A project report on

Small scale cell culture performance of recombinant Chinese Hamster Ovary cells

Submitted by
Mohammed Abdul Majeed
BT06B037

Under the guidance of
Ganesh Reddy
Associate director,
Biologics Development centre,
Dr. Reddy's Laboratories, Hyderabad.

DEPARTMENT OF BIOTECHNOLOGY
Indian Institute of Technology - Madras

CERTIFICATE

This is to certify that project entitled **"Small scale cell culture performance of recombinant Chinese Hamster Ovary cells"** has carried out by **Mohammed Abdul Majeed,** (**BT06B037**), Dual degree, Biotechnology from **Institute of Technology – Madras** under my supervision. This project may be submitted as a part of the curriculum of the Dual Degree **(Biotechnology) Indian Institute of Technology-Madras** as his original work. Any part of this project has not been submitted in any other university's degree or diploma.

Guide

Ganesh Reddy

Associate director,
Biologics Development centre,
Dr. Reddy's Laboratories,
Hyderabad.

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10. BIBLOGRAPHY…….

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Mohammed Abdul Majeed

2. INTRODUCTION

Chinese hamster ovary (CHO) cells were first cultured by Puck (J. Exp. Med. 108,945, 1958) from a biopsy of an ovary from a female Chinese hamster. CHO-K1 cells are a major expression system to produce large and heterogeneously glycosylated proteins of therapeutic use (Gottesman, 1987; Weikert et al., 1999; Bragonzi et al., 2000). CHO-K1 cells have been used for expression of membrane-bound MUC1 at a small scale (Altschuler et al., 2000) indicating that CHO-K1 cells can express MUC1 with cancer-associated core 1-based *O*-glycans, making this expression system highly suitable.

To obtain a process that yields a high quality product, the choice of the culture medium and the method for the production process are of great importance. Most FDA-approved products are produced using serum-free media (Chu and Robinson, 2001). While batch and fed-batch cultures are still commonly used in industrial protein production processes, an increasing number of proteins are produced in continuous perfusion processes, mainly in stirred tanks (Chu and Robinson, 2001). The most important advantages of perfusion cultures are the possibility of controlled nutrient feeding to avoid nutrient limitations and reducing the concentrations of growth inhibiting metabolites (Yang et al., 2000; Konstantinov et al., 1996; Castilho et al., 2002) and the low protease and glycosidase activities in the culture. The latter one results in higher purity and better product quality, especially in case of protease or glycosidase sensitive products (Castilho et al., 2002; Ryll et al., 2000; Garber, 2000). Substrate concentration (e.g. glucose and amino acids) in the medium feed, culture pH and dissolved oxygen concentration are parameters, which have to be optimised for each culture process in order to get the best growth and highest productivity (Jenkins et al., 1996; Goochee and Monica,1990). Especially the oxygen concentration has been shown to be a critical parameter for productivity, cell metabolism and glycosylation (Kunkel et al., 2000; Miller et al., 1987; Zupke et al., 1995). Thus monitoring of oxygen uptake rate (OUR) of the cells during the culture process can give information not only on cell growth, but also on the metabolic demand of the cells, which can be used for an automated optimized feeding (Zhou, 1999; Barnabé and Butler, 2000). This has led to an increase in the quantity of protein required per year, which, in turn, has brought increased pressure to develop clones and processes with yield. The higher protein requirements also raise the question of availability of adequate capacity to produce these therapeutics. Optimizing cell culture systems to increase upstream performance is identified as one of the most important areas to avoid significant capacity constraint in the future.

Biopharmaceuticals play a critical role in the treatment of diseases that affect the human condition. The biological activity of the great majority of these therapeutic proteins depends on correct post translational modifications, best achieved by mammalian expression systems. About 60-70% of all biopharmaceuticals of present market are produced by mammalian cells (Wurm, FM, "*Production of recombinant protein therapeutics in cultivated mammalian cells*", Nat Biotechnol 22, (2004); pp.1, 393-1,398). To date, there are more than 150 monoclonal antibody therapeutics alone in clinical trials at various stages, each dose requires between a milligram to gram of final product (Reichert, JM, Rosenwing, CJ, Faden, LB and Dewitz, M.C. "*Monoclonal antibody successes in the clinic*", Nat Biotechnol 23, (2005); pp.1,073-1,078).

3. MAMMALIAN CELL CULTURE

Mammalian cells are large (10 to 20 µm diameter), slow growing (td ranges from 10-50 h) and very shear sensitive. Product concentration (titer) is usually very low (µg/ml), and toxic metabolites such as ammonium and lactate are produced during growth. These properties of animal cells set certain constraints on the design of animal cell bioreactors.

The process of creating a suitable mammalian cell expression system for protein production, essentially, has four steps:

- Vector construction containing the gene of interest (GOI);
- Transient expression of potential products for preclinical evaluation;
- Cell line development of stable clones; and
- Medium development to optimize nutrient levels for the production clone.

The expression system can then be utilized in process development and scale up studies for current Goods Manufacturing Practices (cGMP). On an average the entire process takes approximately 12-18 months for completion.

Vector design and construction:

Traditional mammalian expression plasmid backbones contain a bacterial origin of replication and a bacterial selection marker enabling vector amplification for plasmid production. Also, an expression cassette containing the GOI and/or a cassette containing a selection marker are inserted into the plasmid. The expression cassette containing a selection marker is inserted into the plasmid. The expression cassette for the therapeutic protein consists of a strong mammalian promoter controlling the transcription of the GOI, which is upstream of the GOI and a polyadenylation signal for the proper messenger ribonucleic acid (mRNA) processing downstream of the GOI. After delivery of the expression plasmid into the host cells, the plasmid integrates into the host genome. The selection and isolation of clones containing the plasmid DNA is achieved using a mammalian selection marker. The selection marker expression cassette usually contains a weak mammalian promoter and a polyadenylation signal.

Protein production in mammalian cells by transient transfection

For large-scale manufacture of recombinant proteins in mammalian cells, the practice of genetically engineering cells to continuously express the protein of interest from a stably integrated transgene remains the standard. Prior to selecting a therapeutic candidate, it is often necessary to produce and evaluate many different proteins for therapeutic potential. Generally stable cell lines can be costly and time consuming, which makes it poorly suited for rapid production of proteins for pre-clinical candidate screening. The industry standard for cell line generation, currently, ranges between six and nine months. To rapidly produce adequate amounts of recombinant protein without the time and investment of developing individual stable cell clones, transient transfection of mammalian cells has become a commonly employed tactic for pre-clinical protein production. In this strategy, the gene expressing the protein of interest is transfected into

a large number of cells. Cells transiently express the recombinant protein, for several days to a week or more, after which the protein is harvested and purified for downstream use. By careful selection of appropriate mammalian cells, culture conditions, expression vectors and transfection agents, mg quantities of protein can be quickly produced with drastically reduced time and effort.

The fundamental components required for protein productions by transient transfection of mammalian cells are cell themselves. These are suitable culture media for growing and maintaining the cells, expression vector to direct the biosynthesis of the protein of interest, and a means of introducing the expression vector into the cells. The cell lines that are commonly used for large-scale transient transfections are the Human Embryonic Kidney (HEK) 293 cells and the CHO cells. To address the rising demand for serum free production, expression systems have been developed to use a serum free, chemically defined culture medium compatible with transfection agents and a cationic lipid based transfection agent.

A versatile system has been developed that is capable of transferring genes of interest into a wide variety of mammalian host cells and offers a number of advantages over other methods. The system, which is referred to as GPEx^{TM} (an acronym for "gene product expression") utilizes the replication defective retroviral vectors, derived from Moloney murine leukemia virus and pseudo typed with vesicular stomatitis virus G protein, to stably insert single copies of genes into dividing cells. Retro vectors deliver genes coded as RNA that, after entering the cell, are reverse transcribe to DNA and integrated stably into the genome of the host cell. Two enzymes reverse transcriptase and integrase, provided transiently in the vector particle, perform this function. These integrated genes are maintained through subsequent cell divisions as if they were endogenous cellular genes. By controlling the retroviral particles accessing the cell, multiple gene insertion (desirable for high yielding cell cultures) can be achieved without any of the traditional amplification steps. This GPEx technology is used for transferring genes into CHO cells for the purpose of consistently producing cell lines with high production levels in a short amount of time (Fig 1).

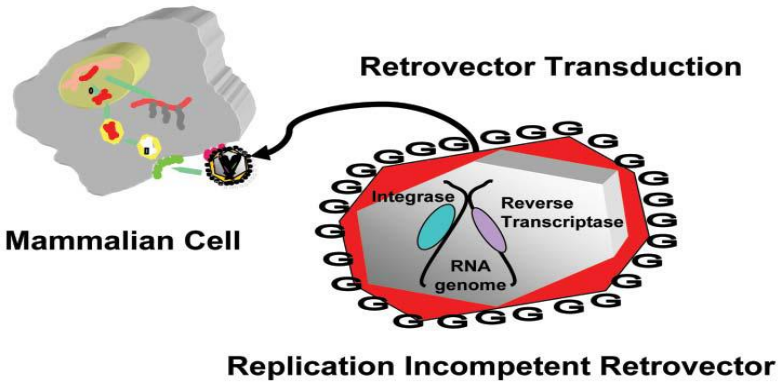


Fig: 1: GPEx cell line engineering

Each of the genomic inserts generated with the GPEx process contains all genetic elements shown in fig: 2

LTR 1 =	Moloney Murine Leukemia Virus Long Terminal Repeat (Non-Protein Coding)
PR =	Moloney Murine Leukemia Virus Viral Packaging Region (Non-Protein Coding)
Promoter =	Cytomegalovirus Immediate Early Gene Promoter Region
RP =	Recombinant Protein Coding Region
RESE =	RNA Export and Stability Element (Non-Protein Coding)
LTR 2 =	Moloney Murine Leukemia Virus Long Terminal Repeat/Poly-Adenylation Signal (Non-Protein Coding)

Fig: 2: Transgene expressions construct.

Cell lines expressing recombinant proteins and antibodies were produced as shown in figure 3.

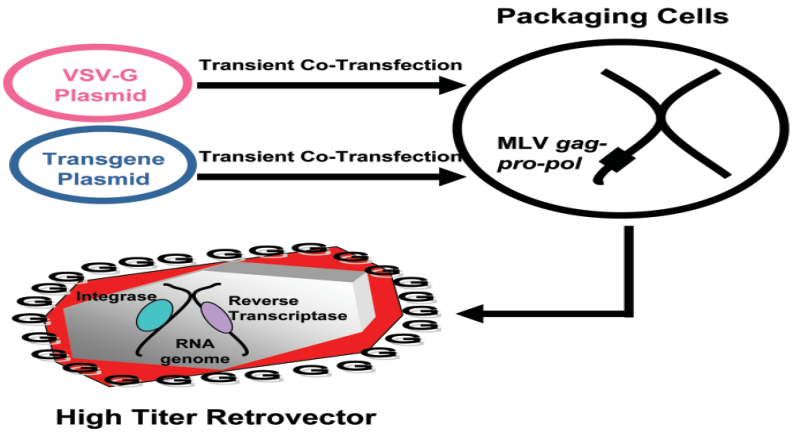


Fig: 3: Retrovector production process.

Cell line development

Irrespective of the host cell line chosen, the development of a production clone expressing the protein of interest follows a common scheme. The GOI is cloned into a suitable expression vector, and the entire plasmid is transfected into the host cells. Following selection for stable integration of the plasmid into the host cell genome, single cells are expanded to obtain clonal populations. Although a serum free medium is commonly used for protein production, the cloning process sometimes has to be carried out in the presence of serum to support cell growth at a single cell level. After an expression analysis, the high producing clones are chosen for further development.

Generation of mammalian stable cell lines expressing recombinant proteins or antibodies have focused primarily on CHO, NSO and more recently, human PER.C6 cell lines. These cell lines have the advantage of being very well characterized and capable of producing recombinant protein levels sufficient for bio-production. Additionally they have been adapted to several media formats, making them easier to use across different experiments. While hybridomas have historically produced lower levels of protein than recombinant, newer technologies enabled hybridomas to achieve comparable yields of stable protein expression for extended periods.

Media development

The origins of modern cell culture media dates back more than 50 years to the work of Morgan (Morgan JF, M.H., and Parker. RC,"*Nutrition of animal Cells in Tissue Culture*. I. Initial studies on a Synthetic Medium", Proc Soc Exp Biol Med 73. (1950); pp. 1-8), Eagle and others. A basal formulation consisting of water, salts, amino acids, carbohydrates, nucleic acid precursors and vitamins was often supplemented with animal derived serum to provide growth promoting factors needed for cell replication in vitro. Over the years, the range of cells able to be grown in vitro has expanded and the applications for cultured cells have become very broad. Undesirable effects of serum supplementation have become more apparent and include high cost, limited availability, lot-to-lot variability, lack of definition and the potential to transmit adventitious agents. Serum is also a problem for manufacturers of vaccines but to do this, requires an understanding of the many functions of serum, which include binding/carrier functions of lipids, hormones, growth factors, attachment factors, cytokines and trace elements, as well as bulk protein functions like protease inhibition, shear protection and buffering (Jayme, DW, Blackman, KE,"*Culture Media for Propogation of Mammalian Cells, Vol.* 5", Alan R. Liss, New York;(1985)).

Media and supplements have several formats to accommodate the diversity of applications. Standard 1X concentration liquid media is commonly used for small-scale experiments and requires the least amount of end-user processing. Dry powder format medium requires reconstitution of several components, pH adjustment, filtration, repackaging and sterility testing by the end-user. This format is generally used for large scale applications and may be used for feed supplements. Liquid media concentrates are made by sub-grouping media components based on the solubility and compatibility characteristics of the individual constituents (Jayme, DW, Blackman, KE,"*Culture Media for Propagation of Mammalian Cells, Vol.* 5", Alan R. Liss, New York;(1985)). In addition to a physical format, media have different chemical compositions. The formats range from the classical basal medium with serum supplementation to a fully CD medium, which is protein-free and furthermore composed solely of biochemical defined low molecular weight components. Gibco, Hyclone, Sigma Aldrich are the leading suppliers of all media formats. The most recent developments include the PF CHO, HYC CP 1027, Excell 302, and MAM-PF. Choice of format ultimately depends on the cell culture, scale of use, and the availability of suitable facilities and equipment to process the format. The controlled optimization methods using defined components are the key to increasing the performance of mammalian expression.

Optimizing the concentrations of every constituent has often derived the precise media formulations. Examples of the different media and their uses are given in the table below.

Table: Different type of culture media and their uses

Media type	Examples	Uses
Balanced salt solutions	PBS, Hanks BSS, Earles salts DPBS ,HBSS EBSS	Form the basis of many complex media
Basal media	MEM	Primary and diploid cultures.
	DMEM	Modification of MEM containing increased level of amino acids and vitamins. Supports a wide range of cell types including hybridomas.
	GMEM	Glasgows modified MEM was defined for BHK-21 cells
Complex media	RPMI 1640	Originally derived for human leukaemic cells. It supports a wide range of mammalian cells including hybridomas
	Iscoves DMEM	Further enriched modification of DMEM which supports high density growth
	Leibovitz L 15	Designed for CO2 free environments

	TC- 100 Grace's Insect Medium Schneider's Insect Medium	Designed for culturing insect cells
Serum Free Media	CHO	For use in serum free applications.
	Ham F10 and derivatives Ham DMEM/F12	NOTE: These media must be supplemented with other factors such as insulin, transferrin and epidermal growth factor. These media are usually HEPES buffered
Insect cells	Sf-900 II SFM, SF Insect-Medium-2	Specifically designed for use with Sf9 insect cells

Basic Constituents of media

- Inorganic salts
- Carbohydrates
- Amino Acids
- Vitamins
- Fatty acids
- Lipids
- Proteins
- Trace Elements
- Buffering System

Each type of constituent performs a specific function as outlined below.

Inorganic Salts

The inclusion of inorganic salts in media performs several functions. Primarily they help to retain the osmotic balance of the cells and help regulate membrane potential by provision of sodium, potassium and calcium ions. All of these are required in the cell matrix for cell attachment and as enzyme cofactors.

Carbohydrates

The main source of energy is derived from carbohydrates generally in the form of sugars. The major sugars used are glucose and galactose however some media contain maltose or fructose. The concentration of sugar varies from basal media containing 1g/l to 4.5g/l in some more complex media. Media containing the higher concentration of sugars are able to support the growth of a wider range of cell types.

Amino Acids

The essential amino acids i.e. those which are not synthesized in the body, are required by the cultured cells with in addition, Cysteine and tyrosine although individual requirements for amino acids will vary from one cell to another. Other non-essential amino acids are often added to compensate either for a particular cell types in capacity to make them or because they are made but lost into the medium. The concentration of amino acids usually limits the maximum cell concentration attainable, and the balance may influence cell survival and growth rate. Glutamine is required by most of the cells, although some cell lines will utilize glutamate, but evidence suggests that glutamine is also used by the cultured cells as an energy and carbon source. (Reitzer *et al.,* 1979)

Vitamins

Serum is an important source of vitamins in cell culture. However, many media are also enriched with vitamins making them consistently more suitable for a wider range of cell lines. Vitamins are precursors for numerous co-factors. Many vitamins especially B group vitamins are necessary for cell growth and proliferation and for some lines the presence of B12 is essential. Some media also have increased levels of vitamins A and E. The vitamins commonly used in media include riboflavin, thiamine and biotin.

Fatty Acids and Lipids

Like proteins and peptides these are important in serum free media since they are normally present in serum. E.g. cholesterol and steroids are essential for specialized cells.

Proteins

These are particularly important in serum free media. The most common proteins and peptides include albumin, transferrin, fibronectin and fetuin and are used to replace those normally present through the addition of serum to the medium.

Trace Elements

These include trace elements such as zinc, copper, selenium and tricarboxylic acid intermediates. Selenium is a detoxifier and helps remove oxygen free radicals.

Whilst all media may be made from the basic ingredients this is time consuming and may predispose to contamination. For convenience most media are available as ready mixed powders or as 10x and 1x liquid media. All commonly used media are listed in the Sigma-Aldrich Life Science Catalogue. If powder or 10x media are purchased it is essential that the water used to reconstitute the powder or dilute the concentrated liquid is free from mineral, organic and microbial contaminants. It must also be pyrogen free (water, tissue culture grade). In most cases water prepared by reverse osmosis and resin cartridge purification with a final resistance of 16-18Mx is suitable. Once prepared, the media should be filter sterilized before use.

Buffering Systems

Most cells require pH conditions in the range 7.2 - 7.4 and close control of pH is essential for optimum culture conditions. There are major variations to this optimum. Fibroblasts prefer a higher pH (7.4 - 7.7) whereas; continuous transformed cell lines require more acid conditions pH (7.0 - 7.4). Regulation of pH is particularly important immediately following cell seeding when a new culture is establishing and is usually achieved by one of two buffering systems; (i) a "natural" buffering system where gaseous CO_2 balances with the CO_3 / HCO_3 content of the culture medium and (ii) chemical buffering using a zwitterion called HEPES. Cultures using natural bicarbonate/CO_2 buffering systems need to be maintained in an atmosphere of 5-10% CO_2 in air usually supplied in a CO_2 incubator. Bicarbonate/CO2 is low cost, non-toxic and also provides other chemical benefits to the cells. HEPES has superior buffering capacity in the pH range 7.2 - 7.4 but is relatively expensive and can be toxic to some cell types at higher concentrations. HEPES buffered cultures do not require a controlled gaseous atmosphere. Most commercial culture media include phenol red as a pH indicator so that the pH status of the medium is constantly indicated by the color. Usually the culture medium should be changed / replenished if the color turns yellow (acid) or purple (alkali).

Physical Properties

pH

Most of the cell lines grow well at pH 7.4. Although optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast cell lines perform but at a pH 7-7.7 and transformed cells perform best at pH 7.0-7.4(Eagle, 1973). Phenol red is commonly used as an indicator. It is red at pH 7.4, becoming orange at pH 7.0, yellow at pH 6.5, lemon yellow below pH 6.5, more pink at pH 7.6 and purple at pH 7.8.

Osmolality

Most of the cultured cells have a fairly wide tolerance for osmotic pressure. Since the Osmolality of human plasma is about 290mOsm/kg, it is reasonable to assume that this is optimum for human cells in vitro, although it may be different for other species. In practice, osmolalities between 260mOsm/kg and 320 mOsm/kg are quite acceptable for

most of the cells, but once selected, should be kept consistent at 10mOsm/kg. Slightly hypotonic medium may be better for Petri dish or open plate culture to compensate for evaporation during incubation. It is usually measured by freezing point depression or elevation of vapor pressure. The measurement of Osmolality is particularly important to monitor of alterations are made in the constitution of the medium.

