Studies on factors affecting the microbial biotransformation of Tetracycline in the presence of various Bacteria and Fungi and Immobilized organisms

Uma Maheswari Kolipaka[1], Bodla R.B[*1]

[1]Research scholar, DIPSAR: Delhi Institute of Pharmaceutical Sciences and Research,

Constituent College of Delhi Pharmaceutical Sciences and Research University (DPSRU),

M.B Road, Pushpa Vihar, Sector-3, New Delhi-110017

[*]Corresponding Author,

Dr.Bodla R.B

Associate Professor,

Department of Pharmaceutical Chemistry, DIPSAR,

New Delhi-110017

E.mail: rameshbodla@gmail.com

Abstract:

Even though numerous antibiotic approaches were suggested that, they can transform antibiotics, little is known about whether or not and how microbiological techniques may degrade antibiotics inside the surroundings. This work involves the Transformation of Tetracycline's using bacteria such as, *Staphylococcus aurous* and *Escherichia coli* and two strains of filamentous fungi, namely *Aspergillums Niger* and *Candida albinos*. And characterized the biotransformation of tetracycline via that microorganisms below various environmental situations. The biotransformation rate was the very best while the initial pH become 9 and the reaction temperature was at 30ºC which may be described by the usage of the Michaelis-Menten model underneath distinct preliminary tetracycline concentrations. Whilst the extra substrate turned into a gift, the substrate that triggered expanded biomass led to a decreased biotransformation rate of tetracycline. Results from this investigation lead to a better estimation of the destiny and shipping of antibiotics to modify/ transformed which will be having more advantages than that of existing forms.

Key words: Biotransformation, Tetracycline; Staphylococcus aureus, E. Coli, *Candida albicans, Aspergillus niger,* Tetracycline.

Introduction:

Biotransformation is a vital process wherein organic compounds undergo a conversion from one form to another, aiming to mitigate the persistence and toxicity of chemical substances. This intricate process relies on a diverse array of microorganisms, including bacteria, fungi, and enzymes. Its significance extends to synthesizing compounds or materials, particularly when conventional synthetic methods pose challenges. Natural transformation, though gradual, nonspecific, and less productive, sets the stage for the burgeoning importance of microbial biotransformation's or microbial biotechnology. These techniques are extensively employed to generate bulk amounts of metabolites with enhanced specificity. This review seeks to evaluate the impact of microbial biotransformation on steroids, antibiotics, various pollutants, and xenobiotic compounds.

Types of Biotransformation:

Biotransformation manifests in two main types: enzymatic and non-enzymatic. Enzymatic transformations further classify into microsomal and non-microsomal categories. Enzymatic elimination occurs through various enzymes in the body. Microsomal biotransformation involves enzymes within the lipophilic membranes of the smooth endoplasmic reticulum, while non-microsomal biotransformation utilizes enzymes found within mitochondria. Examples include alcohol dehydrogenase metabolizing ethanol into acetaldehyde and tyrosine hydrolases converting hypoxanthine into xanthine. Spontaneous, non-catalyzed, and non-enzymatic biotransformation's are relevant for highly active, unstable compounds occurring at physiological pH. Examples include the conversion of chlorazepate into desmethyl diazepam and must in HCl into ethyleneimonium.

Microbial Biotransformation in Various Applications:

Microbial biotransformation stands as a versatile tool in transforming various pollutants and compounds, including hydrocarbons, pharmaceutical substances, and metals. These transformations fall into categories such as oxidation, reduction, hydrolysis, isomerisation, condensation, formation of new carbon bonds, and introduction of functional groups. Over centuries, microbial biotransformation has been instrumental in producing various chemicals crucial for the food, pharmaceutical, agrochemical, and other industries.

In the realm of pharmaceutical research and development, biotransformation studies play a pivotal role in understanding compound metabolism using animal models. Subsequently, the microbial biotransformation phenomenon aids in comparing drug metabolic pathways and scaling up metabolites discovered in animal models for comprehensive pharmacological and toxicological evaluation.

