Cell culture process improvement via supplementation of vitamins and other supplements.

A Dissertation

A Thesis submitted in the fulfillment of the requirement for the award of the degree of

Master of Technologyin

Biotechnology

By

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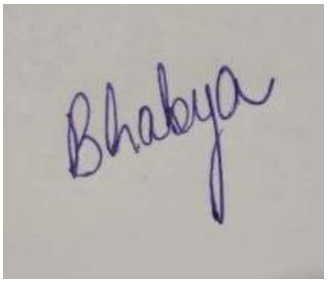
DECLARATION

I hereby declare that the work being presented in the dissertation report entitled Cell culture process improvement via supplementation of vitamins and other supplements submitted by me for the award of the degree of Master of Technology in Department of Biotechnology, TIET University, Patiala is true and original record of my own independent and original research work carried out under the joint supervision of Mr. Ankit Sharma. Further, I declare that no part of this dissertation has been submitted to any other University/Institute for the award of any degree in India or abroad.



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CERTIFICATE

This is to certify that the dissertation titled "CELL CULTURE PROCESS IMPROVEMENT VIA SUPPLEMENTATION OF VITAMINS AND OTHER SUPPLEMENTS", by Bhabya Bhardhwaj, in partial fulfilment for the award of the degree of Master of Technology in Biotechnology Engineering, is a record of candidate's own work carried out by her under the supervision of Mr. Ankit Sharma, Research Scientist, Lupin Research Park. The matter embodied in this dissertation has not been submitted for the award of any other degree or diploma to the best of my knowledge and belief.

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Ankit Sharma
05/06/2024
Signature of Industrial mentor

Mr. Ankit Sharma
Research Scientist
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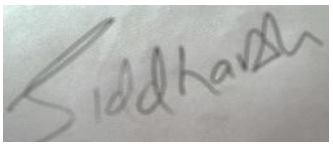
CERTIFICATE

This is to certify that the dissertation titled **"Cell Culture Process Improvement via Supplementation of Vitamins and Other Supplements"**, by Bhabya Bhardhwaj, in partial fulfilment for the award of the degree of Master of Technology in Biotechnology Engineering, is a record of candidate's own work carried out by her under the supervision of Dr. Siddharth Sharma, Professor, Thapar Institute Of Engineering and Technology, Ptiala and Mr. Ankit Sharma, Research Scientist, Lupin Research Park**.** The matter embodied in this dissertation has not been submitted for the award of any other degree or diploma to the best of myknowledge and belief.



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ACKNOWLEDGEMENT

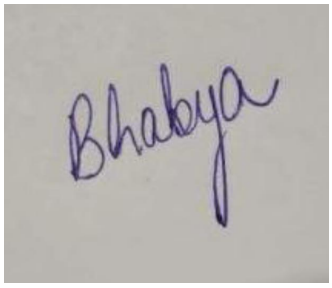
First and foremost, I wish to thank **Dr. Sanjay Tiwari** (Vice President, Lupin Biotech), and my mentor **Mr. Ankit Sharma** (Research Scientist), **Mr. Shivam Saxena** (Research Associate), **Mr**. **Vinmukilan Manokaran** (Research Associate) and **Mr. Vishal Nannavare** (Research Associate) for giving me great opportunity to work in Lupin. It was my great pleasure and privilege to work under their guidance, who has been motivating and encouraging throughout the project and put in every possible effort to make the project a success.

I would like to thank Upstream Mammalian team members for their valuable support and guidance. I am thankful for the suggestions, and meticulous guidance of my divisional mentors who helped me to understand things in a better way and being so supportive and motivating every time.

My special thanks to Lupin Limited (Research Park), Pune and Thapar Institute of Engineering and Technology, Patiala, Punjab for giving me an opportunity to undertake this internship.

I would like to extend my sincere gratitude towards **Dr. M.S. Reddy**, HOD, Biotechnology department, and **Dr. Siddharth Sharma**, Professor for being cooperative to the students of the department and providing relevant guidance in their endeavors.

I thank my family and friends for encouraging me throughout my internship and being with me throughout as a support system.



Bhabya Bhardhwaj

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ABBREVIATIONS

Mabs	Monoclonal Antibodies
CHO Cells	Chinese Hamster Ovary
pH	Potential Of Hydrogen
DO	Dissolved Oxygen
IgG	Immunoglobulin G
CO_2	Carbon Dioxide
VCC	Viable Cell Count
mOsm	milliosmoles

ABSTRACT

Monoclonal antibodies have become a key class of therapeutic agents on the market and are one of the pharmaceutical industry's fastest-growing items right now. About eighty Mabs have received permission for marketing. An ever-growing need exists for the process of developing monoclonal antibodies to be optimized in a way that maximizes cost effectiveness. To do this, a thorough comprehension of the metabolic processes found in mammalian cells is required. This will facilitate the effective optimization of the chemical and physical parameters. The optimization of vitamin and other supplementation for an efficient process for producing monoclonal antibodies is described in this thesis.

In this work, mammalian cell lines were used to test twelve different supplements and ten different vitamins in fed batch method. The trials were conducted in shaking flasks with identical basal media maintained at 34°C. Every day, biochemical analysis, osmo, cell count, and sampling were carried out. The shake flasks were collected and samples were tested for antibody titers using a Cedex biochemical analyzer once the cell viability dropped to 50%.

Cell growth, cell viability, lactate generation, and antibody buildup in the broth were used to analyze the results. Only one additional supplement, out of ten distinct vitamins and twelve different supplements, significantly contributed to the improvements in Titer, VCC, Cell Viability, and reduced lactate buildup, while the vitamins had no discernible impact. Therefore, in the most recent round of the experiment, this additional supplement was used in certain flasks along with others that had been concentrated more. The process was optimized in this way.

Contents

1. INTRODUCTION

1.1 GENERAL

The primary source of several extremely valuable biopharmaceutical products, such as Mabs, viral vaccines, and hormones, is mammalian cell cultures [1]. Recombinant protein therapies are most commonly produced using mammalian cells, such as CHO. Using these expression systems to produce consistent results requires a controlled culture based on parameter ranges specified by the process design space [2]. For an extended period, the majority of antibody treatments necessitate high dosages. Thus, a significant amount of purified product must be generated in order to meet the growing demand for antibodies for a variety of medical treatments. To this end, the pharmaceutical companies have created extremely efficient and reliable methods.

1.2 MONOCLONAL ANTIBODY

B-lymphocytes create antibodies, which are immune system proteins. By identifying and attaching to their targets, or antigens, these proteins carry out their actions. A lone B-cell is the source of a monoclonal antibody (mAb). The first mAb production was started in 1975[3]. Since they are monovalent, or only bind to one particular epitope on the antigen, monoclonal antibodies are all clones of the same parent cells. Commercial monoclonal antibody medications include Actemra, Humira, Rituxan, Synagis, and many others. Two of the four polypeptide chains that make up a monoclonal antibody are heavy chains, and the other two are light chains. Disulfide bonds hold the two heavy chains together such that they form a “Y.”

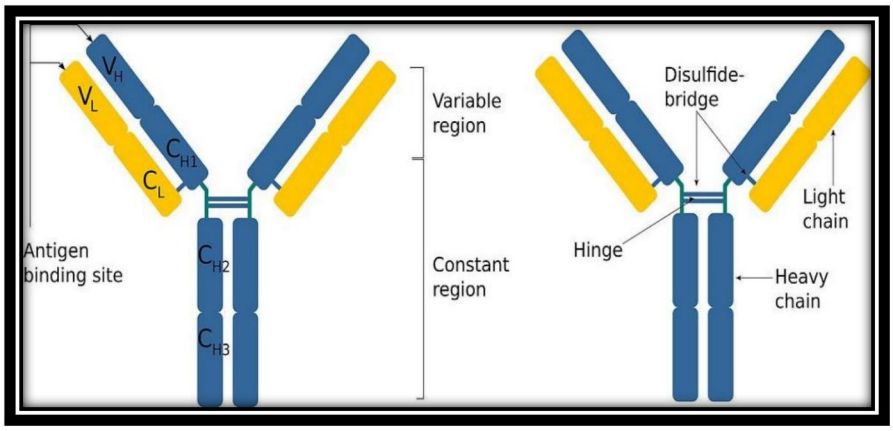


Figure 1.1. Structure of monoclonal antibody.

1.3 CELL CULTURE PROCESS FOR PRODUCTION OF ANTIBODY

The first step in the cell culture procedure for producing antibodies is to thaw the vial and expand the cells using a series of inoculum processes. Before being moved to the production bioreactor, where the Mab is expressed into the media, the cells are further grown in a series of seed bioreactors. Cells and cell detritus are subsequently separated from the cell culture broth using centrifugation and a sequence of filtration procedures.

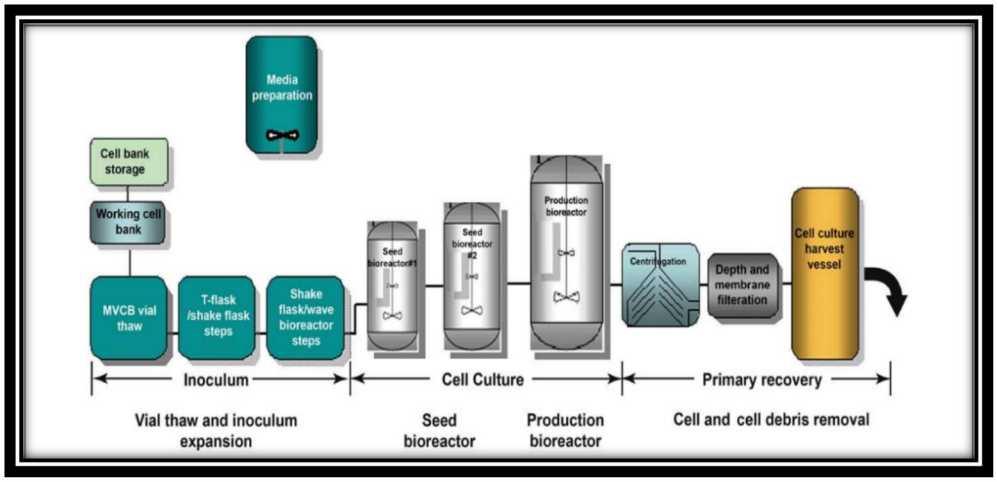


Figure 1.2. mAb upstream process.

1.4 CHO CELLS FOR MONOCLONAL ANTIBODY PRODUCTION

In 1948, CHO cells were employed for breeding in US research labs for the first time. Because CHO cells can carry out the post translational alterations required for these kinds of products, they are frequently employed in biotechnology to produce therapeutic proteins such monoclonal antibodies.

Mammalian cells called CHO cells are generated from the ovary of Chinese hamsters, and they are chosen above other cell lines due to their compatibility with human proteins.

Furthermore, CHO cells have proven to be a dependable and safe host, and they can easily adjust to serum-free medium. They also have a very healthy growth.