Temperature

Apart from the direct effect of temperature on cell growth, it will also influence ph due to the increased solubility of CO_2 at lower temperature and, possibly, due o changes in ionization and pK_a of the buffer. The pH should be adjusted to 0.2 units lower at room temperature than at 36.5ºC.

Mode of cultivation of animal cell culture

Mammalian cell culture has been employed for the production of therapeutic and diagnostic proteins using techniques similar to those used in microbial biotechnology. However there are several distinctions between mammalian cell cultures and microbes that render cultivational technologies inadequate (Randerson, 1985; Liest *et al.*, 1990; Tokashiki and Yokoyama, 1997; Sandig *et al.*, 2005). The following table (table 2) describes the biological characteristics of mammalian cells and their crucial industrial aspects.

Batch mode

This mode of operation is the most simple fermentation system. In this process, medium is charged into the spinners and then inoculated with cells. During the cultivation, no additive medium is supplied ton the vessel and only the cultivation parameters (air, foam, pH and temperature) are controlled. Cells enter the so called lag phase, in which they do not increase in size. During this phase, cells synthesize enzymes and transport systems required for their growth. The length of this phase depends on the physiological state of the cells and the concentration of the inoculum. The cells then enter an exponential phase. In this phase, cells grow exponentially following a first order kinetics. When the medium components are depleted and the concentration of toxic metabolites is increased, the growth is slowed. Cell growth is finally ceased and cells enter a stationary phase. During this phase, cells continue to metabolize, producing energy required for the maintenance of basic cell functions. When the cell's reserves of energy are consumed, they begin to die, following the same first order kinetics as log phase. This phase is known as the decline phase, which continues until all cells are being dead. Batch cultivation is simple regard to system and cultivation conditions, however cell density is usually low (1- $2*10^6$ mL^{-1}) and the product concentration is also low (Pringle, 1992; Tokashaki and Yokoyama, 1997). Nutrient limitation is considered to be a major factor that causes cell death during the decline phase of batch culture. In order to overcome different problems encountered by nutrient limitation during batch cultivation, fed batch and continuous cultivations were developed.

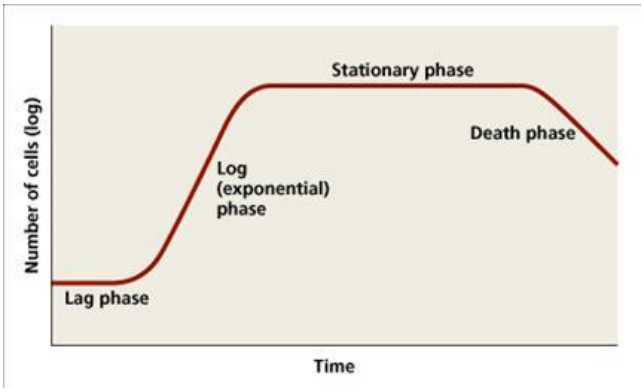


Fig 4: Standard growth curve

Fed batch mode

As the total product yield is proportional to the number of viable cells, the idea of supplementing medium with nutrient solution during fermentation led to the development of the concept of fed batch mode of cultivation. In this system only the limiting nutritional ingredients are intermittently fed to the medium over the course of fermentation (Glacken et al., 1986; Jang and Barford, 2000). In such case, limiting nutrients can be suitably adjusted so that they may not inhibit the growth of the cells, but culture medium is not taken out from the system and the accumulation of waste metabolites proceeds gradually. As a result, growth is inhibited and cells are dead upon reaching a certain level. Compared with batch cultivation where cell growth is inhibited by nutrient limitation, fed batch cultivation enables to grow for a prolonged time reaching a higher cell density and product yield. However, growth inhibition is caused by the accumulation of toxic metabolites (Sandig et al., 2005).

Continuous mode

The continuous mode of operation can be described as a system where sterile nutrient medium is continuously fed into the bioreactor with a simultaneous removal of an equivalent amount of the metabolized medium. This special feeding strategy prolongs the culture and allows an increase in cell concentration to be achieved. The continuous culture can be further subdivided into chemo stat and perfusion culture.

Mammalian cell biotechnology has been an explosively growing field for the last 25 years. Recombinant Chinese hamster Ovary cells now far surpass *E.coli* and all other expression systems for recombinant protein production have gone 100 fold or more, are now often expressed in grams per liter and expected to continue to rise. Mammalian cell culture is the most commonly used system for the production of both marketed recombinant proteins and those undergoing clinical testing. Today, quarter of all new drugs approvals are for biopharmaceuticals. The therapeutics so far is synthesized from the genetically engineered mammalian cell lines like the CHO cell lines. They are needed in large quantities and hence careful study of the underlining biochemical, cell biological and engineering principles for control of production processes are being considered.

These safety concerns have led to the demand for serum-free cell culture media to be developed. There is therefore a need in the art for a cell culture medium which enables animal cells to grow at a suitable rate, but without relying on the presence of serum.

Working of a bioreactor

The basic types of bioreactors are:

1. Reactors with internal mechanical agitation.
2. Bubble column, which rely on gas sparging for agitation
3. Loop bioreactors, in which mixing and liquid circulation are induced by the motion of an injected gas, by a mechanical pump, or by a combination of the two.

The traditional fermenter is stirred-tank reactor, the prime example of a reactor with internal mechanical agitation. The main virtues of the systems are that they are highly flexible and can provide high volumetric mass-transfer coefficient values for gas transfer.

The Stirred tank bioreactor

The reactor used for rCHO cell fermentation is shown in the following figure:

Fig 5: Stirred Tank Bioreactor

Source: Sartorius AG

Laboratory scale bioreactors with liquid volumes of less than 10 liters are constructed out of Pyrex glass. For large reactors, stainless steel is used.

Standard geometry of the Stirred tank bioreactor:

A stirred tank bioreactor will either be approximately cylindrical or have a curved base. A curved base assists in the mixing of reactor contents.

Stirred tank bioreactors are generally constructed to standard dimensions. That is, they are constructed according to recognized standards such as those published by the International Standards Organization and the British Standards Institution.

These dimensions are taken into account both mixing effectiveness and structural considerations. It will typically have the following dimensions as shown in table 3.

Table 3: Standard geometry of the Stirred tank bioreactor

Ratio		**Typical Values**	**Remarks**
Height of liquid in reactor to height of reactor	H_L/H_t	0.7-0.8	Depends on the level of foaming produced during the fermentation
Height of reactor to diameter of tank	H_t/D_t	1-2	European reactors tend to be taller than those designed in the USA.
Diameter of impeller to diameter of tank	D_a/D_t	1/3-1/2	Rushton turbine reactors are generally 1/3 of the tank diameter. Axial flow impellers are larger.
Diameter of baffles to diameter of tank	D_b/D_t	0.08-0.1	
Impeller blade height to diameter of impeller	W/D_a	0.2	
Impeller blade width to diameter of impeller	L/D_a	0.25	
Distance between middle of impeller blade and impeller blade height	E/W	1	

Head space volume:

A bioreactor is divided in a working volume and a head-space volume. The working volume is the fraction of the total volume taken up by medium, microbes and gas bubbles. The remaining volume is called the head space.

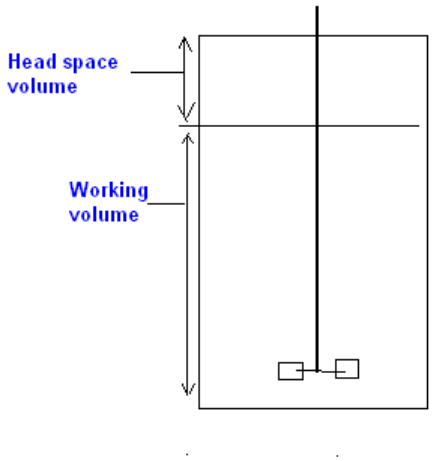


Fig 6: Head space volume

Typically, the working volume will be 70-80% of the total fermenter volume. This calue will however depend on rate of foam formation during the reactor. If the medium of the fermentation has a tendency to foam \, then a larger headspace and smaller working volume will be used.

A Modern mechanically agitated bioreactor will contain:

1. An agitator system
2. An oxygen delivery system
3. A foam control system
4. A temperature control system
5. A pH control system
6. Sampling port

Agitation system

The function of agitation system is to

1. Provide good mixing and thus increase mass transfer rates through the bulk liquid and bubble boundary layers
2. Provide the appropriate shear conditions required for breaking up of bubbles

The agitation system consists of the agitator and the baffles. The baffles are used to break the liquid flow to increase turbulence and mixing efficiency.

The agitator consists of the components shown in the following diagram.

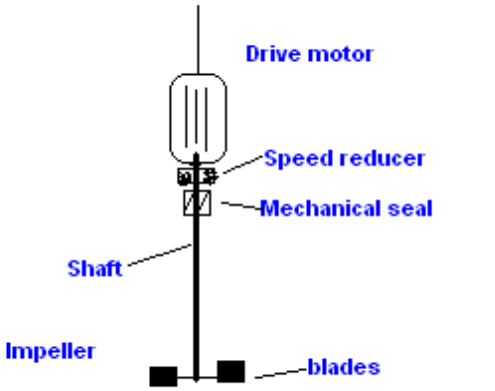


Fig 7: Agitator

The no. of impeller will depend on the height of the liquid in the reactor. Each impeller will have between two and six blades. For industrial mammalian cell culture, 'Pitched blade" impellers are used.

Mechanical Seals

A mechanical seal prevents contaminants from entering the reactor and organisms from escaping thorough shaft. The seal uses vapors from the liquid for lubrications.

Oxygen delivery system

The oxygen delivery system consist of the following

A compressor

A compressor forces the air into the reactor. The compressor generates sufficient pressure to force the air through the filter, sparger holes and into the liquid. Air compressors used for large scale bioreactors typically produce air at 250 kPa. The air should be dry and oil free so as to not block the inlet air filter or contaminate the medium.

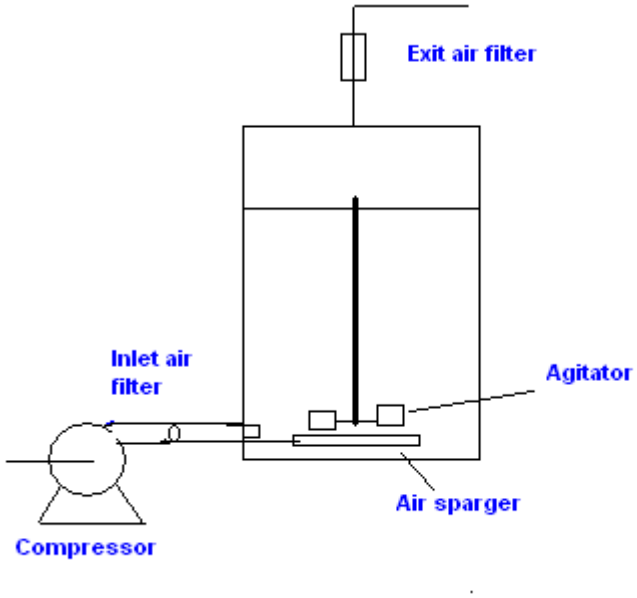


Fig 8: The compressor

Inlet air sterilization system:

This is undertaken to prevent contaminating organisms from entering the reactor. The exit air on the other hand is sterilized to keep contaminants from entering the reactor. A common method of sterilizing the inlet an exit air is filtration. For small reactors (with volume less than 5 liters), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block. For large scale fermenters (upto 1000 liters), pleated membrane filters housed in propylene cartridges are used. By pleating the membrane, it is possible to create a compact filter with a very large surface area for air filtration. Increasing the filtration area decreases the pressure required to pass a given volume of air through the filter.

The condenser is a simple heat exchanger through wich cool water is passed. Volatile materials and water vapour condense on the inner condenser surface. This minimizes water evaporation and the loss of volatiles. Drying the air also prevents blocking of the exit air filter with water.

Air sparger

The air sparger breaks the incoming air into small bubbles. A sparger ring consists of a hollow tube in which small holes have been drilled. It must be located below the agitator and be approximately the same diameter as the impeller. Thus, the bubbles rise directly into the impeller blades, facilitating bubble break up.

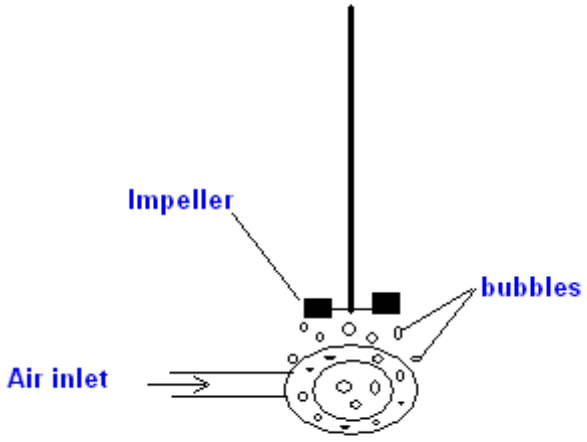


Fig 9: Air Sparger

Foam control system:

This is an essential element of the operation of a sparged bioreactor. Excessive foam formation can lead to blocked air exit filters and to pressure buildup in the reactor. The later can lead to a loss of medium, damage to the reactor and injury to operating personnel. Foam is typically controlled with aid of antifoaming agents based on silicone or vegetable oils. Excessive antifoam addition can however result in poor oxygen transfer rates.

Temperature control system:

The temperature control system consists of

- Temperature probe
- Heat transfer system

Typically the heat transfer system will use a "jacket" to transfer heat in or out of the reactor. The jacket is a shell which surrounds parts of the reactor. The jacket will typically be "dimpled" to encourage turbulence in the jacket and thus increase the heat transfer efficiency.

pH control system:

The pH control system consists of

- a pH probe
- Alkali delivery system
- Acid delivery system

The pH probe is connected to the controller and when pH goes above to the set point, it starts alkali delivery system by pumping alkali. When ph goes down to the set point, it starts acid delivery system by pumping acid.

Sample system

All sampling starts from a sample pipe, which should dip into the bulk of the culture liquid. A length of silicone tubing is connected to the sample pipe (and clamped off until sample is to be taken) which is plugged and covered with at open end. When a sample is to be taken, the clamp on the sample pipe tubing is released. A sterile syringe is coupled to the tubing and air is forced in the tube. Then, The syringe is pulled back to create a partial vacuum in the sample bottle. When the required volume of the sample is taken, the syringe is pushed into clear the line and sample tubing is clamped shut again. The edge of the tube is sprayed with 70% isopropyl alcohol (IPA) and covered with Sample need to be taken for proper monitoring of the culture conditions.

Spinner reactor

Celstir® design is the standard flask type for cell culture. Celstir® flasks feature adjustable blade impeller for increased aeration and dimpled bottom to prevent the accumulation of cells under the impeller (dimpled bottom on 125 mL size and larger). When used with the Micro-Stir®, which can operate at very low speeds, the Celstir® flask provides an optimum environment for most suspension lines, including insect cell cultures. Hybridomas and adapted cell lines can also be grown in these spinner flasks. Sidearms on Magna Flex® and Celstir® vessels 500 mL and larger were widened to accommodate monitoring and control devices or media inlet/outlet lines. The Wheaton Celstir® features an adjustable paddle blade impeller for better mixing. The impeller does not protrude through the top cap, thereby maximizing incubator space and reducing the chance for contamination. The addition of the bottom dimple to flasks 125 mL and larger improves circulation and reduces the accumulation of cells in the center of the flask. Units 500 mL and larger have a 45 mm sidearm to be used as an air vent, media inlet or outlet, inoculation port, pH probe inlet, or for other applications. Celstirs® provide maximum surface interface between culture and flask atmosphere. All flasks are proportioned to provide a head space ratio of 1:1 or greater. The unit consists of a glass flask, Teflon® and glass impeller assembly, and Teflon® and Silicone-lined top cap. It is designed for use with Wheaton Micro-Stir® magnetic stirrers. The entire unit may be autoclaved. Units 500 mL and larger can be used with the Vented Cap (W240751).

Catalog #	Dia x Ht (mm)	Screw Cap Size	Sidearm Size (mm)	Volume (mL)
356873	38 x 122	38-430	15-415	25
356875	38 x 141	38-430	15-415	50
356876	65 x 155	51-400	33-430	125
356879	85 x 175	51-400	33-430	250
356882	110 x 190	100-400	45 mm	500
356884	130 x 250	100-400	45 mm	1000
356887	178 x 341	100-400	45 mm	3000
356889	258 x 404	100-400	45 mm	6000
356890	293 x 445	100-400	45 mm	8000

Ref : http://www.wheatonsci.com

4. Process development and characterization

Aims and strategies

The aims of process development are two fold

1. Maximize process productivity
2. Improve process robustness

Strategies for maximizing process productivity focus on two key aspects

1. Creation and selection of highly expressing cell lines through use of an efficient expression system
2. Development of improved bioreactor processes through control of physicochemical and nutritional environment

Improved process robustness necessitates

1. Elimination of potential source of variability e.g. ill-defined raw materials
2. Maintenance of precise process control

Increasing demand of biopharmaceuticals from mammalian cell culture coupled with capacity limitations necessitates improvement in process productivity and robustness. Process variables can be controlled by improving the physicochemical environment in which the cultivation takes place. In nearly all the processes these variables are Culture pH, temperature and dissolved oxygen tension which have to be controlled very precisely.

Effect of culture pH:

Hydrogen ion concentration (pH affects the activity of enzymes and therefore the cell growth rate. The optimal pH for growth may be different from that of the product formation. The pH optimum for CHO cells ranges from 6.5-7.5.
In most fermentation, pH can vary substantially. Often the nature of the nitrogen source can be important. If ammonia is a sole nitrogen source, hydrogen ions are released into the medium to reduce nitrate to ammonia, resulting in a decrease in pH. If nitrate is the sole energy source, hydrogen ions are removed from the media to reduce nitrate to ammonia, resulting in an increase in pH. Also, pH can change because of the production of organic acids, the utilization of acids or production of bases. The evolution or supply of CO_2 can alter pH greatly in mammalian cell culture.

Reduction of culture pH generally results in reduced specific glucose utilization and lactate accumulation rates for mammalian cell line when maintained within the permissible range for cell growth. A lower specific glucose utilization rate is beneficial as

it reduces the quantity of glucose to be added as part of the fed-batch process, simplifying feed formulation and addition. A lower specific lactate accumulation rate reduces the overall lactate concentration and thereby limits the amount of alkali required to control culture pH, simplifying process operation and improving process robustness.

Culture pH in particular can have a dramatic effect on cell growth and productivity. Responses are cell line specific.

- Changes in maximum viable cell concentration
- Changes in integral viable cell count
- Changes in specific production rate
- Changes in nutrient utilization and catabolite accumulation

Effect of Dissolved oxygen:

Dissolved oxygen (DO) is an important substrate in aerobic fermentation and may be a limiting substrate, since oxygen gas is sparingly soluble in water. At high cell concentrations, the rate of oxygen consumption may exceed the rate of oxygen supply, leading to oxygen limitations.
Above critical oxygen, the growth rate becomes independent of the dissolved oxygen concentration. Oxygen is a growth rate limiting factor when DO level is below the critical DO concentration. Oxygen is introduced to fermentation broth by sparging air through the broth.

Effect of CO2:

The atmospheric CO2 tension will regulate the concentration of dissolved CO2 directly, as a function of temperature. This in turn produces H2CO3 which has a low dissociation constant with most of the available cations, it tends to re-associate, leaving the medium acid. The net result is of increasing atmospheric CO2 is to depress the pH. T*he effect of elevated CO2 tension is neutralized by increasing the bicarbonate concentration, which adjusts the equilibrium to 7.4. Where the culture produces a lot of acid, and the endogenous production of CO2 is high, it is desirable to allow excess CO2 of to escape. HEPES is incorporated into the medium to stabilize the pH.

Effect of Temperature:

Temperature not only effects the cell growth, but also influence the pH due to increased solubility of CO2 as lower temperature, and possibly, due to changes in ionization and pKa of the buffer. The ideal temperature for the growth of mammalian cells is set to 37 C and the pH I checked while making up a media for the first time and throughout the culture.

It has been shown that the shift in temperature to slowing down the metabolism of rCHO cells promotes culture productivity and longevity with good product quality. The typical process involves the shift in temperature after first feed addition from 37 °C to 31 °C in controlled conditions.

Effect of Osmolality:

Osmolalities around 260mOsm/kg are quite acceptable for most cells, but one selected, should be kept consistent at + 10 mOsm/kg. Osmolality is usually measured by freezing-pint depression or elevation vapour pressure. Less concentration of media when compared to cells results in a hypotonic environment. Osmosis causes a net flow of water into cells causing swelling and expansion. Similarly, hypotonic environment leads to the shrinkage of cell contents.