White Biotechnology:

White biotechnology leverages microbial biotransformation for generating valuable products. Living cells such as bacteria, filamentous fungi, animals, plants, algae, yeast, and actinomycetes find applications in this domain. Microbial cells are preferred for biotransformation due to their high surface-volume ratio, faster growth rates reducing biomass transformation time, efficient substrate transformation due to higher metabolism rates, and ease of maintaining sterile conditions.

Procedure for Biotransformation:

The procedure for biotransformation involves utilizing vegetative cells, spores, resting cells, enzymes, and immobilized cells/enzymes. In growing cultures, the strain is cultivated in a suitable medium, and a concentrated substrate solution is added after the culture's suitable growth period (6-24h). Emulsifiers or solvents may be used to solubilize poorly soluble compounds. Large-scale transformations occur under sterile conditions in aerated and stirred fermenters, monitored chromatographically or spectroscopic ally. The process concludes when a maximum titer is achieved. Resting cells, providing advantages like elimination of growth inhibition by the substrate, and immobilized cells, allowing for continuous processes, are commonly employed in transformation processes.

End products of transformation reactions are found extracellular and may be in dissolved or suspended forms. Recovery methods include washing cell material with water or organic solvents to detach the reaction product, followed by various techniques based on product solubility.

Various Applications of Microbial Biotransformation:

Transformation of steroids and sterols

Transformation of non-steroid compounds

Dihydroxyacetone from glycerol

Prostaglandins

L-Ascorbic acid (vitamin C)

Transformation of antibiotics

Transformation of pesticides

Transformation of pollutants

Petroleum biotransformation

Methodology:

The microbial biotransformation of tetracyclines was investigated through submerged fermentation. Strains of Staphylococcus aureus and Escherichia coli were cultured in LB medium, while Candida albicans and Aspergillus niger were grown in PYG medium, each containing 20 mg L -1 tetracycline, at 30°C on a shaker set at 150 rpm. Upon reaching the mid-exponential phase, cells were harvested, washed twice with sterilized physiological saline (0.9% NaCl), and adjusted to OD600nm = 1.00 before being introduced into 50 mL sterilized tetracycline (1%, v/v) in a 250 mL flask [17]. Two experimental series were designed based on preliminary screening results. The first involved testing two bacteria (Staphylococcus aureus, Escherichia coli) and two fungi (Candida albicans, Aspergillus niger). The second series tested immobilized cells of bacteria and fungi.

For both series, the following conditions were upheld:

The initial tetracycline concentration was maintained below 20 mg L-1, with pH levels set at 9.0 and 6.0 for bacteria and fungi, respectively, and temperatures set at 30°C and 26°C, respectively, unless otherwise stated.

All experiments were conducted in triplicate.

Blank controls, without bacteria, fungi, and immobilized cells, were included in all tests.

Flasks were shielded with aluminum foil to prevent photodegradation.

Liquid samples were collected daily for 7 days (bacteria) and every 3 days (fungi up to 15 days), and the parent compound concentration was measured using HPLC.

Rate of Biotransformation of Tetracycline’s:

Submerged fermentation was employed for the biotransformation of tetracycline’s with varying concentrations. The observed decrease in tetracycline concentration was attributed to both hydrolysis and biotransformation in the presence of organisms, including two bacteria and two fungi. In the absence of organisms, the decline in tetracycline concentration was solely attributed to hydrolysis. While not extensively covered in this study, previous literature mentioned various organisms transforming tetracycline’s into multiple derivatives, emphasizing first-order kinetics.

Throughout the two experimental series, an intriguing observation was made: as the tetracycline concentration increased, biomass also increased, yet the transformation rate decreased. Essentially, transformed products were formed in lower concentrations. Notably, immobilized cells exhibited a reduced transformation rate despite maintaining consistent pH and temperature conditions. Among the four organisms tested, Staphylococcus aurous, Escherichia coli, Candida albinos, and Aspergillums Niger, all yielded three derivatives. Immobilized cells similarly produced the same number of derivatives, differing only in the concentration of transformed products.

Factors Affecting Biotransformation:

In our study, we investigated the biotransformation of commercially available tetracycline using various bacteria and fungi. Microbial biotransformation involves the transformation of compounds in the presence of microbes, with enzymes playing a pivotal role. Several factors influence this process, including temperature, pH, substrate concentration, enzyme concentration, product concentration, light, radiation, and time. These factors were systematically examined during the monitoring of tetracycline biotransformation.