1.5 MEDIA AND FEED OPTIMIZATION

CHO cell-based mAb synthesis currently uses chemically specified medium. Additionally, for cell proliferation and the generation of mAbs, an adequate supply of feed containing minerals, particular amino acids, and vitamins is needed[4]. Optimizing media and feed is essential for developing bioprocesses with high cell density and productivity. The goal of this procedure is to minimize production costs while promoting cell growth and efficient protein production by precisely adjusting the feed addition timing and composition of the cell culture media.

1.6 GROWTH CURVE

A graph that illustrates the expansion of the cell population over time is commonly referred to as a growth curve in mammalian cell culture. There are typically four primary phases to this curve: the lag phase, the exponential phase, the stationary period, and the death phase.

LAG PHASE: Little to no cell development occurs during this phase as cells adjust to their new surroundings.

PHASE EXPONENTIAL: During this stage, cells start dividing quickly and the population grows exponentially.

STATIONARY PHASE: As waste builds up and nutrients are exhausted, cell development gradually slows down and ends.

PHASE OF DEATH: During this stage, waste products accumulate and nutrients are insufficient, causing cells to begin to die.

To optimize the environment for cell growth, it is essential to keep an eye on the cell growth curve in mammalian cell culture.

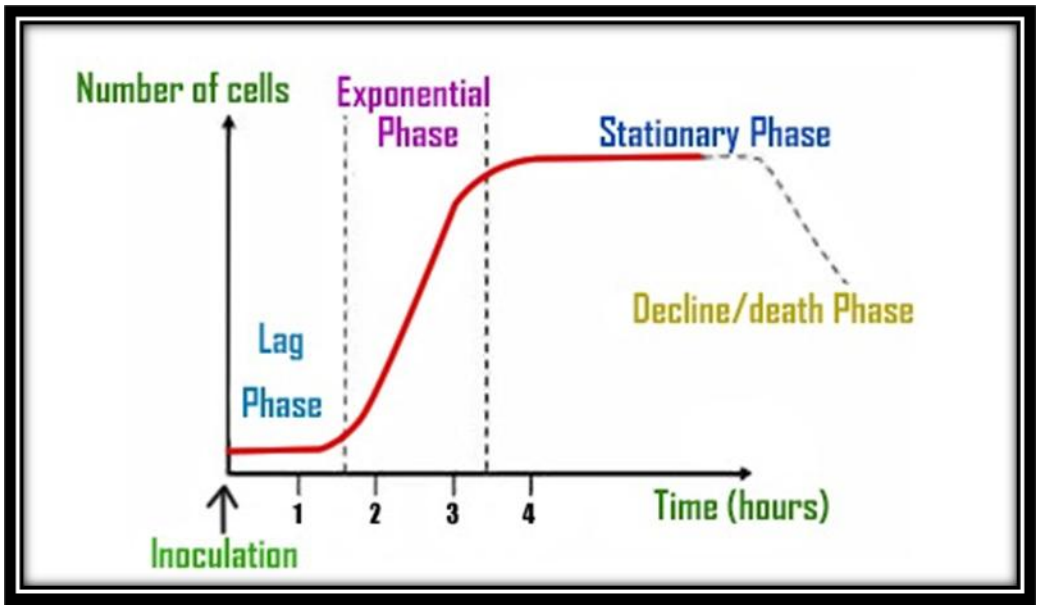


Figure 1.3. Growth Curve.

1.7 AIMS AND OBJECTIVES

To collect seed for this experiment, the cell bank must first be thawed. Next, this seed was cultivated in a glutamine-containing basal medium. This stage aids in the cells' acclimation to the basal medium. The batches are then started using this seed. Feed is a crucial component in giving cells nourishment and raising output. We must optimize both the chemical and physical factors in order to have an effective process.
Whereas feed, feeding technique, and growth media dictate the chemical environment for cells, temperature, pH, agitation, and dissolved oxygen all play significant roles in maintaining an appropriate physical environment to produce protein and grow with efficiency.

To improve the titer and caliber of the protein generated, additional vitamins and supplements are also added. Thus, media screening and feed screening are crucial to the process development of producing monoclonal antibodies.

The objectives of the experiment are as follows:

1. Identification of role of each and every component in cell line.
2. Effect of vitamins and other supplements.
3. Optimal concentration for vitamins and other supplements in cell culture media.

2. REVIEW OF LITERATURE

2.1 MAMMALIAN CELL CULTURE TECHNOLOGY

One of the main areas of contemporary biotechnology is mammalian cell culture technology. Its high application in the pharmaceutical industry is demonstrated by the numerous monoclonal antibodies, vaccines, fusion proteins, and other products that have been created. A mammal's cells that have been isolated is referred to as mammalian cell culture. After being separated from a particular tissue, these cells are grown in an artificial medium that contains all the nutrients required by a mammalian body.

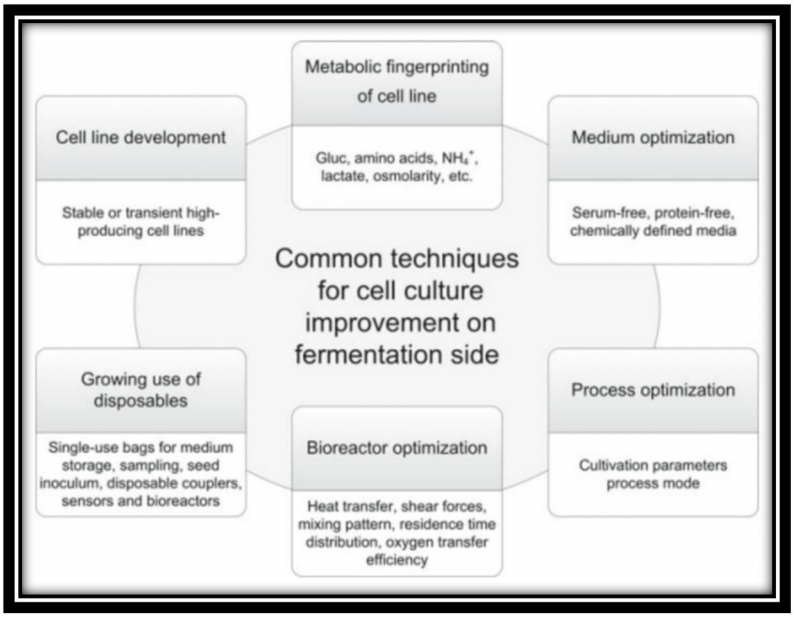


Figure 2.1. Overview of mammalian cell culture technology.

2.2 MONOCLONAL ANTIBODIES

Antibodies that have only one parent are known as monoclonal antibodies. They come from animals that have been inoculated with the drug under investigation, such as Chinese hamsters and murine models. Thus, the fusing of malignant cell lines with B lymphocytes from the inoculated animal results in the production of these cell lines. The cells must be cultivated in vitro tissue culture or by injection into the peritoneal cavity of a suitable organism, such as a mouse, in order to produce the necessary mAbs.

It is generally possible to cultivate antibodies in vitro for large-scale manufacturing. Hybridoma cell lines are generated using this technique as either suspension or immobilized cultures. For this, a variety of bioreactor types are employed on microcarriers. Stirred tank bioreactors, wave bioreactors, roller bottles, shake flasks, and other types of bioreactors are typical examples. Strict precautions are used during in vitro culture to prevent contamination. The entire process must be stopped if it becomes contaminated. To provide the best possible growth and productivity, physical and chemical parameters in this approach must be optimized.

2.3 PROCESS PARAMETERS

2.3.1. Ph:

Mammalian cells have a pH range of 6.7 to 7.4 [5]. Chinese hamster ovary (CHO) cells exhibited significant variation in the glycosylation patterns and specific expression rates of the recombinant protein mouse placental lactogen-I across the extracellular pH range of 6.1 to 8.7. Between pH 7.6 and pH 8, the largest specific mPL-I expression rates were observed [6].

2.3.2. Temperature:

Mammalian cells are typically cultured at a temperature of approximately 37°C in order to replicate the normal body environment. The temperature is kept constant in shake flasks and roller bottles by means of hot air in the CO2 incubator, and it is kept constant in bioreactors by means of a cooling jacket surrounding the vessel. A temperature of roughly 37°C is shown to be the greatest specific growth rate. Mammalian cells are said to grow best in a temperature range of 33°C to 38°C [7].

It is known that different cell lines reach their maximal titer at various temperatures.

2.3.3. Dissolved oxygen:

Both high and low DO hinder cell development. Cells can grow in a nitrogen environment at 0% DO and yet be viable. At low DO, cell viability is increased. At DO exceeding 1% air saturation, there are slight alterations in the rates of glucose, glutamine, and oxygen consumption. Below 1% DO, however, the metabolic uptake rates alter and growth becomes oxygen-limited. When the DO falls from 1% to 0%, the metabolic rates of glucose, glutamine, lactate, and ammonia rise by two to three times [8].

Animal cells need oxygen to function. The synthesis of MAbs, cell development, and metabolism all require oxygen. Thus, one of the essential nutrients for cells is oxygen. Further restricting oxygen transmission is diffusional resistance. Thus, to improve the process, it is crucial to comprehend how cells behave when there is minimal oxygen. It is also known that anaerobic conditions slow down cell development [9].

2.3.4. Osmolality:

Osmolality is a crucial process variable in the in vitro culture of mammalian cells. In order to replicate the osmolarity of serum, which is 290 mOsm/kg, cell culture media is designed to have an osmolality in the range of 260 and 320 mOsm per kg. At high osmolarities, there is a documented decrease in cell proliferation and an increase in antibody synthesis. It appears that osmolality has a cell line-dependent effect on antibody production. Both ionic and non-ionic chemicals can be added to the medium to change its osmolality [10].

Atypical elevation of osmolality can also lead to a rise in the particular cell death rate and a reduction in growth of hybridoma cell lines that generate IgG monoclonal antibodies by more than 50%. Moreover, CHO cells may become less viable as a result of elevated osmolality, primarily through apoptosis.

2.4. BASIC NUTRITIONAL REQUIREMENTS FOR CULTURE MEDIUM

2.4.1. Vitamins:

Vitamins serve as coenzymes in cell metabolism processes. Culture medium contain common vitamins such biotin, riboflavin, and vitamin B12 [11]. Choline is a necessary substrate for the synthesis of phospholipids rather than a cofactor. All phospholipids that include nitrogen come from phosphatidylserine [12]. Cell division and development require a variety of vitamins.

With many enzyme systems, vitamins function as both cofactors and coenzymes. In most cases, co-enzymes can be used in place of parent vitamins. Pyridoxal phosphate, the active form of pyridoxal, functions as the tightly bonded prosthetic group of several enzymes that catalyze amino acid reactions. It was necessary to provide all of the amino acids needed in the culture media when cells were cultivated without pyridoxal [13].