In most of the biopharmaceutical industries rely on fed batch operation with different feed strategies and media development. In this project, process has been characterized in two parts one is growth run and production run. Growth run is basically cultivation of rCHO cells in plain growth medium without any feed where as production run has mean, to add feed (media specific) at day 0 and when IVCC reaches 5-7 mil cells/ml/day as well as temperature shift from 37 °C to 31 °C. This mode of feed strategy gives higher productivity and better quality of product as far as production of monoclonal antibody is concerned.

5. OBJECTIVES

- Being a part of the Up Stream Development team, firstly, evaluation of the growth characteristics of rCHO in various cell culture platforms was done i.e. comparison of the growth performance of recombinant CHO cells in different scale platforms i.e. the spinners (250ml and 500ml) and fermenter. Study the various metabolic profiles of the same.
- Glutamine (present in the pfCHO media) was found to degrade at 37 degC. Determination of degradation kinetics of glutamine and the formation kinetics of ammonia formation.
- Compare the growth performance and the metabolite profiles of recombinant CHO cells when the initial media had various percentages of spent media. i.e. various spinners having different initial fresh to spent media volume ratio were to be started and their growth trend to be observed.
- Compare the growth performance and the metabolite profiles of recombinant CHO cells with different initial glutamine concentration; objective was to determine which is the principle factor; is it ammonia inhibition or is it that the cell density is highest in the high glutamine content culture.

6. MATERIALS

Cell lines used

Chinese Hamster Ovary (CHO) was used in the study. This cell lines have been transfected with genes expressing for a specific recombinant protein products.

Equipments

1. Sterile Pipettes
2. Water bath
3. Biological safety cabinet
4. Centrifuge
5. Wheaton Micro-Stir®

Media used

PFCHO media

Preparation of Media

Materials

Powdered media
Supplements like L-Glutamine, Pluronic F-68, Sodium bicarbonate
WFI
1N NaOH
1N HCl

Equipments

Electronic Weighing balance
Spatula
Magnetic Stirrer
Pipettes
pH meter

Media sterilization

Equipments

Peristaltic pump
Membrane filters
Biological Safety Cabinet

Cell Counting using haemocytometer

Materials

Culture sample
Trypan blue dye

Equipments

Haemocytometer
Pipettes
Microscope

Cell counting using Vi – cell XR

Beckman coulter – cell viability analyzer
Vial , plastic 4 ml (code: 383291)

Fermenters used

Sartorius (5 Lit.)
Wheaton double Sidearm Celstir®

7. ANALYTICAL ASSAYS

Determination of the concentrations of nutrients and metabolites

The partial pressure of dissolved carbon dioxide (pCO2) and several key nutrients such as Glucose, glutamine, and metabolites such as lactate and ammonia were measured using a **Nova analyzer** (Bioprofile 400, Nova, MA).

BioProfile 400 Test Menu

Nutrients/Metabolites	Acid/Base Status	Electrolytes	Calculated Parameters
Glutamine	pH	Sodium	Osmolality
Glutamate	PO_2	Potassium	Air Saturation
Glucose	PCO_2		CO_2 Saturation
Lactate			HCO_3^-
Ammonium			

The results are obtained on a thermo paper roll (as shown below);

BioProfile 400

Operator 123 17 Jul 04 10:50 Analyzer 1

Sample #	1	
Sample ID	89652	
Time of Analysis	17 Jul 04 10:50	
Sample Temperature	37.0°C	
Corrected to 37.0°C		
pH	7.481	
PCO_2	29.5	mmHg
PO_2	183.3	mmHg
Na^+	134.7	mmol/L
K^+	3.92	mmol/L
NH_4^+	6.54	mmol/L
Gluc	11.25	g/L
Gln	4.56	mmol/L
Glu	1.52	mmol/L
Lac	2.56	g/L
Calculated Results		
Osm	452	mOsm/kg
Air Sat	95.0	%
CO_2 Sat	5.1	%

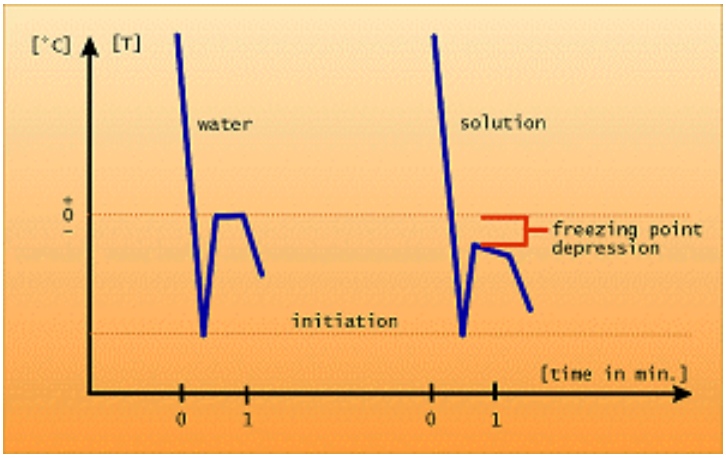
Ref: http://www.novabiomedical.com

Determination of osmolality

Osmomat 030

The total osmolality of an aqueous solution is determined by comparing the freezing point of pure water and the freezing point of the sample.

Water has a freezing point of 0 °C, a solution with saline concentration of 1 Osmol/kg has a freezing point of -1.858 °C.



Ref: http://www.gonotec.com

8. METHODS

Procedure for media preparation

The media components were mixed with WFI in required quantities under constant stirring and allowed to dissolve completely. The pH was adjusted to 7.00 +/- 0.02 with concentrated HCl / NaOH as required. The final volume was made by adding WFI. The Osmolality of the medium was checked using osmolality analyzer. (Gonotech instrument USA) Generally the osmolality of the media range 280-330mOsm/kg.

Cell counting methods

The measurement of overall health of cell cultures requires accurate measurements of both cell concentration and percentage of viable cells. The widely accepted method for cell viability determination was the trypan blue dye exclusion method. In this method, the cell suspension was mixed with trypan blue dye. The non viable cell membrane becomes permeable allowing for the uptake of the trypan blue dye. So, the dead cells appear purple-violet as compared to viable cells. The cell counts were performed by hemocytometer.

Procedure for Cell counting using hemocytometer

Culture samples were aseptically taken from the spinner flask in the biological safety cabinet. Samples were mixed gently with trypan blue dye by vortexing. Cover slip was placed over the hemocytometer slide and the mixture was loaded on the slide. Usually count of the four corner squares was adjusted with dilution so that the each square holds about 50 cells. Number of viable cells (observed bright) and number of cells (stained blue) was counted.

$$\text{Number of viable cells (million cells/ml)} = \frac{\text{number of viable cells} * \text{dilution factor}}{400}$$

$$\text{Number of dead cells (million cells/ml)} = \frac{\text{number of dead cells} * \text{dilution factor}}{400}$$

Total number of cells (million cells/ml) = number of viable cells + number of dead cells

$$\text{Percentage Viability (\%)} = \frac{\text{Number of viable cells} * 100}{\text{Total number of cells}}$$

Delta IVCC (million cells/ml) = (difference in time period) * (viable cells of present time period+ viable cells of previous time period)/2

Integral Viable cell Count (million cells/ml) = present day delta IVCC + previous day IVCC

Sampling from spinner and fermenter

The growing culture was aseptically taken from the spinner in the biological safety cabinet. About 6ml of culture was taken either directly of with the help of sterile pipette into an eppendorf and the cell viability, density of the culture was recorded and the spinner flask was placed in the incubator maintained at 37ºC. For the fermenter, sampling was done via a sampling bottle.

9. RESULTS

Experiment number: 1

The study of growth characteristics and metabolic profiles of rCHO cells in Spinner flask (250ml and 500ml) and fermenter (2l).

Spinner 250ml data table:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml)	Dead Cell Count (x106/ml)	Total Cell Count (x106/ml)	Viability (%)	Delta IvCC (x106.day/ml)	IvCC (x106.day/ml)
1	3/6/08 11:00am	0.0	0.34	0.04	0.38	90.76	0	0
2	4/6/08 11:00am	24.0	0.74	0.02	0.76	98.02	0.54	0.54
3	5/6/08 11:00am	48.0	1.35	0.04	1.39	97.12	1.05	1.59
4	6/6/08 11:00am	72.0	2.35	0.12	2.47	95.15	1.85	3.44
5	7/6/08 11:00am	96.0	2.71	0.08	2.79	97.13	2.53	5.97
6	8/6/08 11:00am	120.0	2.44	0.32	2.75	88.55	2.57	8.54
7	9/6/08 11:00am	144.0	1.95	0.47	2.42	80.75	2.19	10.73
8	10/6/08 11:00am	168.0	1.52	0.59	2.11	72.07	1.74	12.47
9	11/6/08 11:00am	192.0	1.00	0.74	1.74	57.70	1.26	13.73
10	12/6/08 11:00am	216.0	0.61	1.43	2.03	29.89	0.81	14.54

Sample no	pH 250ml	OSM (mOsm/kg) Gonotech	Gln 250ml (mmol/L)	Glu 250ml (mmol/L)	Gluc 250ml (g/L)	Lac 250ml (g/L)	NH4+ 250ml (mmol/L)	Na+ (mmol/L)	K+ (mmol/L)	OSM (mOsm/kg) Nova	PCO2(mm Hg)
1	7.23		3.64	2.13	3.44	0.00	1.65	143	7.6		26
2	7.24	353	3.30	1.05	3.04	0.00	2.39	143	7.8	353	35
3	7	320	1.44	1.16	2.76	0.80	3.51	143	7.7	320	45
4	6.76	358	0.00	1.30	2.14	1.16	4.01	143	7.6	358	68
5	6.61	323	0.00	1.19	1.92	1.43	4.07	143.9	7.6	323	69
6	6.57	322	0.00	1.07	1.68	1.65	4.24	145.5	7.56	322	74
7	6.41	318	0.00	1.27	1.60	1.64	4.31	145	7.7	318	99
8	6.34		0.00	1.21	1.43	2.06	4.34	145	7.8	382	63
9	6.35		0.00	1.17	1.14	1.94	4.59	147	7.8	384	85
10	6.39		0.24	1.24	1.44	2.17	5.68	146	7.7	389	71

Spinner 500ml data table:

Sample no.	Date & Time (dd/mm/yy)	Age (Hrs.)	Viable Cell Count (x106/ml)	Dead Cell Count (x106/ml)	Total Cell Count (x106/ml)	Viability (%)	Delta IvCC (x106.day/ml)	IvCC (x106.day/ml)
1	3/6/08 11:00am	0.0	0.24	0.02	0.25	94.09	0	0
2	4/6/08 11:00am	24.0	0.39	0.02	0.42	94.58	0.32	0.32
3	5/6/08 11:00am	48.0	1.08	0.06	1.13	95.14	0.74	1.05
4	6/6/08 11:00am	72.0	2.18	0.04	2.22	98.19	1.63	2.68
5	7/6/08 11:00am	96.0	2.95	0.10	3.05	96.88	2.56	5.24
6	8/6/08 11:00am	120.0	3.37	0.17	3.54	95.20	3.16	8.40
7	9/6/08 11:00am	144.0	2.63	0.34	2.97	88.54	3.00	11.40
8	10/6/08 11:00am	168.0	1.82	0.71	2.52	72.02	2.22	13.62
9	11/6/08 11:00am	192.0	0.93	0.92	1.84	50.34	1.37	14.99
10	12/6/08 11:00am	216.0	0.76	2.14	2.89	26.19	0.84	15.84

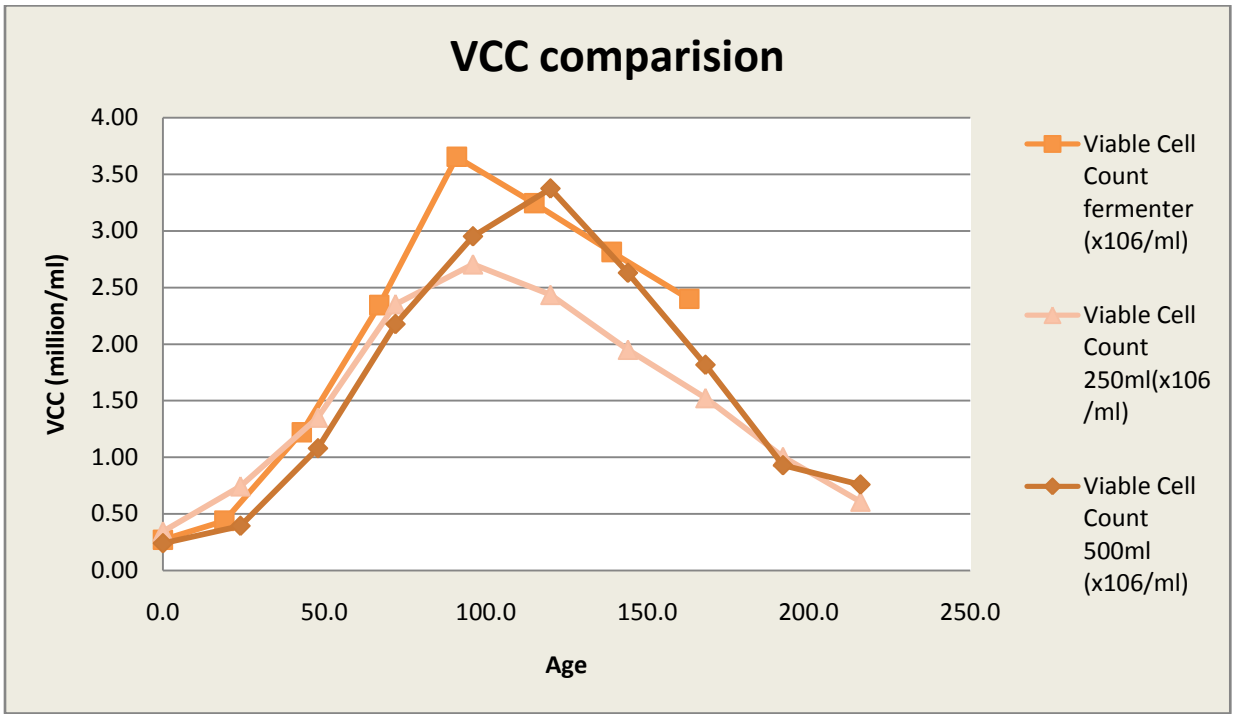
Sample no	pH 500ml	OSM (mOsm/kg) Gonotech	Gln 500ml(mmol/L)	Glu 500ml (mmol/L)	Gluc 500ml (g/L)	Lac 500ml (g/L)	NH4+ 500ml (mmol/L)	Na+ (mmol/L)	K+ (mmol/L)	OSM (mOsm/kg) Nova	PCO2(mm Hg) 500ml
1	7.3		3.81	2.07	3.46	0.00	1.46	144	7.6		24
2	7.42	332	3.82	0.99	3.22	0.00	1.96	145	7.8		35
3	7.08	319	2.63	1.13	3.11	0.33	3.17	145	7.8	372	46
4	6.91	331	0.29	1.23	2.61	1.23	3.71	144	7.5	377	54
5	6.9	319	err	1.17	2.10	1.40	4.06	145.9	7.7	324	67
6	6.64	319	err	1.03	1.93	1.79	4.25	146.1	7.66	324	68
7	6.6		err	1.02	1.57	1.89	4.37	147	7.7	317	100
8	6.52		err	0.82	1.37	1.78	4.24	145	7.6	379	56
9	6.64		err	1.01	1.24	1.77	4.13	145	7.8	379	48
10	6.60		0.21	1.09	1.25	1.97	5.90	145	7.8	383	46

Fermenter data table:

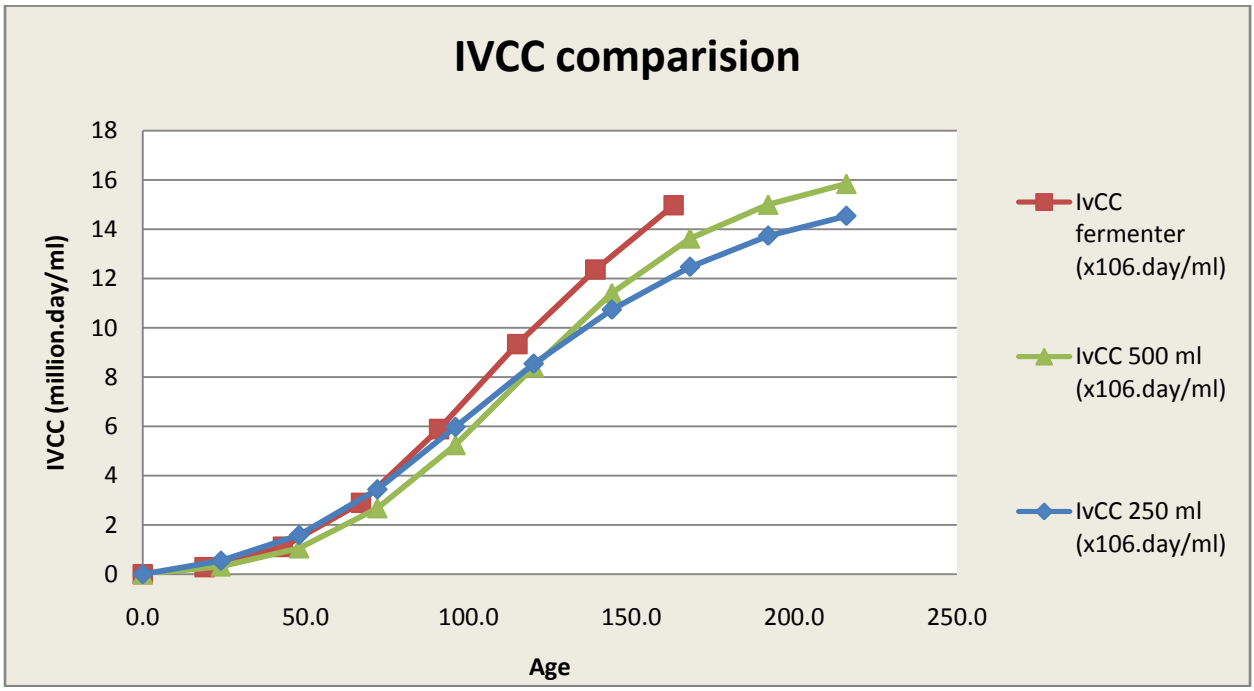
Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml)	Dead Cell Count (x106/ml)	Total Cell Count (x106/ml)	Viability (%)	Delta IvCC (x106.day/ml)	IvCC (x106.day/ml)
1	28/5/08 4:00pm	0.0	0.27	0.03	0.30	90.00	0	0
2	29/5/08 11:00am	19.0	0.44	0.02	0.46	96.15	0.28	0.28
3	30/5/08 11:00am	43.0	1.22	0.05	1.27	96.44	0.83	1.11
4	31/5/08 11:00am	67.0	2.34	0.11	2.45	95.51	1.78	2.89
5	1/6/08 11:00am	91.0	3.65	0.16	3.81	95.93	3.00	5.89
6	2/6/08 11:00am	115.0	3.24	0.56	3.80	85.39	3.45	9.34
7	3/6/08 11:00am	139.0	2.81	0.80	3.61	77.85	3.03	12.36
8	4/6/08 11:00am	163.0	2.40	2.18	4.57	52.43	2.61	14.97

Sample no	pH fermenter	OSM (mOsm/kg) Gonotech	Gln (mmol/L)	Glu (mmol/L)	Gluc fermentor (g/L)	Lac fermentor (g/L)	NH4+ fermenter (mmol/L)	Na+ (mmol/L)	K+ (mmol/L)	OSM (mOsm/kg) Nova	PCO2 fermentor (mm Hg)
1	7.29	321	***		3.47	0.00	2.23	138	7.5		48
2	7.19	305	***		2.92	0.00	2.57	122	6.7		16
3	7.04	302	***		3.36	0.35	3.84	138	7.6		37
4	6.99	313	***		2.54	0.62	4.55	138	7.3	359	48
5	6.9	316	***		2.13	0.96	4.66	138.1	7.3	362	56
6	7.1	315	***		1.52	0.44	4.75	137.2	7.37	351	48
7	6.84	313	***		1.31	1.38	4.93	140	7.6	366	42
8	6.91	325	***		1.09	1.77	4.93	140	7.7	361	39

