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Department of Chemical Engineering

Controlled Microbial Growth for high value Antibiotics Production

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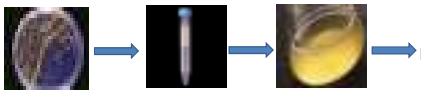
ABSTRACT

The aim of this work is to develop innovative technique for production of antibiotics like Mupirocin, Vancomycin produced from Pseudomonas fluorescence and Amycolatopsis orientalis respectively using simple & cost effective method. Detection technique used is UV-VIS Spectrophotometer and HPLC(High Performance Liquid Chromatography). Different kinetics data was obtained for the growth of Pseudomonas fluorescence and Amycolatopsis orientalis in Luria Broth(LB) and Trypticase Soy Broth (TSB) respectively, at 30°C. Production and detection of Mupirocin and Vancomycin has also been done at lab scale and it has been seen that amount of Mupirocin and Vancomycin have increased to about 3 times using our technique.

1. INTRODUCTION

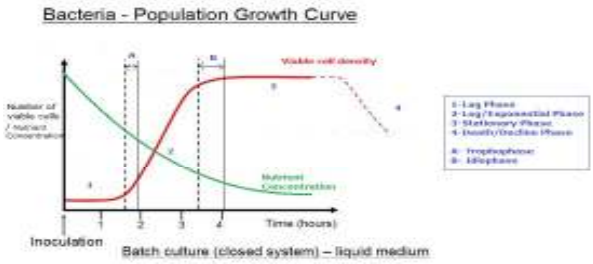
Pseudomonas fluorescence and Amycolatopsis orientalis are gram negative rod shaped bacteria used mainly for the production of Mupirocin and Vancomycin antibiotics. The optimum growth condition is 28-32°C. Mupirocin is a bacteriostatic at low concentration and bactericidal at high concentration. Both antibiotics are active against gram positive bacteria like Staphylococcus Aureus. Both antibiotics have high economical value, so there is a need to develop different technique to increase the productivity of these antibiotics with minimal of resources.

Stages



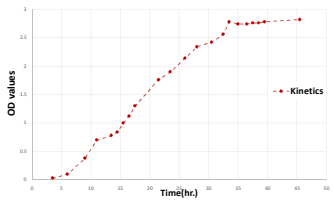
Cell culture Inoculum Fermented Broth

Organism/ Media	Secondary Metabolite	Fermentation Conditions		Detection Parameters (HPLC C18)	
Pseudomonas fluorescence/ LB	Mupirocin	Temperature	30°C	Mobile Phase	27.5:72.5 Acetonitrile and Ammonium Acetate(.05M)
		RPM	200	UV Wavelength	221 nm
Amycolatopsis orientalis/ TSB	Vancomycin	Temperature	30°C	Mobile Phase	30:65:5 Methanol, Water and Ammonium Acetate (0.2 M)
		RPM	240	UV Wavelength	280 nm

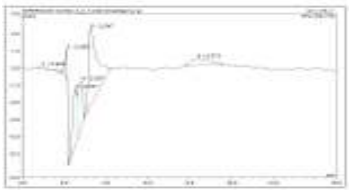


2. MATERIALS & METHODS

Pseudomonas fluorescence was grown in Luria Broth media for about 34 hour which is the onset of idiophase. Bacterial cells were separated using centrifugation technique. Optical density was monitored time to time using UV Vis Spectrophotometer, the product obtained after fermentation was analyzed using High Performance Liquid Chromatography(HPLC) technique.



Kinetic study of Pseudomonas Fluorescence



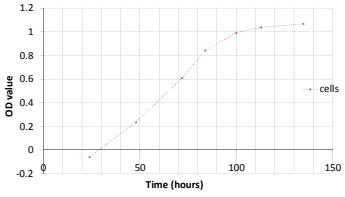
HPLC detection of Mupirocin



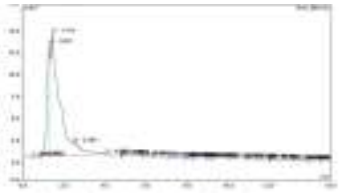
Antibiotics Sensitivity test using Staphylococcus Aureus



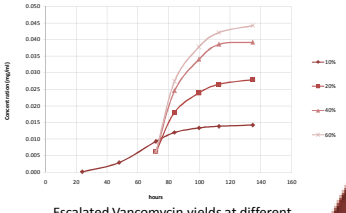
Enlarged view of AST



Kinetic study of Amycolatopsis orientalis



HPLC detection of Vancomycin



Escalated Vancomycin yields at different inoculation percentages

3. RESULT & CONCLUSION

Mupirocin and Vancomycin both were produced at lab scale. Mupirocin content in supernatant crude was 26 µg/ml as measured by HPLC method, its content get increased to 66µg/ml (2.6 times) by inducing stressed condition i.e adding 2x times cell at the onset of idiophase. Vancomycin content in natural (unstressed) conditions was 15 µg/ml which rose to 27, 39 and 43 µg/ml for 2x, 4x and 6x times cell inoculation at the onset of idiophase. A continuous reactor scheme has been designed to harvest these escalated yields in regular cycles.