2.4.2. Amino Acids:

Because they are the building blocks of proteins, amino acids are a necessary component of every known cell growth medium. The culture media must contain essential amino acids since cells are unable to produce them on their own. They are necessary for cell division, and the highest possible cell density is determined by their concentration [14].

The building blocks for the production of proteins in cells are amino acids. Twelve L-amino acids, including arginine, cystine, isoleucine, leucine, methionine, lysine, valine, tyrosine, tryptophan, histidine, threonine, and phenylalanine, are required by all cells [11].

Moreover, glutamine is essential for the process of cell metabolism, and its nitrogen content is crucial for the creation of tri-, bi-, and monophosphate acid glycoside [11].

2.4.3. Other Supplements:

2.4.3.1. Inorganic Salt:

By supplying sodium, potassium, and calcium ions to the cells through the media, inorganic salts in the medium aid in maintaining the osmotic balance and aid in controlling the membrane potential. [14].

2.4.3.2. Fatty Acids and Lipids:

Since they are often found in serum and eventually aid in cell proliferation, they are especially significant in serum-free media [14].

2.4.3.3. Media Supplements:

Certain cell lines require additional components that are not present in the basal media in order to use the complete growth media that is advised. These elements, or supplements, support the maintenance of normal cell metabolism and proliferation [15]. [16].

A few cell lines cannot develop normally without supplements such as hormones, growth factors, and signaling molecules [14].

Additionally, adding additives to growth media alters its shelf life. When compared to the basal media alone, complete media with a protein supplement typically breakdown more quickly [14].

3. Materials and Methodology

3.1 Materials:

3.1.1. Instruments used:

3.1.1.1 Weighing Balance:

An instrument used to calculate an object's weight or mass is a weighing balance. It is offered with various weighing capabilities and a broad variety of sizes.

Fig 3.1.: Weighing Balance

3.1.1.2 Biosafety Cabinet:

When dealing with materials infected with (or potentially contaminated with) pathogens, a biosafety cabinet (BSC)—also known as a biological safety cabinet or microbiological safety cabinet—is an enclosed, ventilated laboratory workspace that requires a specified biosafety level. There are various varieties of BSC, distinguished by the level of biocontainment they offer.

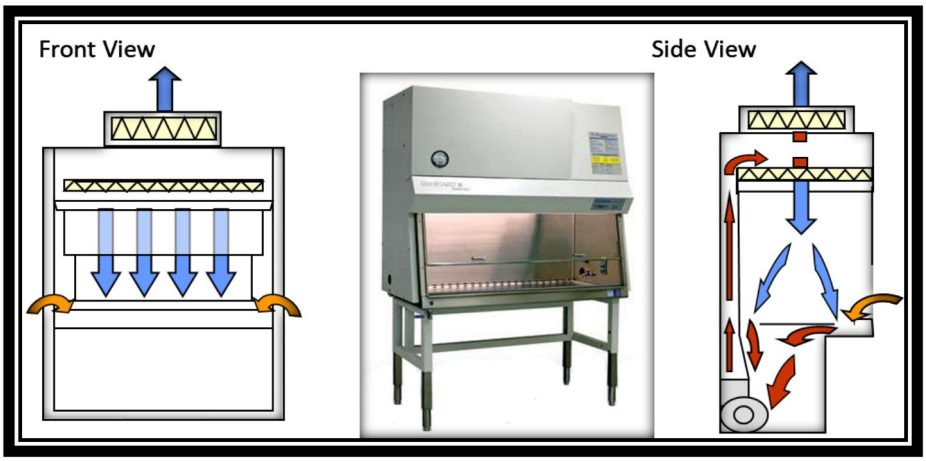


Fig 3.2.: Biosafety Cabinet

3.1.1.3 pH Meter:

One tool for calculating the amount of hydrogen ions in a solution is a pH meter. This shows how acidic or basic the solution is. It calculates the electrical potential difference between the reference electrode and a pH electrode. The pH or acidity of the provided solution is related to the difference in electrical potential.

Fig 3.3.: pH Meter

3.1.1.4 Hemocytometer:

A hemocytometer is a device used for manual cell counting. It is often referred to as a hemocytometer or a cell counting chamber. The hemocytometer was initially developed for the purpose of measuring blood cells, as its name suggests.

In cell culture labs, this modified and calibrated microscope slide design is used to count the number of viable, viable, or dead cells in a mammalian cell culture. To count the quantity of cells in each chamber, it is composed of counting chambers that hold a predetermined volume of cell suspension.

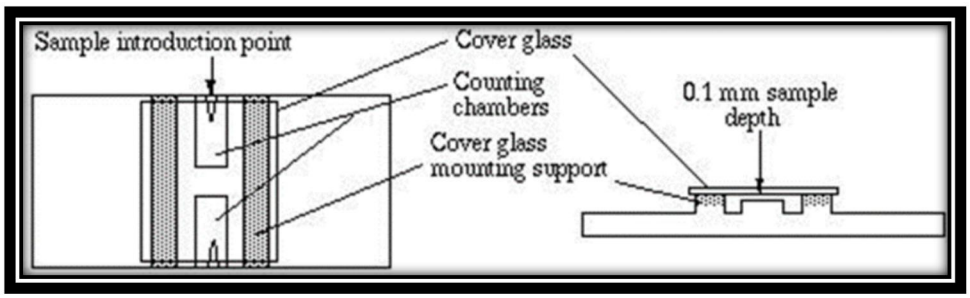


Fig 3.4.: Hemocytometer

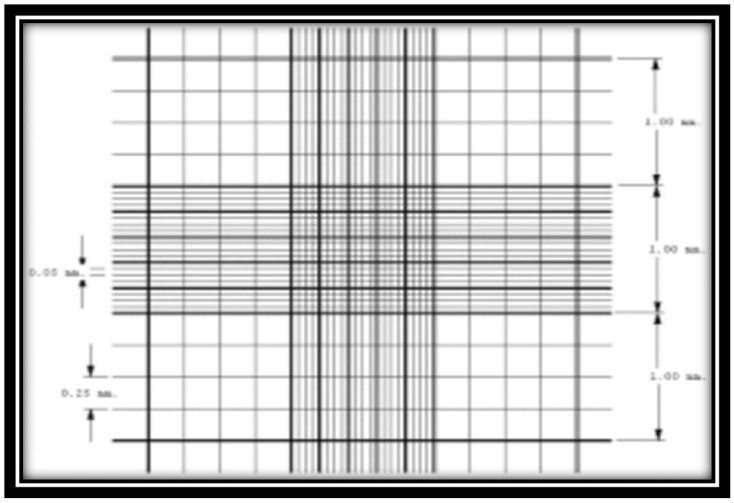


Fig 3.5. Counting grids of hemocytometer

3.1.1.5 CO_2 Incubator shaker:

An environment as natural as possible is produced within a CO2 incubator shaker to support the growth of cell and tissue cultures.

The primary use of CO2 incubators is in vitro culture, which is the process of growing live things. Sample growth and safety are always the top priorities during the several-week process. The temperature, humidity, agitation, and CO2 level must all be as close to the specifications for cell culture as possible in order to ensure both of these factors.

Fig 3.6. CO_2 Incubator Shaker

3.1.1.6 Biochemical Analyzer:

It is a device that measures the vital nutrients and byproducts that mammalian cells generate as they create monoclonal antibodies. With the Cedex bioanalyzer, metabolite levels such as glucose, lactate, glutamine, ammonia, and IgG are analyzed. This device calculates the concentration in the solution by measuring the amount of light absorbance in a fluid using absorption photometry.

Fig 3.7. Cedex Bioanalyzer

3.1.1.7 Osmometer:

This device calculates the osmolality of an aqueous solution using physical parameters such as freezing point; when a solute particle dissolves in a solvent, the freezing point of the resulting solution drops relative to the solvent alone; further solute addition results in even smaller freezing points. By accurately determining the freezing point of the solution, the osmolality of the samples can be measured with great efficiency.

Fig 3.8. Osmometer

3.1.1.8 Microscope:

An effective tool for monitoring cell cultures and ensuring they are operating as intended is the optical microscope. Using an optical microscope, an operator examines cultivated cells to assess their state and decide whether to move on to the next stage of the culture process.

Fig 3.9. Microscope

3.1.2. Software / Tool used:

3.1.2.1 Minitab:

In collaboration with Triola Statistics Company, researchers Barbara F. Ryan, Thomas A. Ryan, Jr., and Brian L. Joiner created the statistics program Minitab at Pennsylvania State University in 1972.

It started off as a simplified version of the National Institute of Standards and Technology's OMNITAB statistical analysis application. In order to take on even the most difficult challenges and possibilities, Minitab Statistical Software can analyze both historical and present data to identify trends, identify and forecast patterns, reveal hidden correlations between variables, and produce eye-catching visualizations.

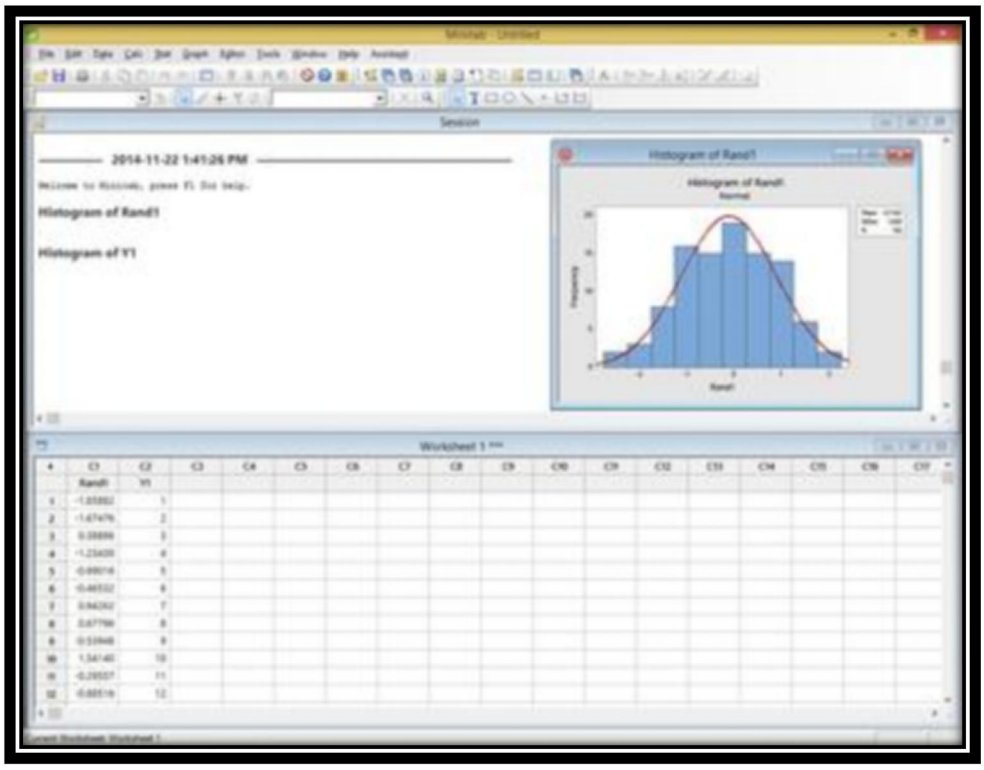


Fig 3.10. Minitab Screenshot

3.1.3. Chemicals and Reagents used:

- Basal Media
- Sodium Hydroxide
- WFI
- Standard feed A
- Standard feed B
- D-Glucose
- L-Glutamine
- Amino acids Solution1
- Amino acids Solution 2
- Vitamin Solution
- 10 Different Vitamins:
  - Vitamin A, Vitamin B, Vitamin C, Vitamin D, Vitamin E, Vitamin F, Vitamin G, Vitamin H, Vitamin J, Vitamin K
- 12 Different Other Supplements:
  - Other supplement A, Other supplement B, Other supplement C, Other supplement D, Other supplement E, Other supplement F, Other supplement G, Other supplement H, Other supplement J, Other supplement K, Other supplement L, Other supplement M.
- 70% Isopropyl Alcohol
- Trypan blue

3.1.4. Miscellaneous:

- Shake flasks
- Pipette gun
- Serological Pipette
- 0.2µm filter
- Vacuum pump
- Centrifuge
- Magnetic stirrer
- Liquid nitrogen container
- Refrigerator
- Water bath
- Microscope
- Vortex

3.2 Methods:

3.2.1. Preparation of seed media and production media:

- Seed media was prepared which consists of basal media along with a few additives which supports the growth of the cells. This media was then filtered aseptically using 0.2 µm bottle top filter.