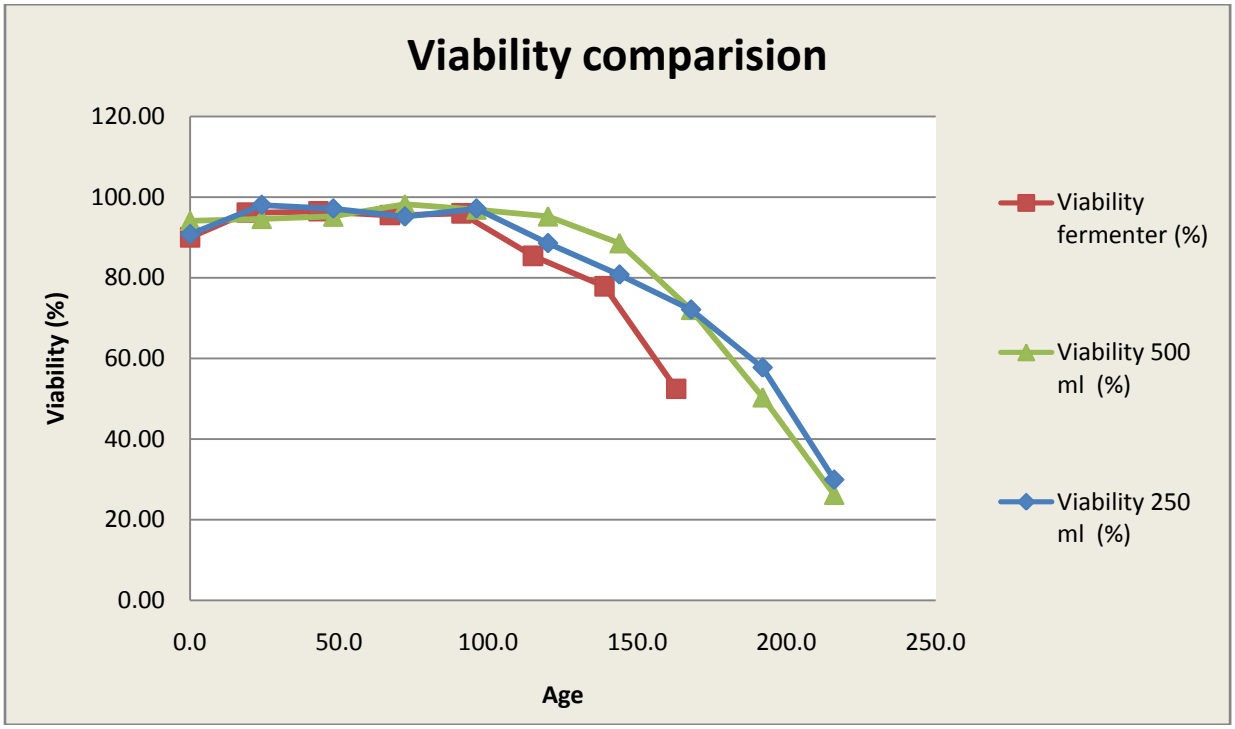
Comparative graph of Viable cell count of spinner (250ml and 500ml) and fermenter:



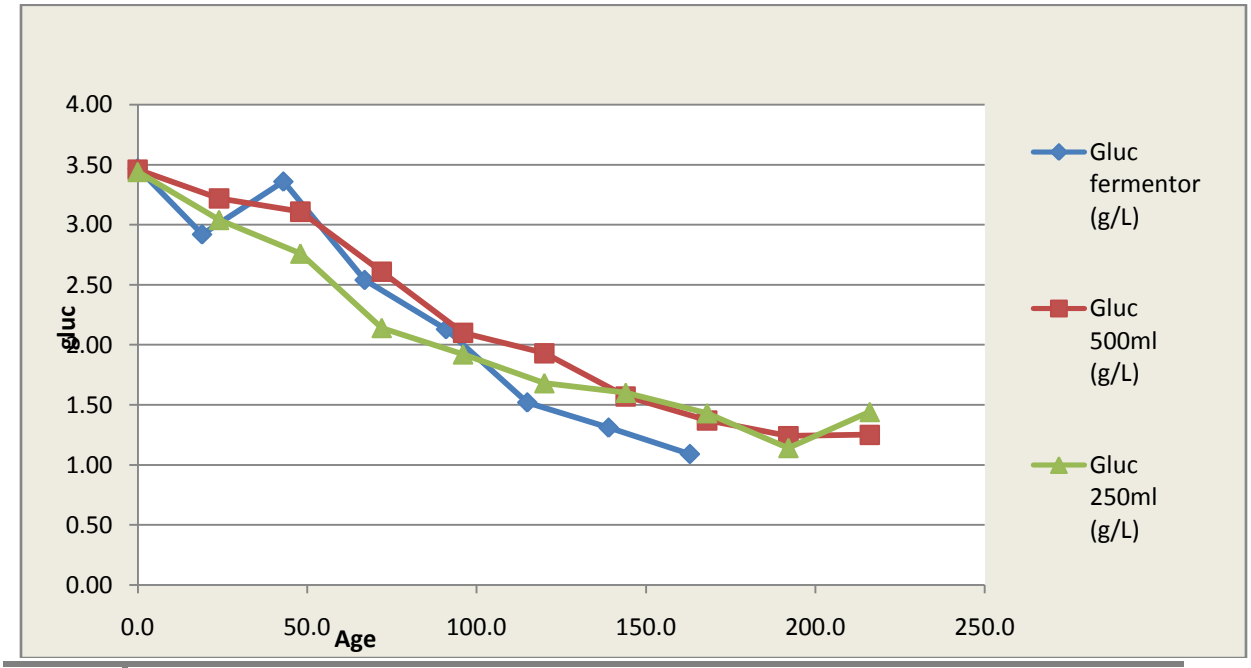
Comparative graph of Integral Viable cell count of spinner (250ml and 500ml) and fermenter:



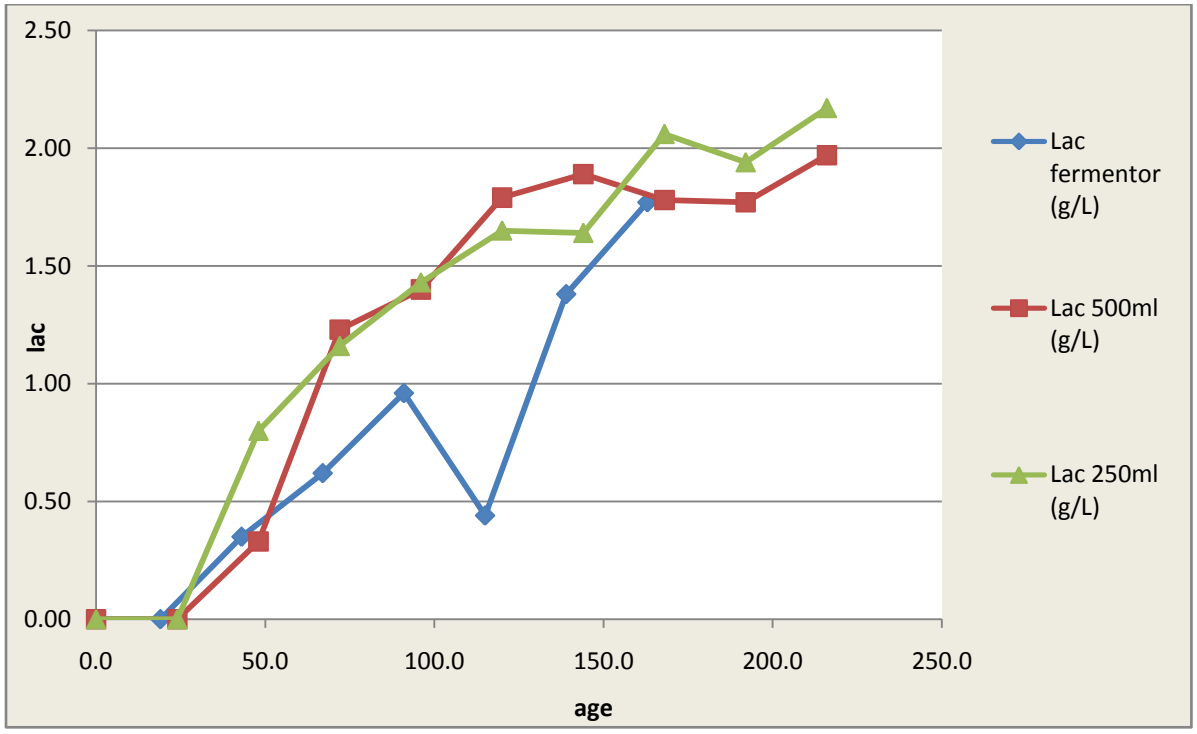
Comparative graph of Viability of spinner (250ml and 500ml) and fermenter:



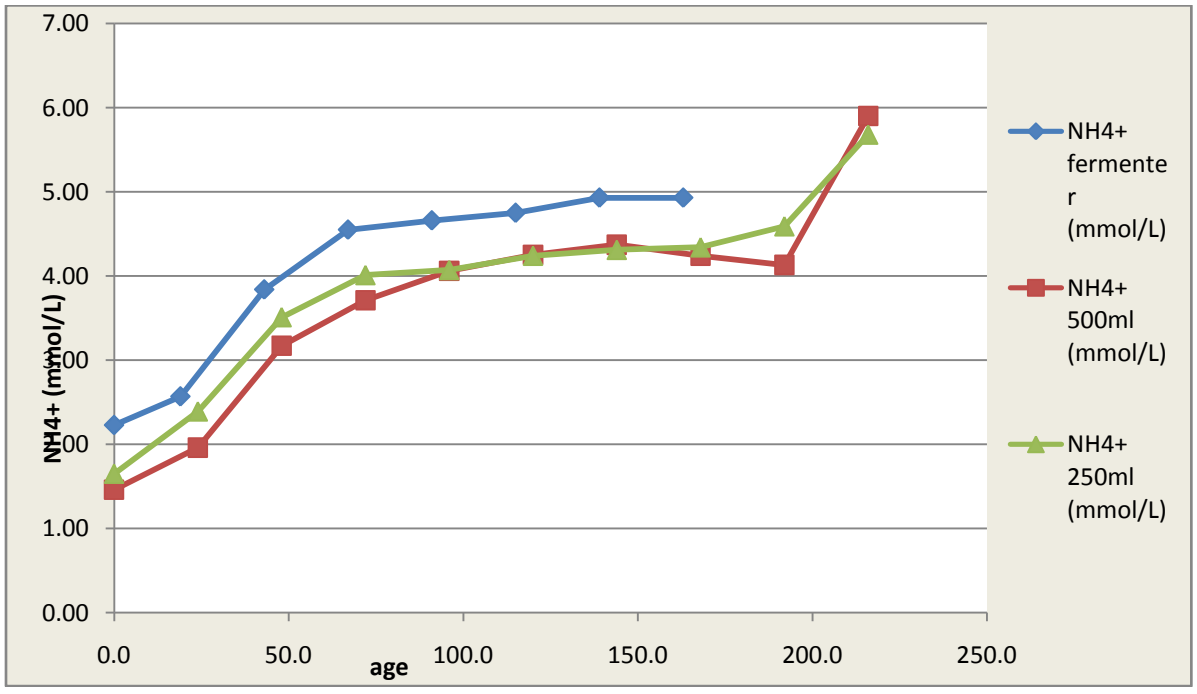
Comparative graph of Glucose concentrations of spinner (250ml and 500ml) and fermenter:



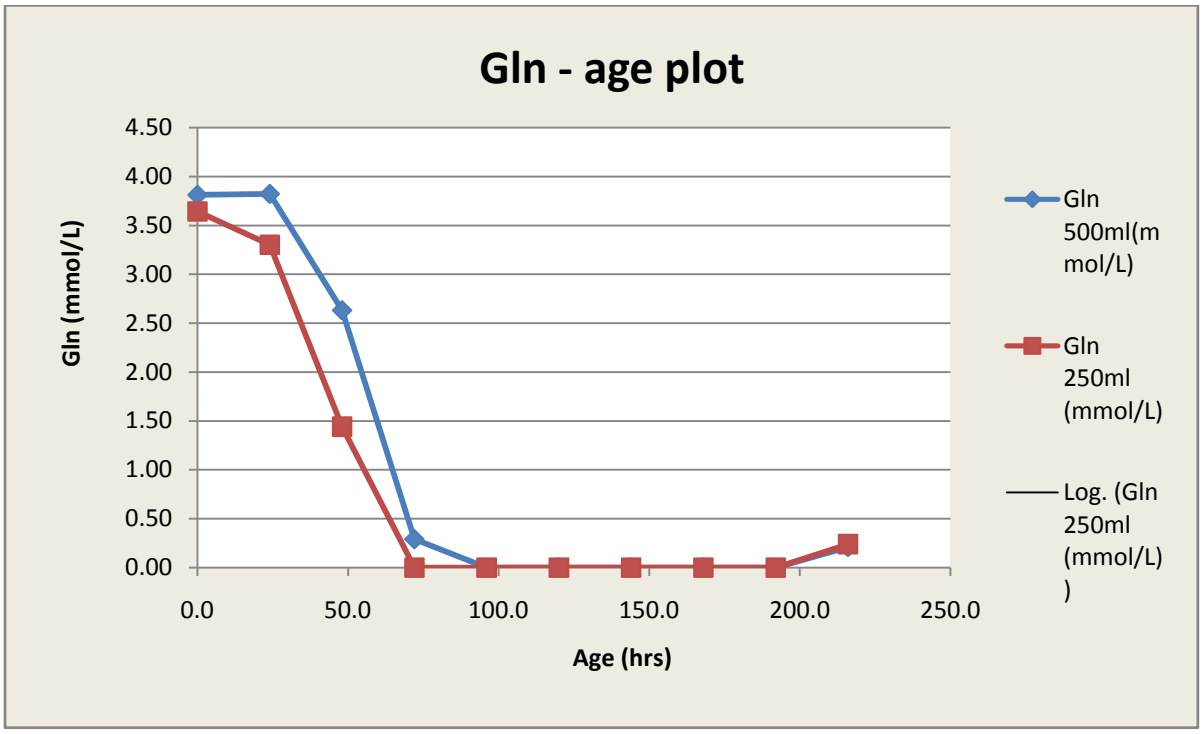
Comparative graph of lactate concentrations of spinner (250ml and 500ml) and fermenter:



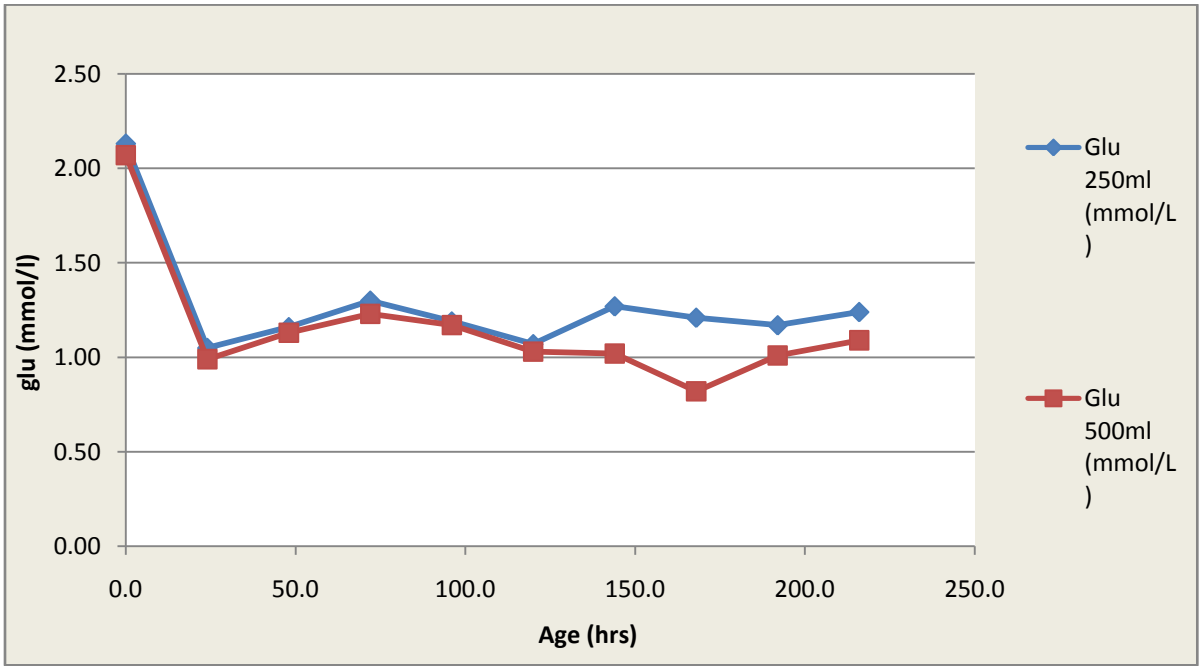
Comparative graph of ammonia concentrations of spinner (250ml and 500ml) and fermenter:



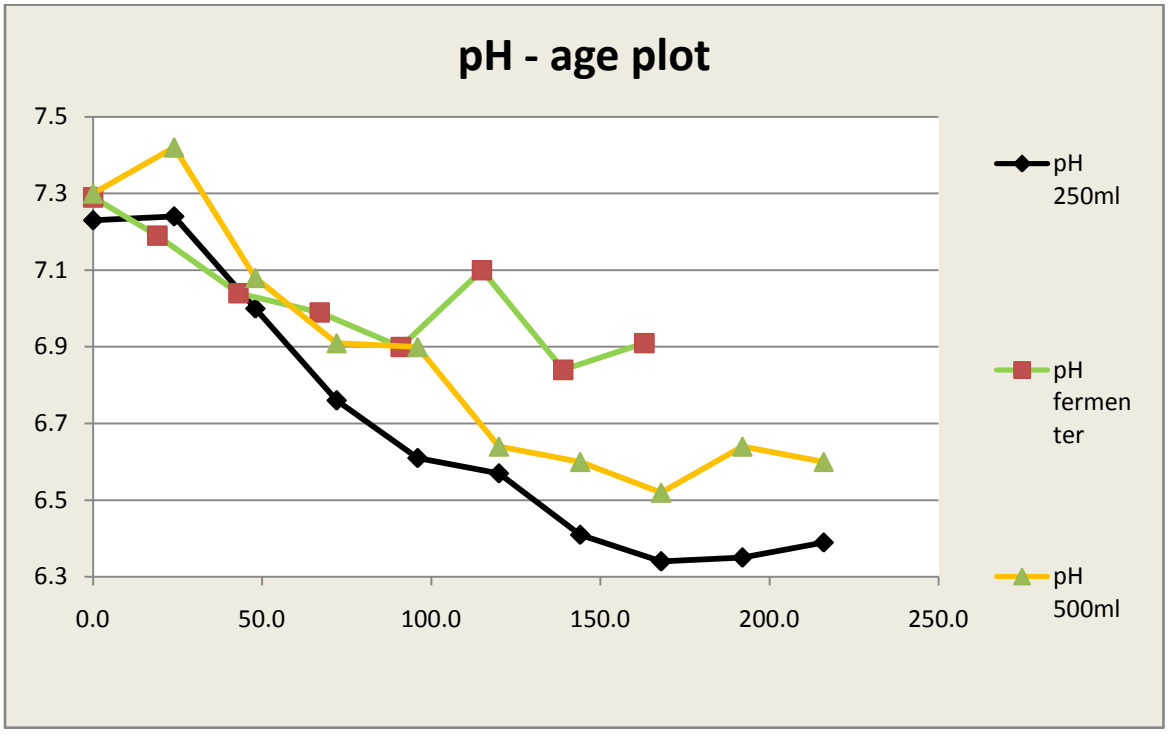
Comparative graph of glutamine concentrations of spinner (250ml and 500ml):



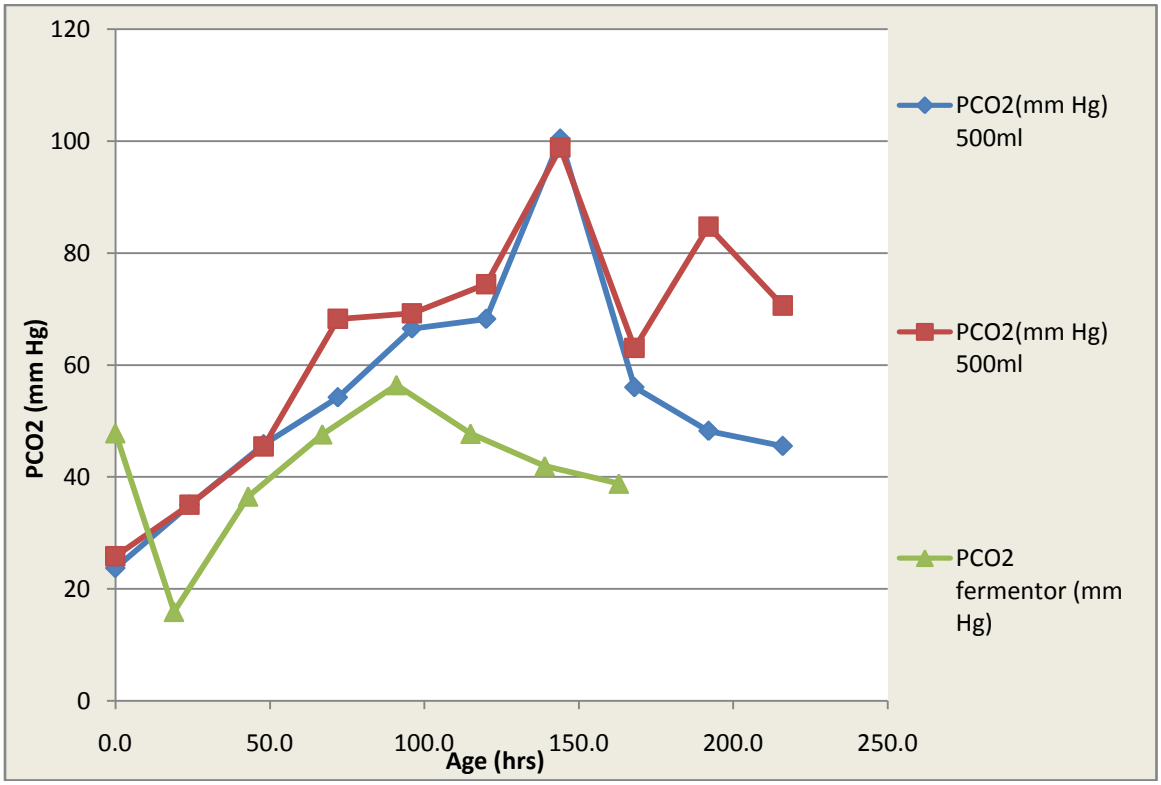
Comparative graph of glutamate concentrations of spinner (250ml and 500ml) and fermenter:



Comparative graph of pH of spinner (250ml and 500ml) and fermenter:



Comparative graph of pCO2 of spinner (250ml and 500ml) and fermenter:



Discussion

The cell count in the fermenter (2 lit) goes up to a maximum of around 3.8 million/ml. The cell count in the spinner(500 ml) goes up to a maximum of around 3.4 million/ml. The cell count in the spinner (250 ml) goes up to a maximum of around 2.8 million/ml. These trends are acceptable as the conditions in the fermenter are more controlled.

