is due to the centrifugal forces generated by the swirling gas stream. However, it can be seen from the particle trajectories in Figure 3.11 that the swirling motion of the gas stream is quite weak, the particles only passing through approximately 1 rotation as they flow along the length of the Turbosep. A comparison of the maximum axial and tangential velocity components in Figures 3.3 and 3.9 respectively show how the axial component is approximately 4 times greater than the tangential component. This explains the low levels of swirl observed and further suggests that only weak centrifugal forces will be generated.
By following the trajectory for each particle it was possible to determine its outcome i.e. whether it was captured or escaped and in the former case where it was captured. It was found that all of the 10 μm diameter particles hit the vortex arrester and of which 81% were captured.

Figure 3.11 CFD predicted trajectories for 10 μm diameter particles injected from 100 locations along the inlet duct at a simulated air flow rate of 500 Lmin⁻¹. The coefficient of restitution was 0.1.

In the case of 2 μm diameter particles, 11% were captured on the walls as they flowed downwards along the length of the Turbosep, 84% hit the vortex arrester and of which proportion 14% were captured. The remainder (5%) of the particles escaped without hitting a wall. The mean velocity in the vicinity of the vortex arrester is very low (< 1ms⁻¹) and is in the direction of the vortex finder (see Figure 3.6). Therefore, the
probability of a particle that hits the vortex arrester being captured is more likely to be based on sedimentation according to Stokes Law rather than any centrifugal force. This theory would also explain why of all the particles that hit the vortex arrester, a much higher proportion of the 10 μm diameter particles are captured compared to the proportion of 2 μm diameter particles.

However, the question remains as to why 10 μm diameter particles are not captured on the walls as they flow down the length of the Turbosep. Agutter (1997) found that the likelihood of an organism to adhere to a wall was inversely proportional to its size. This phenomena is probably due to boundary layer effects. When a fluid reaches a surface the velocity in the neighbourhood of the surface changes, this change is proportional to the perpendicular distance from the surface and is brought about because of the viscous forces acting within the fluid. Thus the boundary layer has a velocity gradient, progressing from zero at the wall through to the bulk of the fluid. A small particle with a diameter less than the thickness of the boundary layer will be completely immersed in the boundary and will be more likely to be captured than a larger particle. Thus the probability of an organism sticking to a wall is not only dependent on the coefficient of restitution but also a function of the particle size.
4. Foam Control Using the Turbosep Mechanical Foam Separator.

Foaming is a common phenomenon in many industrial fermentations and often occurs to such an extent that the process is either severely impaired or even completely arrested. Traditional methods have focused on the elimination rather than the control of foam using antifoam agents, mechanical separators or a combination of both. However, foaming is beneficial in promoting good gas transfer within the fermenter (Cook, personnel communication) and so it would be better to control the extent of foaming rather than eliminate it entirely. The use of antifoam agents is well established and are usually administered when the foam level within the vessel reaches a pre-determined level. However, foam generation can be extremely volatile, and the working volume of the fermenter has to be reduced to prevent the potential loss of product and microorganisms into the exhaust gas stream (Bryant, 1970). A variety of different mechanical foam breaking devices have been developed in an attempt to achieve more cost effective fermentation foam control. Despite all these efforts, most of the techniques have proved ineffective on large scale foam mass, or foams produced at high aeration and agitation rates unless they are combined with the addition of large volumes of antifoam (Zlorkarnik, 1986). Thus, none of the techniques developed so far are entirely suitable or universally applicable to fermentation foams.

The containment of the fermenter exhaust gases is commonly achieved through the use of a chain of filters. However, blinding of the exhaust gas filtration system by contact with liquid or foam has been reported to be a major cause of accidental failure during a fermentation (Leaver and Hambleton, 1992) and increases the probability of microbial release. Thus any foam control strategy must be viewed as more than just a method for controlling foam build-up but also as a requirement for maintaining containment integrity.

The Turbosep (see Section 1.5) offers a unique solution to the problem of fermentation foam control, providing an effective separation of entrained foam, liquids and aerosol from the exhaust gas without inhibiting the foaming process itself. An efficient use of the Turbosep would therefore manage the foaming process rather than eliminating it through the excessive use of antifoam. This should allow light foaming to be harnessed
as a means of effective gas transfer. However, under gross foam out conditions it is possible to exceed the capacity of the Turbosep causing it to overflow. The control strategy employed to prevent foam from passing downstream of the Turbosep has been previously described in Section 1.5.1. The efficiency of the Turbosep has been reported, not to be limited by its foam capacity, but rather by the flow capacity of the liquid return pipe (Rollinson, 1988). By positioning an expansion tank in the liquid return pipe between the Turbosep and the fermenter, any rise in foam or liquid can be detected. A standard foam probe inserted into the expansion tank detects such a build up and uses a conventional antifoam dosing system to inject antifoam directly into the return pipe. The antifoam is then transported into the fermenter where it acts to prevent further foam build-up. However the fouling of the foam probe and continual liquid presence within the expansion tank has been shown to interfere with the normal antifoam dosing strategy (Ridealgh, personnel communication). This causes variation in the total volume of antifoam administered during batches of the same fermentation and leads to problems in the control and reproducibility of each batch. The following sections describe the development of a novel foam control strategy to overcome these problems and demonstrates how the Turbosep can be designed to work as an integral part of the fermentation process, giving greater control and increased productivity.

4.1 Characterisation of a Model Foaming System.

Foaming is an inherent consequence of the aerobic fermentation process and is affected by the hydrodynamic conditions that in turn are affected by the introduction of gas, the nature and composition of the medium, the presence of growing cells and the physical conditions of the fermentation (Duitschaever et al, 1988). Furthermore, the foam load within a fermentation varies with time, with heavy peak production occurring for only relatively short periods. The degree of foam separation facilitated by the Turbosep was examined by challenging it with different foams over varying aeration rates under a defined set of experimental conditions. However, the dynamic complexity of fermentation foams prevents experiments being carried out under standardised conditions. This suggests that a simplified model foaming system was required that would display similar characteristics to a ‘typical’ fermentation foam, but would foam continually and uniformly over the time course of an experiment. The specific
characteristics of the foam that are important within this context of this study are the rate of foam formation ($R_f$) and rate of foam collapse ($R_c$).

Complex fermentation broths have high foaming tendencies, however their foaming characteristics have not been experimentally measured. To ensure that any model foaming system could realistically simulate a fermentation foam a quantitative study on the foaming characteristics of a fermentation broth was performed to obtain base values for $R_f$ and $R_c$ so that comparisons with potential model substrates could be made. Lauria broth (see Section 2.1.2.2) was selected for this purpose because it is a nutrient rich complex medium that can cause severe foaming problems in a variety of different fermentations. *E.coli* RV308 pHKY531 was cultured to stationary phase as described in Section 2.1.2.3.1 and foam characterisation was performed as described in Section 2.3. The experiments were carried out at an aeration rate of 10 Lmin$^{-1}$ (5 VVM) using both sterilised and unsterilised Lauria broth. Table 4.1 suggests that during sterilisation foam stabilising components are formed in Lauria broth, although it should be noted that the differences are not statistically significant.

**Table 4.1** Foam rise and collapse profile in Lauria broth.

<table>
<thead>
<tr>
<th></th>
<th>Rate of foam rise (unsterilised LB broth)</th>
<th>Rate of foam collapse (Unsterilised LB broth)</th>
<th>Rate of foam rise (sterilised LB broth)</th>
<th>Rate of foam collapse (Sterilised LB broth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>17 seconds$^{-1}$</td>
<td>648 seconds$^{-1}$</td>
<td>15 seconds$^{-1}$</td>
<td>743 seconds$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>+/- 1.6</td>
<td>+/- 73</td>
<td>+/- 1.1</td>
<td>+/- 98</td>
</tr>
</tbody>
</table>

Three semi-soluble substrates, egg albumen, bovine serum albumin and soybean flour were used to simulate fermentation foams and 50, 100, 150 and 200 mgL$^{-1}$ concentrations of each were prepared using tap water to simulate fermentation media. Each of these substrates is known to foam intensely, uniformly and constantly when aerated and have previously been used in experimental systems for testing antifoam (Vardar-Sukan, 1992). Each substrate was sterilised before use and foam characterisation was carried out as previously described.
Chapter 4. Foam Control using the Turbosep Mechanical Foam Separator

Figures 4.1 and 4.2 clearly indicate that $R_f$ increases with increasing concentration in each of the simulated biomedia. Notably at a concentration of 200 mg L$^{-1}$ the rate of foam formation in each of the substrates appears to be similar. When the $R_c$ rates are considered it is found that soybean flour produces the most stable foam. This is not unexpected, as soybean flour is used as a foam stabiliser in the food industry (Viestures, 1982). These results demonstrate the dependence of foaming on the nature of the biomedia.

A comparison of the $R_f$ and $R_c$ values for each of the substrates indicates that at an aeration rate of 10 Lmin$^{-1}$ (5 VVM), a 200 mg L$^{-1}$ concentration of egg albumin displays a similar foam rise and collapse profile to that of sterilised Lauria broth. A 200 mg L$^{-1}$ concentration of soybean flour also displays a similar foam rise profile, however the foam produced is approximately 4 times more stable. Therefore it was decided to use 200 mg L$^{-1}$ concentrations of egg albumin and soybean flour, subsequently referred to as Foam A and Foam B respectively as the model foaming systems in this study. Experimentally challenging the Turbosep with Foam A and B over a range of different air flow rates will allow a comparison of the effect of foam stability on the separational performance of the Turbosep to be made.

During a typical microbial fermentation the dissolved oxygen tension of the culture broth is maintained above a critical level with step increases in agitation and aeration rates. The rate of foam formation is known to be a function of this aeration rate and can be visualised as an increased foam burden during the latter stages of a fermentation when aeration rates are normally at their maximum. It follows that over the time course of a fermentation the foam challenge on the Turbosep will increase. To check that the model foams could realistically simulate the foaming dynamics of a fermentation the effects of varying aeration rate on $R_f$ and $R_c$ was measured. Figure 4.3 clearly shows that increasing aeration rate results in a linear increase in the foam formation rate for each model foaming system. Figure 4.4 demonstrates how the rate of foam collapse appears to be constant and independent to the rate of foam formation.
Chapter 4. Foam Control using the Turbosep Mechanical Foam Separator

Figure 4.1 Effect of concentration on the foam formation profile for (●) soyabean flour, (■) egg albumin and (▲) bovine serum albumin. Foam was produced by aerating each substrate at 10 Lmin⁻¹. Each point is the mean of three samples with its standard deviation.

Figure 4.2 Effect of concentration on the foam collapse profile for (●) soyabean flour, (■) egg albumin and (▲) bovine serum albumin. Foam was produced by aerating each substrate at 10 Lmin⁻¹. Each point is the mean of three samples with its standard deviation.
Chapter 4. Foam Control using the Turbosep Mechanical Foam Separator

Figure 4.3 Effect of aeration rate on foam formation profile for (●) 200 mgL⁻¹ soyabean flour and (■) 200 mgL⁻¹ egg albumin. Each point is the mean of three samples with its standard deviation.

Figure 4.4 Effect of aeration rate on the foam collapse profile for (●) 200 mgL⁻¹ soyabean flour and (■) 200 mgL⁻¹ egg albumin. Each point is the mean of three samples with its standard deviation.
As the rate of foam formation is a function of air flow rate, increasing the rate at which air is sparged into the vessel will allow the effect of increasing foam challenge on the separational performance of the Turbosep to be investigated. Furthermore, as the foam formation profiles for each of these model foams is similar, any changes in the performance of the Turbosep in terms of foam separation can be related directly to foam stability.

4.2 Fermentation Foam Control Using the Turbosep.

Antifoam addition into a fermenter fitted with a Turbosep is currently controlled using the expansion tank foam probe methodology. Similar to other foam control strategies this is a reactive system working to prevent the generation of foam, in line with the philosophy that allowing a fermentation to foam promotes loss of control.

As the foam enters the exhaust gas line the differential pressure will increase due to increased resistance to flow through the connecting pipe work, the vanes and the impingement plate. As the duration and severity of foaming are all direct indicators of the state of the fermentation, this information could be harnessed in a more pro-active control strategy; differential pressure being utilised to trigger antifoam addition during periods of heavy foam formation, with the time between injections being dependent on the phase of growth of the organism. During periods of light foaming, no antifoam addition would be required and this would result in improved gas transfer rates within the vessel.

The following section describes a series of experiments that were performed to identify whether differential pressure measurements across the Turbosep could be used as an accurate trigger point for anti-foam addition. The Turbosep was connected to a 250 L stainless steel stirred tank reactor as described in section 2.4.2. Static pressure tapings were positioned across the Turbosep and connected to the two ports of a differential pressure sensor. Foaming was induced by the aeration of 200 L of either Foam A or B varied between 150 - 500 L min$^{-1}$. Differential pressure readings were recorded when foam was first observed downstream of the Turbosep; that is at the onset of foam-over.
Chapter 4. Foam Control using the Turbosep Mechanical Foam Separator

Figure 4.5. Schematic PID for the differential pressure control of antifoam addition.

Figures 4.6 shows the effect of aeration rate on the differential pressure across the Turbosep. When the tank was empty the clean pressure drop, $\Delta P$ increased from 2.4 mBar at an airflow of 160 Lmin$^{-1}$ to 10.1 mBar at 300 Lmin$^{-1}$. During the initial stage of foam-over, $\Delta P_f$ increased from 9.2 mBar to 17.4 mBar for Foam A and from 16.2 to 22.9 mBar for Foam B at identical airflow rates. It is notable that for both model foams the gradient of $\Delta P_f$ is almost identical to the clean pressure drop ($\Delta P$) measured in the empty tank. The fact that the gradients are constant suggests that the increment in differential pressure with a change in airflow is almost entirely due to the movement of air through the Turbosep after the foam has been separated. Furthermore, the extent of the displacement in the differential pressure between the empty tank and Foams A and B is simply due to the weight of foam in the system.
Chapter 4. Foam Control using the Turbosep Mechanical Foam Separator

At the onset of foam-over, when the feed pipe to the Turbosep is full, it can be assumed that the volume of foam contained within the Turbosep, the recycle line and all connecting pipework will be momentarily constant and independent to the rate of further foam formation. At this instant, $\Delta P_r$ will be a function of aeration rate and foam stability. This explains the increased $\Delta P_r$ recorded for the more stable Foam B.

The relatively large errors observed at some of the sampling points can be attributed to the subjective nature of the experiment where the point of foam-over is determined visually. However there is a good correlation between the measured differential pressure and the volume of foam entering the system. This suggests that antifoam addition could be linked to the differential pressure generated as foam is separated and returned to the vessel. Triggering antifoam addition at a pre-determined value below $\Delta P_r$ will prevent the system from becoming overloaded and will also optimise the addition of antifoam. Unfortunately foam stability is related to a series of complex chemical and physical interactions, which effectively precludes mathematical analysis. Moreover, the stability of the foam changes over the course of a fermentation as metabolic products are excreted from the cells. It follows that the differential pressure trigger point would be unique for each fermentation process and would need to be determined experimentally. Typically this could be achieved during the commissioning stage.