- Production media was prepared which consisted of the basal media along with few other components which support the cell growth, cell viability and the was filtered using 0.2 µm bottle top filter.

3.2.2. Preparation of seed:

To start an experiment, the seed needs to be grown in order to obtain the required seed count for further inoculating it into the production media.

The steps followed for the preparation of seeds are as below:

- Cells were thawed in the vial which was cryopreserved in liquid nitrogen at -1960°C and was later transferred to 30ml seed media in a 125ml shake flask. This was then kept in CO_2 incubator shaker at a temperature of 37°C for 72 hours or until we got the desired cell count. After about 72 hours, sampling was done and cell count was taken.
- Passaging and scaling up of the initial culture was done into 150 ml of seed media and was kept in CO_2 incubator for 72 hours or till the desirable cell count was attained. The amount of culture volume required to inoculate 150ml of seed media is given by the following equation

$$Volume\ of\ seed = \frac{(Required\ cell\ density\ \times Total\ Volume)}{Cell\ count\ of\ the\ seed}$$

- Later passaging and scaling up of the culture was done into 250ml of seed media and was kept in CO_2 incubator at 37°C for about 72 hours or till the desirable cell count is attained.

3.2.3. Preparation of feeds for standard feeding:

250 ml Standard feed A, Standard feed B, Glucose, Glutamine, Amino acids Solution1, Amino acids Solution 2, Vitamin Solution were prepared according to the protocol and was then filtered aseptically using 0.2 µm bottle top filter.

3.2.4. Preparation of Vitamins and Other supplements for feeding strategy for screening experiment:

250 ml 10 different vitamins i.e. Vitamin A, Vitamin B, Vitamin C, Vitamin D, Vitamin E, Vitamin F, Vitamin G, Vitamin H, Vitamin J, Vitamin K and 12 different other supplements i.e Other supplement A, Other supplement B, Other supplement C, Other supplement D, Other supplement E, Other supplement F, Other supplement G, Other supplement H, Other supplement J, Other supplement K, Other supplement L, Other supplement M were prepared according to the protocol and was then filtered aseptically using 0.2 µm bottle top filter.

3.2.5. Experiment 1- Vitamins screening experiment:

- 16 sterile 125ml shake flasks were taken and the desired amount of production media was aseptically transferred into each the flasks using pipettes.
- Desired amount of seed was then added aseptically into the media under laminar air flow unit and were kept in CO_2 incubator with set parameters.
- This was run under batch mode in which one flask was set as control in which only standard feeding using standard feed A, standard feed B, glucose, amino acid solution1, amino acid solution 2, vitamin solution and glutamine was done on every day basis starting from day 3 as per the standard protocol on the basis of osmolality.
- In other fifteen flasks the addition of standard feeds using standard feed A, standard feed B, glucose, amino acid solution1, amino acid solution 2, vitamin solution and glutamine was done on every day basis starting from day 3 as per the standard protocol on the basis of osmolality .
- Vitamins in different combinations as per minitab set protocol were supplemented on alternate days starting from day 8 in all the flasks
- Sampling was done every day followed by cell count, vcc, viability, biochemical analysis and osmolality.
- According to the biochemical analysis, glucose addition was done to maintain the glucose levels. This experiment was done to screen out the suitable vitamin for maintaining the cell growth, cell viability and titer.

Flask ID	Vitamin A	Vitamin B	Vitamin C	Vitamin D	Vitamin E	Vitamin F	Vitamin G	Vitamin H	Vitamin J	Vitamin K
1	0	0	0	0	0	0	**0**	0	0	0
2	0	0	0	0	0	0	0	1	1	1
3	0	0	0	1	1	1	1	0	0	0
4	0	0	0	1	1	1	1	1	1	1
5	0	1	1	0	0	1	1	0	0	1
6	0	1	1	0	0	1	1	1	1	0
7	0	1	1	1	1	0	0	0	0	1
8	0	1	1	1	1	0	0	1	1	0
9	1	0	1	0	1	0	1	0	1	0
10	1	0	1	0	1	0	1	1	0	1
11	1	0	1	1	0	1	0	0	1	0
12	1	0	1	1	0	1	0	1	0	1
13	1	1	0	0	1	1	0	0	1	1
14	1	1	0	0	1	1	0	1	0	0
15	1	1	0	1	0	0	1	0	1	1
16	1	1	0	1	0	0	1	1	0	0

Table 3.1: Vitamins feeding strategy

3.2.6. Experiment 2- Other Supplements screening experiment:

- 16 sterile 125ml shake flasks were taken and the desired amount of production media was aseptically transferred into each the flasks using pipettes.
- Desired amount of seed was then added aseptically into the media under laminar air flow unit and were kept in CO_2 incubator with set parameters.
- This was run under batch mode in which one flask was set as control in which only standard feeding using standard feed A, standard feed B, glucose, amino acid solution1, amino acid solution 2, vitamin solution and glutamine was done on every day basis starting from day 3 as per the standard protocol on the basis of osmolality was done whereas in other fifteen flasks the addition of standard feeds using standard feed A,

- standard feed B, glucose, amino acid solution1, amino acid solution 2, vitamin solution and glutamine was done on every day basis starting from day 3 as per the standard protocol on the basis of osmolality was done.
- Other supplements in different combinations were supplemented as per the minitab set protocol on alternate days starting from day8 in all the flasks.
- Sampling was done every day followed by cell count, vcc, viability, biochemical analysis and osmolality.
- According to the biochemical analysis, glucose addition was done to maintain the glucose levels. This experiment was done to screen out the suitable other supplement for maintaining the cell growth, cell viability and titer.

Flask ID	Other Supplement A	Other Supplement B	Other Supplement C	Other Supplement D	Other Supplement E	Other Supplement F	Other Supplement G	Other Supplement H	Other Supplement J	Other Supplement K	Other Supplement L	Other Supplement M
1	0	0	0	0	0	0	**0**	0	0	0	0	0
2	0	0	0	0	0	0	0	1	1	1	1	1
3	0	0	0	1	1	1	1	0	0	0	0	1
4	0	0	0	1	1	1	1	1	1	1	1	0
5	0	1	1	0	0	1	1	0	0	1	1	0
6	0	1	1	0	0	1	1	1	1	0	0	1
7	0	1	1	1	1	0	0	0	0	1	1	1
8	0	1	1	1	1	0	0	1	1	0	0	0
9	0	0	1	0	1	0	1	0	1	0	1	0
10	1	0	1	0	1	0	1	1	0	1	0	1
11	1	0	1	1	0	1	0	0	1	0	1	1
12	1	0	1	1	0	1	0	1	0	1	0	0
13	1	1	0	0	1	1	0	0	1	1	0	0
14	1	1	0	0	1	1	0	1	0	0	1	1
15	1	1	0	1	0	0	1	0	1	1	0	1
16	1	1	0	1	0	0	1	1	0	0	1	0

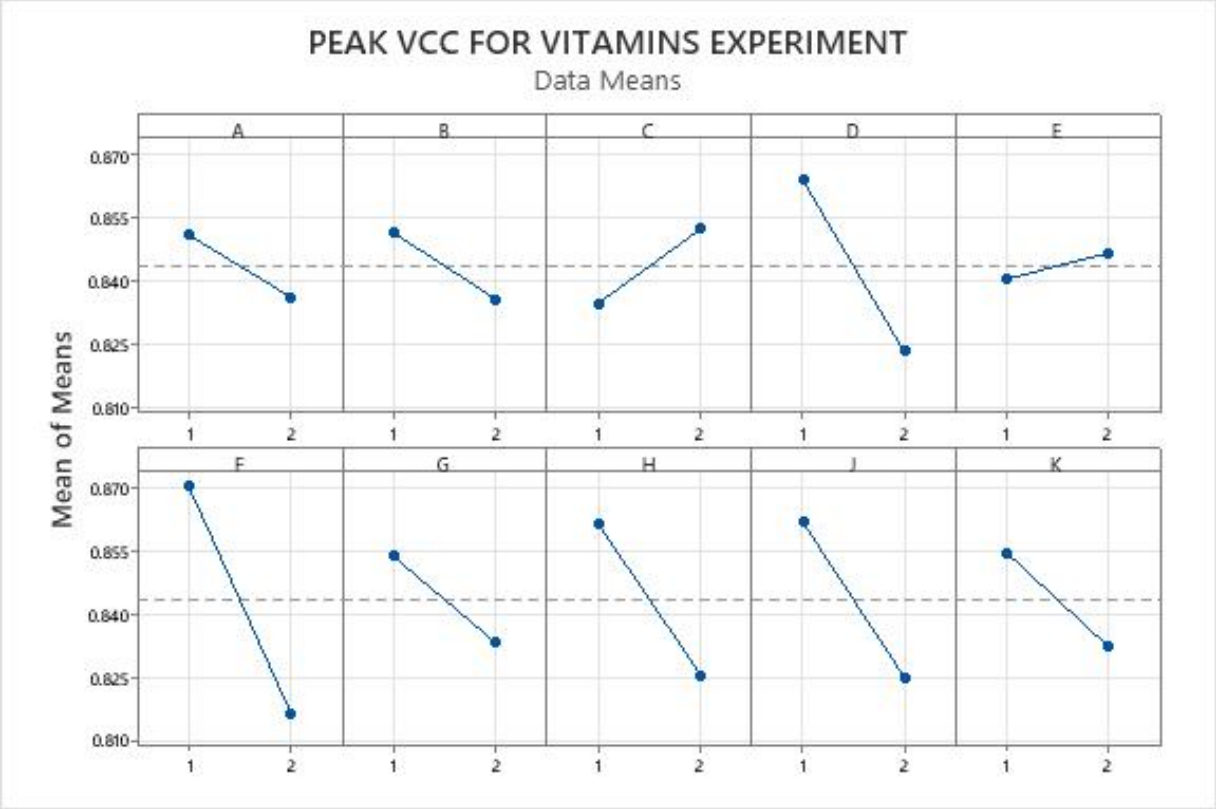
Table 3.2: Other supplement feeding strategy

3.2.7. Harvest:

Once the viability of the batch came down to 50%, the batches were harvested and centrifuged in 50ml falcon tubes. The pellet was discarded and the supernatant was stored in -20℃ for further analysis of their titer and quality.