Viability for the fermenter drops early as compared to the spinners. This can be attributed to the controlled conditions in the fermenter (37 degC and neutral pH and the controlled dissolved oxygen) which trigger for the rapid metabolism rate and thus for the increase in no of dead cells. In the spinners even though the temperature is maintained at 37 degC; the pH falls and thus may be responsible for the decrease in metabolism rate.

The sudden decrease in the glucose levels in the fermenter can be attributed to the high cell concentrations/high rate of cell growth and the controlled conditions which result in high consumption of glucose. As soon as the glutamine got depleted in the reactors, the uptake rate of glucose increased. This can be seen by the two point moving average trend of the glucose profiles.

As observed the accumulation of lactate is less in the fermenter even though the consumption of glucose was maximum in the case of the fermenter. Ammonia accumulation was max in the fermenter coz of the controlled conditions. Also it is obvious that the ammonia production in the 250ml spinner is more as the glutamine consumption is relatively more as compared to the 500ml spinner.

The CO2 in the fermenter is used to maintain the pH of the system and thus the arbitrary trend is coz of the sparger pumping in CO2 to maintain the desired pH. The decrease in the pCO2 in the latter half of the graph is attributed to the decrease in solubility of CO2 in the media as the pH decreases.

At first, the initial cell-specific glucose and glutamine utilization and lactate formation rates were investigated; cells started their exponential growth phase with very high specific glucose and glutamine utilization rates as well as very high lactate formation rates. With increasing cultivation time these specific utilization and formation rates decreased as glucose and glutamine concentrations declined.

Also, when the viability – ammonia versus age plots were studied, it was observed that the viability begins to fall when the ammonia concentration reaches around 4 mmol/l.

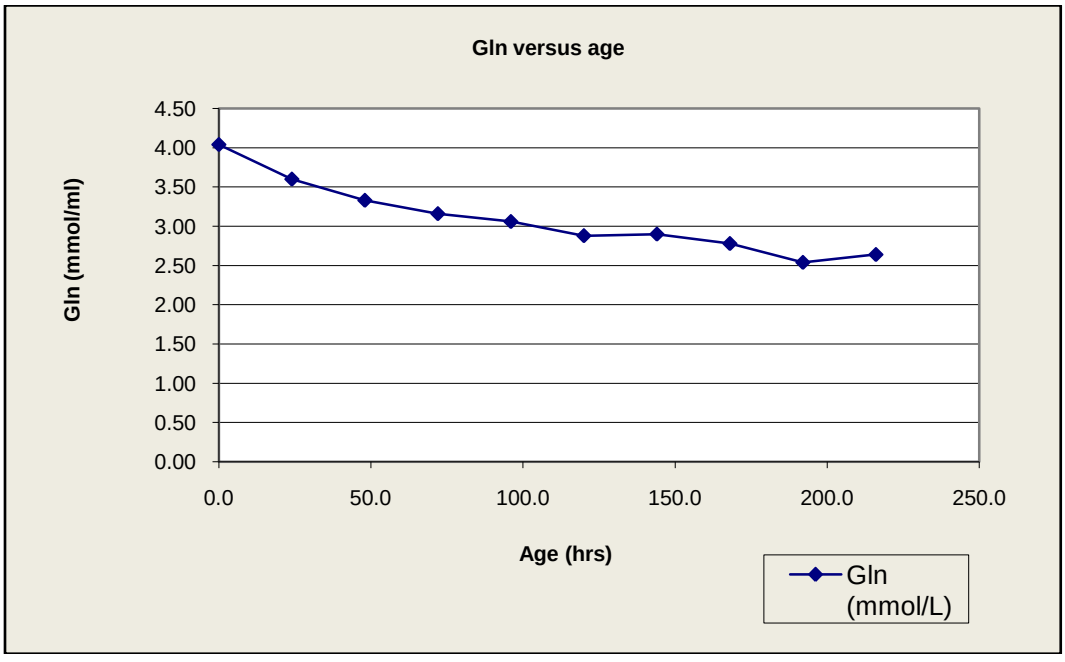
Experiment no: 2

Determination of glutamine degradation rate in a pfCHO media incubated at 37 degC.

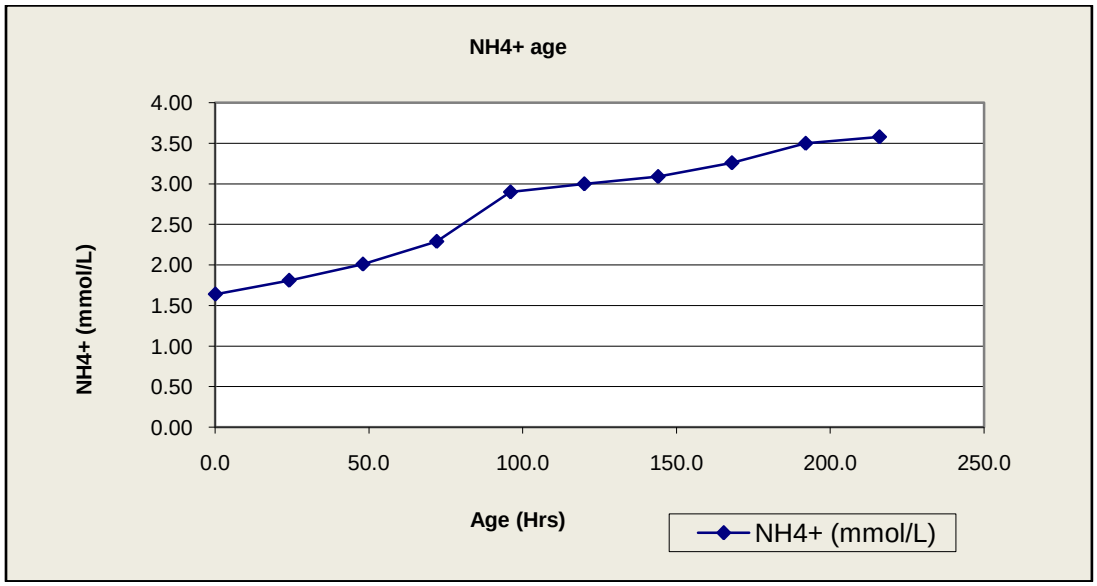
Data table:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Gln (mmol/L)	Glu (mmol/L)	Gluc (g/L)	Lac (g/L)	NH4+ (mmol/L)	Na+ (mmol/L)	K+ (mmol/L)	PCO2 (mmHg)
1	16.6.08 11:00am	0.0	4.04	0.66	3.64	0.00	1.64	141	7.6	18
2	17.6.08 11:00am	24.0	3.60	0.68	3.71	0.00	1.81	142	7.7	19
3	18.6.08 11:00am	48.0	3.33	0.70	3.75	0.00	2.01	141	7.6	19
4	19.6.08 11:00am	72.0	3.16	0.68	3.33	0.00	2.29	141	7.6	18
5	20.6.08 11:00am	96.0	3.06	0.70	3.53	0.00	2.90	140.3	7.5	15
6	21.6.08 11:00am	120.0	2.88	0.73	3.88	0.00	3.00	141.0	7.64	16
7	22.6.08 11:00am	144.0	2.90	0.61	3.45	0.00	3.09	141	7.7	15
8	23.6.08 11:00am	168.0	2.78	0.65	3.28	0.00	3.26	141	7.7	13
9	24.6.08 11:00 am	192.0	2.54	0.63	3.45	0.00	3.50	142	7.6	13
10	25.6.08 11:00am	216.0	2.64	0.68	3.94	0.00	3.58	141	7.6	14

Glutamine profile:



Ammonia profile:

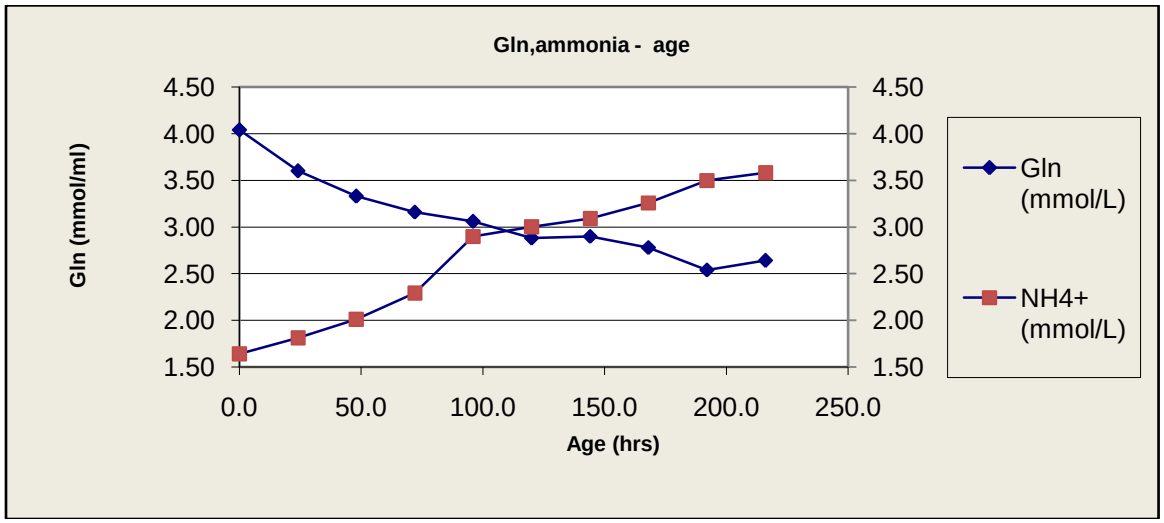


The degradation of glutamine follows a first order kinetics. i.e.

$$d/dt\ (Gln) = -K_1\ (Gln)$$

And ammonia kinetics as $d/dt\ (amm) = K_2\ (amm)$

Also as glutamine degrades, ammonia is formed. So ideally the graphs of both should be a mirror image of each other (as shown below):



Now fitting a trend line for the above graphs we get:
K_1 = 0.0071411 and K_2 = 0.0066044

Experiment no: 3

The study of growth characteristics and metabolite profiles when the initial media had different ratio of fresh to spent media volume.

Spinner no	Initial percentage of new and spent media
A	100% new ; 0% spent
B	90% new ; 10%spent
C	80% new ; 20% spent
D	60% new ; 40% spent
E	40% new ; 60% spent
F	20% new ; 80% spent
G	0% new ; 100% spent

The experiment was carried out in 200 ml bottles with magnetic stirrers.

Data sheet of spinner A:

Sample no.	Date & Time (dd/mm/yy)	Age (Hrs.)	Viable Cell Count A (x106/ml)	Dead Cell Count A (x106/ml)	Total Cell Count (x106/ml) A	Viability (%) A	Delta IvCC (x106.day/ml)	IvCC (x106.day/ml) A
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.74	0.04	0.78	95.18	0.43	0.43
3	22/6/08 11:00am	42.0	1.83	0.08	1.90	96.05	1.28	1.71
4	23/6/08 11:00am	66.0	3.39	0.27	3.66	92.62	2.61	4.32
5	24/6/08 11:00am	90.0	2.86	0.28	3.14	91.08	3.13	7.44
6	25/6/08 11:00am	114.0	3.00	0.59	3.59	83.57	2.93	10.37
7	26/6/08 11:00am	138.0	1.89	2.29	4.18	45.22	2.45	12.82

Gln A (mmol/L)	Glu A (mmol/L)	Gluc A (g/L)	Lac A (g/L)	NH4+ A (mmol/L)	Na+ A(mmol/L)	K+ A(mmol/L)	OSM A (mOsm/kg) Nova	PCO2(mm Hg) A	CO sat (%) A	HCO3 A(mmol/L)
5.68	0.66	3.64	0.00	1.64	141	7.6	367	18		
3.70	0.85	2.97	0.27	2.52	141	7.5	368	45	6.8	16.0
2.29	0.96	2.67	0.82	3.94	142	7.4	369	57	8.6	15.0
0.88	0.82	2.33	1.22	4.90	143	7.4	375	91	14.0	10.7
1.61	0.84	2.07	1.52	5.14	143	7.5	379	51	7.8	11.9
0.27	0.76	1.69	1.73	5.45	145.4	7.6	383	116	17.7	6.7
0.00	0.84	1.50	1.92	5.47	145.4	7.7	385	131	20.0	5.8

Data sheet of spinner B:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count B (x106/ml)	Dead Cell Count B (x106/ml)	Total Cell Count B (x106/ml)	Viability B (%)	Delta IvCC (x106.day/ml) B	IvCC (x106.day/ml) B
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.61	0.03	0.64	95.70	0.38	0.38
3	22/6/08 11:00am	42.0	2.11	0.06	2.17	97.24	1.36	1.74
4	23/6/08 11:00am	66.0	2.79	0.21	3.00	93.00	2.45	4.19
5	24/6/08 11:00am	90.0	2.31	0.38	2.69	85.87	2.55	6.74
6	25/6/08 11:00am	114.0	1.92	0.96	2.88	66.67	2.12	8.86
7	26/6/08 11:00am	138.0	0.61	1.01	1.62	37.65	1.27	10.12

Gln B (mmol/L)	Glu B (mmol/L)	Gluc B (g/L)	Lac B (g/L)	NH4+ B (mmol/L)	Na+ B (mmol/L)	K+ B (mmol/L)	OSM B (mOsm/kg) Nova	PCO2(mm Hg) B	CO sat (%) B	HCO3 (mmol/L) B
5.20	0.83	3.54	0.45	2.56	141	7.6	367	18		
3.22	1.02	2.87	0.72	3.44	141	7.5	369	39	5.6	14.2
2.34	1.14	2.45	1.12	4.65	143	7.4	374	57	8.6	13.3
1.46	1.03	2.12	1.59	5.41	145	7.4	380	94	14.4	8.7
1.72	1.02	1.59	1.90	5.77	145	7.5	384	105	16.0	7.3
1.77	1.07	1.62	2.03	5.81	146.4	7.6	389	109	16.6	5.0
1.91	1.09	1.44	1.97	5.71	145.4	7.84	386	116	17.7	5.6

Data sheet of spinner C:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) C	Dead Cell Count C (x106/ml)	Total Cell Count C (x106/ml)	Viability C (%)	Delta IvCC (x106.day/ml) C	IvCC (x106.day/ml) C
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.99	0.04	1.03	96.59	0.52	0.52
3	22/6/08 11:00am	42.0	2.15	0.09	2.24	95.98	1.57	2.09
4	23/6/08 11:00am	66.0	2.58	0.22	2.80	92.14	2.37	4.46
5	24/6/08 11:00am	90.0	2.08	0.40	2.48	83.87	2.33	6.79
6	25/6/08 11:00am	114.0	1.43	1.07	2.50	57.20	1.76	8.54
7	26/6/08 11:00am	138.0	0.76	1.55	2.31	32.90	1.10	9.64

Gln (mmol/L) C	Glu (mmol/L) C	Gluc 500 C	Lac 500 C	NH4+ C (mmol/L)	Na+ C (mmol/L)	K+ C (mmol/L)	OSM C (mOsm/kg) Nova	PCO2 C (mm Hg)	CO sat (%) C	HCO3 C (mmol/L)
5.98	0.89	3.49	0.49	2.65	141	7.6	368	18		
3.00	1.08	2.82	0.76	3.53	142	7.5	369	37	5.6	14.2
2.31	1.11	2.33	1.19	4.74	143	7.4	376	63	9.6	12.6
1.63	1.00	1.90	1.66	5.42	145	7.4	381	84	12.9	8.3
1.72	0.97	1.65	1.99	5.90	146	7.5	388	111	16.9	6.9
1.41	1.00	1.40	2.05	5.92	146.1	7.7	388	108	16.4	4.7
2.16	1.08	1.42	2.02	5.78	147.0	7.91	389	113	17.2	5.6

Data sheet of spinner D:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) D	Dead Cell Count D (x106/ml)	Total Cell Count D (x106/ml)	Viability (%) D	Delta IvCC (x106.day/ml) D	IvCC (x106.day/ml) D
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.56	0.04	0.59	94.07	0.36	0.36
3	22/6/08 11:00am	42.0	1.68	0.07	1.75	96.00	1.12	1.48
4	23/6/08 11:00am	66.0	2.59	0.26	2.85	90.88	2.14	3.61
5	24/6/08 11:00am	90.0	2.23	0.32	2.55	87.45	2.41	6.02
6	25/6/08 11:00am	114.0	1.60	1.17	2.77	57.76	1.92	7.94
7	26/6/08 11:00am	138.0	1.19	1.52	2.71	43.91	1.40	9.33

Gln (mmol/L) D	Glu (mmol/L) D	Gluc D (g/L)	Lac D (g/L)	NH4+ D (mmol/L)	Na+ D (mmol/L)	K+ D (mmol/L)	OSM D (mOsm/kg) Nova	PCO2 D (mm Hg)	CO sat (%) D	HCO3 D (mmol/L)
4.78	1.03	3.17	0.91	3.98	142	7.6	375	18		
2.80	1.22	2.50	1.18	4.86	144	7.6	377	37	5.6	12.3
2.18	1.25	2.04	1.43	5.79	143	7.6	379	49	7.5	11.4
1.57	1.11	1.67	1.65	6.12	147	7.5	384	80	12.1	9.2
1.59	1.18	1.49	1.99	6.56	145	7.5	386	66	10.1	8.0
1.25	1.06	1.12	2.00	6.51	146.3	7.8	387	92	14.0	5.2
2.00	1.09	1.08	2.02	6.50	146.6	7.89	388	115	17.4	5.6

Data sheet of spinner E:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) E	Dead Cell Count E (x106/ml)	Total Cell Count E (x106/ml)	Viability (%) E	Delta IvCC (x106.day/ml) E	IvCC (x106.day/ml) E
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.46	0.02	0.48	95.31	0.32	0.32
3	22/6/08 11:00am	42.0	1.57	0.08	1.65	95.44	1.01	1.34
4	23/6/08 11:00am	66.0	2.92	0.19	3.11	93.89	2.25	3.58
5	24/6/08 11:00am	90.0	1.85	0.31	2.16	85.65	2.39	5.97
6	25/6/08 11:00am	114.0	1.87	0.84	2.71	69.00	1.86	7.83
7	26/6/08 11:00am	138.0	1.24	1.40	2.64	46.97	1.56	9.38

Gln (mmol/L) E	Glu (mmol/L) E	Gluc E (g/L)	Lac E (g/L)	NH4+ E (mmol/L)	Na+ E (mmol/L)	K+ E (mmol/L)	OSM E (mOsm/kg) Nova	PCO2 E (mm Hg)	CO sat (%) E	HCO3 E (mmol/L)
3.88	1.38	2.70	1.30	5.52	143	7.7	379	18		
1.90	1.57	2.03	1.57	6.40	144	7.7	383	33	5.0	10.2
1.81	1.42	1.65	1.68	6.90	146	7.8	387	47	7.2	9.8
1.74	1.33	1.44	1.67	7.05	146	7.6	385	73	11.1	9.1
1.74	1.32	1.32	1.97	7.46	146	7.6	388	76	11.6	8.0
1.89	1.24	0.97	1.98	7.41	147.5	7.7	390	94	14.4	5.6
2.07	1.16	0.99	1.97	7.45	148.4	8.01	392	113	17.3	6.1