Figure 4.7 shows the effect of aeration rate on the differential pressure measured between the fermenter headspace and a point downstream of the Turbosep. The increased differential pressure observed compared to that in Figure 4.6 is due to the extra pressure drop, principally due to the weight of foam in the connecting pipe work. Most industrial fermenters have an in-situ pressure sensor fitted in the headspace that could be exploited when upgrading to the differential pressure control strategy, thus both simplifying and reducing the capital costs of the retro-fit.
Figure 4.6 Effect of aeration rate on the differential pressure measured across the Turbosep when ‘foaming over.’ (■) clean pressure drop, (●) Foam A and (▲) Foam B. Each point is the mean of four samples with its standard deviation.

Figure 4.7 Effect of aeration rate on the differential pressure measured between the headspace and downstream of the Turbosep when ‘foaming over.’ (■) clean pressure drop, (●) Foam A and (▲) Foam B. Each point is the mean of four samples with its standard deviation.
Rollinson (1988) described how the separational efficiency of the Turbosep was limited to the flow capacity of the liquid return pipe. It follows that increasing the working volume of the liquid return pipe would effectively increase the foam capacity of the Turbosep and allow an increased rate of foam challenge without foam-over. Increased capacity would also reduce the antifoam requirements of the process thus improving the performance of the fermentation. The previous differential pressure experiments were repeated to determine how the installation of an expansion tank, which increased the effective volume of the liquid return pipe by 190 fold (see Appendix A for dimensions) could improve the degree of foam separation facilitated by the Turbosep.

Figures 4.8 and 4.9 shows the effect of aeration rate on the differential pressure ($\Delta P_f$) during the initial stage of foam-over. When the Turbosep was challenged with the less stable Foam A, no foam-over was observed. In this instance the rate of foam separation and return must have exceeded the rate of foam formation in the vessel. Additionally, $\Delta P_f$ for Foam B was reduced by 1-2 mBar compared to that in the absence of an expansion tank.

![Figure 4.8](image)

**Figure 4.8** Effect of aeration rate on the differential pressure measured across the Turbosep fitted with an expansion tank when ‘foaming over.’ (■) clean pressure drop and (▲) Foam B. Each point is the mean of four samples with its standard deviation.
Figure 4.9 Effect of aeration rate on the differential pressure measured between the headspace and downstream of the Turbosep fitted with an expansion tank when ‘foaming over.’ (■) clean pressure drop, (●) medium A foam and (▲) medium B foam. Each point is the mean of four samples with its standard deviation.

If the volume of foam entering the Turbosep is constant an increase in the volume of the return pipe would allow more time for the foam to collapse and is sufficiently long to prevent foam A from exceeding the capacity of the Turbosep. Unfortunately it was not possible to measure the actual degree of foam separation, which the Turbosep achieves. This is due to the dynamic nature of foams where generation, coalescence and collapse processes are functions of time and the applied physical forces. However, during these experiments it was observed that foam was continually being generated at the broth surface. As the density of foam approaches that of air it is reasonable to assume that the rate of foam formation will be similar to the sparged air flow rate into the vessel. Thus at an aeration rate of 150 L min\(^{-1}\) it can be assumed that an equivalent volume of foam is generated. The total capacity of the Turbosep, expansion tank and associated pipe work is 9.46 L and the natural collapse rate of Foam A estimated from Figure 4.4 is 0.2 L min\(^{-1}\). Based on these figures and in the absence of any applied physical forces, foam-over would be expected in 4 seconds. However as no foam-over was observed with
Foam A when the expansion box was fitted, this suggests that the Turbosep was providing a high degree of foam separation.

4.3 Optimisation Antifoam Addition with the Turbosep.

Anti-foam agents have been shown to have a number of deleterious effects on the productivity of a fermentation (Kawase and Moo-Young, 1987). Therefore, their use should be limited to gross foam out situations and during such periods only minimal volumes dosed. Antifoam is currently added into the Turbosep liquid recycle pipe. This has an immediate effect controlling the foam before it becomes a problem. However, there is no evidence that the return pipe is the optimum position for administering antifoam.

Experiments were performed to identify the optimal position to administer antifoam into a vessel in terms of total volume applied with time. Antifoam was added either directly into the expansion tank or into the vessel headspace. The Turbosep was challenged with Foam B at an aeration rate of 300 L min⁻¹ and the differential pressure was measured directly across the Turbosep. In both cases an expansion box to improve separational capacity was fitted. Antifoam addition was triggered at a differential pressure of 19 mBar, a value 10% lower than ΔP, to allow time for the antifoam to be dispersed.

Figure 4.10 shows how the position of antifoam addition can have a substantial impact on the volumes required to control excessive foaming. Approximately 29 % less antifoam was required over a 120 minute period when dosed directly into the headspace compared to the expansion tank. Antifoams generally have a high viscosity and their rate of dispersion is known to be a function of their chemical composition. This suggests that antifoam dosed into the expansion tank is poorly transported into the vessel so limiting its effect on reducing further foam build-up and challenge to the Turbosep. Dosing directly into the headspace instantaneously reduces the foam challenge on the Turbosep and appears to provide sufficient time for the foam in the return pipe to drain.
Figure 4.10 The volumes of anti-foam required to prevent the Turbosep from ‘foaming over’ when antifoam was added either (○) into headspace or (■) into the expansion tank. The Turbosep was challenged with Foam B at an aeration rate of 300 L min\(^{-1}\). Each point is the mean of 3 samples with its standard deviation.

4.4 Industrial Scale Fermentation Foam Control with the Turbosep

A case study was performed to identify how the Turbosep could increase the productivity of an industrial scale fermentation. \textit{E.coli} RV308 pHKY531 was cultured in a 6000 L fermenter at Eli Lilly & Company, Speke Operations according to their control strategy. The Turbosep was used to control the foam level in the tank, and the differential pressure across it linked to the control of antifoam addition. The increased productivity provided by the Turbosep is summarised in Table 4.2 (data supplied by Cook, personnel communication). The Turbosep was determined to increase the total productivity of the fermentation by 16\% without changing any of the process operating parameters.

Unfortunately a detailed cost benefit analysis cannot be determined as the capital, consumable and operating costs of the fermentation are not known. However, extrapolating these results to a production year of 350 batches (based on one successful
fermentation per day with a 14 day down time) the total yearly yield could be increased by 56 fold without any increase in the operating cost of the fermentation.

Table 4.2 Increased fermentation productivity provided by the Turbosep.

<table>
<thead>
<tr>
<th>Product</th>
<th>Process organism</th>
<th>Working volume</th>
<th>Decrease in antifoam usage</th>
<th>% increase in Kla</th>
<th>% increase in yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td><em>E. coli</em> RV308</td>
<td>5000 L</td>
<td>9 to 3 litres</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>Somatotropin</td>
<td>pHKY531</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The Turbosep was determined to reduce the antifoam requirements of this particular fermentation by approximately 66%. Antifoam has been shown to have a detrimental effect on the oxygen transfer rates achieved during a fermentation (Koch, 1995). Thus decreasing the total volume of antifoam administered will generally result in an increase in yield and or an ability to reduce the agitation and aeration rates with the subsequent reduction in power consumption while maintaining the same microbial growth rates.

It has been stated that the performance of a fermentation can be optimised by the adequate control of the fermentation process. The results presented here demonstrated how the Turbosep could play a key role in this aim especially when the differential pressure across it is linked to the provision and regulation of antifoam addition. Using the Turbosep in conjunction with the differential pressure control strategy allowed the addition of antifoam to be totally automated and dependent on the requirements of the fermentation at any time. An increased working volume was also possible as foam was allowed to pass into the exhaust gas pipe without comprising the fermentation, thus further increasing the productivity of the fermentation.
Chapter 5. The Containment of Fermenter Exhaust Gases

5. The Containment of Fermenter Exhaust Gases.

The requirements for contained bioprocesses are driven by international safety and environmental legislation (Dickson, 1996). With the increasing use of genetically modified organisms to produce novel therapeutic products, it is important to show the containment of bioprocesses. The design of effective containment systems has received much consideration (Hambleton et al, 1991; Vranch, 1992). However, since the recommendations have generally been made in the absence of quantitative performance data, there is some question over the validity of the design solutions (Titchener-Hooker et al, 1993). Fermentations are frequently assumed to be contained, through the use of a chain of filters in series on the exhaust gas line. However filter systems are expensive and may be susceptible to failure through incorrect operation.

Fermentations have the potential to release micro-organisms either accidentally or incidentally during the course of their operation. Most attention has been focused on the release of micro-organisms in aerosols, since, in this state the released organisms may pose a threat to health and the environment and can not be easily detected or contained (Hambleton et al, 1992). The enumeration of the released micro-organisms is often based on a culture method. However, the number of culturable cells recovered from aerosols may under-estimate the total viable number present because of the effects of sampling stress (Alvarez et al, 1995). Additionally, it is now accepted that a significant proportion of water borne viable cells may exist in a non-culturable state (Colwell et al, 1985), and that these viable but non-culturable cells can retain their pathogenicity (Colwell et al, 1990).

Recent research has shown that PCR can be used to detect airborne bacteria (Alvarez et al, 1994, Alvarez et al, 1995; Roll and Fujioka, 1995). Additionally, several workers (Mahon and Lax, 1993; Leser, 1995; Lee et al, 1996) have shown that quantitative PCR, using a competitive internal standard (Becker-Andre and Hahlbrock, 1989; Gilliland et al, 1990) can be employed to measure the number of bacteria in the environment. Furthermore QPCR has advantages over other culture based techniques for the enumeration of micro-organisms as it can detect non-viable bacterial pathogens (Josephson et al, 1993) and is unaffected by sampling stress (Alvarez et al, 1995).
Noble et al (1997) employed the QPCR methodology to quantify the incidental release of *E. coli* cells into the exhaust gas during a fermentation. The authors found that in general there was an increase in the rate of microbial release into the exhaust gas as the fermentation proceeded. However there was no simple relationship between cell density in the fermentation broth and the number of cells aerosolised. There are a series of factors that might be implicated in the observed variation in release rate as the fermentation proceeds. For instance, Pilancinski et al (1990) showed that the aerosol formation from a fermentation broth was influenced by several factors such as aeration rate, agitation rate and the rheological properties of the broth.

Assessing the performance of an exhaust gas containment system in terms of its effectiveness in containing the process organism requires determination of the quantity of cells that are released during its operation. This chapter describes a series of experiments that were performed to quantify the influence of fermentation operating parameters and the use of a Turbosep foam separator on the microbial load in the sampled gas stream of the exhaust gas.

### 5.1 Exhaust Gas Sampling Using a Cyclone-QPCR methodology

The methodology used for the capture and enumeration of process cells has been previously described in Sections 2.4 and 2.2 respectively.

Release of cells into the exhaust gas stream was measured by disconnecting the exhaust gas filter and rapidly connecting the cyclone. As the exhaust gas stream was piped directly into the cyclone the proportion of aerosolised cells collected will be largely a function of the cyclone capture efficiency. Upton et al (1994) has reported that the Aerojet General Cyclone has a high collection efficiency for particles above 2 μm diameter. Bradley (1999) reported that it was possible to recover in excess of 90% of cells released as a suspension into the Aerojet General Cyclone inlet at an air throughput of 375 L min\(^{-1}\). Therefore the number of cells captured and enumerated can be reasonably estimated as greater than 90% of those released.

The samples obtained from the cyclone were stored overnight at 4 °C and were assayed by QPCR within 24 hours. The concentration of cells in the background was subtracted...
from the subsequent exhaust-gas cyclone sample. An example of a calculation of the number of cells released at a given point into the exhaust gas is shown in Tables 5.1 and 5.2.

In order to interpret the results of the *E.coli* exhaust gas sampling it is important to know at any sampling point, the plasmid copy number of the cell. A QPCR target sequence is held in the DNA of each plasmid. Noble *et al* (1997) found the plasmid copy number varied with time during the exponential phase, however at the onset of stationary phase a steady state was achieved. During stationary phase cell division and DNA synthesis are much reduced and the number of plasmids per cell therefore remains stable. The plasmid copy number was determined to be 40 +/- 1.0 plasmids per cell at stationary phase (Bradley, 1999). For this reason all exhaust gas sampling was carried out during stationary phase.

**Table 5.1** Typical calculation used to determine mean cell release rates of *E.coli* RV308 pHKY531

<table>
<thead>
<tr>
<th>Sample plasmid concentration (mL⁻¹)</th>
<th>Plasmid copy number</th>
<th>Sample volume (mL)</th>
<th>Total cells released</th>
<th>Cell release rate (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>b</td>
<td>C</td>
<td>d</td>
<td>e</td>
</tr>
<tr>
<td>Measured</td>
<td>measured</td>
<td>Measured</td>
<td>(a / b) * c</td>
<td>d / 15</td>
</tr>
</tbody>
</table>

The calculation for determining mean release rates in *S.cerevisiae* is simplified as the QPCR target sequence is held in the single chromosome and therefore remains constant.

**Table 5.2** Typical calculation used to determine mean cell release rates of *S.cerevisiae* S150 Δhsp82

<table>
<thead>
<tr>
<th>Sample cell concentration (mL⁻¹)</th>
<th>Sample volume (mL)</th>
<th>Total cells released</th>
<th>Cell release rate (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>b</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>Measured</td>
<td>measured</td>
<td>(a / 1) * b</td>
<td>c / 15</td>
</tr>
</tbody>
</table>
5.2 Measurement of Cellular Release into Unfiltered Fermenter Exhaust Gas.

Large numbers of aerosols are dispersed from the liquid surface during an aerated and agitated fermentation. The aerosol dispersion is considered to be primarily affected by the rates of agitation and aeration (Baron and Willeke, 1986; Ohta et al, 1991). The dispersed aerosol concentration and size distribution depend on the rheological properties of the liquid (Pan et al, 1990; Pilacinski et al, 1990; Szewczyk et al, 1992). In this study the influence of agitation rate, aeration rate and the effect of antifoam addition on the release rates of process micro-organisms has been investigated at a 2 L scale. *E.coli* RV308 pHKY531 and *S.cerevisiae* S150Δhsp82 were cultured to stationary phase according to the control strategies detailed in sections 2.1.2.3.1 and 2.1.2.3.2.

Pilacinski et al (1990) has previously described the mechanism of droplet formation that causes an aerosol to form above a liquid. This description is however less appropriate for industrial processes where there is often a foam layer above the liquid surface. Therefore to simulate an industrial fermentation a head of foam at least 30mm in height above the broth surface was allowed to develop prior to sampling the exhaust gas. The foam was generated by the aeration of the broth and the height of the foam layer measured through the glass walls of the fermenter.

Figure 5.1 shows a comparison of the number concentration of cells released at a fixed aeration rate of 2 L min⁻¹ and agitation rates ranging from 0 to 1900 rpm. The measured cell release rate was found to increases with increasing agitation. At agitator speeds of 1500 and 1900 rpm the cell release rates were almost 25 % higher than those below 500 rpm.