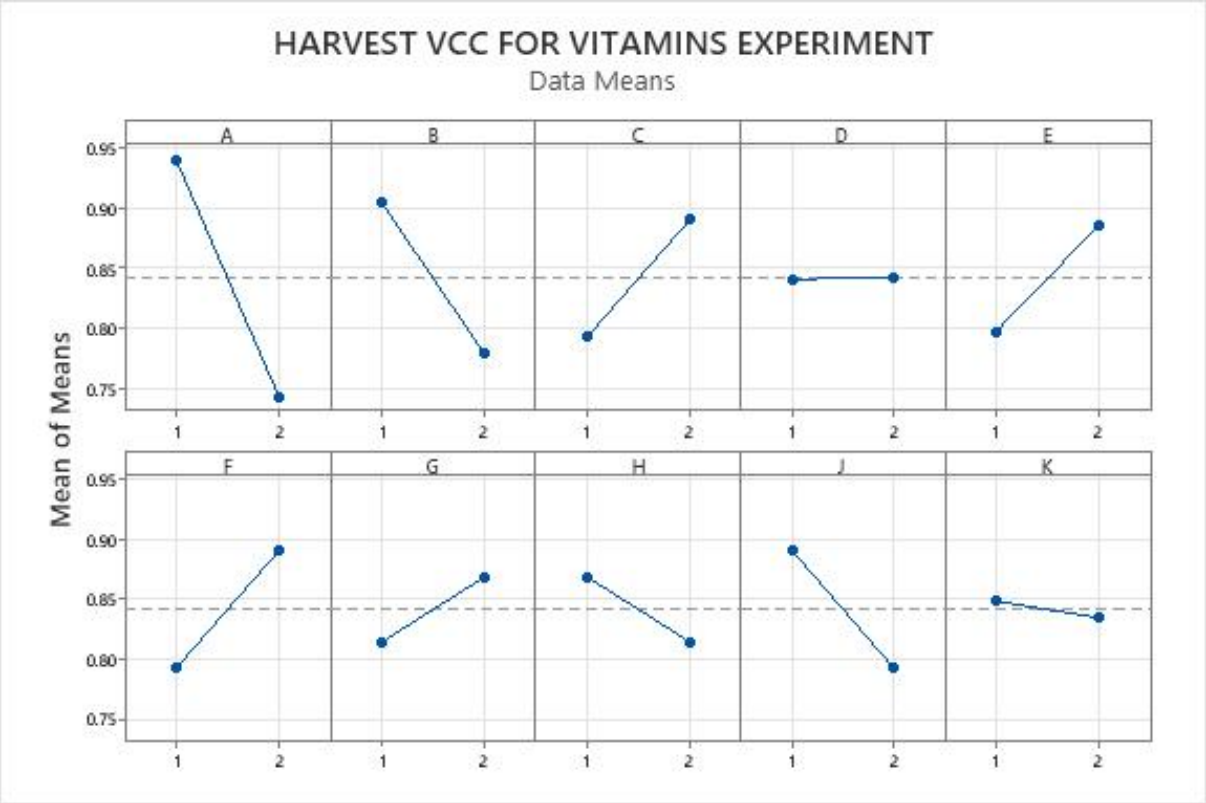
4.Results And Discussions

4.1 Results for experiment 1-Vitamins screening experiment:



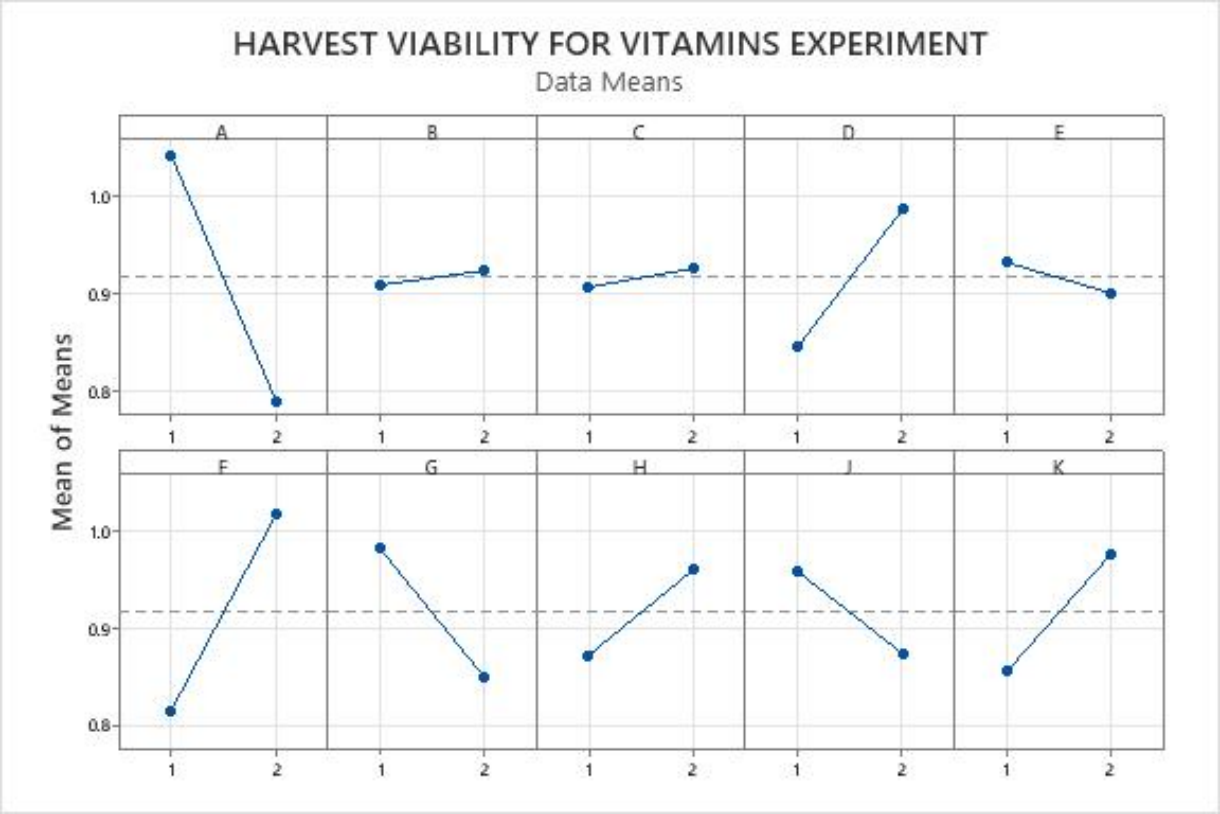
4.1.1. Graphical representation of the effect of Vitamins on Peak VCC.

- In graph 4.1.1. , the X- axis represents the concentration of the Vitamin added and the Y- axis represents the Peak VCC.
- The graph indicates that Vitamin C has a positive impact on the Peak VCC whereas Vitamins A, B, D, F, G, H, J and K have a negative impact on the Peak VCC.
- Vitamin E has no significant impact on the Peak VCC.
- Here, Vitamin C has improved the Peak VCC very efficiently as compared to other Vitamins.



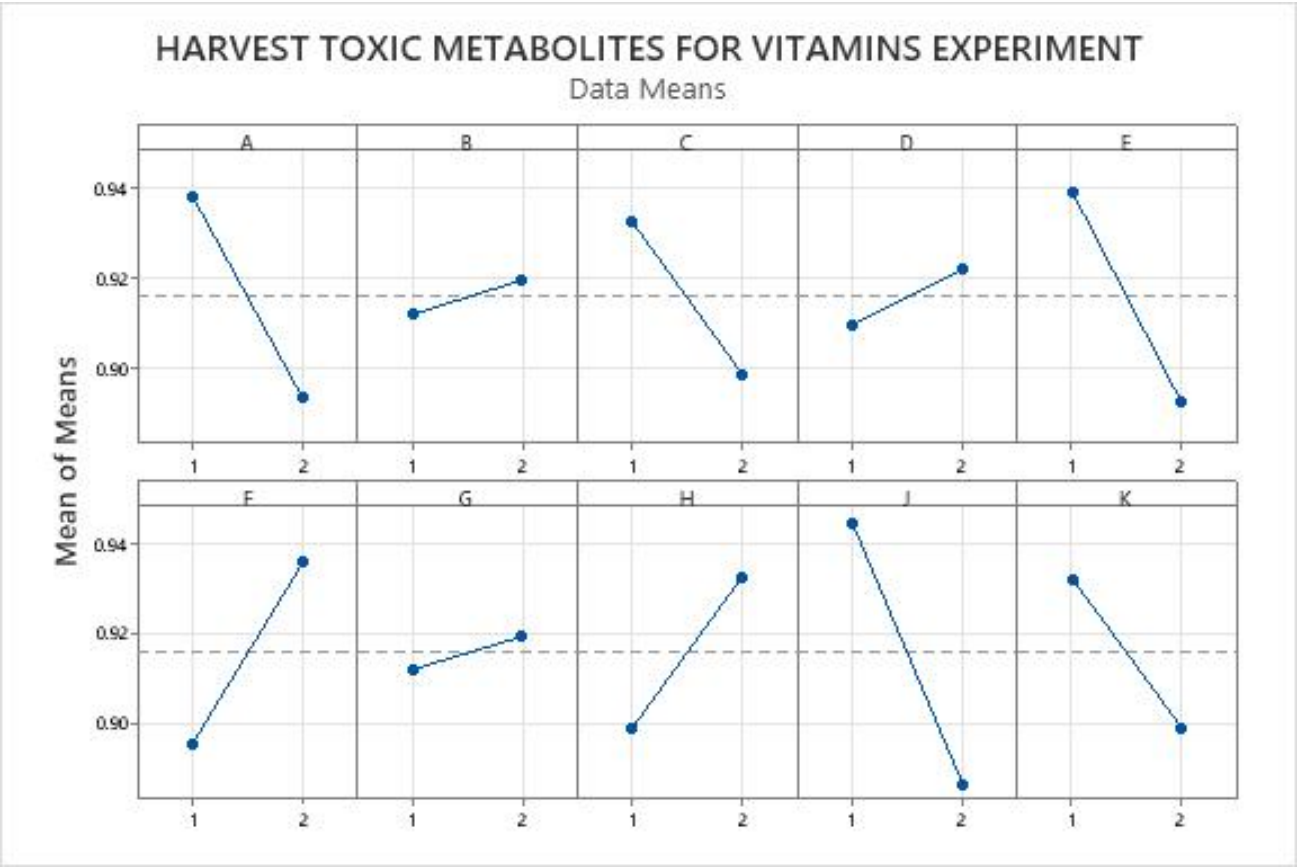
4.1.2. Graphical representation of the Vitamins on Harvest VCC

- In graph 4.1.2. , the X- axis represents the concentration of the Vitamin added and the Y- axis represents the Harvest VCC.
- The graph indicates that Vitamin C, E, F and G have a positive impact on the Harvest VCC whereas Vitamins A, B, H, J and K have a negative impact on the Harvest VCC.
- Vitamin D has no significant impact on the Harvest VCC.