Data sheet of spinner F:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) F	Dead Cell Count F (x106/ml)	Total Cell Count F (x106/ml)	Viability (%) F	Delta IvCC (x106.day/ml) F	IvCC (x106.day/ml) F
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.53	0.03	0.55	95.02	0.35	0.35
3	22/6/08 11:00am	42.0	1.56	0.07	1.62	95.99	1.04	1.39
4	23/6/08 11:00am	66.0	2.21	0.25	2.46	89.84	1.88	3.27
5	24/6/08 11:00am	90.0	1.30	0.22	1.52	85.53	1.76	5.02
6	25/6/08 11:00am	114.0	0.17	0.55	0.72	23.61	0.74	5.76
7	26/6/08 11:00am	138.0	0.09	1.72	1.81	4.97	0.13	5.89
8	27/6/08 11:00am	162.0	0.00	1.59	1.59	0.00	0.05	5.93

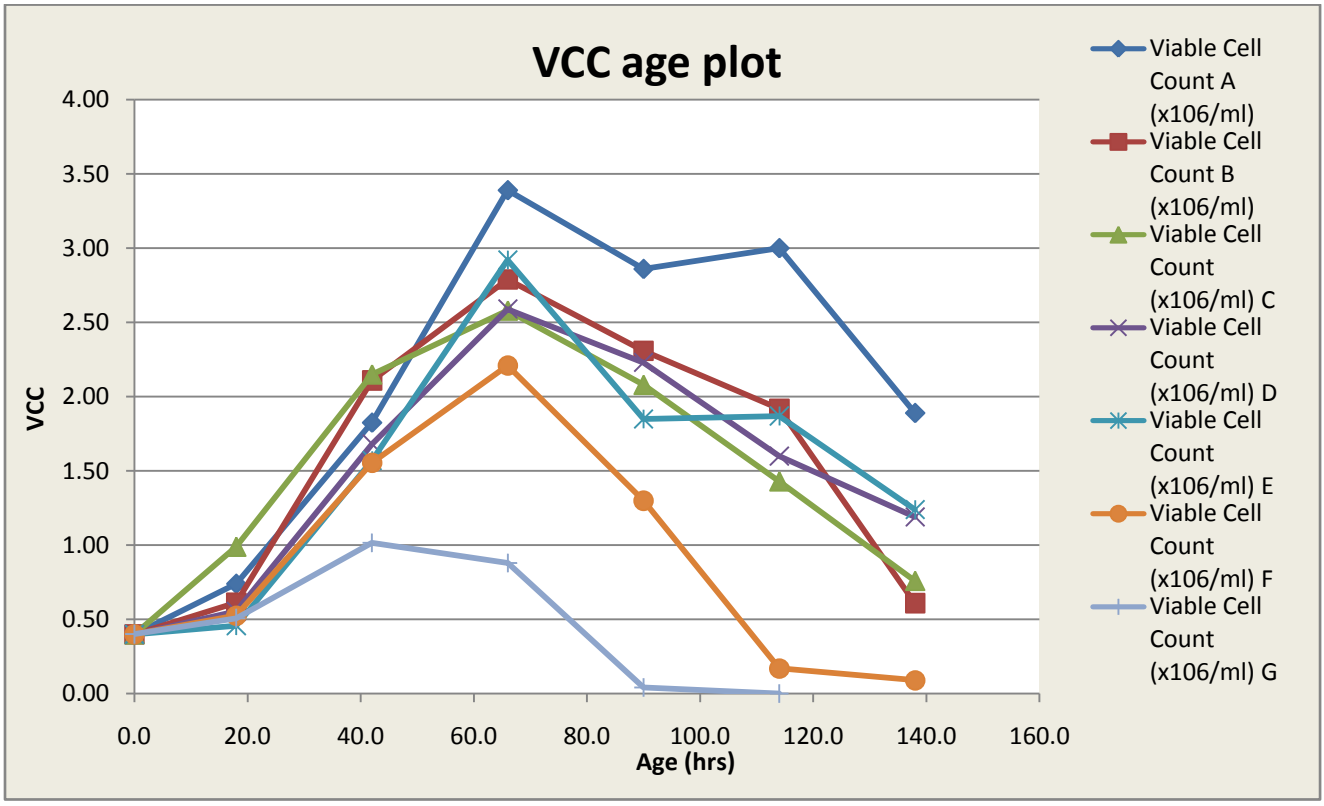
Gln (mmol/L) F	Glu (mmol/L) F	Gluc F (g/L)	Lac F (g/L)	NH4+ F (mmol/L)	Na+ F (mmol/L)	K+ F (mmol/L)	OSM (mOsm/kg) Nova F	PCO2 F (mm Hg)	CO sat (%) F	HCO3 F (mmol/L)
3.54	1.41	2.39	1.55	6.66	143	7.6	383	18		
1.56	1.60	1.72	1.82	7.54	144	7.7	386	29	4.4	8.7
1.77	1.52	1.39	1.87	7.81	145	7.7	386	41	6.2	8.5
1.88	1.46	1.27	1.79	7.88	146	7.6	385	63	9.7	9.1
1.79	1.52	1.31	1.86	8.22	147	7.6	391	80	12.2	9.2
1.69	1.44	0.87	1.93	8.26	147.0	7.9	390	84	12.8	6.2
2.20	1.39	1.01	1.98	8.05	146.5	8.02	390	86	13.1	6.7

Data sheet of spinner G:

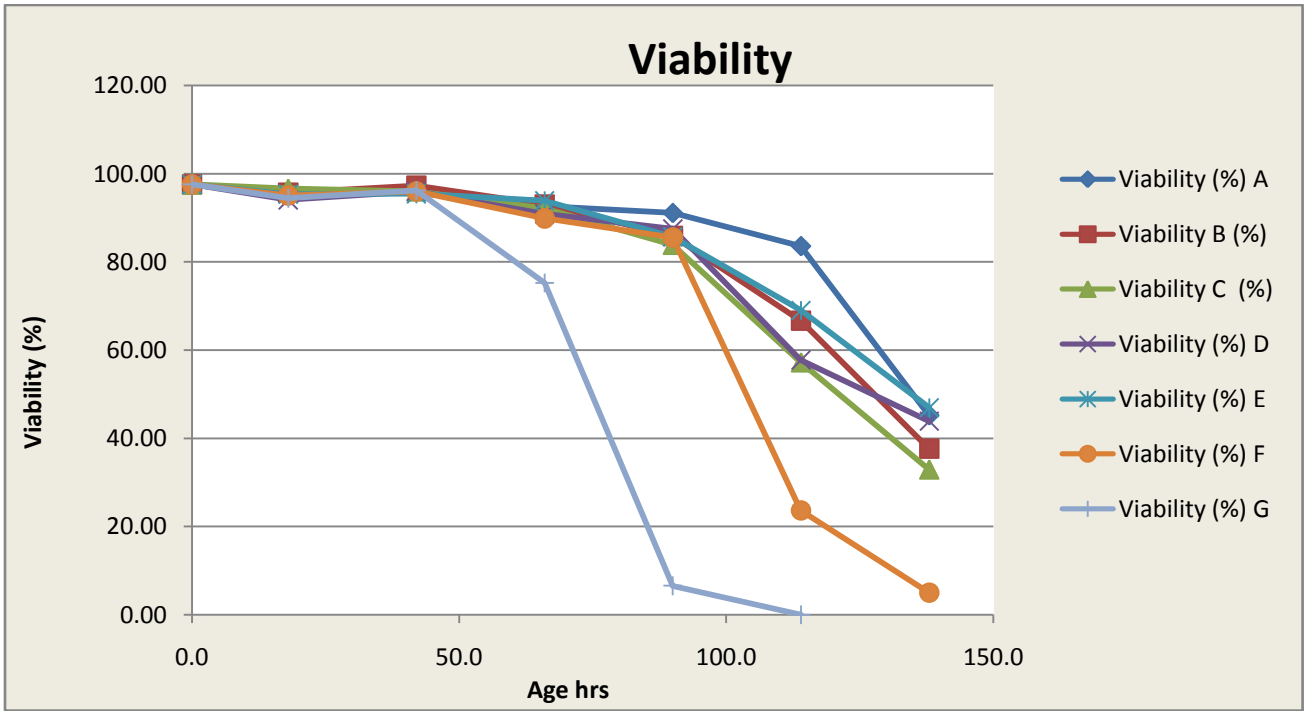
Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) G	Dead Cell Count (x106/ml) G	Total Cell Count (x106/ml) G	Viability (%) G	Delta IvCC (x106.day/ml) G	IvCC (x106.day/ml) G
1	20/6/08 5 :00pm	0.0	0.40	0.01	0.41	97.56	0	0
2	21/6/08 11:00am	18.0	0.51	0.03	0.54	94.42	0.34	0.34
3	22/6/08 11:00am	42.0	1.02	0.04	1.06	96.21	0.76	1.10
4	23/6/08 11:00am	66.0	0.88	0.29	1.17	75.21	0.95	2.05
5	24/6/08 11:00am	90.0	0.04	0.57	0.61	6.56	0.46	2.51
6	25/6/08 11:00am	114.0	0.00	0.61	0.61	0.00	0.02	2.53

Gln (mmol/L) G	Glu (mmol/L) G	Gluc G (g/L)	Lac G (g/L)	NH4+ G (mmol/L)	Na+ G (mmol/L)	K+ G (mmol/L)	OSM (mOsm/kg) Nova G	PCO2 G (mm Hg)	CO sat (%) G	HCO3 (mmol/L) G
3.20	1.48	1.99	1.77	7.72	144	7.6	387	18		
2.40	1.67	1.32	2.04	8.60	145	7.7	389	30	4.5	7.2
1.61	1.62	1.19	2.05	8.58	144	7.8	388	32	4.9	7.2
2.27	1.63	1.12	2.02	8.89	145	7.9	388	51	7.8	7.1
2.55	1.71	1.14	2.23	9.10	146.30	8	394.6	55	8.4	6.2
2.50	1.56	1.00	2.10	8.91	145.0	7.9	390	51	7.7	5.6

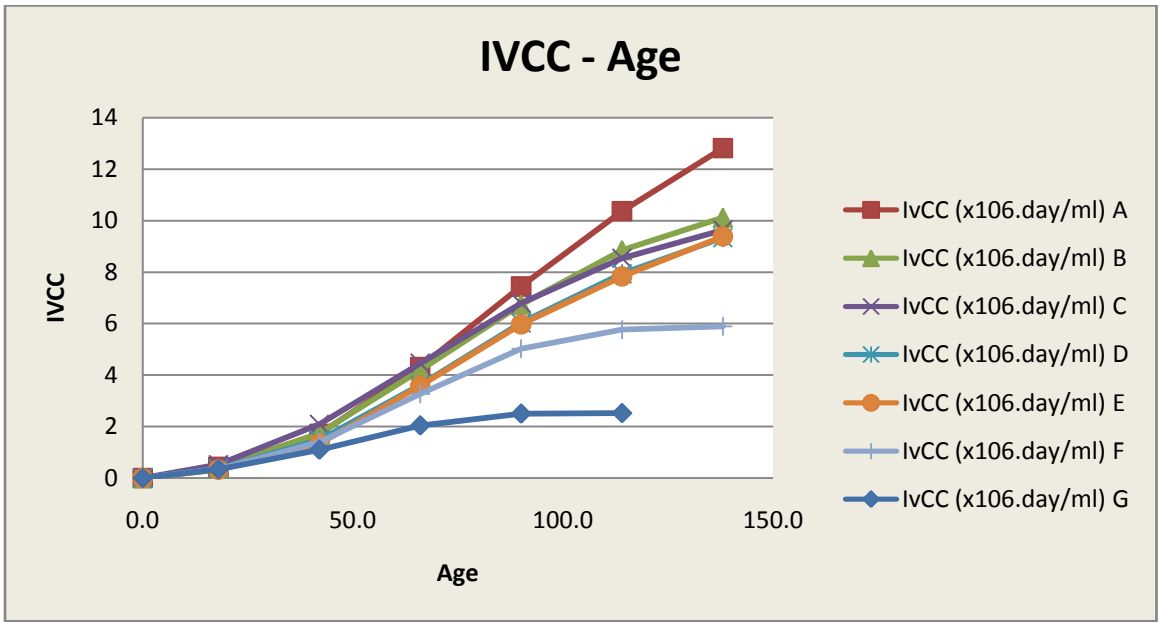
Comparison of Viable cell count profile of the spinners



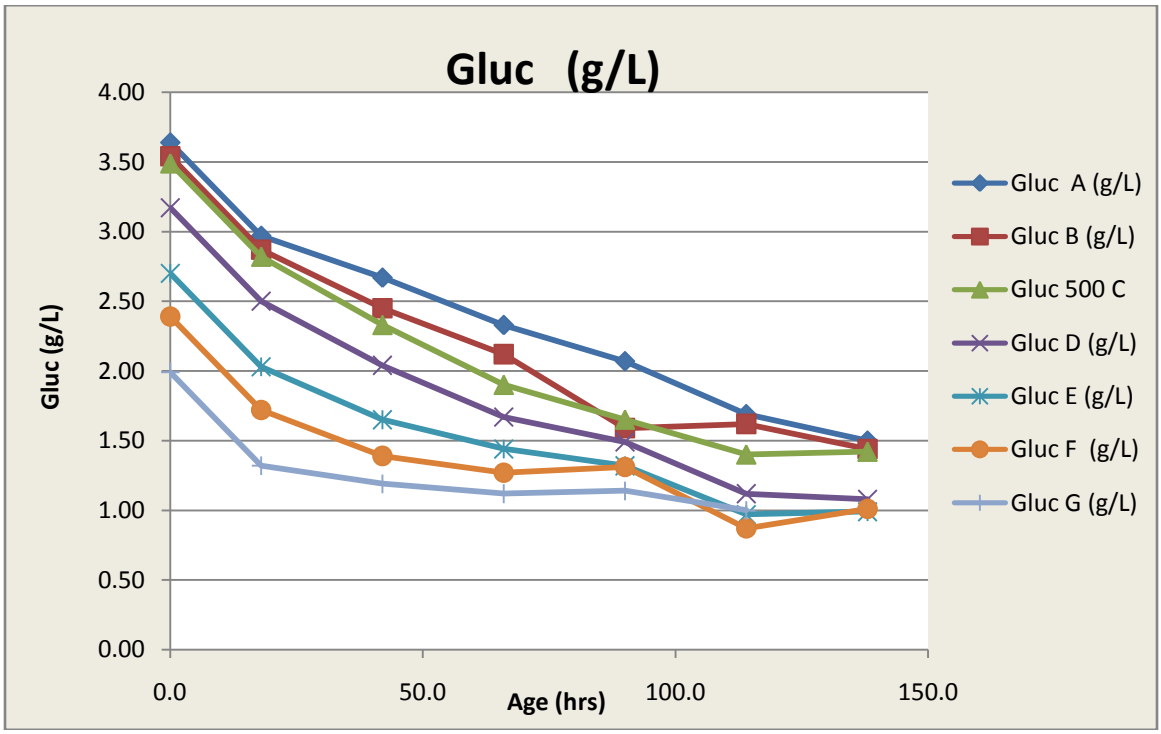
Comparison of Viability profile of the spinners



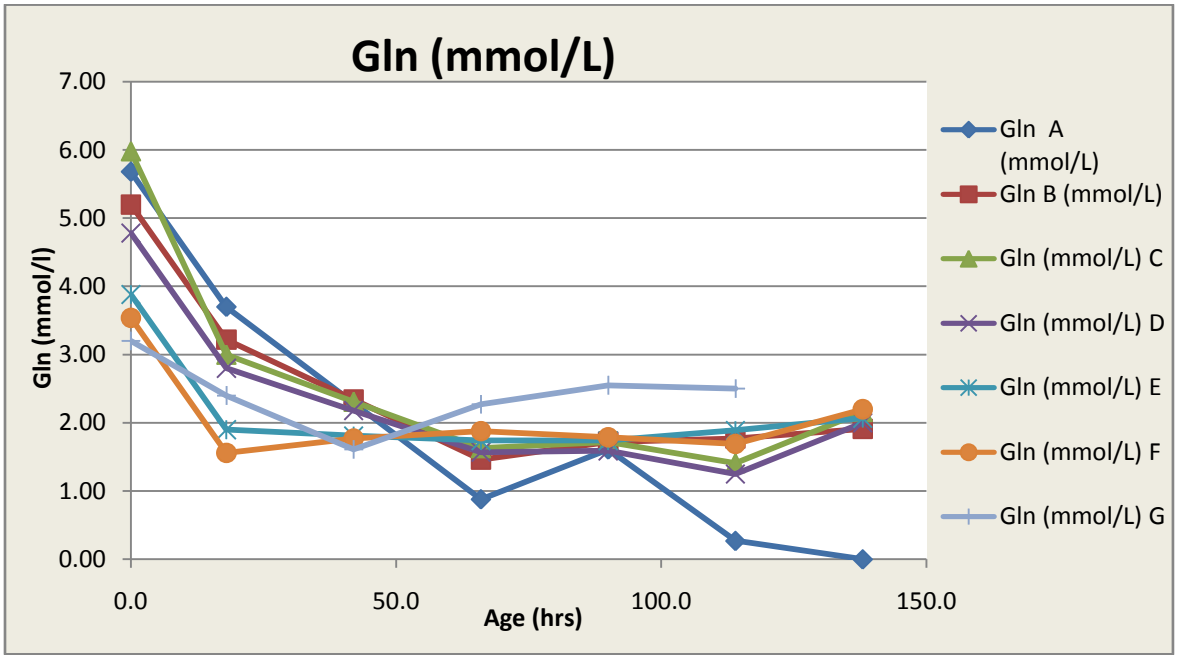
Comparison of integral viable cell concentration profile of the spinners



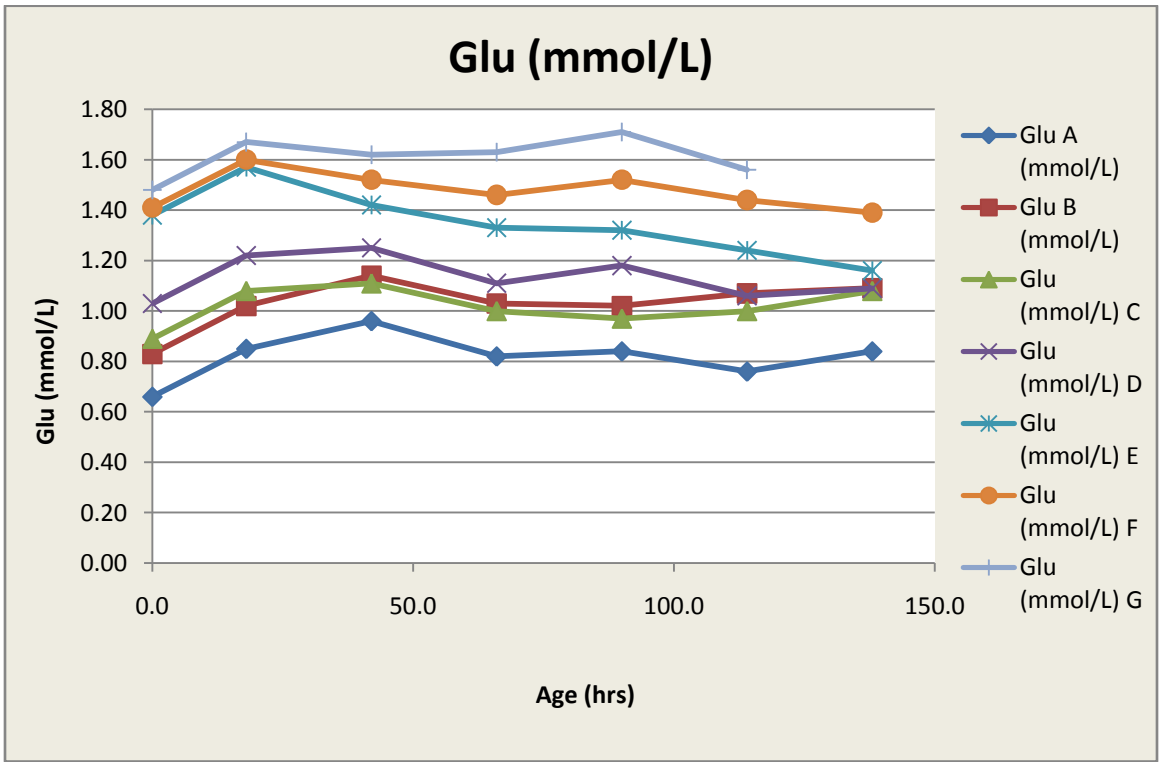
Comparison of Glucose concentration profile of the spinners



