

CARBON DI OXIDE SEQUESTRATION DISCERNMENT: ENDEAVORING BIOMIMETIC CALCIUM CARBONATE FOR PRODUCTION OF TYROSINASE AND LACCASE WITH EVALUATION OF ENZYME SYNERGY FOR EFFICIENT HUMIFICATION

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ABSTRACT

Abstract: The sequential rise in the atmospheric carbon dioxide concentration due to anthropogenic emission of this gas has resulted in an elevation of the average mean global temperature resulting in drastic effects circumventing climate change. Carbon dioxide sequestration via mineralization is one of the methods that have the capability to efficiently store carbon dioxide in a stable form. The biomimetic approach involving carbonic Anhydrase has presented a thought-provoking rationale for sequestering carbon dioxide into calcium carbonate which is being concurrently practiced for mushroom production. Tyrosinase and Laccases extracted from

mushroom purified and then subsequently used for the conversion of phenolic waste from a different source to humus. Previous studies on abiotic and biotic catalysis have focused on polyphenols, amino acids, and sugars, while no work has been done on lipids, one of the most refractory components of plant materials and a significant contributor to the humin fraction of soil organic matter. Use of advanced analytical instrumentation, i.e., synchrotron-based X-ray absorption spectroscopy, Spectromicroscopy and infrared spectroscopy, atomic force microscopy, multi-dimensional nuclear magnetic resonance spectroscopy, and the same should shed light on the mystery of environmental humic substances and their complexes with mineral particles.

INTRODUCTION

Carbon dioxide (CO₂) is one of the most important greenhouse gases (GHG). The most dominant source of anthropogenic CO₂ contributing to the rise in atmospheric concentration since the industrial revolution is the combustion of fossil fuels. These emissions are expected to result in global climate change with potentially severe consequences for ecosystems and mankind. In this context, these emissions should be restrained in order to mitigate climate change. (Joris Maarten Koornneef, 2011)

Atmospheric concentration of carbon dioxide (CO₂) has been increasing due to human induce (anthropogenic) activities. There was a general consensus among the scientists that high concentrations of CO₂ in the atmosphere cause global warming. Predictions show that the atmospheric concentrations of CO₂ will continue to increase if the production and release of CO₂ to the atmosphere is not controlled (Ramanathan, 2006). The Kyoto Protocol signed in 1997 required that the industrialized nations need to reduce their CO₂ emissions to 95% of their 1990 levels by 2012 (Bolin, 2005). Thus, many researchers have focused on the development of methods for the mitigation of the CO₂. One of the strategies to control the CO₂ concentration in the atmosphere is to reduce its production and release into the atmosphere. For this purpose, less carbon intensive energy sources such as wind energy, solar energy, and nuclear energy, could be used. The other strategy could be to increase the efficiency of the energy use, from production to its end use (Reichle et al., 2009). However, these two options are not practically possible. Therefore, a third option is required which is to capture and dispose the produced CO₂ in a safe manner (sequestration) (Reichle et al., 2009).

Carbon capture and sequestration (CCS) techniques have been perceived as one of the main solutions to the increasing threat of anthropogenic emissions. CCS techniques, methods, and technologies mainly involve the use of a carbon sink, wherein carbon dioxide can be diverted from the atmosphere whilst being stored in a secure and reliable manner. These CCS methods can be classified into three main categories according to their sinks—namely oceanic, terrestrial, and geologic. Oceanic carbon sequestration involves either injecting CO₂ deep into the ocean or promoting photosynthetic CO₂ fixation by aquatic organisms to

sequester CO₂, whereas soil and vegetation (such as forests) are considered the sinks for terrestrial carbon sequestration. On the other hand, geologic carbon sequestration utilizes rock formations and deep land traps, seams, and aquifers to securely store carbon. Most of these methods are highly efficient as carbon capture and sequestration can reach up to 100% efficiency for most processes; however, some of these technologies are energy-intensive and cost-ineffective, and thus, cannot be used for industrial application. (Bernard et al., 2019).