Temperature:

The optimal temperature for the most significant biotransformation of tetracycline occurred at 30°C in the presence of Gram-positive and Gram-negative bacteria such as Staphylococcus aureus and E. coli. Deviations from this temperature range, either higher or lower, resulted in a decreased transformation of tetracycline. Fungi, specifically Candida albicans and Aspergillus niger, demonstrated optimal transformation at 26°C.

pH:

Bacteria exhibited maximal tetracycline transformation at pH 9, while fungi achieved optimal transformation at pH 6. Any alterations in pH, whether an increase or decrease, led to a diminished transformation of tetracycline.

Concentration of Enzyme:

Enzyme concentration, assessed through two microbial types—whole microbes (bacteria and fungi) and immobilized microbes—demonstrated a direct correlation with the rate of tetracycline transformation. Increasing the inoculum in accordance with culture medium and substrate concentration resulted in enhanced transformation, indicating the proportional relationship between enzyme concentration and transformation rate.

Substrate Concentration:

Tetracycline concentration acted as the substrate, and its increased concentration initially led to an augmented transformation. However, beyond a certain concentration threshold, further increases did not correspond to a rise in the transformation rate. This trend was represented by a rectangular hyperbola plot, with Tetracycline concentration on the X-axis and the rate of Biotransformation on the Y-axis.

Product Concentration:

Observations during the biotransformation of tetracycline revealed that an increase in the concentration of a transformed compound, after a specific period, resulted in a decrease in the rate of transformation.

Light and Radiation:

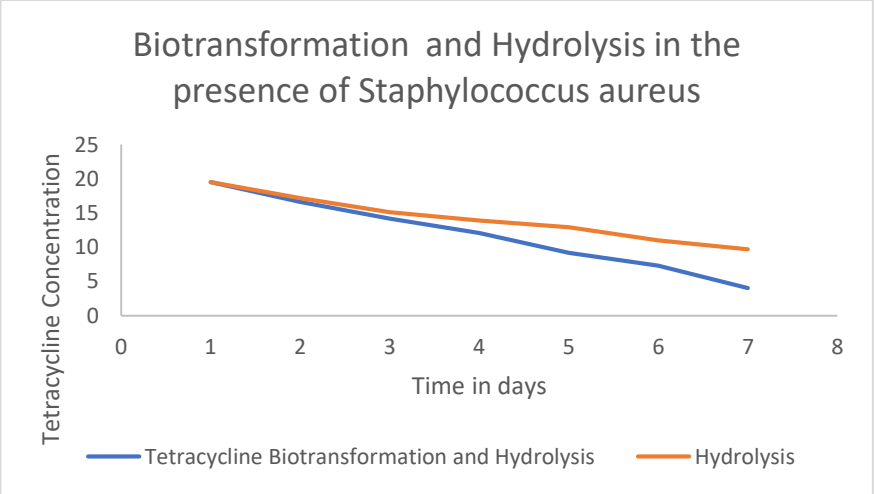
Light and radiation had a significant impact on the transformation of tetracycline. Exposure to UV light led to the lowest transformation, while visible light resulted in a higher concentration of transformed compounds. Changes in light conditions affected the enzymes involved in the transformation process.

Time:

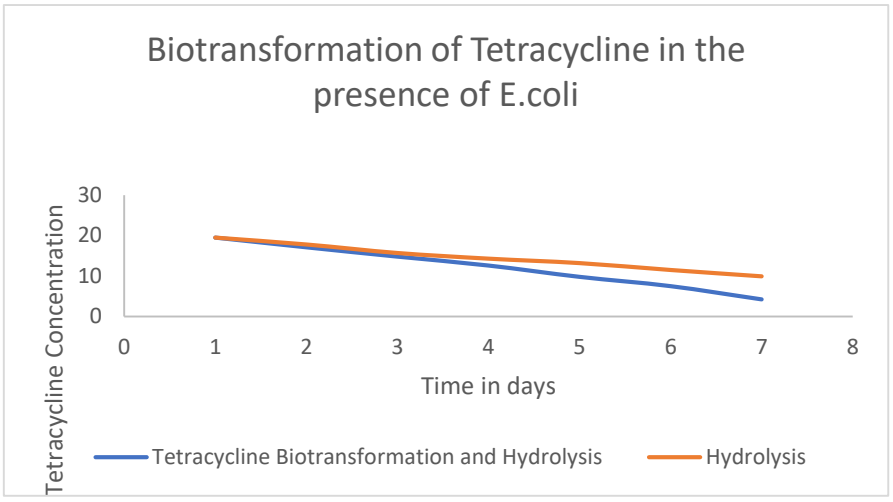
Under suitable environmental conditions, including optimal pH, temperature, microbial concentration, tetracycline concentration, and light exposure, the transformation of tetracycline was achieved in a relatively short time. Bacterial transformation commenced within 24 hours, whereas fungal transformation required approximately 6 days. Graphical representations given below in scheme 1 to schem 6.