The agitators in a stirred tank bioreactor perform a wide range of different functions including air break-up from the sparger to give a high surface area of contact between air and the broth. At a fixed aeration rate the degree of air break-up is a function of agitator speed.
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Figure 5.1 Effect of agitation rate on the release of *E. coli* cells into the unfiltered exhaust gas from a 2L fermenter. The fermenter was aerated at 1.5 L min⁻¹ and each point is the mean of three samples with their standard deviation.

In a well-dispersed low viscosity system, at a constant airflow rate the bubbles once dispersed are noncoalescing (Nienow, 1990) with a narrow range of sizes (1 mm or less). These bubbles expand as they rise due to decreasing hydrodynamic pressure and burst at the surface releasing a large number of jet droplets into the headspace. If the jet droplets are greater than 1 μm in diameter, the minimum reported diameter of a single *E. coli* cell then microbial release into the headspace may occur. However, if the airflow rate is too high for the agitation conditions, the airflow dominates the bulk flow and the air is poorly dispersed, a condition known as flooding. This results in an increased breakaway frequency from the agitator and the bubble size at breakaway leads to enhanced bubble coalescence (Nienow, 1990), so that a smaller number of relatively large bubbles burst at the broth surface. At agitation speeds of 500 rpm or higher, the air flow was completely dispersed and this indicates that in addition to bubble bursting due to aeration, which will be constant at a fixed aeration rate, bioaerosol jet droplets larger than 1 μm diameter were also generated due to mechanical disruption of the liquid. As the agitation rate was reduced to zero the degree of mechanical disruption of the air flow was similarly reduced and the impellers flooded. The reduced number of cells collected indicates that the number of jet droplets generated was less than a fully agitated system.
Figure 5.2 shows a comparison of the number concentration released at a fixed agitation rate of 500 rpm and aeration rates ranging from 0 to 5 L min\(^{-1}\). The measured cell release rate was found to increase with increasing aeration. At a constant agitation rate, the number of jet droplets released due to mechanical disruption of the broth is constant. Therefore the increase in aerosolised cell numbers collected with increasing aeration rate must be caused by an increase in the number of 1 \(\mu\)m or larger diameter jet droplets generated at the liquid surface. When the aeration rate was reduced to zero, \(7.2 \times 10^4\) cells min\(^{-1}\) were released and this can be attributed solely to the mechanical disruption of the broth. Furthermore at an agitation rate of 0 rpm and an aeration rate of 1.5 L min\(^{-1}\), equivalent to 1 VVM (Figure 5.1) the number of cells released is approximately 50% greater than that at an agitation rate of 500 rpm and an aeration rate of 0 L min\(^{-1}\) (Figure 5.2). This shows that the influence of aeration rate is more significant than agitation rate on the release of \(E.\)\(coli\) cells form a stirred tank reactor.

When the experiments were repeated using \(S.\)\(cerevisiae\) a release rate of \(2 \times 10^4\) cells min\(^{-1}\) was observed at an aeration rate of 5 L min\(^{-1}\), however, no release was detected at any other sampling point. The total number of cells released was found to be approximately one order of magnitude lower than the observed release rates for \(E.\)\(coli\) under identical experimental conditions. The increase in the settling velocity which occurs according to Stokes Law as the particle diameter increases (at a fixed density difference between the particle and the air) suggests that at aeration rates below 1.5 L min\(^{-1}\) the settling velocity of a single \(S.\)\(cerevisiae\) cell is greater than the rising velocity i.e. the velocity of the gas leaving the liquid surface (Appendix F).

At aeration rates below 5 L min\(^{-1}\) the probability of microbial release from the headspace was much reduced and any release that occurred was below the limit of sensitivity for the \(S.\)\(cerevisiae\) QPCR assay. This calculation is based on the assumption that cells aerosolise form the liquid/foam surface as a single cell.
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Figure 5.2 Effect of aeration rate on the release of *E. coli* cells into unfiltered exhaust gas from a 2L fermenter. The fermenter was agitated at 500 rpm and each point is the mean of three samples with their standard deviation.

However, Ferris (1995) measured the particle size distribution of both *E. coli* and *S. cerevisiae* aerosolised from an atomiser. Particles larger than 5 \(\mu\)m and 2 \(\mu\)m diameter were observed for *S. cerevisiae* and *E. coli* respectively. The increase in size distribution is probably due to cells clumping together, aided by any solids that are present in the fermentation media. Clumps of cells are unlikely to escape from the liquid surface due to their increased size and larger settling velocity.

Other studies have investigated the effect of agitation rate and aeration rate on the release of aerosols from a stirred tank reactor. Pilacinski *et al* (1990) found that the fraction of particles large enough to potentially carry micro-organisms increased with increasing agitation and air flow rate. Szewczyk *et al* (1992) carried out similar measurements using an *E. coli* culture. The authors found that the particle size distribution was practically independent of agitation and aeration rate below a diameter of 2 \(\mu\)m. However for particles larger than 2 \(\mu\)m diameter the concentration was found to increase with an increase in the air flow and agitation rate. Due to equipment limitations the particle size distribution of the aerosolised cells could not be measured, however, similar trends are observed here to both the Pilacinski and Szewczyk studies.
During a fermentation process, metabolic products are generally excreted into the broth. These products cause changes in the rheological properties of the broth, such as kinematic viscosity and surface tension, especially if surface active compounds are produced (Georgiou et al, 1992). As liquid dispersion is dependent on the rheological properties of the broth, it was thought likely that changes in these properties would affect the release of process cells from the fermenter. Figure 5.3 shows that the addition of up to 2.5 % v/v of 30 % polypropylene glycol (PPG) antifoam which decreases the surface tension of the broth had no significant effect on the release rate of *E. coli*.

During an *E. coli* fermentation, the metabolism of glucose produces ethanol and carbon dioxide and well as lactic, acetic, formic and succinic acids (Porter, 1946). In addition the metabolism of tryptophan, adds more organic acids into the medium. This results in an increase in the surface tension of the medium and an increase in the foaming tendency of the broth. Huang *et al* (1994) demonstrated that under foaming conditions a higher effluent aerosol concentration can be measured in the headspace compared to that under non-foaming conditions. During this study, the antifoam was dosed to control excessive foam formation but not added in sufficient volumes to completely collapse the foam layer. This suggests that although surface tension may have a direct influence on the rate of cellular release, it is in fact the presence of a foam layer, an indirect function of surface tension that has a more significant effect. This was most evident after the addition of 100 mL of antifoam (Figure 5.3) which collapsed foam layer and resulted in a 2.5 fold reduction in the number of cells released. Further evidence for the effect of foam was presented by Noble *et al* (1997), who found an increase in the rate of microbial release into the exhaust gas on the first occurrence of foam.

Expression of the rate of release of cells in terms of the exhaust gas volume allows a comparison of results with other researchers who have used different methods to measure cell concentrations in the fermenter head space. The data presented here shows a release rate of $1 \times 10^8$ cells per m$^3$ at an agitation rate of 500 rpm and an aeration rate of 1.5 L min$^{-1}$. 
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Winkler (1987) has reported that in the fermenter headspace there are about $10^6$ contaminated particles per m$^3$ of gas. Since each contaminated particle contains at least one viable cell then this describes only the minimum number of cells present. Additionally it is well known that a large proportion of viable cells are not culturable (Colwell et al, 1985) and that in the aerosolised state the culturable portion is likely to be very low (Neef et al, 1995). Neef and co-workers (1995) have reported that less than 1 % of cells collected by filtration from an aerosol were able to grow as minicolonies compared to over 90 % culturability of cells directly filtered out from the suspension used to generate the aerosol. Since a PCR methodology rather than a culture based method was used to detect cells, it is difficult to directly compare results with those of Winkler (1987).

However, based on the findings of Neef and co-workers (1995) it is reasonable to estimate that the total number of cells as opposed to contaminated particles in the headspace of the fermenter to be in excess of $10^8$ cells m$^3$. There would then be a degree of correlation with the results of these studies. Other studies on aerosols produced by fermentation have concentrated on aerosol particle diameter rather than cell...
concentration in the aerosol. Pilancinski *et al* (1990) measured over $10^9$ particles per m$^3$ of which between 30-40% exceeded 2μm in diameter above a complex broth stirred at 130 rpm. Szewczyk *et al* (1991) measured the particle concentration at approximately 15 cm above the fermentation broth and found that the level decreases from greater than $6 \times 10^9$ particles per m$^3$ at inoculation to $2.5 \times 10^8$ particles after microbial growth at a stirrer speed of 450 rpm. As the air flow rate is increased over the time course of a fermentation, the generated particles are distributed in an increasing volume of air leading to a decrease in concentration and this may account for the fall in numbers detected in the study by Szewczyk and co-workers. Unfortunately, it is not possible to compare findings with these workers as there is no direct correlation between the number concentration of particles and cells in the aerosol, as the latter depends on the particle size range and the fermentation conditions.

Noble *et al* (1997) monitored the release of *E. coli* throughout a fermentation using a cyclone-QPCR methodology and found that samples taken during stationary phase contained $1.1 \times 10^8$ process cells m$^{-3}$ of exhaust gas. During this study the aeration rate was maintained at 1.5 L min$^{-1}$ while the agitation rate was varied to maintain broth DOT above 20 %. However it was reported that during the later stages of the fermentation the stirrer speed was in the range 800 - 1000 rpm. Thus there appears to be a very good correlation with the results presented here. Over the course of a 5.5 hour fermentation period a total of $3 \times 10^7$ process cells were released. Comparison of this figure with the total number of cells in the broth, $1.8 \times 10^{10}$ cells at stationary phase, reveals that the number of cells released is a very small proportion of the total. Noble *et al* (1997) determined that the total number of cells released was contained in less than 2 μL of fermentation broth at the time of harvest.
5.3 Bioaerosol Containment Provided by the Turbosep Foam Separator.

The previous section has demonstrated that process micro-organisms are released into the exhaust gas stream of a fermenter in only very low numbers. However in the case of recombinant micro-organisms the current safety regulations, described in section 1.1.3 require that any release is either minimised or prevented depending on the pathogenicity of the cultured micro-organism. In the case of containment levels B2 or higher exhaust gas containment is necessary to prevent release. This is normally achieved through filtration, however the consumable cost of filters is high particularly on larger scale vessels and their effectiveness has been shown to vary with the type of process they contain. Thus there is a need for a high performance but more cost effective containment system. CFD studies on the particle transport in the Turbosep (see Section 3.2.2.3) suggests that it is efficient in removing small particles from the exhaust gas stream in addition to separating foam. The following section details a series of experiments that were performed to quantify the bioaerosol collection efficiency of the Turbosep.

The Turbosep was connected to a 250 L stirred bioreactor as described in Section 2.4.2. Aliquots of an E.coli RV308 pHKY531 glycerol stock were re-suspended using a high shear mixer (Silverson) into 200 L of a minimal salt buffer containing per litre 2g KH$_2$PO$_4$ and 4g NaHPO$_4$ to a final cell concentration of either 1 x 10$^9$ or 1 x 10$^{10}$ cells mL$^{-1}$. The minimal salt buffer ensured that cell viability was maintained during the subsequent experiments. The only vessel in the Advanced Centre for Biochemical Engineering (ACBE) that was of the appropriate design and size to be used with the Turbosep was the 250 L bioreactor. However, this vessel was not equipped with any sensors, gauges or temperature control facility, required during a fermentation. This meant that micro-organisms had to be cultured elsewhere (see Section 2.1.2.3) and transferred to the 250 L vessel when required.

After the suspension had been transferred to the 250 L vessel, the particle size distribution of the liquid phase was measured (see section 2.4.3.1) to ensure that the size range was comparable to that of a stationary phase E.coli fermentation. The vessel was agitated at 440 rpm, the maximum rotational speed of the agitator and aerated with
varying airflow rates. Any bioaerosol generated was collected in the headspace and both upstream and downstream of the Turbosep using the cyclone.

The number of cells detected at each sampling point in terms of cells released per minute, using two different cell concentrations is shown in Figures 5.4 and 5.6. Notably the number of cells released downstream of the Turbosep that were escaping into the external environment was found to decrease with increasing rates of aeration. Expressing the data in terms of the number of cells released per m$^3$ of exhaust gas allows a more standardised comparison of the effect of aeration on the level of containment achieved by the Turbosep (Figures 5.5 and 5.7). In this case a reduction in the number concentration of cells released was observed with increasing aeration rate.

The percentage reductions in the measured cell number concentration observed between the headspace and Turbosep inlet is greater at lower air flow rates than the percentage reduction in cell numbers achieved within the Turbosep. There are a number of mechanisms that might be implicated in this reduction of cell count on entry to the Turbosep. The airflow is highly turbulent, even at the lowest aeration rate (Re = 11176 at an air flow rate of 150 L min$^{-1}$). The turbulent eddies generated within the bulk flow would transport the bioaerosol particles to the inner wall of the connection pipe and if the particles are small enough they would be captured within the stagnant turbulence boundary layer. Bioaerosol particle coalescence would also result in the formation of a reduced number of larger particles that together with the naturally occurring larger particles would sediment according to Stokes law. If this occurred then the sampling population will be skewed.

The data presented here demonstrated that the Turbosep exhaust gas containment system provided at least a 4 fold reduction in the microbial loading of the exhaust gas when challenged under non-foaming conditions.
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Figure 5.4. Microbial containment expressed as mean release rate per minute provided by the Turbosep when challenged with a low concentration bioaerosol in a 250 L bioreactor, where each point is the mean of two samples with their standard deviation.

Figure 5.5. Microbial containment expressed as mean release rate per m$^3$ provided by the Turbosep when challenged with a low concentration bioaerosol in a 250 L bioreactor, where each point is the mean of two samples with their standard deviation.
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Figure 5.6. Microbial containment expressed as mean release rate per minute provided by the Turbosep when challenged with a high concentration bioaerosol in a 250 L bioreactor, where each point is the mean of two samples and their standard deviation.

Figure 5.7. Microbial containment expressed as mean release rate per m² provided by the Turbosep when challenged with a high concentration bioaerosol in a 250 L bioreactor, where each point is the mean of two samples and their standard deviation.
The effect of broth cell concentration on the number concentration of cells aerosolised into the headspace, under defined agitation and aeration rates is shown in figure 5.8 (data taken from Figures 5.4 and 5.6). It can be seen that the higher the broth cell concentration the greater the proportion of cells released, although no simple relationship between the two appears to exist. Huang et al (1994) stated that a greater degree of cell aggregation is likely at higher concentration and therefore the proportion of cells released would be lower. Larger clumps of cells may not be lifted into the aerosol due to their size not being compatible with the particle size distribution of the aerosol. Although some degree of cell aggregation would be expected the similar trends in the measured microbial release rates into the bioreactor headspace observed at two different cell concentrations (see Figures 5.4 and 5.6) suggests that any differences in the degree of aggregation is not significant. The reason for the variation in release rates can probably be explained by the number of cells neighbouring a jet droplet during the period of its formation. Assuming a well mixed homogenous broth, the higher the cell concentration the higher the probability that one or more cells will be caught within in the aerosolised jet droplet.