A number of CO₂ sequestration options have been proposed. These were placement in the deep oceans; placement in geologic formations such as deep saline aquifers, abandoned oil or gas reservoirs and unmineable coal seams, and consumption via advanced chemical and biological processes (Reichle et al., 2009). However, none of these options has been proven to be ideal for CO₂ sequestration and are still under investigation. Nevertheless, CO₂ has to be pure in these sequestration processes (Bachu, 2000; Gentzis, 2000). That is, the CO₂ has to be captured from flue gases, to be compressed, to be transported, and finally, to be injected into the desired sequestration sites, which all together requires increasing the electric bill to the customers. The sequestration cost could be reduced if the CO₂ is captured and sequestered directly at the production sites. Furthermore, the production of valuable products from the CO₂ could be possible such as the nano-sized precipitated CaCO₃, which make the sequestration process economically desirable, and even profitable. In order to capture CO₂ from the flue gases, the CO₂ should undergo a number of transformations such as the dissolution in an aqueous phase, hydration by water, ionization, and carbonate formation. Among these, the hydration of the CO₂ is the slowest step. The hydration of CO₂ can be enhanced by Carbonic Anhydrase (CA) (Bond et al., 2001). However, there are some disadvantages using the free enzyme in solution such that the stability of the enzyme is low, its repeatable usage is limited, and recovery from the reaction environment generally won't be possible. These disadvantages can be eliminated by immobilizing the enzyme. There are some enzyme immobilization techniques such as entrapment in matrices, adsorption on the solid surfaces, covalent bonding, and cross-linking within polymeric networks (Shuler and Kargi, 2002). Carbonic anhydrase has been immobilized on some solid and polymeric supports such as acrylamide, chitosan, chitosan-alginate bead (Liu et al., 2005), porous silica and graphite bead, within the poly (acrylic acid-co-acrylamide) hydrogen (Cheng et al., 2008), and on a hydrophobic adsorbent of Sepharose 4B via hexyl, octyl, dodecyl, and palmitic glycidyl ethers couplings (Hosseinkhani & Gorgani, 2003).

The concept of generating biomimetic humus; conceives the sequestration of CO₂ through a novel approach that involves conversion of CO₂ into H⁺ and HCO₃⁻ ions mediated by bacterial carbonic Anhydrase, the product can be utilized through different pathways resulting in the formation of dihydric phenol. These compounds can be converted into polyphenolic catecholcatalyzed by mushroom tyrosinases.

The biomimetic approach involves the sequestration of carbon di oxide into calcium carbonate although an environmentally benign product it requires a sheltered place to dump, however scarcity of land in developing country like India encourages utilization of resources in a cyclic pathways.

The assortment of calcium carbonate and calcium sulphate, known as gypsum is used in different concentration for the production of mushroom spawn, irrespective of variety of mushroom involved. This new approach thus advocates the utilization of biomimetically formed calcium carbonate for production of mushroom followed by extraction of enzyme tyrosinases and its further application in conversion of phenols and carboxylated phenol to humus. A successful demonstration of this process can be followed by characterization and mass production of enzyme for commercial application of the process. Value added products like laccases are wide spread in basidiomycetes and along with tyrosinase form a unique complex of bioactive substances and are involved in the degradation of lignocellulosic substances. The lignolytic enzyme complex including laccase are secreted into environment where they participate in lignin degradation play crucial role alongside tyrosinase in synthesis and degradation of humic substances.

CURRENT CONCEPTS ON THE NATURE OF HUMIC SUBSTANCES

Humic substances (HS) are viewed as an inherent component of soil organic matter, which plays multiple life-sustaining functions in the environment. The complexity of the molecular ensemble of humic substances is reflected in singular physicochemical features and results in uniquely broad and diverse interactions with both abiotic components and living organisms directed towards adaptation of life to its abiotic environment. The reported examples of mediating roles of HS in soil range from the transport of available nutrients in soil solution (Olaetxea et al. 2018) to the remediation of contaminated soils (Perminova & Hatfield 2005)

Traditionally humic substances (HS) were viewed as heterogeneous, high molecular weight polymers, for example, the widely accepted definition according to Gillett “HS are a category of naturally occurring, biogenic, heterogeneous organic substances that can be generally characterized as being yellow-to-black in colour, of high molecular weight, and refractory”. (Temperinic et al., 2005) provided a broader definition for the term: “HS refers to a category of naturally occurring materials found in or extracted from soils, sediments, and natural waters”.

They result from the decomposition of plant and animal residues. Despite the important role of HS in the sustainability of life, their chemical nature and reactivities still remain poorly understood and there remains some contention with regards to their molecular structures. Another widely known school of thought states that HS are formed by polymerization and polycondensation of simple biomolecules derived from the degradation of biological residues. However, there is no conclusive evidence to disprove either view. A number of reports support the polymer-sorption model for HAs. Sorption nonlinearity in the undissolved phase is attributed to polymer properties of the sorbent; hysteresis and conditional effects can up to now only be explained with the polymer analogy.

According to the chemical terminology of the International Union of Pure and Applied Chemistry (IUPAC), a macromolecule (polymer molecule) is a molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass (The Royal Society, 2005). Polymer molecules do not have a definite formula since they

consist of chains of different lengths. The IUPAC definition of a supramolecule is a system of two or more molecular entities held together and organized by means of intermolecular (noncovalent) binding interactions. Macromolecules as well as small molecules tend to form supramolecular structures, the properties of which largely determine the chemical and physical nature of the whole material (Silverman et al., 2009).

