IMPROVING K_{MO} VIA ENZYME ENGINEERING FOR INDUSTRIALLY COMPETITIVE OXIDASES

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To date, protein engineering has enabled the optimization of many existing enzymes, as well as the creation of entirely new biocatalytic reactions that were previously unknown in nature. This, however, is only the first step towards the achievement of industrially competitive biocatalysts[1].

Oxygen-dependent enzymes are among the most interesting biocatalysts for biocatalytic chemical synthesis due to their versatility and ability to selectively oxidize a broad range of chemicals in a high selectivity fashion[2]. However, the utilization of molecular oxygen in biocatalytic chemical synthesis, especially at a large scale, presents a few physicochemical constraints:

1) The low transfer rate of oxygen from the gas to the aqueous phase[3]
2) The low solubility of O_2 in aqueous solutions at conditions where most industrial processes operate and the enzyme performance is best[4]
3) The low affinity that some enzymes have towards oxygen[5]

The combination of these factors often makes oxygen availability the bottleneck for oxidative reactions.

This work intends to use novel technologies such as the Tube-in-Tube reactor to understand the kinetic constants of oxidases (K_{MO}), under continuous operational conditions, in more detail[6]. We will intend to further improve the oxygen-related constants by performing enzyme engineering on several oxidases by employing directed evolution. Furthermore, the data collected from the continuous system will be analyzed by using Python-based data analysis and machine learning, which will then become an important tool for understanding and improving the productivity and efficiency of protein-based industrial processes.

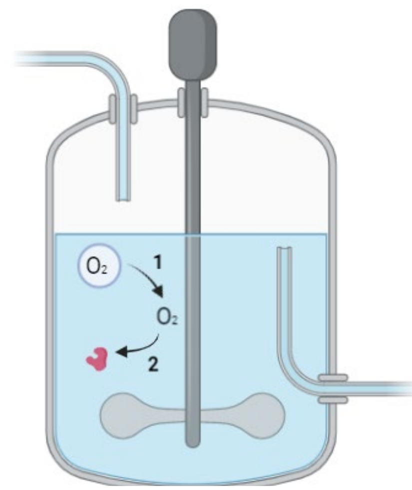

Figure 1: Process of solubilization of oxygen until it is utilized by the enzyme. Oxygen in the air bubbles solubilizes into the media (1). Once solubilized, O_2 can be used by the enzyme (2).

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