

Figure 1. Biodiesel Production in the U.S.

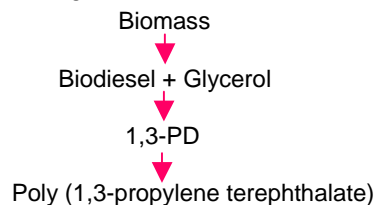


Figure 3. Proposed Value-Added Biodiesel Production

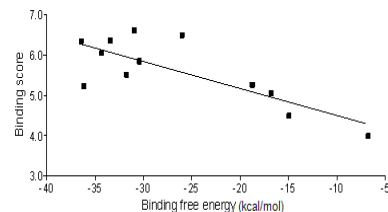
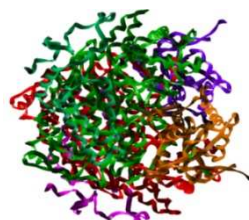


Figure 2. Binding Score vs. Binding Free Energy of B12-dependent GDHs



- Red and light green chains are α subunits
- Purple and pink chains are β subunits
- Orange and green chains are γ subunits

Figure 4. B12-dependent GDH from SYBYL

Table 1. Binding Scores of B12-dependent GDHs

Organisms	Binding free energy (kcal/mol)	Binding score
<i>Salmonella enterica</i>	-18.722	5.26
<i>Mycobacterium sp. KMS</i>	-14.937	4.48
<i>Thermoanaerobacter sp.</i>	-6.736	3.98
<i>Shigella sonnei</i>	-25.998	6.49
<i>Clostridium perfringens</i>	-31.733	5.51
<i>Mycobacterium smegmatis</i>	-36.186	5.22
<i>Klebsiella pneumoniae</i>	-34.295	6.05
<i>Listeria welshimeri</i>	-30.942	6.62
<i>Salmonella enterica</i>	-36.424	6.34
<i>Mesorhizobium loti</i>	-30.407	5.84
<i>Streptococcus sanguinis</i>	-16.752	5.05
<i>Lactobacillus reuteri</i>	-33.423	6.34

Table 2. Binding Scores of B12-independent GDHs

Organisms	Binding free energy (kcal/mol)	Binding score
<i>Clostridium butyricum</i>	-19.162	6.64
<i>Pelobacter carbinolicus</i>	-4.270	4.64
<i>Roseburia inulinivorans</i>	-23.485	6.48
<i>Epulopiscium sp</i>	-22.825	4.23
<i>Clostridium difficile</i>	-17.194	4.36

Table 3. Number of H-bond Interactions and Binding Energy between Subunits of GDHs

Subunits of enzymes	Organisms	Percentage of subunit surface buried in the interface	No. of H bonds	Total binding energy (kcal/mol)
Between two α	<i>Klebsiella pneumoniae</i>	12.0% of each α	72	-3904.11
Between α and β	<i>Klebsiella pneumoniae</i>	5.0% of α ; 15.6% β	31	-1075.00
Between α and γ	<i>Klebsiella pneumoniae</i>	6.5% of α ; 33.5% of γ	54	-1760.67
Between two A	<i>Clostridium butyricum</i>	33.1% of each A	8	-8,200.74

The tremendous growth of biodiesel in Europe and the U.S. (Fig.1) has significantly affected the glycerol market prices. One of the economically viable ways to valorize glycerol from the biodiesel industry is the conversion of glycerol into a high-priced chemical such as 1,3-PD (Fig. 3). It is of importance to identify the properties of enzymes that are involved in the reactions for microbial production of 1,3-PD using renewable feedstock as substrates. The two enzymes, glycerol dehydratases (GDHs) and NADH-linked 1,3-PD dehydrogenase, play a critical role in reducing glycerol to 1,3-PD. GDHs are classified into two categories: B12-dependent and -independent GDHs as shown in Fig. 4 & 5. Sequence research (Fig. 6) and phylogenetic analysis were carried out for both B12-dependent and -independent GDHs. Molecular docking simulations were performed with the 3D structural models of GDHs generated on the basis of the available crystal structures in the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) in order to study the binding features of glycerol to B12-dependent and -independent GDHs (Tables 1 & 2). The enzymes that have high values of binding score and binding energy are considered to have prominent product binding properties. The binding scores evaluated by Surflex-Dock in SYBYL for B12-dependent GDHs showed a satisfactory correlation with the binding energies (Fig. 2). The interactions between subunits were calculated based on the structure information in the RCSB PDB by using SYBYL (Table 3). The enzymes with high subunit binding energies are considered to have stronger chain interactions.

Conclusions and Future Work:

Estimated total binding energy between subunits states that the interactions between two dimers in B12-independent GDHs are stronger than those in B12-dependent GDHs (Table 3). The binding scores and binding energies calculated states that the four B12-dependent GDHs as well as two B12-independent GDHs have the best substrate-product binding properties (Table 1 & 2). In order to optimize the biosynthesis of 1,3-PD from glycerol, kinetic properties of the proposed reaction pathways will be estimated using the ONIOM (Our own N-layered Integrated molecular Orbital + molecular Mechanics) method in Gaussian03.

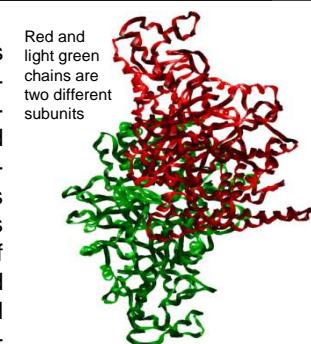


Figure 5. B12-independent GDH from SYBYL

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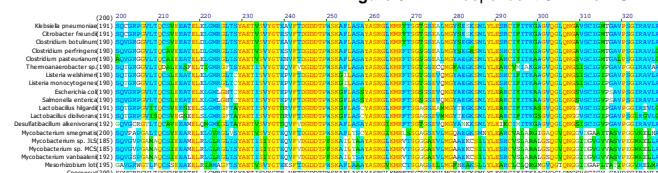


Figure 6. Sequence Analysis from VectorNTI