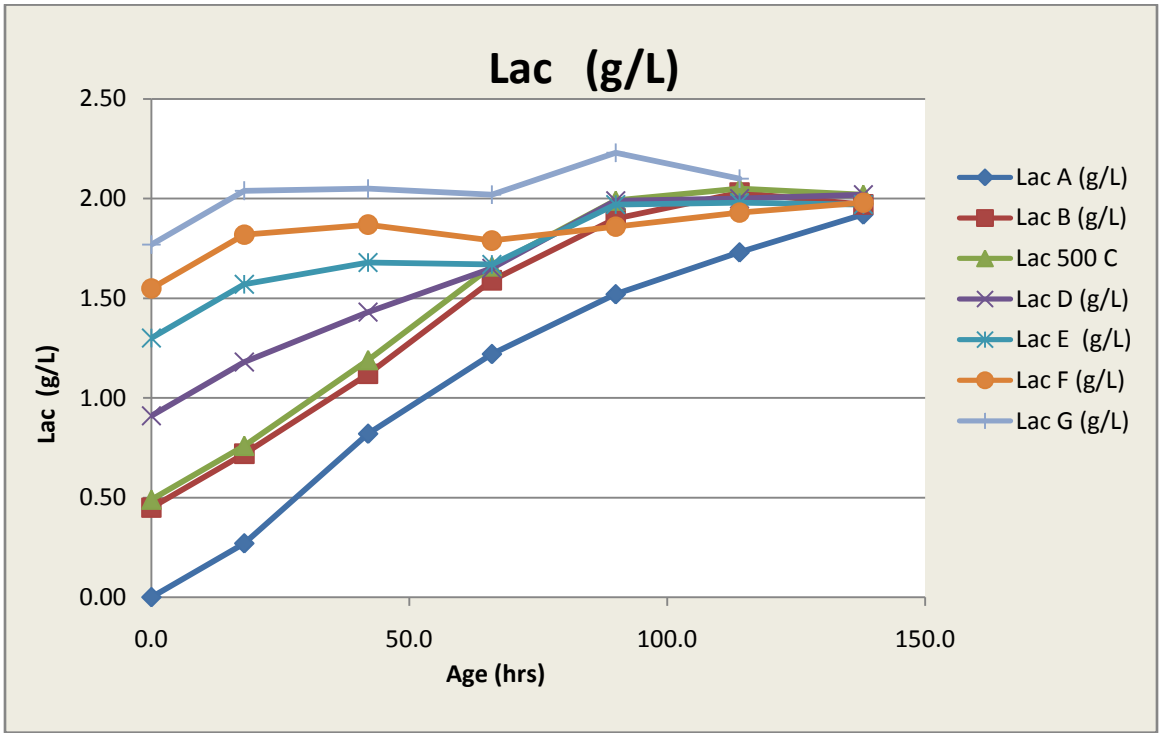
Comparison of Glutamine concentration profile of the spinners



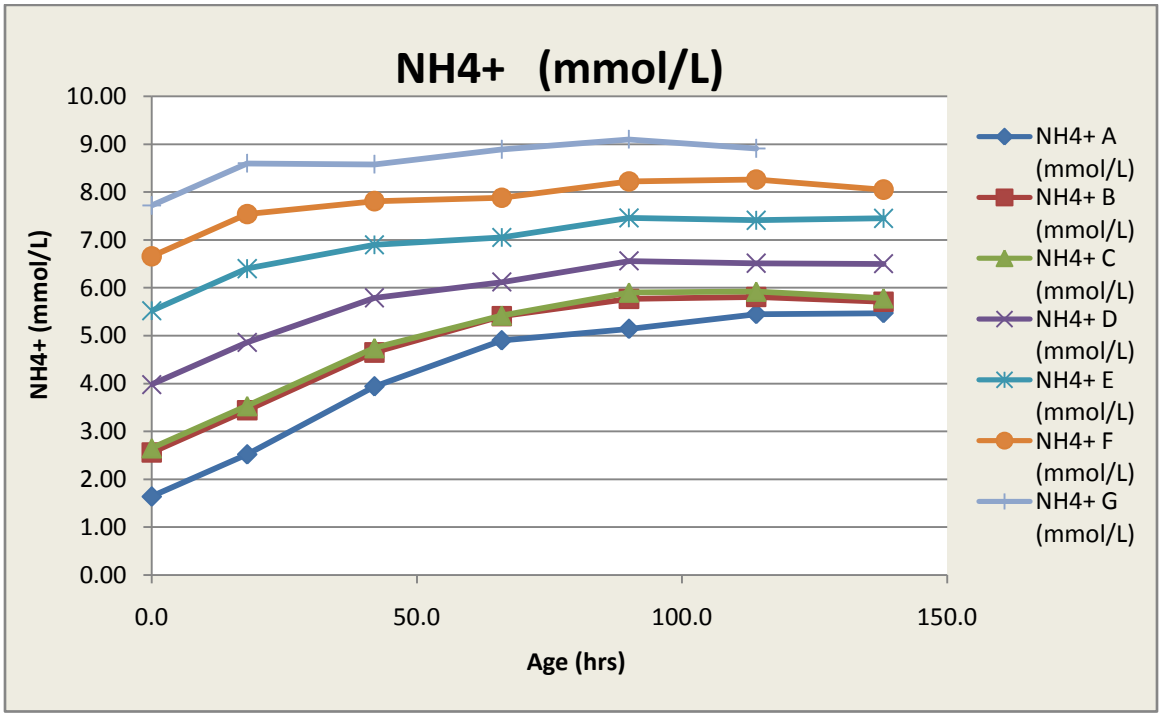
Comparison of Glutamate concentration profile of the spinners



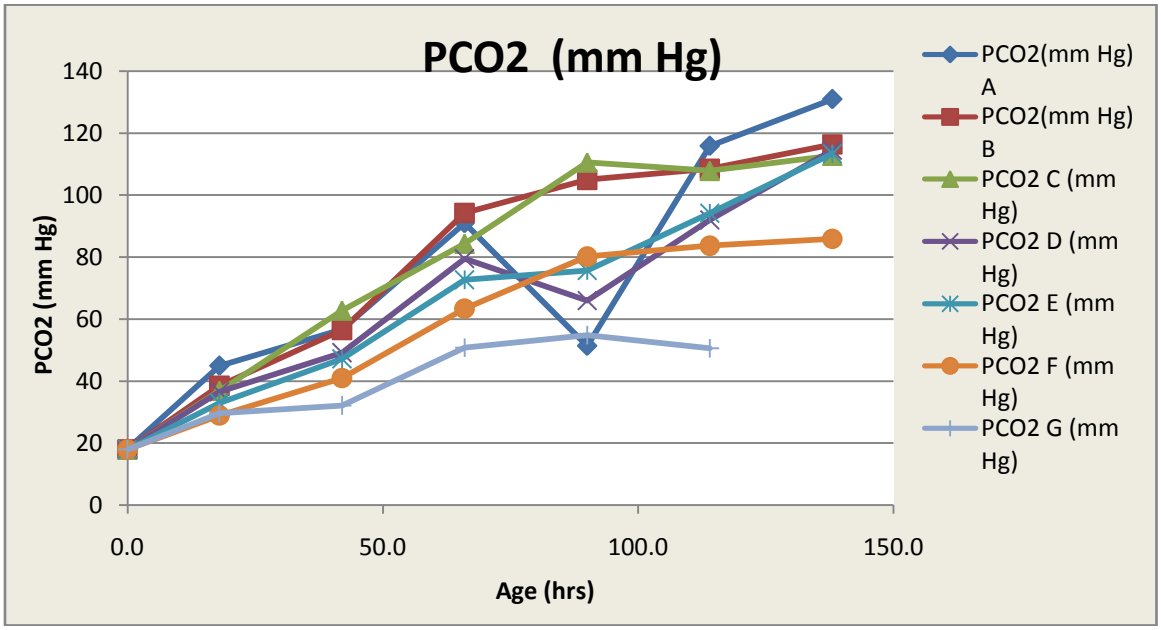
Comparison of Lactate concentration profile of the spinners



Comparison of Ammonia concentration profile of the spinners



Comparison of pCO2 profile of the spinners



Discussion

The Viable cell count profiles are as expected i.e. the maximum cell count was in the order of the quantity of fresh media present initially. Interestingly the count for the spinner F (with 20% fresh media initially) went up to 2.2 million cells/ml which was high as compared to the one with only spent media (spinner G) (1.02 million cells/ml) . The VCC for the 100% fresh media (spinner A) went up to 3.39 million cells/ml.

The Spinner G (100% spent media) was able to sustain the cells at a good viability only for 42 hrs. It suddenly crashed on the 66th hrs reading. The viability began to drop for all the spinners after 90 hrs but the rate of decrease of viability was in the order of initial quantity of fresh media i.e. spinner A has a low rate while spinner F has the highest.

The glutamine profile for the spinner F initially decreased till the 42 hrs period which was the growing phase of the culture for the same; whereas for the other spinners the glutamine degradation was until the 66th hrs period. The ammonia profiles of the various spinners were relatively parallel to each other.

It is observed that when lactate concentration reaches a value of 2 (g/l) ; the viability begins to fall. The pCO2 profiles were in the order of spinner A being highest and spinner G being lowest, since the CO2 is released in the medium during the period in which the cells are alive.

Experiment no: 4

The study of growth characteristics and metabolite profiles when the initial media had different initial glutamine concentration.

Spinner no	Initial concentration of glutamine
A	4 mmol/l gln
B	8 mmol/l gln
C	16 mmol/l gln

The experiment was conducted in Wheaton spinners of 500ml volume.

Data Sheet of spinner A:

Sample no.	Date & Time (dd/mm/yy)	Age (Hrs.)	Viable Cell Count A (x106/ml)	Dead Cell Count A (x106/ml)	Total Cell Count (x106/ml) A	Viability (%) A	Delta IvCC (x106.day/ml)	IvCC (x106.day/ml) A
1	3/6/08 11:00am	0.0	0.17	0.02	0.18	91.78	0	0
2	4/6/08 11:00am	24.0	0.33	0.02	0.35	93.53	0.25	0.25
3	5/6/08 11:00am	48.0	1.12	0.06	1.17	95.30	0.72	0.97
4	6/6/08 11:00am	72.0	2.30	0.04	2.34	98.29	1.71	2.67
5	7/6/08 11:00am	96.0	3.05	0.10	3.15	96.98	2.68	5.35
6	8/6/08 11:00am	120.0	3.29	0.17	3.46	95.08	3.17	8.52
7	9/6/08 11:00am	144.0	2.60	0.34	2.94	88.42	2.94	11.46
8	10/6/08 11:00am	168.0	1.82	0.71	2.53	72.08	2.21	13.66
9	11/6/08 11:00am	192.0	0.77	0.92	1.68	45.54	1.29	14.96
10	12/6/08 11:00am	216.0	0.59	2.14	2.72	21.51	0.68	15.63

pH A	OSM (mOsm/kg) Gonotech	Gln A (mmol/L)	Glu A (mmol/L)	Gluc A (g/L)	Lac A (g/L)	NH4+ A (mmol/L)	Na+ A(mmol/L)	K+ A(mmol/L)	PCO2(mm Hg) A	CO sat (%) A	HCO3 A(mmol/L)
7.3		3.81	2.07	3.46	0.00	1.46	144	7.6	24	2.1	19.6
7.42	332	3.82	0.99	3.22	0.00	1.96	145	7.8	35	2.1	19.6
7.08	319	2.63	1.13	3.11	0.33	3.17	145	7.8	46	7.0	15.7
6.91	331	0.29	1.23	2.61	1.23	3.71	144	7.5	54	3.6	13.4
6.9	319	err	1.17	2.10	1.40	4.06	145.9	7.7	67	6.4	9.4
6.64	319	err	1.03	1.93	1.79	4.25	146.1	7.66	68	4.9	7.4
6.6		err	1.02	1.57	1.89	4.37	147	7.7	100	6.6	6.3
6.52		err	0.82	1.37	1.78	4.24	145	7.6	56	4.9	7.2
6.64		err	1.01	1.24	1.77	4.13	145	7.8	48	7.3	6.6
6.60		0.21	1.09	1.25	1.97	5.90	145	7.8	46	6.9	6.2

Data Sheet of spinner B:

Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count B (x106/ml)	Dead Cell Count B (x106/ml)	Total Cell Count B (x106/ml)	Viability B (%)	Delta IvCC (x106.day/ml) B	IvCC (x106.day/ml) B
1	10/6/08 11:00am	0.0	0.18	0.01	0.19	94.74	0	0
2	11/6/08 11:00am	24.0	0.22	0.00	0.22	98.85	0.20	0.20
3	12/6/08 11:00am	48.0	0.51	0.01	0.52	98.06	0.36	0.56
4	13/6/08 11:00am	72.0	0.96	0.04	1.00	95.98	0.73	1.29
5	14/6/08 11:00am	96.0	1.93	0.04	1.97	97.97	1.44	2.73
6	15/6/08 11:00am	120.0	2.28	0.05	2.33	98.06	2.11	4.84
7	16/6/08 11:00am	144.0	1.53	0.04	1.56	97.76	1.90	6.74
8	17/6/08 11:00am	168.0	0.81	0.59	1.40	57.86	1.17	7.91

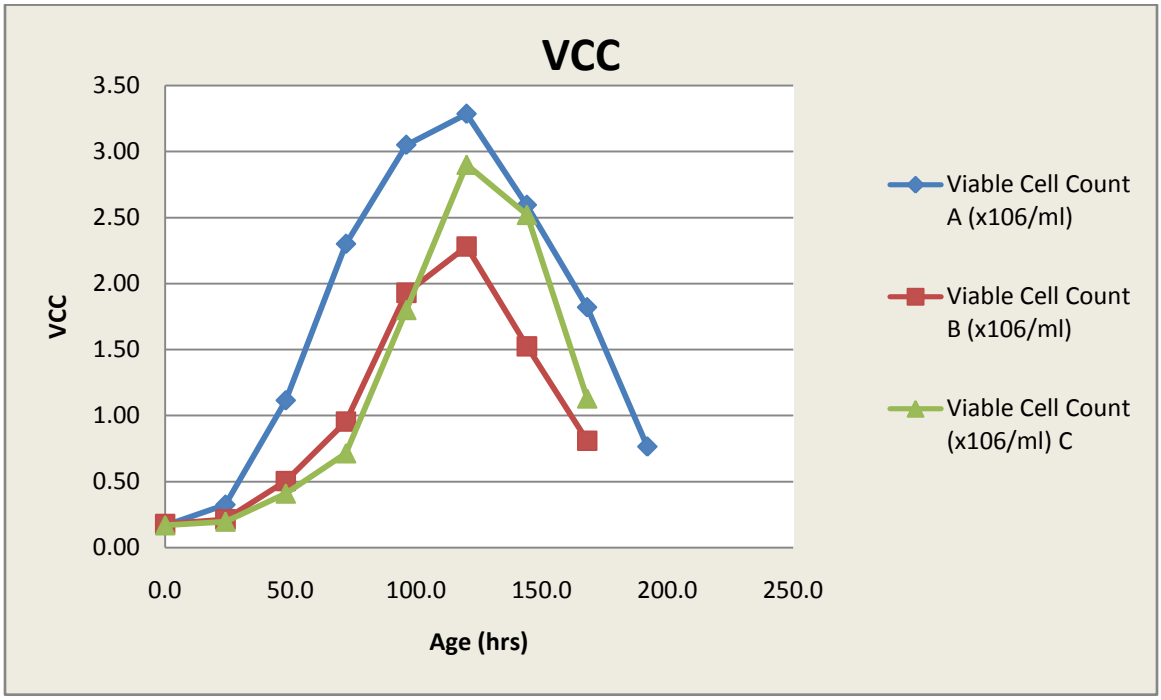
pH B	Gln B (mmol/L)	Glu B (mmol/L)	Gluc B (g/L)	Lac B (g/L)	NH4+ B (mmol/L)	Na+ B (mmol/L)	K+ B (mmol/L)	OSM B (mOsm/kg) Nova	PCO2(mm Hg) B	CO sat (%) B	HCO3 (mmol/L) B
7.35	9.90	0.58	3.98	0.00	1.33	146	7.7		27	4.4	21.5
7.44	6.29	0.80	3.48	0.00	2.42	147	8.0		30	4.5	20.4
7.22	5.59	1.16	3.43	0.32	3.60	146	7.9	376	31	4.8	18.5
7.03	4.36	1.17	3.19	0.63	4.90	148	7.7	383	58	8.9	17.6
6.8	3.61	1.50	2.81	1.26	6.49	146.6	7.7	389	39	5.9	12.8
6.69	1.90	1.33	1.86	1.60	7.42	146.4	7.70	389	35		
6.73	3.62	1.44	1.77	2.00	7.14	148	7.8	395	73		
6.58	1.99	1.66	1.46	2.07	7.26	147	7.9	393	57		

Data Sheet of Spinner C:

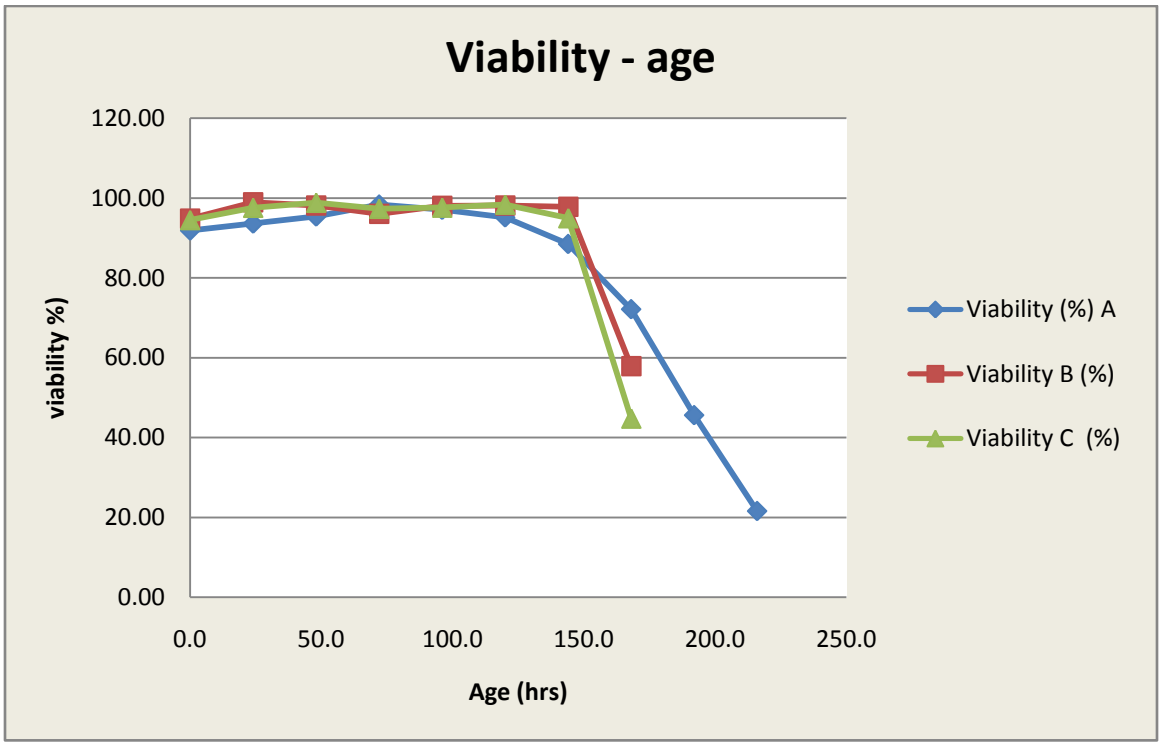
Sample no.	Date & Time (mm/dd/yy)	Age (Hrs.)	Viable Cell Count (x106/ml) C	Dead Cell Count C (x106/ml)	Total Cell Count C (x106/ml)	Viability C (%)	Delta IvCC (x106.day/ml) C	IvCC (x106.day/ml) C
1	10/6/08 11:00am	0.0	0.17	0.01	0.18	94.44	0	0
2	11/6/08 11:00am	24.0	0.20	0.01	0.20	97.53	0.18	0.18
3	12/6/08 11:00am	48.0	0.41	0.01	0.42	98.80	0.30	0.49
4	13/6/08 11:00am	72.0	0.72	0.02	0.74	97.28	0.56	1.05
5	14/6/08 11:00am	96.0	1.80	0.05	1.85	97.56	1.26	2.31
6	15/6/08 11:00am	120.0	2.90	0.05	2.95	98.31	2.35	4.66
7	16/6/08 11:00am	144.0	2.52	0.14	2.66	94.92	2.71	7.37
8	17/6/08 11:00am	168.0	1.13	1.40	2.53	44.75	1.83	9.19

pH C	Gln (mmol/L) C	Glu (mmol/L) C	Gluc 500 C	Lac 500 C	NH4+ C (mmol/L)	Na+ C (mmol/L)	K+ C (mmol/L)	OSM C (mOsm /kg) Nova	PCO2 C (mm Hg)	CO sat (%) C	HCO3 C (mmol/L)
7.38	21.00	0.63	3.96	0.00	1.30	146	7.8		28	4.3	21.3
7.32	13.68	0.75	3.50	0.00	2.87	146	8.0		37	5.6	19.5
7.24	13.50	1.04	3.45	0.20	4.55	145	7.9	374	31	4.8	19.3
7.1	10.20	1.13	3.28	0.50	6.19	147	7.7	383	52	7.9	18.9
7.03	8.62	1.49	3.12	0.92	8.58	145.5	7.7	388	39	6.0	16.8
6.77	6.24	1.37	2.01	1.67	10.45	144.9	7.63	391			
6.92	5.17	1.57	1.34	2.23	10.36	144	7.8	394	73		
6.74	4.09	1.83	0.99	2.44	10.81	144	7.9	396	40		