Conclusion:

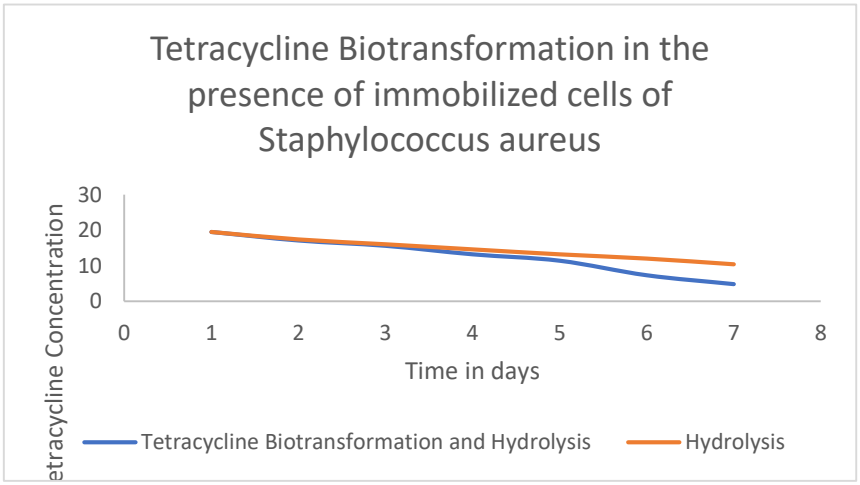
In conclusion, biotransformation, the fundamental process of life, plays a crucial role in various applications. Microbes, extensively employed for steroid biotransformation to produce specific derivatives, offer an alternative to traditional synthetic methods. Furthermore, biotransformation proves effective in addressing environmental challenges, such as the degradation of xenobiotic and petroleum hydrocarbons. In our review, temperature and pH emerged as pivotal factors significantly influencing microbial biotransformation of tetracycline. Immobilized organisms exhibited similar responses to these factors as whole organisms. Hence, microbial biotransformation stands as a valuable asset for the contemporary world, showcasing its versatility across a wide range of applications.



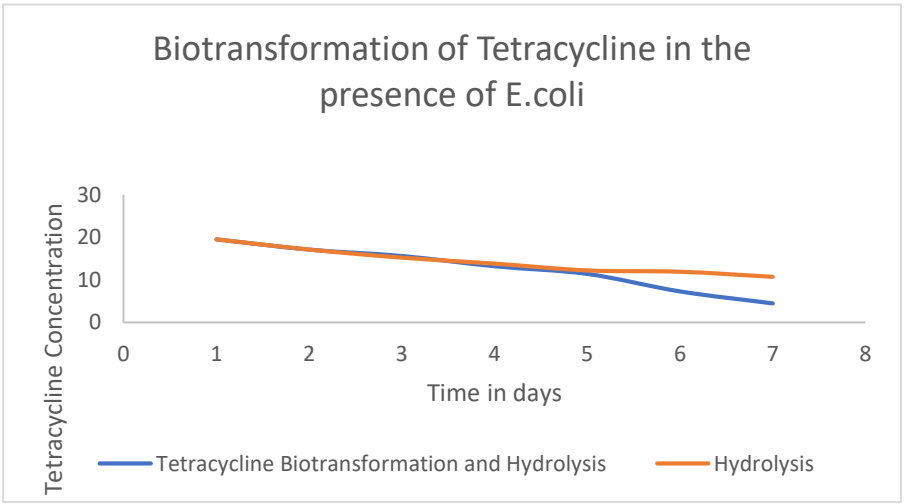
Scheme:1 Residual Concentration of Tetracycline in the presence and absence of Gram-Positive Bacteria *Staphylococcus aureus* at P^H 9 and Temperature 30^0C.



