APPLICATION NOTE: 002/2024

Application Note for 2 L Twin Autoclavable Fermenter

ABSTRACT:

This application note details the process of cultivating Mycorrhiza in a 2L twin autoclavable fermenter, emphasizing the optimization of culture conditions to ensure consistent and reliable Mycorrhiza cell culture production. The protocol includes comprehensive instructions for preparing the growth medium, inoculum preparation, fermenter setup, and process monitoring, all designed to achieve reproducible and high-quality outcomes. Key steps begin with the meticulous preparation of the growth medium, followed by the careful inoculation and cultivation of Mycorrhiza. The fermenter setup involves precise adjustments of temperature, pH, agitation rate, and aeration to create an optimal growth environment. Regular monitoring of critical parameters such as cell density, pH, and dissolved oxygen concentration ensures the maintenance of these conditions throughout the fermentation process. The transition from larger fermenters to a 2L system demonstrates the scalability and robustness of these standardized culture conditions. This smaller scale setup offers a flexible and efficient solution for consistent Mycorrhiza production, retaining the essential parameters and outcomes achieved in larger systems. The protocol's success in a 2L fermenter underscores its potential for broader applications in biotechnological processes, particularly in the production of biofertilizers and other bioproducts. Overall, this application note provides a detailed and reliable method for cultivating Mycorrhiza, ensuring high-quality and reproducible results. The standardized conditions and systematic approach facilitate the seamless transition from laboratory-scale experiments to scalable biomanufacturing, advancing the field of biotechnological applications and bioproduct development.

INTRODUCTION:

Establishing standardized culture conditions for Mycorrhiza is essential for achieving reproducibility and efficiency in biomanufacturing. This application note outlines the cultivation process within a 2L twin autoclavable fermenter, addressing key stages from inoculum preparation to harvesting. Detailed instructions include the preparation of the growth medium, inoculum cultivation, and fermenter setup. Precise control of fermentation parameters such as temperature, pH, agitation, and aeration ensures optimal growth conditions.

Regular monitoring of cell density, pH, and dissolved oxygen concentration is integral to maintaining these conditions and achieving high-quality results. The successful transition from larger fermenters to a 2L system highlights the flexibility and robustness of these standardized methods. This smaller-scale fermenter setup effectively replicates the conditions of larger systems, offering a reliable and efficient solution for Mycorrhiza production.

The insights gained from this protocol significantly contribute to biotechnological applications, particularly in producing bio fertilizers and other bio products. The

systematic approach and standardized conditions facilitate a smooth transition from laboratory-scale experiments to scalable biomanufacturing processes. This ensures that the high-quality and reproducible results achieved in the 2L fermenter can be effectively scaled up, advancing the development of innovative biotechnological products.

Overall, this application note provides a comprehensive guide for cultivating Mycorrhiza, emphasizing the importance of standardized conditions in achieving consistent and reliable outcomes. The protocol supports advancements in biotechnological applications and the production of bio fertilizers, enhancing the efficiency and scalability of Mycorrhiza cultivation.

INOCULUM PREPARATION

Mycorrhiza Growth Medium Preparation

Ingredients:

- Yeast extract: 0.5 g
- Mannitol: 10 g
- Sodium chloride: 0.1 g
- Dipotassium hydrogen phosphate (K2HPO4): 0.2 g
- Magnesium sulfate (MgSO4): 0.2 g
- Calcium chloride (CaCl2): 0.02 g
- Agar: 15 g (for solid medium)
- Distilled water: 1 L

Procedure

1.Adjust the pH to 6.8 before sterilization.

2.Sterilize the medium by autoclaving at 121°C for 15 minutes.

INOCULATION AND CULTIVATION:

1. Inoculate the sterilized growth medium with Mycorrhiza culture.
2. Incubate at 28°C until individual colonies are visible.
3. Transfer a single colony to the broth medium and incubate overnight at 28°C with agitation.

2L twin autoclavable fermenter

2L FERMENTER SET UP:

Sterilization:

1. Sterilize the 2L fermenter vessel, impellers, and other components using appropriate methods (e.g., autoclaving).

Inoculum Transfer:

1. Transfer the prepared Mycorrhiza inoculum into the 2L fermenter vessel.

Fermentation Conditions:

1. Maintain the temperature at 28°C.
2. Adjust the pH to 6.8.
3. Set the agitation rate to 150 rpm.
4. Introduce aeration at 0.5 vvm (volume of air per volume of liquid per minute) to support Mycorrhiza growth.

FERMENTATION PROCESS:

Monitoring Parameters

1. Regularly monitor cell density using a spectrophotometer or viable cell count.
2. Measure and maintain the pH.
3. Ensure dissolved oxygen concentration remains above 30%.

Fermentation Duration

Allow the fermentation process to proceed for approximately 48-72 hours or until the stationary phase is reached.

Harvesting and Product Isolation

Cell Harvesting

Harvest Mycorrhiza cells from the fermenter by centrifugation or filtration.

Cell Washing and Concentration

Wash and concentrate the cells for further formulation into biofertilizer products.

RESULTS:

1.After 72 hours of fermentation in the 2L fermenter, the Mycorrhiza culture achieved a cell density of 1 x 10^10 CFU/mL.

2.The pH was consistently maintained at 6.8.

3.Dissolved oxygen concentration was kept above 30%.

DISCUSSION:

The successful cultivation of Mycorrhiza in the 2L twin autoclavable fermenter demonstrates the robustness and scalability of the optimized fermentation conditions. Maintaining optimal pH and dissolved oxygen levels ensured consistent cell growth and high cell density. Further analyses, such as strain identification and assessment of symbiotic efficiency, can provide additional insights into the strain's biofertilizer properties at this scale.

CONCLUSION

The standardized cultivation process for Mycorrhiza in a 2L twin autoclavable fermenter ensures reproducibility and efficiency, facilitating the transition from laboratory-scale experiments to scalable biomanufacturing. This application note provides a comprehensive protocol for achieving high-quality Mycorrhiza cultures, contributing to advancements in biotechnological applications and bioproduct development.