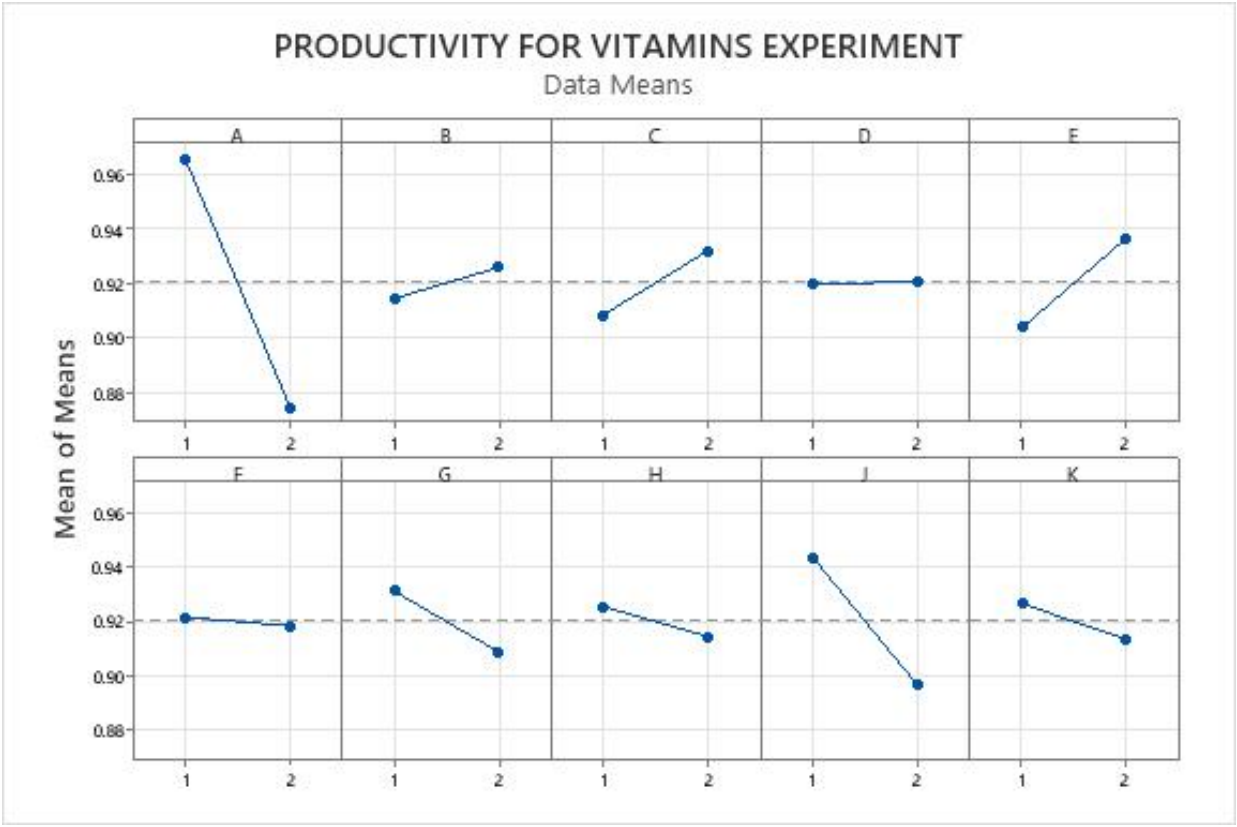
4.1.3. Graphical representation of the effect of Vitamins on Harvest Viability.

- In graph 4.1.3. , the X- axis represent the concentration of the Vitamin added and the Y- axis represents the Harvest Viability.
- The graph indicates that Vitamin D, F, H and K have a positive impact on the Peak VCC whereas Vitamins A, E, G and J have a negative impact on the Harvest Viability.
- Vitamin B and C have no significant impact on the Harvest Viability.
- Here, Vitamin has improved the Harvest Viability very efficiently as compared to other Vitamins.



4.1.4. Graphical representation of the effect of Vitamins on Toxic Metabolites.

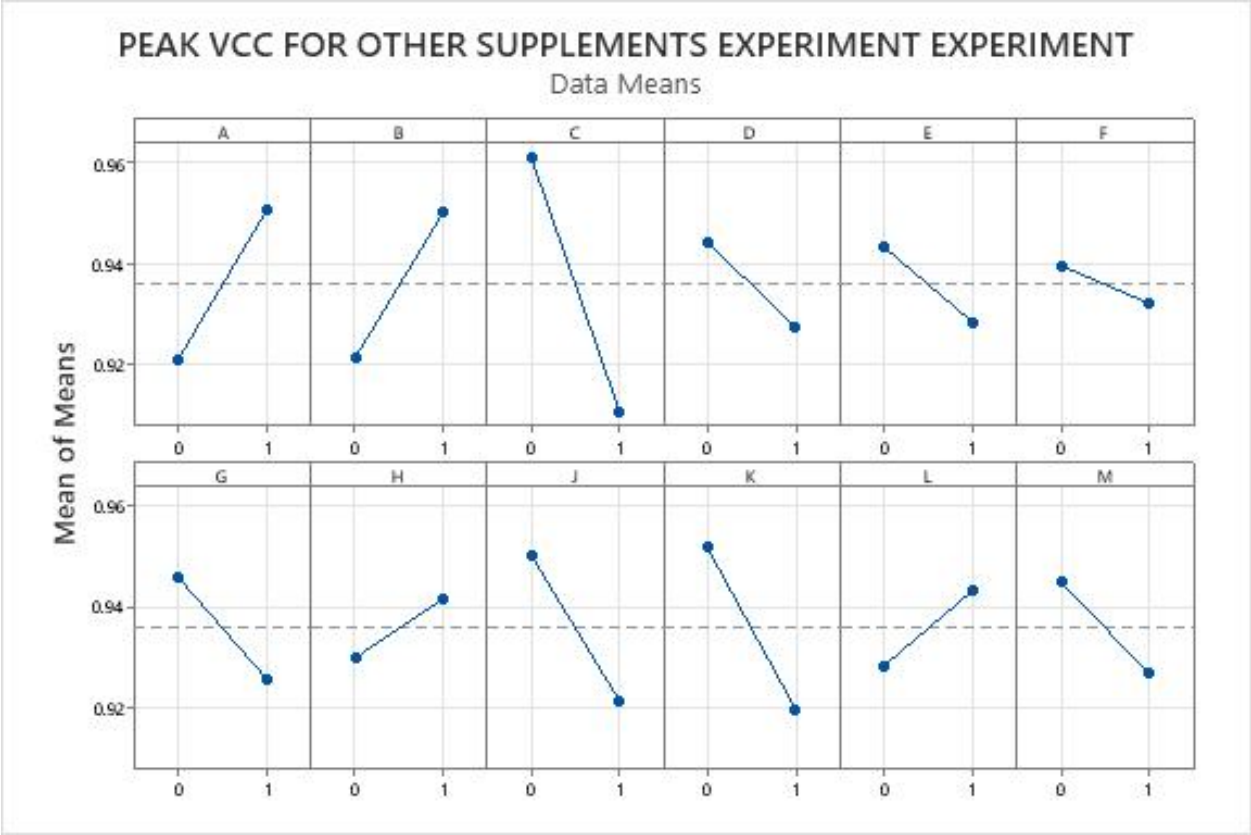
- In graph 4.1.4. , the X- axis represents the concentration of the Vitamin added and the Y- axis represents the Production of Toxic Metabolites.
- The graph indicates that Vitamin A, C, E, J and K have a positive impact on the production of toxic metabolites on the harvest day whereas Vitamins D, F and H have a negative impact on the Production of the toxic metabolites on the harvest day.
- Vitamin B and G have no significant impact on the Production of the toxic metabolites on the harvest day .
- Here, Vitamin J has reduced the production of toxic metabolites very efficiently as compared to other Vitamins.



4.1.5. Graphical representation of the effect of Vitamins on Productivity

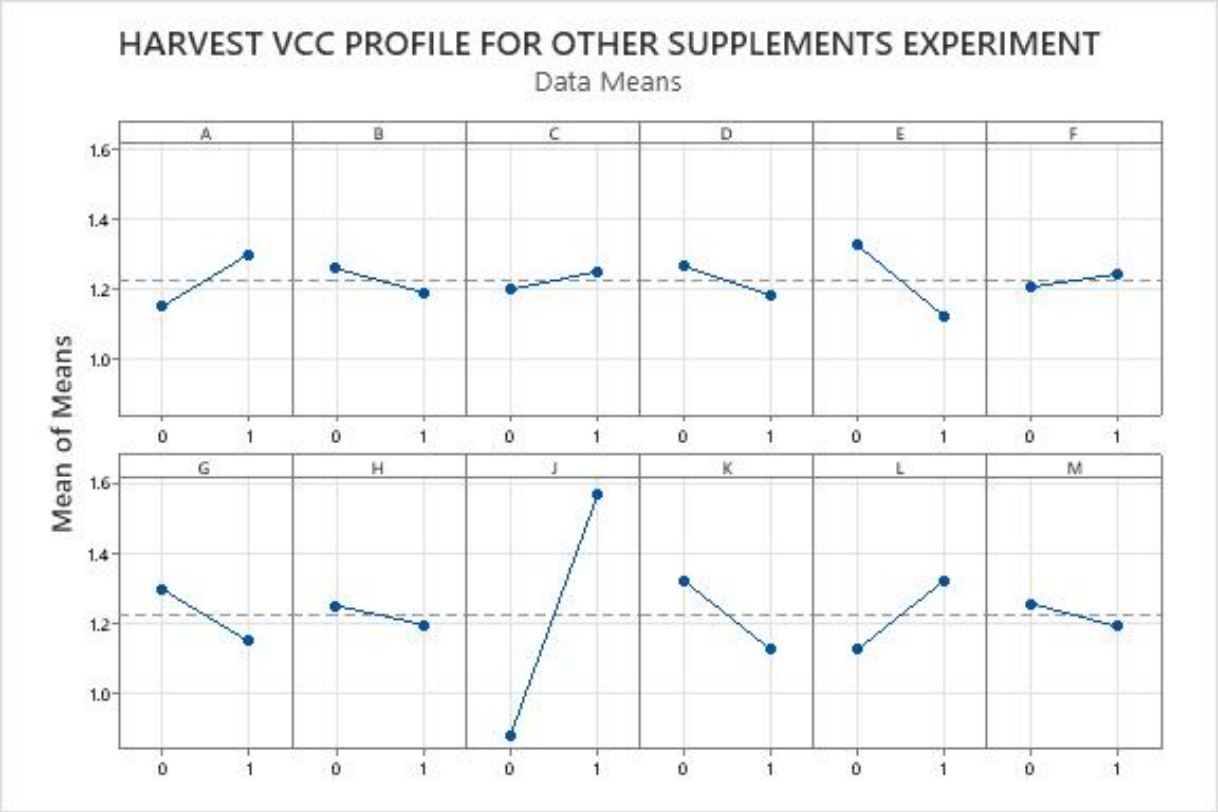
- In graph 4.1.5. , the X- axis represents the concentration of the Vitamin added and the Y- axis represents the Productivity.
- The graph indicates that Vitamin B, C and E have a positive impact on the Productivity whereas Vitamins A, G, H, J and K have negative impact on the Productivity.
- Vitamin D and F have no significant impact on the Productivity.
- Here, Vitamin E has improved the productivity very efficiently as compared to other Vitamins.