Although the supramolecular model has not explicitly been shown for unfractionated dissolved organic matter (DOM) and unaltered humic substances including humin, the combination of all studies suggests supramolecular as well as macromolecular characteristics of natural organic matter (NOM). Neither macromolecules nor supramolecules can be excluded in solid and dissolved NOM. Recent studies have shown that the soil biotic community is able to disintegrate any organic matter of natural origin including black C unless it is physically inaccessible and/or chemically protected. Therefore, mere associations of biological residues and metabolites would not be able to make up the stable environmental HS and to account for their darkness in color.

Mere hydrophobic interactions and hydrogen bonding between colorless biomolecules such as lipids, proteins, and polysaccharides cannot provide a logical explanation of the browning reaction and the resultant dark color of HS.

Browning of biomolecules during oxidative degradative processes is produced by five known biochemical pathways in nature:

- The Maillard reaction (condensation reaction of reducing sugars and amino compounds to produce melanoidins),
- Oxidation of polyphenols to produce quinones and subsequent polymerization reactions of quinones (enzymatic and non-enzymatic reactions),
- Quinone-amine polycondensation reactions
- Ascorbic acid browning, and
- Oxidative lipid-protein polycondensation reactions (Silverman et al., 2005).

Most of these browning reactions involve carbonylamine reactions which result in the formation of highly colored high and low molecular weight polymers (Wasser, 2002). Quinones are produced during the decomposition of lignin and by the oxidation of polyphenols; they are highly unstable and very reactive in aqueous media (Waid, 1999). They readily combine with 10 amine, sulfhydryl, phenol, and indole and imidazole groups of amino acids, peptides and proteins to give even more intensely colored products than simple quinones or phenol polymers. Abundant research evidence at the molecular level shows that biomolecules such as amino acids, sugars, and polyphenols, derived from the breakdown of biological residues and from biological metabolites, undergo polymerization and/or polycondensation especially by catalysis of enzymes and mineral particles (clay minerals, short-range ordered Mn, Fe, and Al oxides and (oxy)hydroxides, and primary minerals) These reactions evidently account for the browning reaction and the darkness in color of HS. Furthermore, HS are known to contain free radicals, as shown by electron paramagnetic resonance studies (Whitford, 2005). Free radicals drive polymerization reactions with other biomolecules and organic pollutants. Black C is another possible contributor to the color of

HS in soils, which forms as a result of condensation of aromatic structures of organic residues during the burning process.

The sequential rise in the atmospheric carbon di oxide concentration due to anthropogenic emission of this gas has resulted in elevation of the average mean global temperature resulting in a drastic effects circumventing to climate change. Biomimetic approach involving carbonic Anhydrase has provided an interesting rationale for sequestering carbon di oxide into calcium carbonate which will be concurrently used for mushroom production. Tyrosinase and Laccases extracted from mushroom purified, and then subsequently used for the conversion of phenolic waste from different source to humus.

Our knowledge on the intrinsic mechanisms of environmental processes pertaining to the genesis of humic substances in nature remains to be advanced. Previous studies on abiotic and biotic catalysis have focused on lipids, amino acids and sugars, while no work has been done on polyphenols, one of the most refractory components of mushrooms and a significant contribution to the humic fraction of soil organic matter. The interactions of lipids, proteins and polyphenols in the presence of these catalysts remain to be studied. Our understanding of the influence of pedogenic factors and anthropogenic activities on the transformation of biological constituents to humic substances and the nature and properties of the resultant mineral-humus complexes are still very limited. A vast majority of environmental organic matter is associated with mineral particles. More research should be conducted to uncover the impact of physical chemical-biological interfacial reactions on biogeochemical reactions, which, in turn, govern the humification processes and the formation of mineral-humus complexes. Also additional research about the type of organisms responding to the transformation of humic substances as well as their role is needed. Use of advanced analytical instrumentation, i.e., synchrotron-based X-ray absorption spectroscopy, Spectro microscopy and infrared spectroscopy and the like should shed light on the mystery of environmental humic substances and their complexes with mineral particles.

Fundamental understanding of this subject matter at the molecular level and the impacts on the ecosystem would facilitate our development of innovative management strategies to regulate the behavior of the ecosystem on a global scale. Future research on this extremely important and exciting area of science should be stimulated to restore as well as sustain ecosystem integrity.

CONCLUSION

Fundamental understanding of this subject matter at the molecular level and the impacts on the ecosystem would facilitate our development of innovative management strategies to regulate the behavior of the ecosystem on a global scale. Future research toward this extremely significant and exciting area of science should be stimulated to restore as well as sustain ecosystem integrity. The present study forecasted a holistic approach with rational insinuation foretelling the course of sustainable development mediated by an efficient carbon management strategy corresponding to low economic input along with protractible output about an efficient carbon dioxide sequestration process.

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