A comparison of the results presented in Figures 5.2 and 5.6 allows the incidental release of E.coli at different scales to be investigated. Based on a constant volumetric flow rate of 1 VVM and broth cell concentration of 1 x 10^10 cell mL, release rates of 1.5 x 10^5 and 1.6 x 10^6 cells minute^1 were determined in the 2 L and 250 L vessels respectively. The aerosolisation of process cells is largely a function of the rate of foam bursting at the liquid surface. Assuming the rate of bursting is constant per unit surface area, then it follows that the greater surface area in the 250 L vessel may explain the variation in the results. This is evident when the release rates per unit surface area were normalised; values of 1.9 x 10^8 and 4.8 x 10^8 cells m^2 were calculated for the 2 L and 250 L vessels respectively, providing a much better correlation. The cause of the remaining variation can probably be attributed to the different design of the two vessels.
Figure 5.8 Comparison of the mean % release rate of E.coli RV308 pHK531 from (■) a low concentration broth, $1 \times 10^6$ cells mL$^{-1}$ and (●) high concentration broth, $1 \times 10^8$ cells mL$^{-1}$ into the unfiltered exhaust gas from a 250 L bioreactor. Each point is the mean of two samples and their standard deviation.

The efficiency of the Turbosep at collecting micro-organisms released in the form of an aerosol is shown in Figure 5.9. The cell concentration in the air stream was measured on entry and exit from the Turbosep (data obtained from Figures 5.4 and 5.6). A comparison with the collection efficiency of the high efficiency Aerojet General Cyclone is also given. For the Turbosep, collection efficiency increases as the aerosolised cell concentration entering the Turbosep increases, although similar shaped total collection efficiency curves are obtained.

The reason for the variation in collection efficiencies is probably due to a greater degree of particle aggregation at higher cell concentrations within the Turbosep. This is most likely to be caused by the compression of the airflow as it passes through the narrow orifice below the impingement plate. As previously discussed the collection efficiency of the Turbosep increases with the increasing diameter of the challenging particles.

At the maximum experimental airflow rate of 500 L min$^{-1}$ the Aerojet cyclone is approximately 10 % more efficient in particle capture compared to the Turbosep. At 375
L min⁻¹, the optimum operating air flow rate for the Aerojet cyclone the collection efficiency of both designs is very similar. However, the collection efficiency of the Turbosep is not as sensitive to a reduction in airflow rates as the Aerojet. At the lowest experimental air flow rate of 150 L min⁻¹ the collection efficiency of the Turbosep is reduced to approximately 50 %, while the efficiency of the Aerojet is reduced to under 15 %.

![Graph showing collection efficiency vs airflow rate for Turbosep and Aerojet](image)

The variation in the collection efficiency between the two designs can be explained by the two different mechanisms of microbial capture. In the Aerojet cyclone, similar to other standard design sampling cyclones, airborne micro-organisms are thrown to the outer walls by the centrifugal forces generated due to the high rotational movement of the air and are collected in the recirculating scrubbing liquid. Thus the collection efficiency of the Aerojet cyclone can be seen as a direct function of the centrifugal forces generated which are in turn a function of the tangential velocities of the air stream. It follows that reducing the air velocity at the tangentially shaped inlet section will reduce the total collection efficiency attained.
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CFD analysis of the particle trajectories within the Turbosep (see Section 3.2.2.3) indicates that the cells are generally not collected on the outer walls, the centrifugal forces required to throw the particles outwards are probably not large enough, rather collection efficiency is dependent on the sedimentation of the cells after they hit the vortex arrester. The fact that the collection efficiency of the Turbosep is not as sensitive to changes in air flow rate suggests that this design is more suitable for the containment of industrial scale fermenters, where the aeration rate used is often varied over the time course of the fermentation.

The collection efficiency of the Turbosep was further confirmed through the use of a particle size analyser (see Section 2.4.3.2), set to sample aerosol from the Turbosep inlet and overflow sections. The aerosol was generated by the aeration and agitation of an E.coli fermentation broth, concentration 1x 10^9 cells mL^-1 cultured by the method previously described (section 2.1.2.3.1).

Figure 5.10 shows that the collection efficiency for the micro-organisms within the Turbosep follows the same pattern as that for the larger (>5.0 μm diameter) particles. This suggests that the majority of particles containing micro-organisms collected by the Turbosep are larger than 5.0 μm in diameter. If this were not the case then the collection efficiency of the particles would tend to be greater than that of the micro-organisms for the same airflow rate.

The collection efficiency of the smaller particles (>0.5 μm diameter) ranges from 6 % to 27 % at airflow rates of 150 L min^-1 to 500 L min^-1 respectively. Based on the study of Szewczyk et al (1992) it would be expected that between 60 -70 % of the aerosol generated at the liquid surface would have a diameter smaller than 2 μm. As the collection efficiency of the Turbosep is a function of cell size (see Figure 3.10), the observed % reduction in the number of aerosol particles collected can be explained by a reduction in the efficiency of the Turbosep in capturing sub 2 μm diameter particles.
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Figure 5.10 Turbosep collection efficiency when challenged with low concentration of aerosolised E.coli RV308 pHKY531 (■), particles >0.5 μm diameter (▲) and particles > 5.0 μm diameter (●), where each point is the mean of two (■) or twenty (▲, △) samples with their standard deviation.

Unfortunately it was not possible to experimentally determine the precise $d_{50}$ cut point for the Turbosep. However from the data presented in Figure 5.10 a $d_{50}$ of 3 to 4 μm could be expected, a similar value to that predicted by CFD. Moreover, and for the reasons previously discussed the percentage reduction of particles between the headspace and the Turbosep inlet appears to be higher than the collection efficiency within the Turbosep itself. It follows that increasing the vertical position of the Turbosep above the vessel top-plate would result in a reduction of total particle number at the inlet and thus reduce the microbial challenge on the Turbosep. However a critical balance needed to be observed; increasing the height of the Turbosep would also increase the pressure drop between the headspace and Turbosep inlet section and if this exceeded the total pressure drop across the re-cycle pipe then the bulk flow would reverse direction.
5.4 Turbosep Exhaust Gas Containment Under Foaming Conditions

The following section describes a series of experiments that were performed to quantify the level of microbial containment that could be provided by the Turbosep under continual foam challenge. The Turbosep in conjunction with a pro-active control strategy has been shown to prevent excess fermentation foam formation and to increase the productivity of the process (see Chapter 4). The Turbosep has also been shown in Section 5.3 to provide a level of exhaust gas containment when challenged with bioaerosols. However for the Turbosep to form an integral part of an industrial scale fermentation it is important that it provides an equivalent level of containment when challenged with foams containing biological material. Traditional filters can become blinded if they come into contact with quantities of foam or liquid that can effectively shut down the fermentation. To reduce the rate of blinding it is usual to install in series a condenser, foam catch pot and re-heater upstream of the terminal filter. However, the increased complexity of this system increases the probability of unit failure and the subsequent accidental release of process organisms.

As pure liquids do not foam aliquots of an E.coli RV308 pHKY531 glycerol stock were re-suspended using a high shear mixer (Silverstone) into a foaming buffer containing per litre 2g KH$_2$PO$_4$, 4g NaHPO$_4$ and either 200 mg L$^{-1}$ egg albumen (Foam A) or 200 mg L$^{-1}$ soybean flour (Foam B). Any bioaerosol released downstream of the Turbosep was captured and enumerated using a cyclone-QPCR methodology (see Sections 2.4 and 2.2).

The number of cells detected at each sampling point in terms of cells released per minute, using two different cell concentrations is shown for Foam A in Figures 5.11 and 5.13. Notably the number of cells released downstream of the Turbosep was found to increase with increasing rates of aeration. Expressing the data in terms of the number of cells released per m$^3$ of exhaust gas allows a more standardised comparison of the effect of aeration on the level of containment achieved by the Turbosep (figures 5.12 and 5.14). In this case a reduction in the number concentration of cells released was observed with increasing aeration rate. When the experiments were repeated using the more stable Foam B (Figures 5.15 to 5.18) identical trends were observed.
Figure 5.11 Microbial containment expressed as mean release rate per min⁻¹ provided by the Turbosep when challenged with Foam A containing a low cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.

Figure 5.12 Microbial containment expressed as mean release rate per m³ provided by the Turbosep when challenged with Foam A containing a low cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.
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Figure 5.13 Microbial containment expressed as mean release rate per min$^{-1}$ provided by the Turbosep when challenged with Foam A containing a high cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.

Figure 5.14 Microbial containment expressed as mean release rate per m$^3$ provided by the Turbosep when challenged with Foam A containing a high cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.
Figure 5.15 Microbial containment expressed as mean release rate per min\(^{-1}\) provided by the Turbosep when challenged with Foam B containing a low cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.

Figure 5.16 Microbial containment expressed as mean release rate per m\(^3\) provided by the Turbosep when challenged with Foam B containing a low cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.
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Figure 5.17 Microbial containment expressed as mean release rate per min\(^{-1}\) provided by the Turbosep when challenged with Foam B containing a high cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.

Figure 5.18 Microbial containment expressed as mean release rate per m\(^3\) provided by the Turbosep when challenged with Foam B containing a high cell concentration in a 250 L bioreactor. Each point is the mean of two samples with its standard deviation.
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The data presented here demonstrates that the Turbosep and its associated pipework provides approximately a 6 order of magnitude reduction in the microbial loading per unit volume in the overflow compared to what it was at the inlet when challenged under foaming conditions. Furthermore, the collection efficiency in all cases was found to be in excess of 99.9999%, demonstrating the high level of exhaust gas containment that can be provided by the Turbosep during periods of foam formation.

During these experiments foam was continually being re-cycled through the Turbosep, therefore it is reasonable to assume that the total volume of foam passing through the Turbosep during sampling would be similar to the volume of air sparged into the vessel over the same time period. Errington and Powell (1969) described how the injection of a scrubbing liquid into cyclone could increase the microbial collection efficiency. In this case the large volumes of foam passing through the Turbosep is probably providing the scrubbing action washing any cells released cells back into the vessel. Additionally, as very few cells were collected downstream of the Turbosep this suggests that the volume of foam or separated liquid lost to the precession currents was very small.

The level of containment provided by the Turbosep is dependent on the foaming conditions within the vessel and therefore will vary during the course of fermentation. However the findings presented here demonstrate that the incidental release of process organisms downstream of the Turbosep is low irrespective of the degree of foaming. In the case of category B1 processes or processes that do not require any special treatment of the exhaust gas, the Turbosep could provide a sufficient level of containment to minimise any release without the need of additional filters. For higher categories of containment the Turbosep could function as a pre-filter and will be discussed further in Chapter 7.

In the design of large-scale processes the correct balance needs to be found between the low levels of incidental release observed in this study, and the much higher levels that follow a single accident. Sampling of the exhaust gas downstream of a filter showed that no release was detectable. Within this study at an air flow rate of 500 L min\(^{-1}\) and a broth cell concentration of \(1 \times 10^{10}\) cells mL\(^{-1}\) approximately \(1 \times 10^7\) cells min\(^{-1}\) were released downstream of the Turbosep in the absence of foaming. Under foaming conditions this
release rate was reduced to approximately $6 \times 10^6$ cells min$^{-1}$. However, an accidental foam-out, which released 1 mL of fermentation broth, would allow approximately $1 \times 10^{10}$ cells to escape into the processing environment. Based on a typical fermentation time of 12 hours, the release from this single accident is equivalent to the cumulative incidental release from 1666 fermentations. This calculation is based on the assumption that all incidental release will occur from the Turbosep and that the fermentation was foaming continually.

It is presently unclear whether the long-term cumulative release of a small number of micro-organisms exposes the operator and the environment to a greater hazard than the occasional release of a large number of organisms. However the potential for accidents should be a key feature of risk assessment in industrial processes. Currently, only a microbial risk assessment needs to be undertaken. However it is well known that simplicity of engineering reduces the risk of accidents (Kletz, 1991) This aspect of containment should be given greater priority compared to the increasing complexity necessary to prevent the release of micro-organisms as an incidental release from the process.

Most of the previous research on both large industrial and small scale sampling cyclones has been based on experimental characterisation studies (Kim and Lee, 1990; Patankar, 1980), however the literature citations are often incomplete only focusing on one or two design parameters. Furthermore, as different authors have based their studies on different designs of cyclone, a comparison between findings is difficult.

As with other cyclone designs there are eight parameters that can be used to characterise the design of the Turbosep. The following study, which is based on both a CFD model and a practical investigation of microbial capture, describes the effects of the major Turbosep design parameters on fluid flow, pressure drop and particle collection. CFD has previously been used to model the collection performance of the Turbosep (see Chapter 3). Performance curves were produced with approximately the same shape and $d_{50}$ as those obtained by experiment. Furthermore, the predicted pressure drops were in excellent agreement with the measured data. Although the CFD model is a reliable and relatively inexpensive method of examining design changes on the performance of the Turbosep, and one that provides an alternative to the usually expensive experimental characterisation studies, it is nevertheless important to validate the major findings experimentally.

6.1 The Effects of Geometry on the Performance of the Turbosep.

The primary role of the Turbosep is as a foam separator, but it also removes microorganisms from the exhaust gas of a fermenter thus minimising any biological release into the environment (see Chapter 5). Unfortunately due to the complexity of foams CFD cannot analyse the foam separation process or predict the particle capture in multi-phase flow. Moreover, a process of redesign that was to investigate the effects of changes in all possible parameters would be prohibitively time consuming. Therefore the re-design will focus on optimising the particle collection efficiency and pressure drop performance when challenged with a single-phase gaseous flow.
There are two groups of parameters that can influence the particle collection characteristics of the Turbosep. Operating parameters depend only on the process to which the unit is applied and include the feed flow rate, feed composition and particle size distribution (Bradley, 1965). Design parameters include the aperture sizes and body dimensions. The effect of the major design parameters will be considered in relation to two performance criteria; particle collection efficiency and pressure drop.

There is a wide variation in the size distribution of process organisms. *E.coli* is considered to have an average diameter of 2-3 µm, *S.cerevisiae* an average diameter of 5 µm while the streptomycetes are often filamentous and may have a length of 10 µm or above. In these studies an arbitrary particle diameter of 10 µm was used in most of the simulations. The particle collection mechanics of all small particles is likely to be very similar, and the general trends observed for 10 µm diameter particles as the geometric dimensions are scaled should be equally applicable to smaller sized particles, even though the predicted levels of collection efficiency will differ.

The CFD analysis assumed a simulated air flow rate of between 150 and 500 L min⁻¹, the latter being the maximum rated capacity of this size of Turbosep. Only one parameter was re-scaled at a time. The numerical predictions were then compared with literature reports, where available, on similar design changes in more ‘traditional’ cyclones.