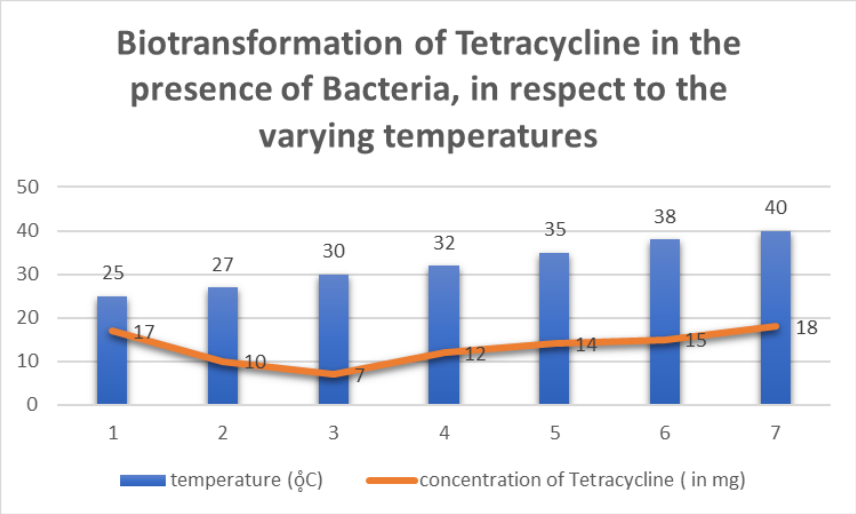
Scheme:2 Residual Concentration of Tetracycline in the presence and absence of Gram-negative Bacteria *Escherichia Coli* at P^H 9 and Temperature 30^0C.



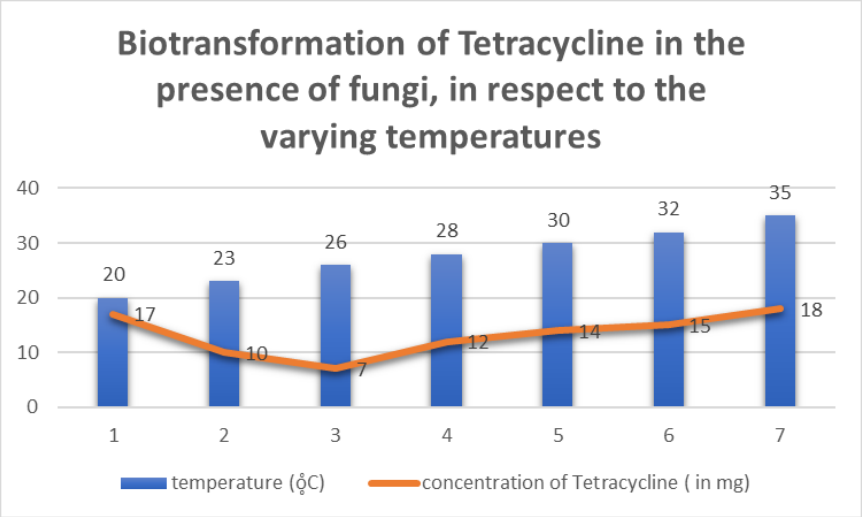
Scheme:3 Residual Concentration of Tetracycline in the presence and absence of Gram-negative Bacteria Immobilized Cells of *Staphylococcus aureus* at P^H 9 and Temperature 30^0C.



Scheme:4 Residual Concentration of Tetracycline in the presence and absence of Gram-negative Bacteria Immobilized Cells of *Escherichia Coli* at P^H 9 and Temperature 30^0C.



Scheme:5 Residual Concentration of Tetracycline in the presence and absence of Bacteria Immobilized Cells of Bacteria at varying temperatures.



Scheme:6 Residual Concentration of Tetracycline in the presence and absence of fungi, Immobilized Cells of fungi, at varying temperatures.

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