Meat Production System Model for INVITROS

Our meat production system can be divided into 4 sectors; the growth of meat, the production of haemoglobin, the production of vitamin B12, and the recycling of carbon dioxide (CO_2).

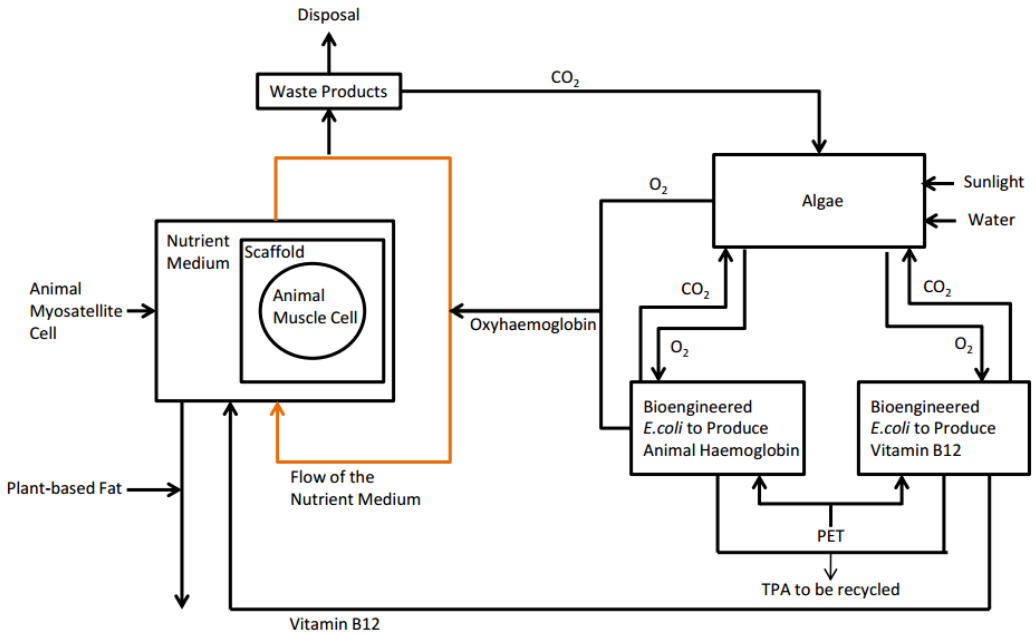

Figure: The proposed model for *in vitro* meat production system. The animal myosatellite cell (muscle stem cell) will be isolated and grown on a scaffold, in the nutrient medium. The orange arrow shows the flow of the nutrient medium. Vitamin B12 produced by bioengineered *Escherichia coli* is added to the bioreactor containing muscle cells. The gaseous exchange is carried out at bioreactors containing algae, which produce oxygen via photosynthesis. Bioengineered *E.coli* produce animal hemoglobin for oxygen transport. Waste products such as lactic acid had been disposed of. The grown meat is harvested and plant-based fatty acid is added to improve texture and flavour..

The Growth of Meat

Muscle cell samples are taken from the animal and myosatellite cells are isolated. The isolated myosatellite cells are grown on a porous and edible scaffold, to where the muscle cells attach and through where the serum-free nutrient medium flows in a circular motion. This type of bioreactor is called direct perfusion bioreactor. The waste products (e.g. lactic acid, old and unuseful cell components) are disposed of, except CO_2 which is recycled. The new oxyhaemoglobin are added continuously into the stream of nutrient medium to transport oxygen for respiration of the muscle cells.

Production of Haemoglobin and Vitamin B12

It has been clear that the haemoglobin is required for oxygen transport of the oxygen towards the growing muscle cell. (Shen et al., 1997) have successfully produced human haemoglobin using *E.coli.* Although haemoglobin of any farm animals has been produced by using the same method (Zhao,

Zhou, Du and Chen, 2021), bovine haemoglobin has the highest potential for being produced by *E.coli* as it has the most similar protein sequence (88% for subunit-α and 85% for subunit-β) and gene sequence (73% for subunit- and 81% for subunit-) wth human haemoglobin.

Vitamin B12 is an essential vitamin responsible for healthy brain and muscle function. It is found exclusively on meat and has only been produced by certain species of gut-colonising bacteria. Industrial production of vitamin B12 has been carried out by growing *Pseudomonas denitrificans* and *Propionibacterium freudenreichii* in the lab. (Fang et al., 2018) has successfully produced vitamin B12 using *E.coli* by inserting the 32 genes responsible for vitamin B12 synthesis.

E.coli can be bioengineered to break down PET into ethylene glycol and TPA by inserting the plasmid containing genes for enzyme PETase (Shi et al., 2021) and MHETase (Janatunaim and Fibriani., 2020). *E.coli* has the genes for consuming ethylene glycol for its survival. Therefore, by overexpressing those genes, an efficient breakdown of PET can be achieved. TPA can be isolated and recycled to produce plastic again.

Recycling of Carbon Dioxide

The carbon dioxide produced from the respiration of the cells has been absorbed by algae bioreactor and converted into the oxygen to be used in respiration again. Algae has been used instead of trees because the growth of algae is easier to control and it can capture more carbon dioxide than trees. Under optimum conditions, the carbon capture of algae *Oscillatoria* can be up to 75%. (Anguselvi, Ebhin Masto, Mukherjee and Kumar Singh, 2019)

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