The flow of 10 µm diameter particles through the standard Turbosep (see Figure 3.11) was taken as the “base line” for the studies. This was simulated in the CFD analysis with the release of 100 spherical particles at random positions at the inlet plane (see Section 3.2.2.3). Their trajectory was followed, and the capture efficiency was taken as the proportion of the total that did not emerge from the overflow pipe. Particles with different shapes are likely to have a different flow pattern and therefore their collection efficiency may differ from that of the spherical particles.
Chapter 6. A Parameter Investigation on the Influence of the Turbosep Geometry on Collection Efficiency

There are two component mechanisms responsible for the particle collection performance of the Turbosep; the first is a function of the centrifugal force which throws smaller particles to the outer wall where they are collected, the second is dependent on the sedimentation of the cells after they hit the vortex arrester and fall into the quiescent zone beneath. Simulations performed on the original Turbosep design using 10 μm particles injected at a simulated air flow rate of 500 Lmin⁻¹ (see Figure 3.11), highlight how in this instance the later mechanism is dominant, with all of the particles striking the vortex arrester, irrespective of whether they are captured or escape.

6.1.1 The Effect of Free Height on the Performance of the Turbosep.

The influence of overall length or free height was investigated by altering the length of the cylindrical section between the vortex finder and vortex arrester. All other dimensions remained unchanged. The numerically predicted collection efficiency was found to decrease with increasing free height (Figure 6.1). This is in line with most of the current research on 'traditional' cyclone designs, where a reduction in free height generally leads to an increase in the particle collection efficiency (Rietema, 1961). In this case, the increase is due to a decrease in the wall surface area, which decreases the wall friction acting on the flow, and results in an increase in the swirling velocity component of the fluid.

As the vertical length in-between the vortex finder and vortex arrester was increased the percentage of particles that were found to flow towards the base of the Turbosep and strike the vortex arrester was reduced. As the probability of capture for these particles is dependent upon sedimentation below the plane of the vortex arrester, then this explains the observed reduction in collection efficiency. Increasing the free height by 0.24 m resulted in a 17 % decrease in collection efficiency.

It appears that reducing the free height had a negligible effect on the pressure energy requirements of the Turbosep, the very slight decrease being attributed to a decrease in wall friction.
Figure 6.1 Numerically predicted effect of changing the free height of the Turbosep on (•) particle collection efficiency and (■) pressure drop when challenged with 10 μm diameter particles at a 500 L min⁻¹ air flow rate. The free height of the standard design is 0.515 m.

6.1.2 The Effect of the Vortex Finder Length on the Performance of the Turbosep.

The predictions for different vortex finder lengths (Figure 6.2) show that the particle collection efficiency increases as the vortex finder length decreases and is in fact highest with no vortex finder.

Complete absence of a vortex finder has been reported (Bradley, 1965) to give a low value for $d_{50}$ but gives rise to the loss of coarse particles to the overflow, presumably via the short circuit flow. However, as we have seen in this study, reducing the vortex finder length increases the collection efficiency of 10 μm diameter particles, suggesting that particles of this size are too small to be caught up in the short circuit flow path. At a vortex finder length of 0.515 m all of the particles hit the vortex arrester irrespective of whether they
were captured or escaped. Reducing the vortex finder length would therefore allow more time for the re-entrainment of these particles in the return stream and consequently would result in an increase in capture efficiency.

![Graph showing the effect of vortex finder length on particle collection efficiency and pressure drop.](image)

Figure 6.2 Numerically predicted effect of changing the vortex finder length on (●) particle collection efficiency and (■) pressure drop in the Turbosep when challenged with 10 μm diameter particles and a 500 L min⁻¹ air flow rate. The length of the vortex finder length in the standard design is 0.125 m.

**6.1.3 The Effect of Vortex Finder Diameter on the Performance of the Turbosep.**

The effect of reducing diameter of the vortex finder is immediately apparent in the CFD analysis of the flow of particles through the device (Figure 6.3). The predictions for the different vortex finder diameters show that both particle collection efficiency and pressure drop decrease as the vortex finder diameter is decreased (Figure 6.4). Reducing the vortex finder diameter from 0.046 m to 0.02 m resulted in a 21% decrease in collection efficiency.
Chapter 6. A Parameter Investigation on the Influence of the Turbosep Geometry on Collection Efficiency

Figure 6.3 Numerically predicted trajectories of particles injected at a simulated air flow rate of 500 L min\(^{-1}\) from 100 locations along the inlet plane when the vortex finder diameter was reduced to 0.02 m. Particles were 10\(\mu\)m in diameter and had a coefficient of restitution of 0.1
Chapter 6. A Parameter Investigation on the Influence of the Turbosep Geometry on Collection Efficiency

![Graph showing the effect of vortex finder diameter on collection efficiency and pressure drop.](image)

Figure 6.4 Numerically predicted effect of changing the vortex finder diameter on (●) particle collection efficiency and (■) pressure drop in the Turbosep when challenged with 10 μm diameter particles and a 500 L min⁻¹ air flow rate. The vortex finder diameter in the standard design is 0.046 m.

6.1.4 The Effect of the Inlet Diameter on the Performance of the Turbosep.

Figure 6.5 illustrates the effect of reducing the feed inlet diameter at a constant aeration rate of 500 L min⁻¹. This results in an increasing entry velocity at the inlet. As the diameter of the inlet is decreased in size from 0.0254 m (1 inch) to 0.0127 m (0.5 inch) collection efficiency increases by 4%.

It is quite evident that an increased inlet velocity would give rise to increased swirl as the flow passes over the vanes. This will lead to the generation of increased centrifugal forces acting on the particles. At an inlet diameter of 0.0127 m, a small number of particles were caught on the outer wall in-between the vortex finder and vortex arrester. However, most of the particles were still found to hit the vortex arrester.
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The optimum feed inlet diameter should take into account both changes in collection efficiency and pressure drop. Although reducing the inlet diameter by 50% gives rise to increased particle collection efficiency, this is accompanied by an 8.4 fold increase in the pressure drop. A critical balance needs to be observed between collection efficiency and pressure drop. If the differential pressure across the Turbosep exceeds the total pressure drop across the re-cycle pipe then the bulk flow will reverse direction. The critical value for this differential pressure is dependent upon the process to which the Turbosep is connected, but such a sharp increase suggests that a 0.0254 (1 inch) feed inlet diameter is probably optimal.

![Figure 6.5 Numerically predicted effect of changing the feed inlet diameter on particle collection efficiency and pressure drop](image)

Figure 6.5 Numerically predicted effect of changing the feed inlet diameter on (●) particle collection efficiency and (■) pressure drop in the Turbosep when challenged with 10 µm diameter particles and a 500 L min⁻¹ air flow rate. The feed inlet diameter in the standard design is 0.0254 m.

6.1.5 The Effect of Underflow Diameter on the Performance of the Turbosep.

Figure 6.6 shows the numerically obtained particle collection efficiency as a function of the diameter of the underflow. The size of the underflow appears to have a minimal effect on
Chapter 6. A Parameter Investigation on the Influence of the Turbosep Geometry on Collection Efficiency

the Turbosep's particle collection performance, efficiency decreasing slightly as the underflow diameter is decreased. Furthermore, the underflow diameter appears to have little or no effect on the pressure energy requirements of the Turbosep. The only reported role of the underflow is in controlling the flow ratio between the overflow and underflow apertures (Bradley, 1965).

![Graph showing the effect of underflow diameter on collection efficiency and pressure drop](image)

Figure 6.6 Numerically predicted effect of changing the underflow diameter on (●) particle collection efficiency and (■) pressure drop in the Turbosep when challenged with 10 μm diameter particles and a 500 L min⁻¹ air flow rate. The underflow diameter in the standard design is 0.0254 m.

6.1.6 The Effect of Vane Angle on the Performance of the Turbosep.

On entry into the Turbosep the flow is directed through a set of static angled vanes, which impart a swirling motion to the flow. As the vane angle is increased about the horizontal axis i.e. less steep, particle collection efficiency increases but with no significant increase in pressure drop (Figure 6.7). Increasing the vane angle by 5 ° over the original design results in a 5 % increase in the predicted particle collection performance.
Figure 6.7 Numerically predicted effect of changing the vane angle on (●) particle collection efficiency and (■) pressure drop in the Turbosep when challenged with 10 μm diameter particles and a 500 L min⁻¹ air flow rate. The vane angle in the standard design is 45°.

6.1.7 The Effect of the Impingement Plate on the Performance of the Turbosep.

The impingement plate has been designed to accelerate natural foam collapse and separation processes. Visual observations of the flow across the impingement plate in a Perspex Turbosep have shown that it causes the flow to change direction and a phase separation of the liquid/foam and gas phases to occur (Ridealgh, personnel communication). The separated liquid droplets coalesce into larger droplets as the liquid drains over the slope of the impingement plate.

However, the impingement plate was thought likely to hinder the development of the swirling component of the flow and also to increase the pressure energy requirements due to the compression of the fluid as it passes through the narrow orifice between the impingement plate and the outer wall of the Turbosep.
To quantify the effect of the impingement plate on the pressure drop and particle collection characteristics of the Turbosep, a simulation was performed on a computation grid in which the impingement plate boundary had been deleted. Removal of the impingement plate resulted in an 87% predicted particle collection efficiency and a 15.1 mBar decrease in pressure drop. Figure 6.8 illustrates the effect of this design change on the predicted trajectories of 10 μm diameter particles. The absence of an impingement plate led to an increased swirling motion of the fluid compared to the original design (see Figure 3.11).

The results highlight the benefits of removing the impingement plate in terms of particle capture and pressure drop. However, its removal is likely to reduce the foam separational efficiency of the Turbosep. This will need to be examined experimentally.
Figure 6.8. Numerically predicted trajectories of particles injected at a simulated air flow rate of 500 Lmin$^{-1}$ from 100 locations along the inlet plane in the absence of the impingement plate. Particles were 10 μm in diameter and had a coefficient of restitution of 0.1.
6.1.8 The Effect of the Vortex Arrester on the Performance of the Turbosep.

The Vortex Arrester acts to halt the swirl of the gas stream and creates a quiescent zone. This theoretically serves two functions; firstly it convert the kinetic energy of the swirling flow to potential energy thus reducing pressure losses within the Turbosep and secondly it prevents the re-entrainment of the separated material allowing it to drain through the underflow.

To estimate the impact of the vortex arrester on the pressure drop and particle collection characteristics of the Turbosep a simulation was performed on a computation grid, which had the vortex arrester boundary removed. The effect of this design change on the predicted particle trajectories was unexpected. It is apparent that the swirling velocity component below the vortex finder increases (Figure 6.9) as the fluid travels along the axial length of the Turbosep and results in an increased particle hold-up in the region above the underflow aperture.

This design change resulted in the observed particle collection efficiency increasing almost to 100% for 10 µm diameter particles at 500 Lmin⁻¹ airflow rate (Figure 6.10), coupled with only a 4.8 mBar increase in the predicted pressure drop. The pressure at the underflow aperture is slightly negative (See Figure 3.8). This negative pressure gradient will draw particles towards the underflow and the increased particle hold-up can be described by force balance between the suction effect at the underflow and the bulk flow movement upward towards the overflow. The fact that this flow phenomenon is not observed when the vortex arrester is present suggests that the negative pressure gradient is localised below the plane of the vortex arrester and can only influence the trajectories of particles that are in the vicinity of the underflow aperture.

Remove the vortex arrester also improves the collection efficiencies for smaller particles (Figure 6.10).
Figure 6.9. Numerically predicted trajectories of particles injected at a simulated air flow rate of 500 Lmin$^{-1}$ from 100 locations along the inlet plane in the absence of the vortex arrester. Particles were 10 μm in diameter and had a coefficient of restitution of 0.1.
Figure 6.10. The effect of removing the vortex arrester on the CFD predicted particle collection efficiency of the Turbosep at 150 Lmin⁻¹ and 500 Lmin⁻¹ airflow rate. (▼) With vortex arrester at 150Lmin⁻¹ and (▲) 500 Lmin⁻¹, (●) without vortex arrester at 150 Lmin⁻¹ and (■) 500 Lmin⁻¹.

6.1.9 The Influence of Body Diameter on the Performance of the Turbosep.

Several authors (Kim and Lee, 1990; Dirgo and Leith, 1985) have reported the effects of the cyclone body diameter. The authors recognised that the smaller the diameter the cyclone the smaller the d₅₀. This is paid for by an increase in pressure drop at the same flow rate or by the need to accept a smaller flow rate at the same pressure requirement.

The effects of body diameter were not investigated within this study. The body dimensions of the Turbosep have previously been sized specifically to withstand a pre-defined volumetric foam challenge (Ridaelgh, personnel communication). Reducing the diameter of the Turbosep to increase particle collection efficiency would inevitably reduce the foam capacity of the Turbosep.
6.2 Summary of Design Parameters.

It is evident from the individual parameter investigations that the performance of the Turbosep is a function of a series of complex geometrical interactions. Each parameter investigated appears to have an effect on the performance of the Turbosep. Furthermore, when attempting to optimise both the particle collection efficiency and pressure drop the results often appear to be in conflict, for example, reducing the diameter of the feed inlet to 0.0127m (0.5 inches) resulted in a 4 % increase in collection efficiency, but this was coupled with a 8.6 fold increase in the pressure energy requirements. A balance needs to be reached in achieving the greatest level of particle collection whilst maintaining the pressure drop within pre-defined limits. A summary of the effect of the different design parameters on the particle collection characteristics and pressure drop in the Turbosep is shown in table 6.1.

Of all the geometric parameters investigated, perhaps most interesting was the effect of removing the vortex arrester. This single design change lead to the capture of all of the simulated particles, with only a minimal 4.8 mBar increase in the pressure drop.

Table 6.1 Summary of the effects of design parameters of the performance of the Turbosep.

<table>
<thead>
<tr>
<th>Design Variable</th>
<th>Particle Collection Efficiency</th>
<th>Pressure Drop</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in Free Height</td>
<td>Decrease in Efficiency</td>
<td>Increase in Pressure Drop</td>
</tr>
<tr>
<td>Increase in Vortex Finder Length</td>
<td>Decrease in Efficiency</td>
<td>Increase in Pressure Drop</td>
</tr>
<tr>
<td>Increase in Vortex Finder Diameter</td>
<td>Increase in Efficiency</td>
<td>Increase in Pressure Drop</td>
</tr>
<tr>
<td>Increase in Feed Diameter</td>
<td>Decrease in Efficiency</td>
<td>Decrease in Pressure Drop</td>
</tr>
<tr>
<td>Increase in Underflow Diameter</td>
<td>Increase in Efficiency</td>
<td>Decrease in Pressure Drop</td>
</tr>
<tr>
<td>Increase in Vane Angle</td>
<td>Increase in Efficiency</td>
<td>Increase in Pressure Drop</td>
</tr>
<tr>
<td>Removal of the Vortex Arrester</td>
<td>Increase in Efficiency</td>
<td>Increase in Pressure Drop</td>
</tr>
<tr>
<td>Removal of the Impingement Plate</td>
<td>Increase in Efficiency</td>
<td>Decrease in pressure Drop</td>
</tr>
</tbody>
</table>

A number of authors (Dirgo and Leith, 1985; Kim and Lee, 1990;) have suggested semi-empirical methods for dimensioning a cyclone. However, as an optimum design will
depend upon the operating parameter such as feed size, inlet velocity and the size distribution there is probably no such thing as an optimum design applicable to all duties.