4.2. RESULTS FOR EXPERIMENT2- OTHER SUPPLEMENTS SCREENING:



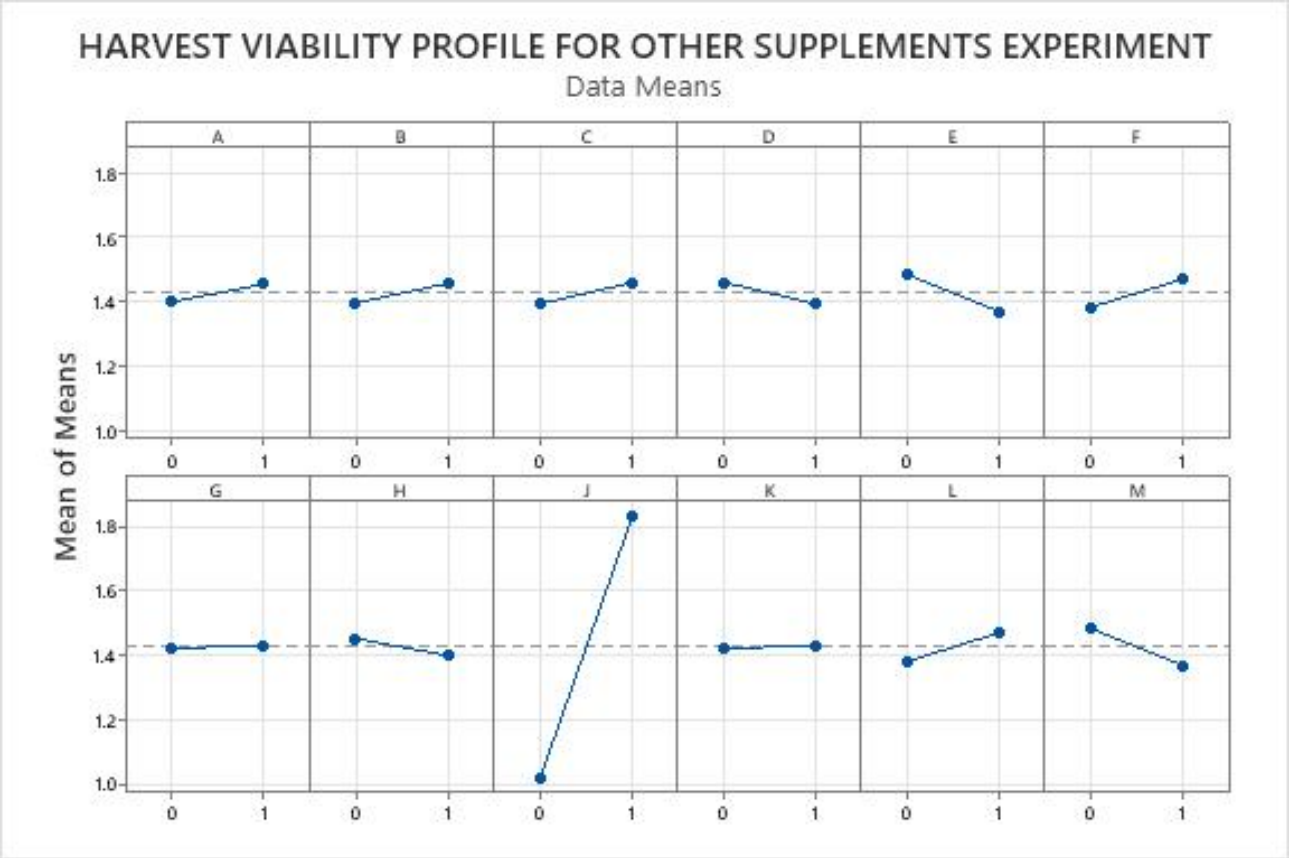
4.2.1. Graphical representation of the effect of Other Supplements on Peak VCC.

- In graph 4.2.1. , the X- axis represents the concentration of the other supplement added and the Y- axis represents the Peak VCC.
- The graph indicates that Other Supplement A, B, H and L have a positive impact on the Peak VCC whereas Other Supplement C, D, E, G, J, K and M have a negative impact on the Peak VCC.
- Other Supplement F has no significant impact on the Peak VCC.



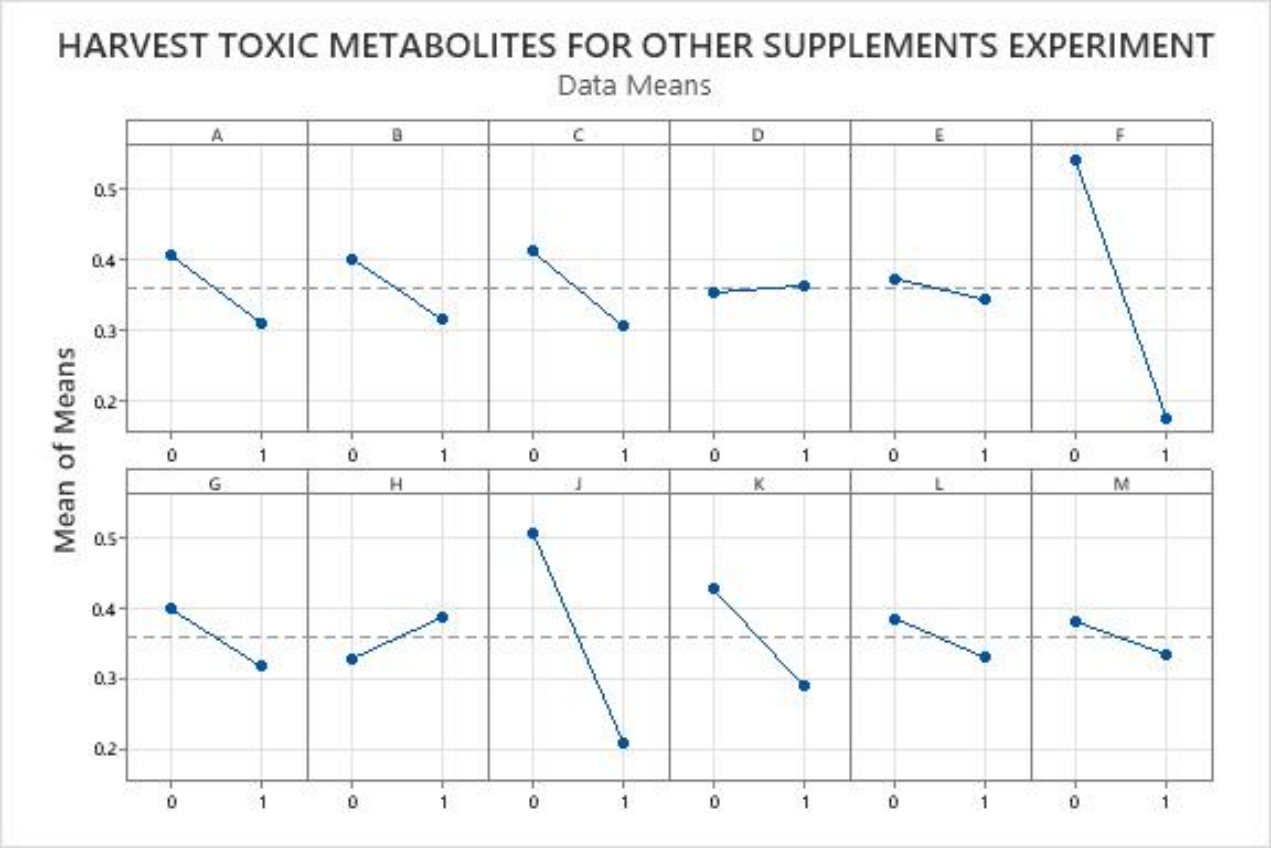
4.2.2. Graphical representation of the effect of Other Supplements on Harvest VCC.

- In graph 4.2.2. , the X- axis represents the concentration of the Other Supplement added and the Y- axis represents the Harvest VCC.
- The graph indicates that Other Supplement A, J and L have a positive impact on the Harvest VCC whereas Other Supplement E, G and K have a negative impact on the Harvest VCC.
- Other Supplement B, D, F, H and M have no significant impact on the Harvest VCC.
- Here, Other Supplement J has improved the Harvest VCC very efficiently as compared to other Other Supplements.