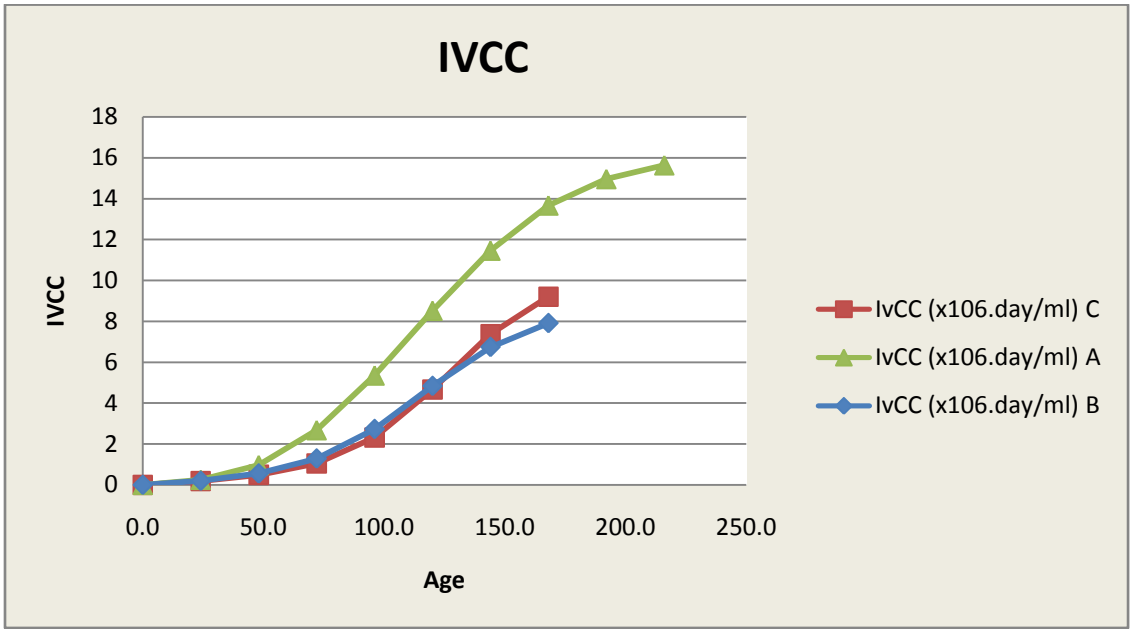
Comparison of viable cell counts of the spinners



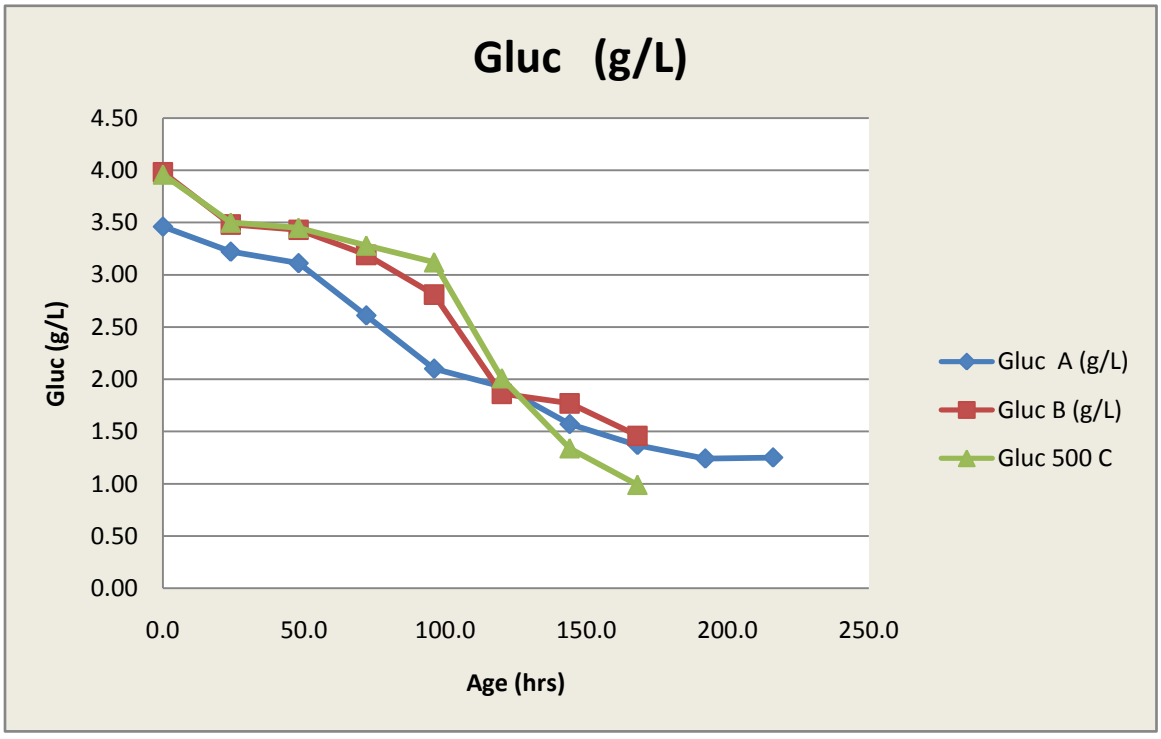
Comparison of viability of the spinners



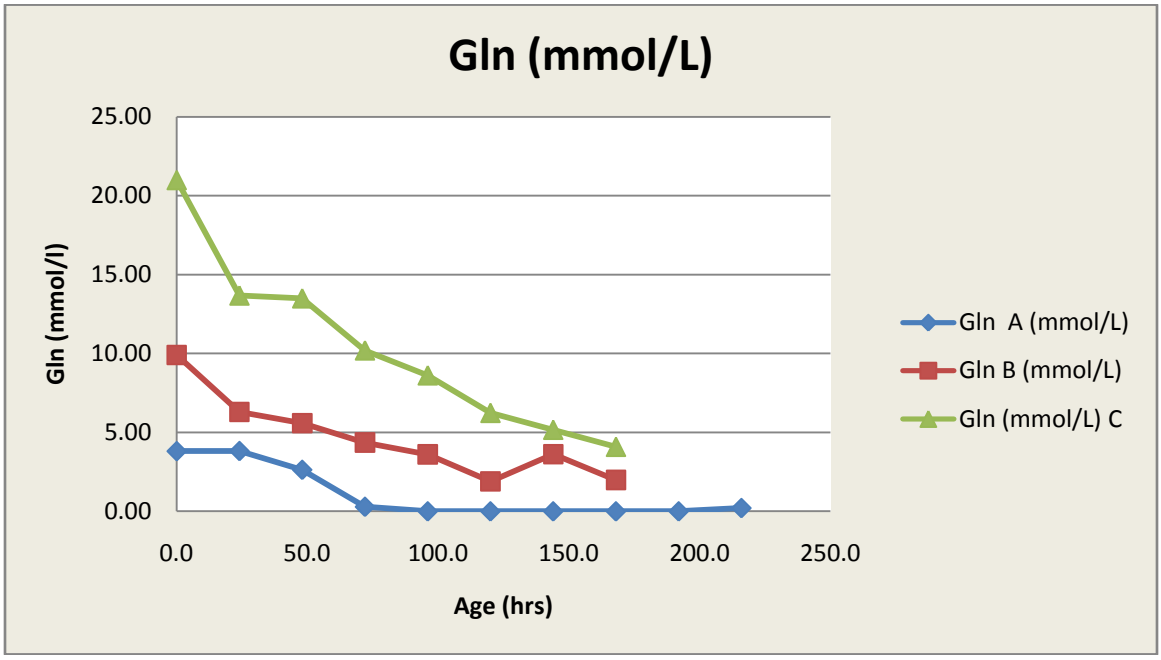
Comparison of integral viable cell count of the spinners



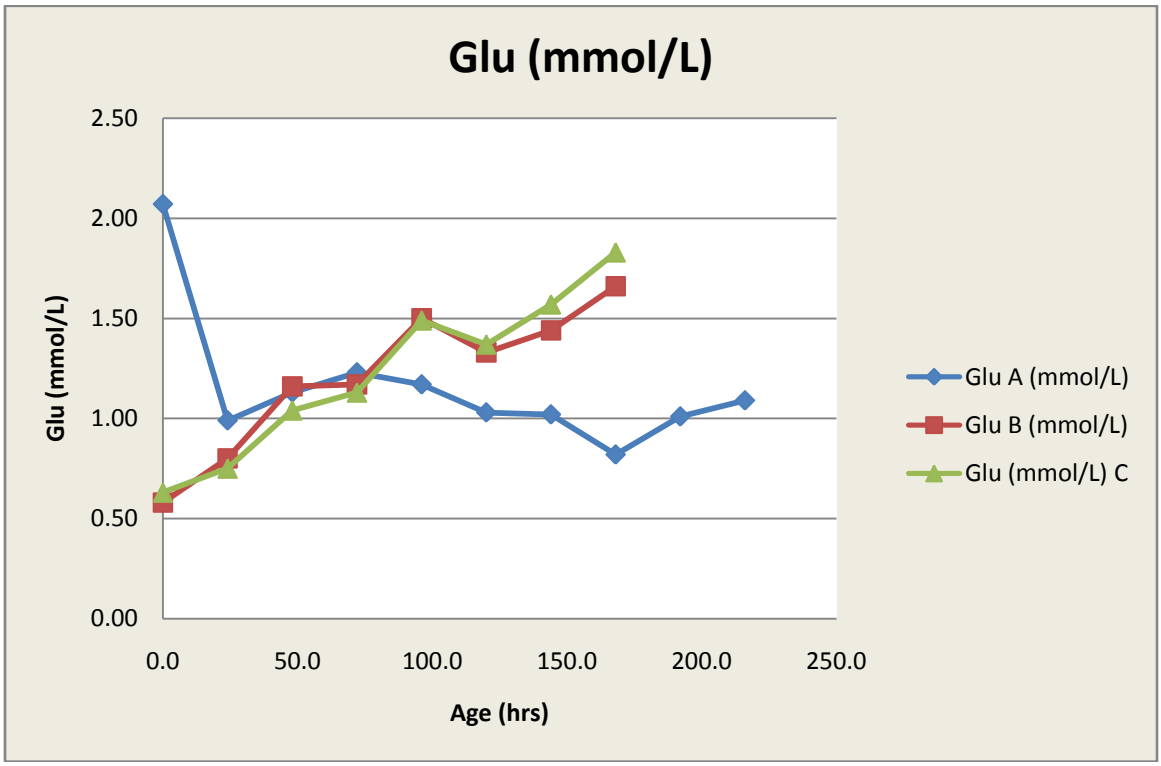
Comparison of glucose profile of the spinners



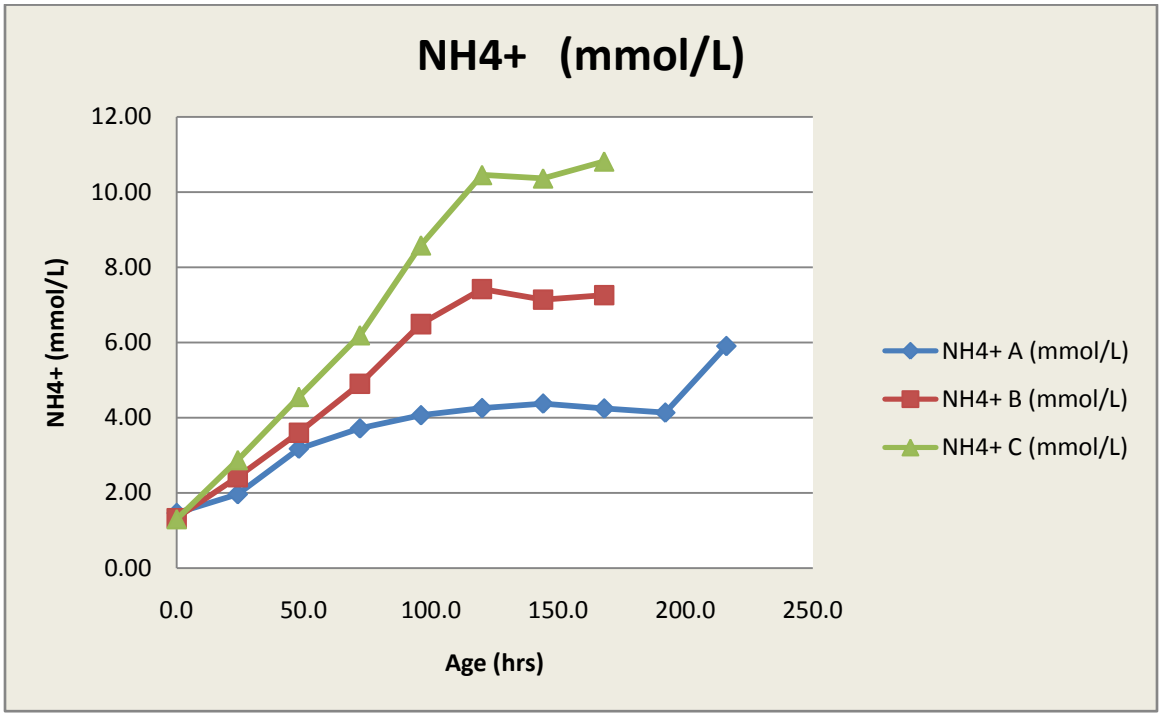
Comparison of glutamine profile of the spinners



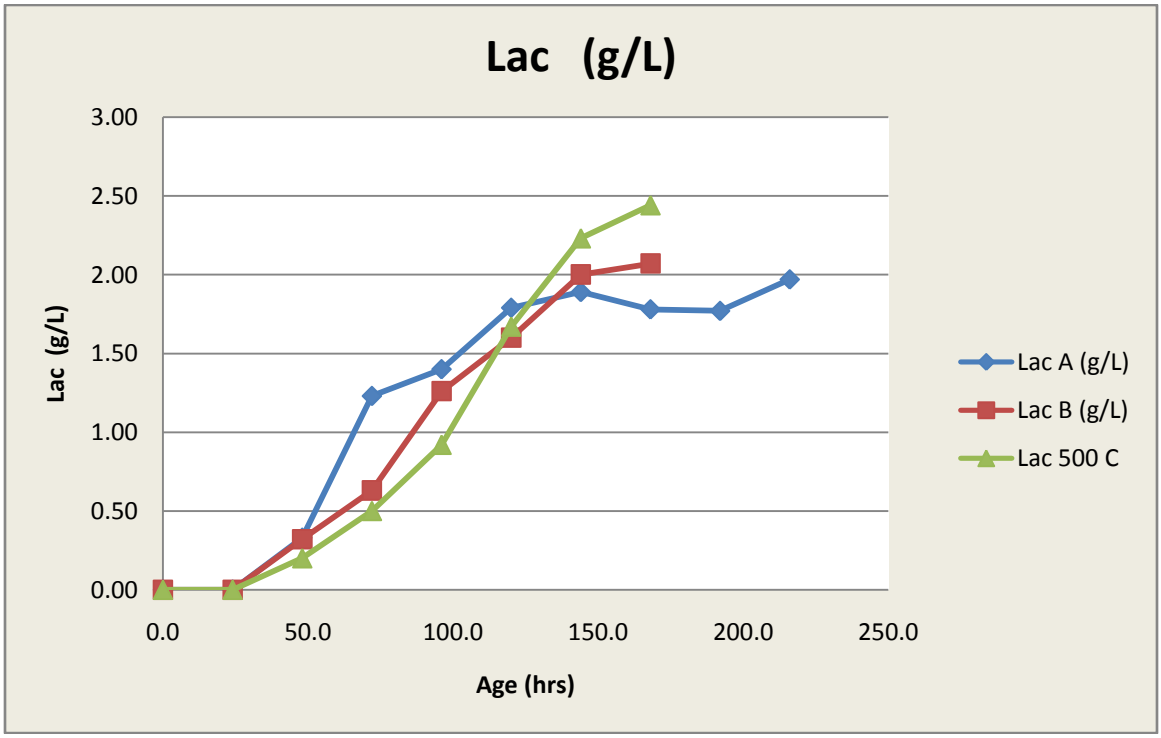
Comparison of glutamate profile of the spinners



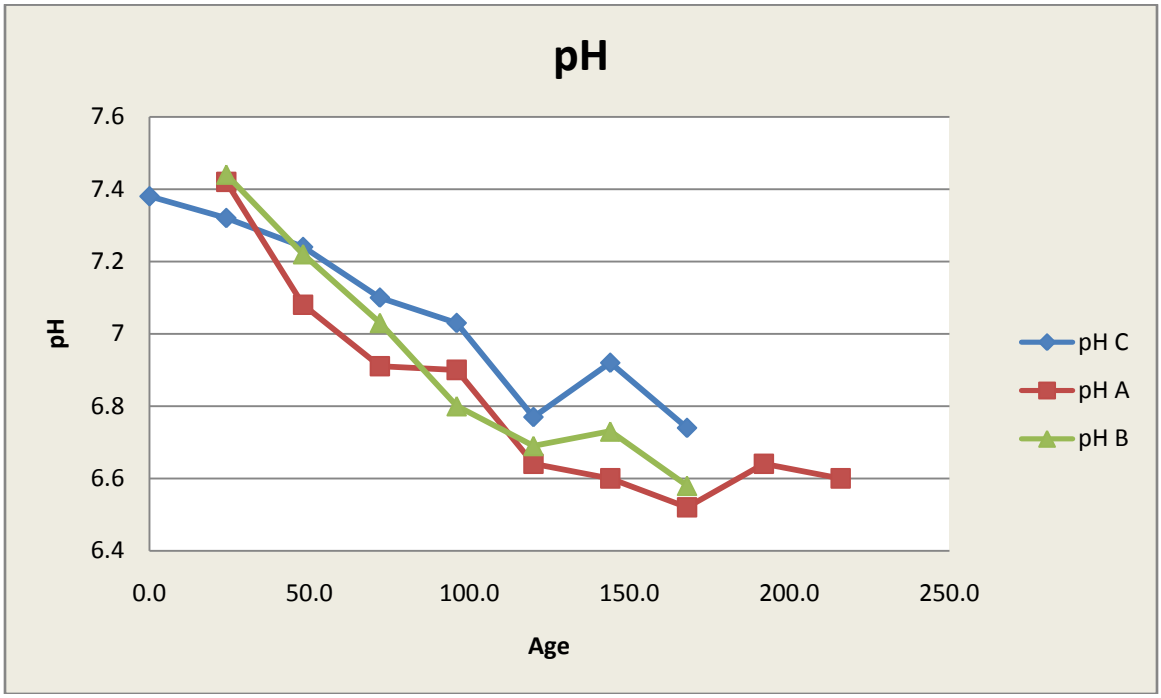
Comparison of ammonia profile of the spinners



Comparison of lactate profile of the spinners



Comparison of pH profile of the spinners



Discussion

The cell count in the spinner A goes up to a maximum of around 3.2 million/ml. The cell count in the spinner B goes up to a maximum of around 2.2 million/ml. The cell count in the spinner C goes up to a maximum of around 2.9 million/ml. These trends can be attributed to the combine effect of inhibition by ammonia at higher initial concentrations of glutamine and growth maintenance due to presence of glutamine.

The viability of the spinner A begins to fall at 96 hrs and gradually falls; whereas in the case of the spinners B and C, the viability falls quite rapidly at 144 hrs.

The glutamine profile is fairly as expected i.e. it decreases gradually for all the spinners but reaches different final glutamine concentrations in order of the initial glutamine concentrations. The glucose consumption increased for the spinner A when the glutamine content reached low concentrations. The lactate accumulation is of the order spinner C > spinner B> spinner A. This can be attributed to the glucose consumption order i.e. spinner C> spinner B > spinner A.

The consumption of glucose is maximum in case of spinner C; but still spinner A has a relatively larger stationary phase; this can be attributed to the inhibition by ammonia in spinner C. Hence, the VCC trend.

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