6.3 Combination Effects.

Of the individual design parameters investigated in the previous sections it is evident that free length, vortex finder length, inlet diameter, vortex finder diameter and vortex arrester all have a significant effect on the observed particle collection performance of the Turbosep. However, the diameter of the vortex finder and feed inlet already appears to be optimal, decreasing the length of the former results in a decrease in particle collection efficiency (Figure 6.4) while reducing the diameter of the latter results in the pressure energy requirements increasing to an inoperable level (Figure 6.5).

The greatest improvement in collection efficiency resulted from the removal of the vortex arrester, therefore, this design change was studied in combination with a limited number of other design changes. These were the vortex finder length and the free height of the Turbosep.

It can be seen in Figure 6.11 that the collection efficiency for 10 μm diameter particles in all three test designs showed a significant improvement over the original. On a linear scale these improvements are greatest for smaller particles, but the absolute efficiencies do not approach 100% as they do for the larger particles. It is also striking that when the vortex arrester is removed, a reduction in the vortex finder length reduces the collection efficiency, rather than increasing it as it does when the vortex arrester is in place (see Figure 6.2). The effect of changes in the overall length of the Turbosep remains as before (see Figure 6.1), although obviously there is little room for improvement in the capture of the 10 μm diameter particles.

The highest level of collection efficiency resulted from the removal of the vortex arrester and so all further studies were based on this design.
Figures 6.11 A comparison of the numerically obtained particle collection efficiency of the original design of Turbosep (●) with a design that had the vortex arrester removed (▼), a design that had the vortex arrester removed and the length of the vortex finder reduced to 0.042 m (▲) and a design that had the vortex arrester removed and the free height reduced to 0.3 m (■).

6.4 Experimental Validation of Removal of the Vortex Arrester.

The experimental validation of the new design was performed essentially as described Sections 2.4 and 2.2. When challenged with an aerosol containing *E. coli* the collection efficiency of the new design was consistently higher than the original design. It can be seen in Figure 6.12 that the most significant improvements in performance occurs at airflow rates of 250 Lmin⁻¹ or below, although it is only at the higher flow rates that the efficiencies approach 100%.

When the experimental data is compared with the equivalent data from the CFD analysis in Figure 6.10 it is clear that the actual collection efficiencies are similar to the values predicted for 10 μm diameter particles. This provides an objective validation for the choice of this particle size in the earlier CFD analyses (Figures 6.1 to 6.10).
Furthermore, the particle collection performance of the new design when challenged with foam containing an *E.coli* suspension was found to be equivalent to the original design. At airflow rates ranging from 150 to 500 L min\(^{-1}\) the microbial load per unit volume in the overflow was 6-orders of magnitude lower than it was at the inlet to the Turbosep.

![Figure 6.12](image-url) A comparison of the Turbosep's collection efficiency when challenged with *E.coli* RV308 pHKY531 bioaerosol (broth cell concentration \(1 \times 10^{10}\) cells mL\(^{-1}\)) in a 250 L bioreactor. (○) Original Turbosep design and (■) new Turbosep design.

**6.5 The Effect of Scale on the Particle Collection Performance of the Turbosep.**

The CFD characterisation studies performed so far have been based on the smallest sized Turbosep. This unit has a rated operational air flow range of 150–500 L min\(^{-1}\). However, the Turbosep range consists of 9 units that between them operate over an air flow range of 150 L min\(^{-1}\) to 140K L min\(^{-1}\). Each unit has been scaled on constant geometric similarity, using standard pipe sizes, such that the inlet velocities of each unit over a defined airflow range are identical.
Ideally the performance of each of the units in terms of particle collection efficiency and foam separation would be identical. Unfortunately the size of the fermenters in the ACBE was prohibitively small to allow experimental characterisation on any of the larger units. Therefore, scale-up investigations were performed using CFD. The flow of particles through the 0.0254 m (1 inch), 0.0508 m (2 inch) and 0.1016 m (4 inch) inlet diameter units was modelled as described in Chapter 3. All simulations were based on the new design which had the vortex arrester removed while all other dimensions were unchanged.

Before discussing the results it should be noted that grid independence could not be achieved in the 0.1016 m (4 inch) inlet diameter unit. The number of control volumes required to achieve independence was in excess of the memory available on the RS6000 Workstation. Therefore the accuracy of this simulation is questionable. Figure 6.13 shows a comparison between each of the units, with only a small decrease in efficiency with increasing scale.

Figure 6.13. A comparison of the effect of scale on the particle collection efficiency of 10 μm equivalent diameter particles in the Turbosep with a (Δ) 0.1254m diameter inlet, (■) 0.0508m diameter inlet and (●) a 0.1016m diameter inlet.
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Based on traditional cyclone theory, where body diameter is inversely proportional to particle collection efficiency (Dirgo and Leith, 1985), it would be expected that efficiency would decrease with increasing diameter. However, the collection efficiency of 10 μm diameter particles in the Turbosep is virtually independent to the generated centrifugal force (see Section 3.2.3.2) and will therefore be less influenced by an increase in body diameter.

A fraction of smaller particles are collected on the outer walls on the Turbosep and so it would be expected that as the diameter of the Turbosep is increased the collection efficiency of these smaller diameter particles would be reduced.

However, in terms of particle collection efficiency the new design has been shown to be significantly better than the original design. Even if collection efficiency was reduced with increasing scale, the levels of particle capture in the new design would still be significantly higher than that observed in the original design.
7. Summary and Conclusions.

This chapter will begin by drawing together the findings of the thesis and will then discuss some of the issues raised. The question of incidental versus accidental release will be considered together with the role of the Turbosep in containing the exhaust gas from a fermenter. The application of CFD for tracking the movement of particles in the Turbosep will also be discussed.

7.1 Summary of Key Results.

There is much published data on the fermenter design for containment (Pennman, 1989; Hambleton et al, 1991; Lever and Hambleton, 1992), however, few, if any of these studies use a quantitative approach. Therefore it was apparent that there was a need for a programme to quantitatively assess the release of micro-organisms from a fermentation or from other process equipment (Ferris et al, 1995; Noble et al, 1997; Noble et al, 1999). Noble et al (1997) monitored the release of *E. coli* into the fermenter exhaust gas from a 2 L fermenter using a sampling cyclone-QPCR methodology. It was found that over the time course of a 5.5 hour period, $3 \times 10^7$ process cells were released into the exhaust gas stream. If there were more data available on the scale of accidental and incidental release from a process then performance criteria could be written more confidently and as a consequence contained bioprocesses designed with reference to a measurable set of performance parameters.

The influence of the operating parameters of a fermentation on the release of micro-organisms into the exhaust gas was investigated at different scales. It was found that *E. coli* release rates increased with increasing agitation and aeration and were highest in the largest scale (250 L) vessel. The total number of *S. cerevisiae* cells released was found to be approximately one order of magnitude lower than the observed release rates for *E. coli* under identical experimental conditions. The increase in the settling velocity that occurs according to Stokes Law as the particle diameter increases (at a fixed density difference between the particle and the air) suggests that at the air flow rates employed *S. cerevisiae* cells are too large to be effectively aerosolised.
Throughout this study it was found that micro-organisms were released into the exhaust gas in only very low numbers. At an agitation rate of 500 rpm and an aeration rate of 1.5 L min\(^{-1}\), approximately 1.5 \(\times 10^5\) \(E.\) coli cells were released per minute from a 2L fermenter. Based on this result it can be estimated that 5 \(\times 10^7\) cells will be released into the exhaust gas stream over the course of a 5.5 hour fermentation. This is equivalent to the loss of 3.3 \(\mu\)L of broth liquid at harvest. The estimated number of cells released is slightly higher than that observed by Noble \textit{et al} 1997. However, the estimate was based on the extrapolation of release measured at stationary phase, when the cell concentration is at its highest and it has been shown (Figure 5.8) that the higher the broth cell concentration, the larger the number of cells released.

The installation of the Turbosep onto a 250 L fermenter was found to reduce the microbial concentration in the exhaust gas by approximately 6 orders of magnitude whilst recirculating foam. In the absence of foam the microbial concentration in the exhaust gas was reduced by approximately 4 orders of magnitude. The number of cells released from the Turbosep per m\(^3\) of exhaust gas was approximately 6 \(\times 10^6\) whilst recirculating foam and 1 \(\times 10^7\) in the absence of any foam. As the number released is low, particularly in relation to the total process volumes concerned, containment systems should be designed to reduce the accidental release rather than the incidental. An accidental foam-out, which released 1 mL of fermentation broth, would allow 1 \(\times 10^{10}\) cells to escape into the processing environment. Based on a fermentation time of 5.5 hours, the release from this single accident is equivalent to the cumulative incidental release from 764 fermentations.

The Turbosep mechanical foam separator has been shown to be an efficient device for separating foams emerging from highly aerated fermentation processes. However the fouling of the foam probe interferes with the normal antifoam dosing strategy and causes variation in the volumes of anti-foam administered between batches of the same fermentation. A novel control strategy for the addition of anti-foam into a fermenter fitted with a Turbosep has been described. Differential pressure measurements across the Turbosep were linked to the provision and regulation of antifoam addition. Using the Turbosep in conjunction with the differential pressure control strategy allowed the addition of antifoam to be totally automated and dependent on the requirements of the
fermentation at any time. The Turbosep was determined to reduce the antifoam requirements of a 6000 L \textit{E.coli} fermentation by approximately 66\%, leading to a 16\% increase in productivity without changing any of the process operating parameters. As the Turbosep manages the foaming process rather than eliminating it through the excessive use of antifoam, light foaming can be harnessed as a means of effective gas transfer. An increased working volume is also possible as foam is allowed to pass into the exhaust gas pipe without compromising the fermentation, thus further increasing the productivity of the process.

Computational fluid dynamics (CFD) has been proven to be an effective way to model the performance of the Turbosep in terms of particle collection efficiency. Performance curves were produced with approximately the same shape and $d_{50}$ as those obtained by experiment. Furthermore the predicted pressure drops were in excellent agreement with the measured data. The CFD model was able to predict the salient features of the Turbosep flow field, thus providing a better understanding of the fluid dynamics of the device. It was originally believed that the particle collection mechanics of the Turbosep would be similar to that of any other cyclone. However, CFD has demonstrated that particle capture is more likely to be based on sedimentation according to Stokes Law rather than centrifugal force. The use of the CFD technique to predict the fluid flow and particle penetration allowed a systematic investigation of the influence of the main design parameters on the Turbosep performance to be investigated in a very cost effective manner and provide valuable guidance in the process of optimising the design of the Turbosep. Of the new designs considered, the greatest improvement in collection efficiency resulted from the removal of the vortex arrestor. On a linear scale this improvement was greatest for smaller particles, but the absolute efficiencies did not approach 100\% as they did for the larger particles. The predicted particle collection efficiency of the new design was at least 30\% higher than the original design.

### 7.2 Incidental versus Accidental Release.

At the present time the biosafety regulations use qualitative statements to define quantitative events, such as minimising or preventing a release (HSE, 1992). This has resulted in a common approach to containing a unit operation through increasing its primary containment, for example, use of double (in series) hydrophobic filters to
contain the exhaust gas (Hambleton et al (1991). However, single exhaust gas filters are considered sufficient to minimise release and therefore comply with containment categories B1 and B2 (Lever and Hambleton, 1992). The addition of a second filter adds another degree of complexity, which in turn could bring with it an increased chance of miss-operation potentially resulting in an accident (Dowell and Hendershot, 1997) and furthermore, may do nothing to reduce the occurrence of release.

The measured levels of incidental release observed during the course of this study are very small compared with the total process volume. Furthermore, should the provision of an exhaust gas filter contribute to a major accident then its use might be questioned. For instance, this might occur as a result of plugging by foam escaping from the fermenter causing an increase in the pressure within the fermenter leading to a bursting disk rupture. Although accidental release has a lower probability of occurring, it has been noted that no engineering plant and no structure is entirely risk free and there is no logical way of differentiating between 'credible' and 'incredible' accidents (Farmer, 1967). When assessing risk, the frequency of occurrence and the consequences of the failure must be considered. The failure for some industrial activities has been calculated by Israeli (1986) and Jefferis and Schlager (1986). A summary of their findings is presented in Table 7.1

**Table 7.1** The failure probabilities of some industrial items and operations.

<table>
<thead>
<tr>
<th>Item or activity</th>
<th>Failure probability (per hour of operation)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human error</td>
<td>$10^{-2} - 10^{-3}$</td>
<td>Israeli, 1986</td>
</tr>
<tr>
<td>Failure of sensing elements</td>
<td>$10^{-3} - 10^{-5}$</td>
<td>Israeli, 1986</td>
</tr>
<tr>
<td>Failure of diaphragm valve</td>
<td>$10^{-5}$</td>
<td>Jefferis and Schlager, 1986</td>
</tr>
<tr>
<td>Failure of mechanical seal</td>
<td>$10^{-5}$</td>
<td>Jefferis and Schlager, 1986</td>
</tr>
<tr>
<td>Failure of steam valve</td>
<td>$10^{-6}$</td>
<td>Jefferis and Schlager, 1986</td>
</tr>
<tr>
<td>Failure of containment</td>
<td>$10^{-6}$</td>
<td>Jefferis and Schlager, 1986</td>
</tr>
</tbody>
</table>
From Table 7.1 it can be seen that the biggest source of failure in a process is human operator. Human error can be split into two categories; those relating to the specific operations, and those relating to general activities (Tweedale, 1992), where specific activities encompass plant maintenance and general activities relate to the management and supervisory weaknesses that cause mistakes.

Miller and Bergman (1993) put forward a view that the public could perceive the over design of a containment facility as exhibiting a lack of confidence in a particular operation or facility. Whilst it should be acknowledged that some bioprocesses require high containment, more appropriate engineering approaches such as described by Brookes and Russell (1986) should be advocated. When adopting a unified approach to equipment design it should be noted that a sterile environment is one in which no microorganisms exist that are capable of growth, compared with a containment system where no transmission of material across a process boundary is possible. This type of unified design can lead to a more economic process as long as the difference between containment and sterility is recognised.

Since levels of incidental release appear to be low, the threat posed by accidental release needs to be addressed. The subject of risk and risk analysis is vast and has been discussed by Montague (1990), reviewed by Sherif (1992) and applied to the biotechnology industry by Kastelein (1992). Kastelein concludes that the quantification of risks associated with biotechnology are considerably more complicated than for conventional chemical industries. Predominantly this difficulty stems from the lack of information on incidental and accidental process release. Through the generation of quantitative data a hazard can be identified and then the risk it poses assessed and managed. An unidentified hazard cannot be managed or its effect only be made manageable by chance (Schumacher et al, 1997). In a perfectly contained process there is a balance between the procedures, training and design that enables containment to be achieved and maintained. This has been described as a biocontainment triangle (Miller and Bergmann, 1993) where a weakness in any of the three sides of the triangle results in a loss of containment.