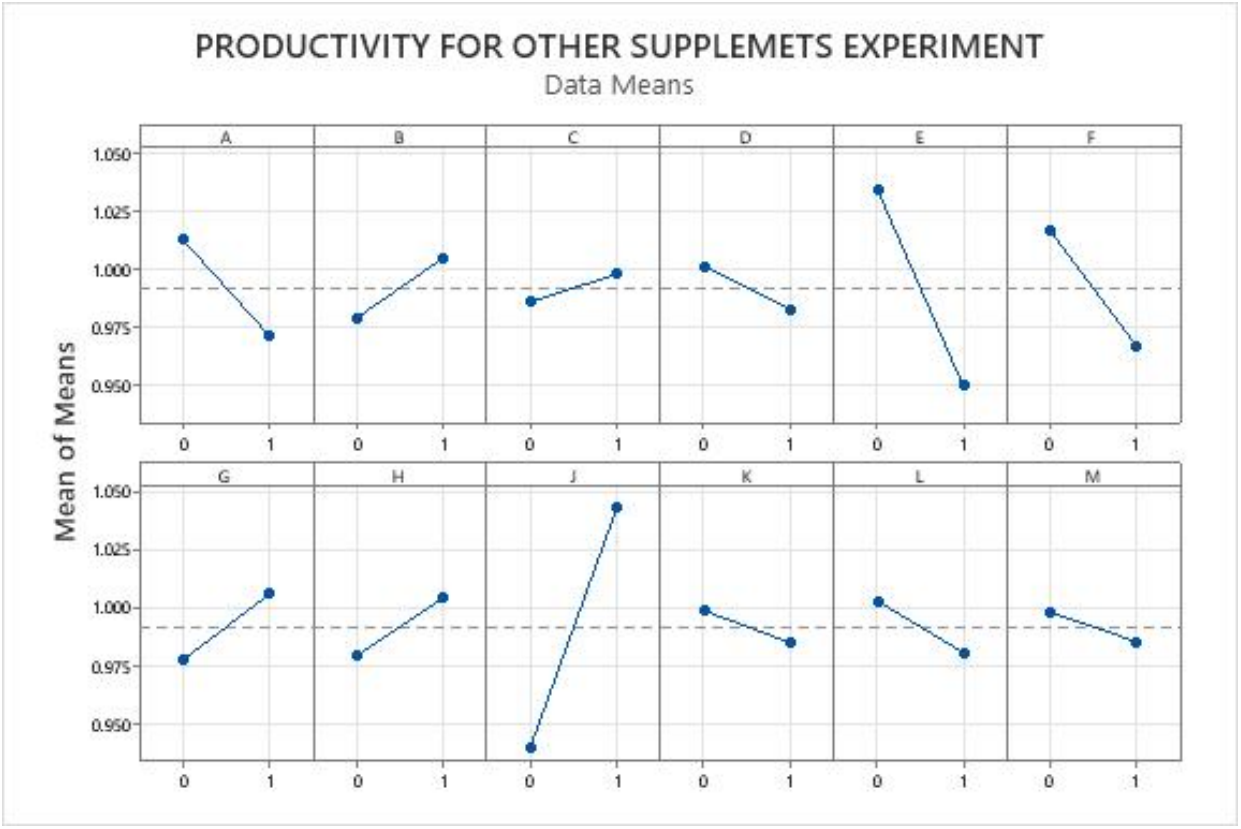
4.2.3. Graphical representation of the effect of Other Supplements on Harvest Viability.

- In graph 4.2.3. , the X- axis represent the concentration of the Other Supplement added and the Y-axis represents the Harvest Viability.
- The graph indicates that Other Supplement F, J and L have a positive impact on the Peak VCC whereas Other Supplement E and M have a negative impact on the Harvest Viability.
- Other Supplement A, B, C, D, G, H, and K have no significant impact on the Harvest Viability.
- Here, Other Supplement J has improved the Harvest Viability very efficiently as compared to other Other Supplements.



4.2.4. Graphical representation of the effect of Other Supplements on Toxic Metabolites .

- In graph 4.2.4. , the X- axis represents the concentration of the Other Supplement added and the Y- axis represents the Production of Toxic Metabolites.
- The graph indicates that Other Supplement A, B, C, F, G, J, K, L and M have a positive impact on the production of toxic metabolites on the harvest day whereas Other supplement H has a negative impact on the Production of the toxic metabolites on the harvest day.
- Other Supplement D and E have no significant impact on the Production of the toxic metabolites on the harvest day .
- Here, Other Supplement F has reduced the production of toxic metabolites very efficiently as compared to other Other Supplements.



4.2.5. Graphical representation of the effect of Other Supplements on Productivity.

- In graph 4.2.5. , the X- axis represents the concentration of the Other Supplement added and the Y- axis represents the Productivity.
- The graph indicates that Other Supplement B, G, H and J have a positive impact on the Productivity whereas Other Supplement A, D, E, F and L have negative impact on the Productivity.
- Other Supplement C , K and M have no significant impact on the Productivity.
- Here, Other Supplement J has improved the productivity very efficiently as compared to other Other Supplements.

5. CONCLUSION AND FUTURE SCOPE

The results of the experiment provide valuable insights into optimizing cell culture conditions and assessing viability. Through vitamins screening, we evaluated various vitamins formulations to identify the most suitable one for cell line. Vitamin screening was done using 10 different Vitamins and we found out that no vitamin played a very significant role in the process optimization.

On the other hand, through other supplements screening, we evaluated various other supplements formulations to identify the most suitable one for cell line. Other supplements screening was done using 12 different Other supplements and we found out that one Other supplement i.e. Other Supplement J played a very significant role in the process optimization. The selection of the suitable Vitamin and Other supplement was done on the basis of Cell viability, VCC, their titer, Peak VCC and the production of toxic metabolites. This process allowed us to select an Other supplement that supported optimal cell growth, viability, enhanced cell proliferation and improved the overall growth. The optimized addition of Other supplement led to enhanced cell growth and sustained viability, indicating its efficacy in providing the necessary nutrients for robust cell culture. Moreover, the viability checks conducted throughout experiment help us monitor viability of cultured cells. By employing correct parameters such as cell membrane integrity, metabolic activity. The results consistently demonstrated high cell viability throughout the culture period.

The optimized conditions help to enhance cell growth, improved viability and maintains cell growth which are crucial for various applications, including research, production and drug discovery. These findings contribute to the advancement of the cell culture techniques and provide foundations for the future studies. During the experiment, numerous additional parameters, such as the build-up of different metabolites, were noticed. Using a biochemical analyser, the concentrations of these metabolites were examined. The findings indicate that a key factor in the reduction of cell viability was the build-up of different metabolites. Increased sugar consumption can result in aerobic glycolysis, which produces lactate build up and an acidic pH. Increased consumption of amino acids has the potential to produce high concentrations of ammonia.
As a result, the feeding of sugar and amino acids should be controlled in accordance with requirements.

Reduced cell viability and count can be a result of increased osmolality. Osmolality should thus be low in order to have a high-quality end product.

Optimization of Culture Conditions: While our experiments have successfully optimized the cell culture conditions, there may be room for further refinement. Future studies can explore different combinations of other supplement J along with different other supplements, vitamins, amino acids to fine-tune the culture conditions and achieve even better results for cell growth, viability, and productivity.

Scale-up and Bio production: Once the optimal culture conditions have been established at the laboratory scale, the next step could involve scaling up the cell culture process. This would involve transferring the optimized conditions to larger bioreactors to support increased cell density and higher productivity. Evaluating the scalability and reproducibility of the optimized conditions is essential for potential industrial applications.

Long-Term Stability and Maintenance: Future studies can focus on evaluating the robustness of the culture system over extended period of time, investigating the effects of prolonged cultivation on cell viability, and identifying strategies to maintain the optimal conditions consistently.

Evaluating and selecting high quality yet cost effective raw materials for cell culture media and feed can impact both quality as well as the cost. Exploring alternative sources of Other supplements, Vitamins and amino acids can help reduce expenses without compromising the cell productivity and quality of the product. Future research can also explore the integration of novel technologies, such as tissue engineering approaches, or gene editing tools, to enhance the cell culture systems , their performance, functionality, and application potential.

Application in Disease Modelling and Drug Discovery: Once the optimized culture conditions are established, they can be used to create more physiologically relevant in vitro models for disease research and drug discovery. Future studies can also focus on utilizing the optimized cell culture system to develop disease models, study disease mechanisms, screen drug candidates, and evaluate therapeutic efficacy. These future scopes will help in the advancement of the field of cell culture and contribute to various applications, including regenerative medicine, biotechnology, and pharmaceutical research.

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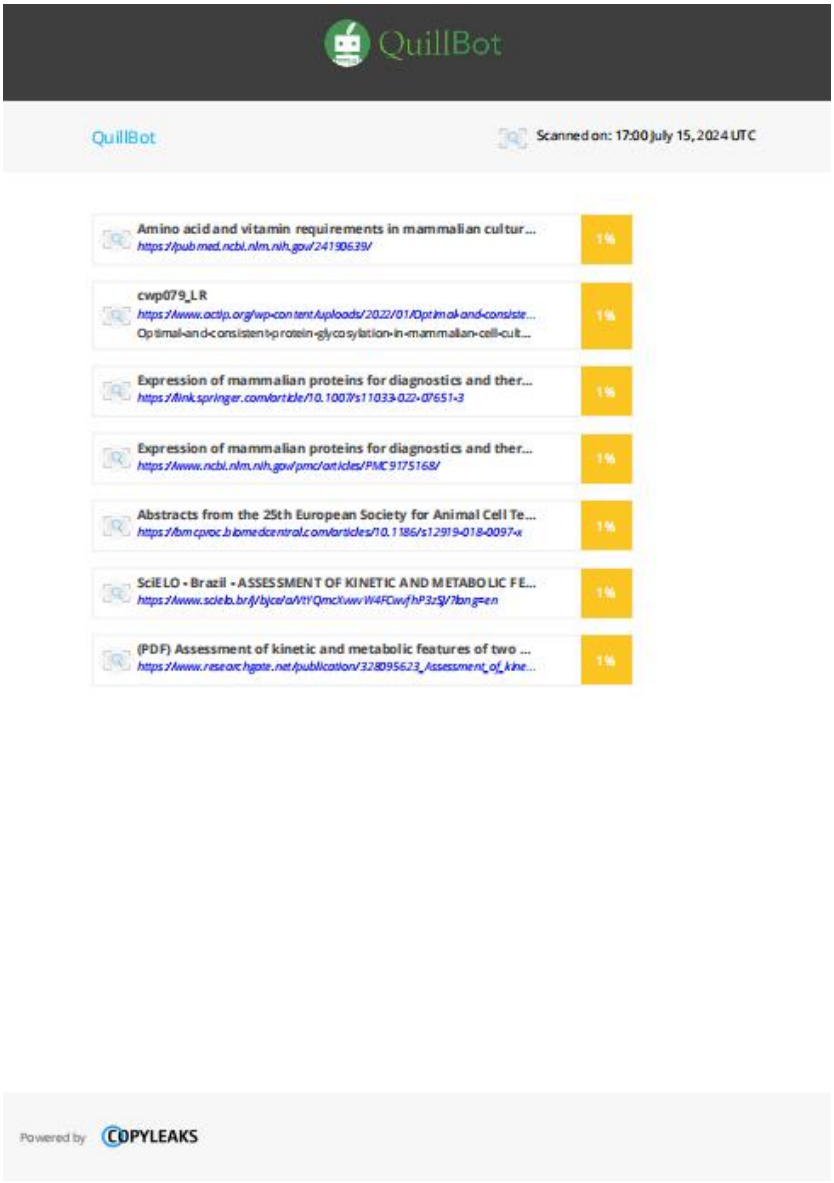

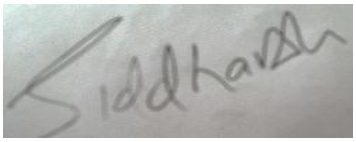

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