In addition to release considerations, exposure assessment also needs to be taken into consideration. Winkler and Parke (1992) gave an example of an exposure assessment. In
Chapter 7. Summary and Conclusions

This scenario, it is calculated that the release of micro-organisms into the environment from the fermenter exhaust gas, using a siphon to reduce the microbial burden, is less than 100 micro-organisms second$^{-1}$ for a 50 m$^3$ fermentation. The dilution of the released aerosol in the atmosphere by horizontal and vertical diffusion was calculated according to a single plume model. It was found that even at low wind speed the plume spread to a height of 300-400 m and a width of 800 m. The dilution that was calculated was sufficient to reduce releases of 1000 micro-organisms second$^{-1}$ to a concentration of less than 1 micro-organism m$^{-3}$ at ground level at a distance of less than 100 m. In addition, it was calculated that only a few micro-organisms sediment per m$^2$ hour$^{-1}$. Since continuous variations in wind speed and direction will prevent sedimentation at identical sites it was concluded that niches at ground level or on plants are generally only reached by single organisms. The likelihood of survival and multiplication in the environment is further reduced by the inability of many micro-organisms to survive aerosolisation and the competition that will be encountered from indigenous micro-organisms at the site of deposition.

A problem of the current legislation is that at containment levels B3 and B4 release of micro-organisms must be prevented. Since there is always a risk of a credible accident, absolute prevention of release is impossible. If these risks are not acknowledged, then it is possible that processes will be designed where accidental releases are ill considered and incidental releases are prevented by complex and costly engineering. In the chemical industry, where operating conditions are more hazardous than those used in bioprocessing, user friendly (forgiving) design has taken hold in the concept of inherently safer design (Kletz, 1996).

7.3 The Effect of the Turbulence Model and Coefficient of Restitution.

Due to turbulent flow in the Turbosep, the key to success in CFD lies with the accurate description of the turbulent behaviour. A number of turbulence models are available ranging from the industry standard k-ε model to the more complex Reynolds Stress model. However, the k-ε model has shown to be inadequate for the calculations of flows with swirl (Boysan et al, 1982) because it leads to excessive levels of turbulent viscosity and unrealistic tangential velocity distributions. The Reynolds Stress model performs
much better than the k-ε model in swirling flows, but it has the disadvantage of being computationally expensive. Turbulence models based on statistical rather than continuum mechanics such as the RNG k-ε model (Yakhot and Smith, 1992) have been reported to have the mathematical simplicity of the k-ε model whilst having the accuracy of the Reynolds Stress model. Within this study the RNG k-ε model was found to provide an excellent correlation with the experimental data. The standard k-ε model performed less well, the false predictions being attributed to the generation of anisotropic Reynolds stresses caused by the swirling motion of the fluid.

The question of an organisms coefficient of restitution has been raised during the course of this study. In other work it has been suggested that micro-organisms would be expected to adhere to walls (Agutter, personnel communication). The results of this study have enabled a comparison of experimental and CFD results that indicate that organisms have a coefficient of restitution of 0.1, so it is worth considering the question of how particles adhere to walls further.

The likelihood of an organism being resuspended into the bulk flow after impacting a wall is dependent upon several factors (Vincent, 1989):

- Organisms will bounce if the velocity of the organism normal to the surface exceed some critical threshold (Dahneke, 1971; Dahneke, 1973). This phenomenon is a function of both the organism size and surface properties. Thus bounce would be expected to be greater in regions of aerodynamic stagnation. Alternatively, organisms will be re-entrained into the bulk air flow if the drag force of the organism in a sheared boundary layer is greater than a critical value, which is determined by local friction forces which are trying to retain the organism (Vincent, 1989). In situations where the velocities of the organisms are low enough to preclude bounce, re-entrainment may be the predominant mechanisms for removing them from surfaces.
- The magnitude and direction of local aerodynamic forces acting upon the organism.
- The 'stickiness' of the organism
Also the effects of electrostatic charge must be considered since it is known that during aerosol generation particles become electrostatically charged, the mechanism of charging being dependent upon the method of aerosol generation (Vincent, 1989). It has been reported that relative humidity and electrostatic charge play a significant part in the adhesion forces holding particles and surfaces in contact (Com and Stein, 1965; Pich, 1966). The charge of both the particle and the wall has an effect and the magnitude of this effect increases as particle size increases. The repulsive effect of similarly charged particles is greater than the attractive effects of oppositely charged particles (Vincent, 1989). Hence, for an aerosol with an equal number of positively and negatively charged organisms, the net effect of an electrostatic charge will always be for fewer organisms to adhere to a wall.

This phenomena is extremely complex and factors such as thermal gradients complicate the matter further. In these studies the coefficient of restitution has been used simply as a means of describing the various levels of adhesion to walls and a value of the coefficient of restitution which best fits the experimental data has been provided. No attempt has been made to describe the complex mechanisms described above or to model the electostatically charged organisms.
7.4 Future Work.

1. The Containment of Fermenter Exhaust Gases

All of the studies to date on the collection efficiency of the Turbosep have focused on cells (E.coli and S. cerevisiae) that are assumed to be approximately cylindrical and have a collective particle size distribution between 1 – 10 μm in diameter. This work should be extended to include other organisms of different shapes and sizes. Streptomycetes, for example are often filamentous and may have a length of 10 μm or above, while phage particles are normally sub-micro in size. When extending the work to include these organisms the following issue should be noted:

- A QPCR assay will need to be developed for each new organism studied.
- Due to the length of the Streptomycetes they would be more susceptible to shear damage in the sampling cyclone.
- The sampling cyclone is inefficient in collecting sub-micro sized particles and therefore a new sampling method would need to be developed for phages. Theoretically phages could be captured and recovered from a filter.

2. The Coefficient of Restitution.

Throughout this thesis no attempt has been made to model the deposition and bounce of particles onto a surface. Instead a value of the coefficient of restitution that best fits the experimental data has been used. To provide a further validation of the CFD work presented in this thesis it would be useful to experimentally determine the coefficient of restitution for different micro-organism. This could be achieved by using high-speed magnified photography to measure the deposition and bounce of different sized cells, at different impact velocities and angles onto a surface.

3. Blinding of the HEPA Filter Downstream of the Turbosep.

Work presented in Appendix G has shown that a HEPA filter positioned upstream of the Turbosep is more susceptible to blinding compared to when it is protected by a
hydrophobic pre-filter. The cause of this blinding is thought to be due to condensate forming in the pipe work and filter body. Comparative experiments should be performed using different combinations of Turbosep, condenser and pre-filter to identify the optimal arrangement for the protection of the HEPA filter.


Work presented in Appendix H has shown that the DNA from process organisms released into the environment can be detected for up to eight weeks. These ‘rough and ready’ experiments were prone to sampling error and as a result this work should be repeated using a more controlled and defined set of experimental conditions. Furthermore, it would be interesting to compare the persistence of E.coli and S.cerevisiae with other commonly used process organisms for example the streptomycetes.


Since levels of incidental release observed in this study appear to be low, the threat posed by accidental release needs to be addressed. Risk analysis techniques such as fault tree analysis commonly used in the chemical industry could be adapted to suit bioprocessing requirements and used to estimate the frequency and cause of accidents.
Appendix A. Detailed Drawings of the Turbosep and Expansion Tank.

Figure A1. The 150 – 500 Lmin$^{-1}$ Turbosep.
The expansion tank was connected directly to re-cycle line in between the Turbosep and the bioreactor. Foams and liquids from the Turbosep underflow enter at point a and pass into the bioreactor at point b. Antifoam was added into the Expansion Tank at point c.
Appendix B. List of Suppliers.

Adolf Kuhner Ag, Basel, Switzerland
Air Control Installations, Chard, Somerset, UK
Alfa-Laval, Camberley, Surrey, UK
Anachem, Luton, Bedfordshire, UK
Bassaire Ltd, Swanwick, Southampton, UK
Beckman Instruments, High Wycombe Buckinghamshire, UK
Bioengineering Ag, Wald, Switzerland
Biolog Inc., Hayward, California, USA
Broadley James Corporation, Santa Ana, California, USA
Carr Separations Inc., Easton, Massachusetts, USA
Celsis Ltd, Cambridge, UK
Chemap, France
Constant Systems Ltd, Warwick, UK
Costar, Cambridge, Massachusetts, USA
Digitron Instruments Ltd, Hereford, UK
Domnick Hunter, Birtley, Co. Durham, UK
Elwyn E. Roberts Isolators Ltd
Fisons Scientific Equipment, Loughborough, Leicestershire, UK
Fisons Instruments, Middlewich, Cheshire, UK
Gelman, Ann Arbor, Michigan, USA
Gibco BRL Life Technologies, Uxbridge, Middlesex, UK
Glen Creston Ltd, Stanmore, Middlesex, UK
Hays Chemical, Leeds, UK
Hybaid, Teddington, Middlesex, UK
Ilford, Mobberley, Cheshire, UK
Incelltech (UK) Ltd, Reading, UK
MDH Ltd, Andover, Hampshire, UK
MSE, Crawley, Sussex, UK
New Brunswick, Edison, New Jersey, USA
New England Biolabs, Beverley, Massachusetts, USA
Nikon, Kingston upon Thames, Surrey, UK
Oxoid, Unipath Ltd., Basingstoke, Hampshire, UK
Perkin Elmer, Foster City, California, USA
Pharmacia, Milton Keynes, Bedfordshire, UK
Promega, Madison, Wisconsin, USA
Qiagen, Chatsworth, California, USA
Sigma, Poole, Dorset, UK
Sigma Laboratory Centrifuges, Osterode, Harz, Germany
Silverson Machines Ltd., Chesham, Bucks, UK
Sinclair Stainless Steel Fabrications, UK
Soham Scientific, Soham, Cambridgeshire, UK
Stratagene, La Jolla, California, USA
Th Goldschmidt Ltd, Milton Keynes, Bedfordshire, UK
UVP Ltd, Cambridge, UK
Warren Spring Laboratories, now at AEA Technology, Harwell, Didcot, Oxon, UK
Watson-Marlow, Falmouth, Cornwall, UK
Weber Scientific International Ltd, Teddington, Middlesex, UK
Appendix C. Calculation of the PCR Product Concentration.

The internal standards have a linear range of quantification described by the following regression analysis.

**For S. cerevisiae (S150Δhsp82)**

Log (peak area ratio (IS(Y)1)) = 1.30 - (0.67 × Log [S150Δhsp82])
Log (peak area ratio (IS(Y)2)) = 2.78 - (0.72 × Log [S150Δhsp82])
Log (peak area ratio (IS(Y)3)) = 3.91 - (0.69 × Log [S150Δhsp82])

**For E.coli (RV308 pHKY531)**

Log (peak area ratio (IS(B)1)) = 3.04 - (0.85 × Log [pHKY531])
Log (peak area ratio (IS(B)2)) = 5.55 - (0.97 × Log [pHKY531])
Log (peak area ratio (IS(B)3)) = 7.88 - (0.95 × Log [pHKY531])

The following example illustrates how the products of a QPCR reaction can be used to calculate the cell concentration of *E.coli* RV308 pHKY531 in a sample. An identical procedure is used for calculating the concentration of *S.cerevisiae* S150Δhsp82.

**Agarose gel electrophoresis.**

Once the PCR cycle is completed the product mix is run on an agarose gel to separate the individual components.

Figure C1. Agarose gel showing the results of a QPCR of a sample containing an unknown concentration of pHKY531. Lane 1 contains IS(B) 1 and the sample, Lane 2 contains IS(B) 2 and the sample, Lane 3 contains the molecular weight marker (PCR Marker, Sigma).
From Figure C1 it can be seen that the plasmid concentration in lane 2 is not in the range of IS(B) 2 as there is no distinct band visible for the internal standard. Lane 1 is chosen for densitometry analysis as both bands are clearly visible on the gel.

**Densitometry Analysis.**

Densitometry provides a method of analysis of the gel that can give the ratio of pHKY531 and IS(B) 1 band intensities. A spectrum of band intensity for the lane under analysis is produced and the peak area for the bands determined.

**Table C1. Densitometry analysis of lane 1.**

<table>
<thead>
<tr>
<th>Band Identity</th>
<th>Peak area (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHKY531</td>
<td>1100</td>
</tr>
<tr>
<td>IS(B) 1</td>
<td>235</td>
</tr>
</tbody>
</table>

**Calculation of the pHKY531 concentration.**

The standard IS(B) 1 has a linear range for quantification described by the following regression analysis.

\[
\log (\text{peak area ratio (IS(B):pHKY531)}) = 3.04 - (0.85 \times \log [\text{pHKY531}])
\]

Therefore the log (peak area ratio (IS(B):pHKY531)) = -0.67

Substituting this value into the regression analysis for IS(B) 1 and solving for pHKY531 concentration gives

\[
\log [\text{pHKY531}] = (3.04 - (-0.67))/0.85
\]

Therefore \( \log [\text{pHKY531}] = 4.36 \)

Therefore \( [\text{pHKY531}] = 2.3 \times 10^4 \) molecules in 10 µL of sample.
Appendix D. The Navier-Stokes Equations.

Continuity equation:

\[
\frac{\partial \rho}{\partial t} + \frac{\partial (\rho u)}{\partial x} + \frac{\partial (\rho v)}{\partial y} + \frac{\partial (\rho w)}{\partial z} = 0
\]

Momentum equations:

\[
\frac{\partial (\rho u)}{\partial t} + \frac{\partial (\rho u^2)}{\partial x} + \frac{\partial (\rho uv)}{\partial y} + \frac{\partial (\rho uw)}{\partial z} = -\frac{\partial p}{\partial x} + \frac{\partial \tau_{yx}}{\partial y} + \frac{\partial \tau_{yz}}{\partial z} + \delta \frac{\partial u}{\partial x} + \delta \frac{\partial T}{\partial x} + \delta \frac{\partial \gamma}{\partial x} + \delta \frac{\partial w}{\partial x}.
\]

\[
\frac{\partial (\rho v)}{\partial t} + \frac{\partial (\rho v^2)}{\partial y} + \frac{\partial (\rho uv)}{\partial x} + \frac{\partial (\rho vw)}{\partial z} = -\frac{\partial p}{\partial y} + \frac{\partial \tau_{xy}}{\partial x} + \frac{\partial \tau_{xz}}{\partial z} + \delta \frac{\partial v}{\partial y} + \delta \frac{\partial T}{\partial y} + \delta \frac{\partial \gamma}{\partial y} + \delta \frac{\partial w}{\partial y}.
\]

\[
\frac{\partial (\rho w)}{\partial t} + \frac{\partial (\rho w^2)}{\partial z} + \frac{\partial (\rho vw)}{\partial y} + \frac{\partial (\rho ww)}{\partial x} = -\frac{\partial p}{\partial z} + \frac{\partial \tau_{zy}}{\partial y} + \frac{\partial \tau_{zz}}{\partial z} + \delta \frac{\partial w}{\partial z} + \delta \frac{\partial T}{\partial z} + \delta \frac{\partial \gamma}{\partial z} + \delta \frac{\partial w}{\partial z}.
\]

Energy equation:

\[
\frac{\partial (\rho h)}{\partial t} + \frac{\partial (\rho h u)}{\partial x} + \frac{\partial (\rho h v)}{\partial y} + \frac{\partial (\rho h w)}{\partial z} = -\frac{\partial p}{\partial x} + \frac{\partial \tau_{yx}}{\partial y} + \frac{\partial \tau_{yz}}{\partial z} + \frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} + \frac{\partial w}{\partial z} + \frac{\partial T}{\partial x} + \frac{\partial T}{\partial y} + \frac{\partial T}{\partial z} + \delta \frac{\partial T}{\partial x} + \delta \frac{\partial T}{\partial y} + \delta \frac{\partial T}{\partial z} + \delta \frac{\partial \gamma}{\partial x} + \delta \frac{\partial \gamma}{\partial y} + \delta \frac{\partial \gamma}{\partial z} + q.
\]

Equation of State:

\[p = \rho RT\]
Appendix E. Experimental Grade Efficiency Curve.

Experiments to obtain a grade efficiency curve for the 150 – 500 L min⁻¹ Turbosep had previously been performed by Domnick Hunter. A summary of the experimental protocol is summarised below.

A known weight of sized particles (with a high tolerance) were aerosolised in a compressed air stream and the flow directed through the Turbosep. Particles were collected both at the underflow and overflow (iso kinetically) apertures. At the end of the experiment the weight of particles collected at both apertures was measured and the grade efficiency calculated. The apparatus used is shown in Figure E.1.

This procedure was repeated for a range of different particles sizes to produce the grade efficiency curve shown in Figure 3.10.

Figure E.1 The experimental apparatus used to obtain a grade efficiency curve
Appendix F. Calculation of the Settling Velocity of a Micro-Organism.

Stokes Law can be used to calculate the settling velocity of a single *S. cerevisiae* or *E. coli* cell. This settling velocity can be compared to the velocity of the gas leaving the broth surface. If the settling velocity of the cell is greater than the rising velocity, the cell will not become aerosolised and will not leave the fermenter in the exhaust gas line. If the settling velocity is less than the rising velocity the cell will become aerosolised and will be released from the fermenter.

Stokes Law, \[ u = \frac{\Delta \rho \cdot d^2 \cdot g}{18 \mu} \]

**Settling Velocity for *E. coli* Cells.**

Density, \( \rho = 1400 \text{ kgm}^{-3} \)
Diameter, \( d = 2 \times 10^{-6} \text{ m} \) (E.coli cells assumed to have a diameter of 2 \( \mu \text{m} \) and a spherical shape)
Viscosity, \( \mu = 189 \times 10^{-7} \text{ Ns.m}^{-2} \) (at 37 °C)

Velocity, \[ u = \frac{9.81 \times 1400 \times (2 \times 10^{-6})^2}{18 \times (189 \times 10^{-7})} \approx 1.6 \times 10^{-4} \text{ m.s}^{-1} \]

**Settling Velocity for *S.cerevisiae* Cells.**

\( \rho = 1400 \text{ kg.m}^{-3} \)
\( d = 5 \times 10^{-6} \text{ m} \) (S. cerevisiae cells assumed to have a diameter of 5 \( \mu \text{m} \) and a spherical shape))
\( \mu = 185 \times 10^{-7} \text{ Ns.m}^{-2} \) (at 28 °C)

Velocity, \[ u = \frac{9.81 \times 1400 \times (5 \times 10^{-6})^2}{18 \times (185 \times 10^{-7})} \approx 1 \times 10^{-3} \text{ m.s}^{-1} \]
Velocity of Gas Leaving Broth Surface.

Velocity = \( \frac{\text{Volumetric flow rate}}{\text{Cross-sectional area}} \)

Where

Volumetric flow rate = \( 2.5 \times 10^{-5} \text{ m}^3 \text{s}^{-1} \)

(1.5 Lmin\(^{-1}\) or 1 VVM sparged into the vessel)

Cross-sectional area = \( \pi \times 0.12 \text{ m}^2 \)

So, velocity = \( 8 \times 10^{-4} \text{ m.s}^{-1} \)
Appendix G. The Role of the Turbosep in Exhaust Gas Containment.

One of the differences between traditional antibiotic producing fermentations and processes involving genetically manipulated micro-organisms is that in the latter, the fermentation exhaust gas has to be treated to reduce the microbial load prior to discharge into the environment. For processes that do not require any special containment features, the Turbosep could be used to reduce the microbial burden of the exhaust gas without any additional containment measures. However, for processes where the regulations stipulate that microbial release should be either minimised or prevented the data presented within this thesis suggests that the Turbosep alone could not provide a sufficient level of containment and would require a terminal HEPA filter to be connected downstream for additional security.

As previously described in section 1.2.4 HEPA filters can become blinded if they come into contact with quantities of foam or liquid, resulting in containment being compromised and a reduced gas flow capacity, effectively shutting down the fermenter. Therefore, it is common practice to position a hydrophobic pre-filter or condenser upstream of the HEPA filter to condition the gas stream so that continuous and effective filtration can be assured. Although the Turbosep has been shown to be effective in removing small particles and foam from the exhaust gas it was not known if it could function as a pre-filter, preventing liquid droplets from passing downstream or whether some additional form of pre-treatment of the gas stream would be required.

An experimental study was performed to quantify the level of protection that the Turbosep could provide to a HEPA filter. A HEPA filter was connected to a 250 L bioreactor as described in Section 2.4.2. A simulated fermentation broth consisting of 200 L of 200 mgL\(^{-1}\) egg albumen and 1 gL\(^{-1}\) NaCl was aerated at 250 L min\(^{-1}\) and the rate of filter blinding determined by measuring the increase in differential pressure across it. Figure G.1 shows a comparison of the rate of filter blinding when either a hydrophobic pre-filter or the Turbosep was connected upstream of a HEPA filter. Additionally, the rate of filter blinding in the absence of any pre-treatment of the exhaust gas is given.
From Figure G.1 it can be seen that the Turbosep provides a high degree of protection to the HEPA filter, although it is not as effective as the hydrophobic pre-filter. The cause of the filter blinding will need to be investigated further, however, it is most likely due to condensate forming in the pipework and in the body of the filter. Positioning a re-heater downstream of the Turbosep to heat up the gas stream should prevent the exhaust gas from condensing and blinding the filter.

**Figure G.1** A comparison of the rate of filter blinding when either (●) a Turbosep, (■) or a hydrophobic pre-filter is connected upstream of a HEPA filter (▲) or when the exhaust gas is piped directly into the HEPA filter.
Appendix H. The Detection of DNA from Process Micro-Organisms Released into the Environment.

Most of the previous research on the release of process micro-organisms has focussed on the routes used by the organisms to escape from a unit operation (Hambleton et al, 1991) and the effect of exposure to these released organisms (Winkler and Parke, 1992). However, little attention has been paid to the survival of released organisms within the environment external to a process. Colwell et al, 1995 has shown how a significant proportion of water borne viable cells may exist in a non-culturable state (Colwell et al, 1995), and that these viable but non-culturable cells can retain their pathogenicity (Colwell et al, 1990).

One advantage of the QPCR reaction is its ability to estimate any release without concern for the viable state of the cells. With this in mind a series of semi-quantitative experiments were performed to test whether it would be possible to detect any release that had occurred over the time course of a fermentation and for how long after the end of the fermentation the organism might still be detected in the local environment.

H.1 Detection of Standard DNA Samples.

A sample from an E.coli RV308 pHKY531 fermentation (Section 2.1.2.3.1) was diluted to a concentration of $2 \times 10^9$ cells/mL as determined by a visual count under a microscope (Section 2.1.4.1). Samples (50 µL) of serial dilutions of the cell suspension containing between $2\times10^9$ and 200 cells/mL were plated onto a 1cm diameter area of phenol-formaldehyde resin (Formica) benchtop. After the elapsed time a sample was recovered by scraping the contaminated benchtop with the end of a sterile cotton wool bud wetted in phosphate buffer. The bud was then shaken with 0.2mL of phosphate buffer, from which a 10mL sample was used for a QPCR assay (Section 2.2).

After two weeks, E.coli was detected in all but the most dilute sample (Table H.1), and this was derived from the patch where only 10 organisms were plated onto the bench. The measurements taken after two weeks are only qualitative due to the degradation of the internal standard used in the PCR assay.
The sensitivity of the QPCR assay, in the format that we use it, has a lower limit of about 250 molecules of the target DNA per sample (Noble et al, 1997). Assuming some 40 to 50 plasmids per cell (Bradley, 1999) this is the equivalent of about 5 cells, or 100 on the swab taken from the bench.

After longer periods (4 to 8 weeks) the response is more variable, and there may be none at all from a patch which later responds positively (Table H.1). Nothing was detected at later times. For these samples the number of cells collected was roughly estimated. The trend in the estimates generally follows the number of cells applied to the patches on the bench, but the actual values could not be used to determine the surface concentration of the *E.coli* cells. In a few cases the results were at the limit of detection and there is a poor correlation between the number of organisms applied to the bench and the number recovered. This is hardly surprising given the very simple method used to convey the cells from the surface into the tubes for the QPCR assay. However the nature of the response does suggest that, with care, a genuine correlation could be established, perhaps as good as the one which exists when the cells are sampled from aerosols (Noble *et al*, 1997; Noble *et al*, 1999).

**Table H.1** Detection of DNA from standardised contamination of surfaces with *E.coli* pHKY531.

<table>
<thead>
<tr>
<th>Cells plated onto surface</th>
<th>after 2 weeks</th>
<th>after 4 weeks</th>
<th>After 6 weeks</th>
<th>after 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^8$</td>
<td>detected</td>
<td>$34 \times 10^3$</td>
<td>$66 \times 10^3$</td>
<td>790</td>
</tr>
<tr>
<td>$10^7$</td>
<td>detected</td>
<td>$6.2 \times 10^3$</td>
<td>$3.3 \times 10^3$</td>
<td>Nd</td>
</tr>
<tr>
<td>$10^6$</td>
<td>detected</td>
<td>Nd</td>
<td>$9.5 \times 10^3$</td>
<td>$\sim 100$</td>
</tr>
<tr>
<td>$10^5$</td>
<td>detected</td>
<td>$4.5 \times 10^3$</td>
<td>280</td>
<td>Nd</td>
</tr>
<tr>
<td>$10^4$</td>
<td>detected</td>
<td>Nd</td>
<td>nd</td>
<td>Nd</td>
</tr>
<tr>
<td>$10^3$</td>
<td>detected</td>
<td>790</td>
<td>$\sim 100$</td>
<td>Nd</td>
</tr>
<tr>
<td>$10^2$</td>
<td>detected</td>
<td>Nd</td>
<td>nd</td>
<td>Nd</td>
</tr>
<tr>
<td>10</td>
<td>nd</td>
<td>Nd</td>
<td>nd</td>
<td>Nd</td>
</tr>
</tbody>
</table>

(nd is equivalent to <100 cells in the 0.2mL sample).

The same swabbing procedure was used to test surfaces on and surrounding several fermenters at various time intervals after an *E.coli* RV308 pHKY531 (see Section 2.1.2.3.1) fermentation. The sample was recovered by scraping the contaminated area of the processing environment with the end of a cocktail stick wetted in phosphate buffer. The end of the stick was then shaken with 0.2mL of phosphate buffer, from which a 10 μL sample was used for a QPCR assay (Section 2.2). Cells were detected up to 6 weeks after the fermentation, but were not found on a 2L fermenter after 9 months (Table H.2). The highest level of contamination was detected on and near to the sampling valve. This suggests that release was partly caused by aerosols formed during the sampling valve.

During the 6-week period the fermenters had been used for the culture of other organisms and had passed through several cleaning and sterilisation cycles. Moreover, the floor of the fermentation hall had been antiseptically scrubbed in the interim period, indicating that the contamination is remarkably persistent. Once present its removal owes more to natural decay than to hygiene in the process environment. However their detection with the QPCR does not mean that the cells are viable; only the target DNA need be present for the assay to respond positively.

The level of the contamination is difficult to determine from the data, but it would be consistent with the loss of about 50 μL of fermentation broth at harvest containing perhaps 2 x 10^{10} cells mL^{-1} spread across an area of 30cm square (10^7 cells on the 1cm diameter disk in Table H.1). This is not an unreasonable quantity to find deposited underneath the sampling valve of a large fermenter where aerosols accumulate.

These results have a number of implications on the potential health effects on plant operators. The main hazards arising from a release of a micro-organism will be due to inhalation, ingestion and skin contact (Norris, 1994). Most common is the allergic response to an organism or product. Workers may also suffer other toxic effects from unprotected exposure to micro-organisms, products or bi-products. This is particularly true for people working with antibiotics. Although these health effects can be avoided through suitable protective clothing and training, the results presented here suggest that
the hazard although probably much reduced after the operation has ceased is not reduced to zero and precautions still should be taken during periods of down time.

**Table H.2** The Detection of *E.coli* pHKY531 DNA in the processing environment.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Sample Position</th>
<th>Sample Time</th>
<th>Cells collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 L</td>
<td>sample valve</td>
<td>1 month</td>
<td>nd</td>
</tr>
<tr>
<td>75 L</td>
<td>surface below sample valve</td>
<td>1 month</td>
<td>670</td>
</tr>
<tr>
<td>75 L</td>
<td>floor 30 cm from sample valve</td>
<td>1 month</td>
<td>3300</td>
</tr>
<tr>
<td>450 L</td>
<td>sample valve</td>
<td>1 month</td>
<td>9500</td>
</tr>
<tr>
<td>450 L</td>
<td>surface below sample valve</td>
<td>1 month</td>
<td>280</td>
</tr>
<tr>
<td>450 L</td>
<td>transfer port</td>
<td>1 month</td>
<td>nd</td>
</tr>
<tr>
<td>450 L</td>
<td>floor 30 cm from transfer port</td>
<td>1 month</td>
<td>6000</td>
</tr>
<tr>
<td>450 L</td>
<td>sample valve</td>
<td>1 month</td>
<td>nd</td>
</tr>
<tr>
<td>250 L</td>
<td>floor below sample valve</td>
<td>6 weeks</td>
<td>2400</td>
</tr>
<tr>
<td>250 L</td>
<td>floor below sample valve</td>
<td>6 weeks</td>
<td>480</td>
</tr>
<tr>
<td>250 L</td>
<td>top plate</td>
<td>6 weeks</td>
<td>250</td>
</tr>
<tr>
<td>2 L</td>
<td>support table</td>
<td>9 months</td>
<td>nd</td>
</tr>
<tr>
<td>2 L</td>
<td>top plate</td>
<td>9 months</td>
<td>nd</td>
</tr>
</tbody>
</table>

(nd is equivalent to <100 cells in the 0.2mL sample).
References.


References


Bikerman, J.J. (1938). The Unit of Foaminess. Trans. Farad. Soc. 34, 634


References


References


References


