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MODELLING OF MICROALGAE DRYING PROCESS USING COMPUTATIONAL METHODS: A FIRST STEP

Robby B. Manrique[1a], Aristotle T. Ubando[1b,3], Alvin B. Culaba[1c,3], Al Rey C. Villagracia[2d], Melanie Y. David[2e], Nelson B. Arboleda Jr
[1] [1]*Mechnical Engineering Department, De La Salle University Manila*
[2]*Physics Department, De La Salle University Manila*
[3]*Center for Engineering and Sustainable Development Research (CESDR), De La Salle University, Manila*
[a]robby_manrique@yahoo.com
[b]aristotle.ubando@dlsu.edu.ph
[c]alvin.culaba@dlsu.edu.ph
[d]al.villagracia@dlsu.edu.ph
[e]melanie.david@dlsu.edu.ph
[f]nelson.arboleda@dlsu.edu.ph

Abstract: Biofuels are biomass derived fuels which is considered to have a low carbon emitting characteristics. Biodiesel is one type of biofuels that was introduced in the public and plays a crucial role in the global energy demand particularly in the transport sector. Challenges in making biodiesel commercialization include minimization of the energy inputs on the every process particularly the drying process. Drying microalgae is one of the post harvesting process in converting algal biomass to biodiesel which accounts for 20-30% of the total production cost and energy consumption. A Search for a drying method that requires less energy intensive is needed to address a more efficient production of oil from microalgae for biofuels. This paper is the first step in modelling the drying process wherein a nanoscale understanding on the structure and components of microalgae will be discussed. The main objective of this paper is to model the topology (molecular geometry) of microalgae particularly the lipid bilayer using GROMACS software. The lipid bilayer is made up of phospholipid molecules arranged parallel to each other which serves as a barrier of the many eukaryotic cells. The modelling of the topology includes energy minimization and equilibration which is needed to avoid erroneous results on the potential energy prior to performing molecular dynamics of the entire system. Corresponding results may transpire a new perspective in extracting water from microalgae instead of the traditional drying methods which are highly energy intensive.

Key Words: Microalgae; Drying; biofuels; Molecular dynamics; GROMACS

1. INTRODUCTION

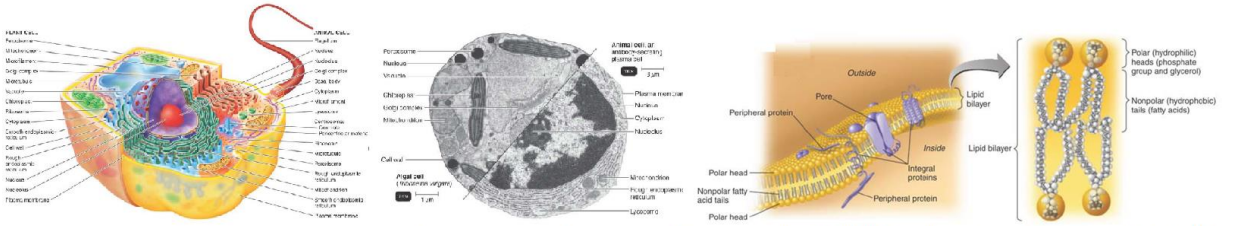
Microalgae as source of biodiesel

Extensive usage of fossil fuels as form of energy has been a major contributor of global warming. Approximately 80% of the global energy demand for transportation, electricity and energy generation is being shouldered by fossil fuels (Hallenbeck and Bennemann, 2002). In 2008, the world energy consumption registered a total of 533 Exajoule (EJ) while the corresponding CO2 emissions of 30.2 billion metric tons (IEO,2011). The energy consumption is expected to rise to 812 EJ and 43.2 billion metric tons in 2035 respectively. The continuous reliance on fossil fuels is unsustainable considering its

rapid depletion and its major contribution to global warming. With the consequences of fossil fuel utilization prompted many countries to engage in the development of renewable energy sources that can completely replace conventional petroleum based fuels.

Biofuels is one of the approaches to mitigate CO2 emissions while delivering sustainable energy. Biofuels are biomass derived fuels which is considered to have a low carbon emitting characteristics. Biodiesel, one type of biofuels which corresponds to spark ignition technologies plays a crucial role in the global energy demand especially in the transport sector. Biodiesel is one of the first alternative fuels introduced in the public. It originates from various oil crops which is defined as monoalkyl esters of long chain fatty acids derived from chemical reaction called transesterification. Currently, biodiesel derived from oil crops such as palm, rapeseed and soybean were available in the market (Amaro, 2010). However, their mass production remains to be questionable due to the arable land it requires for cultivation as well as the total energy input in the entire production chain of biodiesel. On the contrary, the emergence of microalgae as another source had satisfied the challenges encountered with other oil crops. Microalgae are categorized as *eukaryotic cells* (Figure 1) and defined as a photosynthetic organism that requires light, sugars, CO_2, N and P. They can produce lipids, carbohydrates, and proteins which can be processed into biofuels and other valuable co – products (Owende and Brennan, 2010). Its advantages from other oil crops are: higher rate of producing lipids, easier to cultivate, requires less arable land and is relatively low cost in harvesting and transporting compared to other oil crops (Chisty,2007).

The production of biodiesel from microalgae undergoes processes such as cultivation, harvesting, drying, oil extraction and transesterification. The challenge is to minimize the energy input in each process to make the commercialization of biodiesel a viable option. According to the data of Yanfen et al. (2012), drying process has the highest energy input among other processes and accounts for 20-30% of the total production cost and energy consumption (Richmond, 2000). Drying algal biomass up to 10% (Prakash, 1997) or less of its moisture content is necessary to avoid several downstream in the process of lipid extraction and transesterification process.



1a. Schematic diagram of Eukaryotic cell

Figure 1b. Electron micrographs of plant and animal cell

Figure 1. Eukaryotic cell structure (Tortora, 2010)

Figure 2a. Lipid bilayer structure

Figure 2b. Phospholipid molecules in lipid bilayer

Figure 2. Lipid bilayer of plasma membrane

Eukaryotic cells

Plasma Membrane

The plasma membrane of eukaryotic cells is relatively similar to prokaryotic cells in terms of its function and basic structure though there are differences in types of proteins which can be found in them. Figure 2a and 2b shows the structure of a plasma membrane of a typical eukaryotic cell that consists of a lipid bilayer which is made up of phospholipid molecules arranged parallel to each other. Each phospholipid molecules has a polar head (phosphate group) and glycerol which are hydrophilic or soluble in water and non polar chains of fatty acids which are hydrophobic or insoluble in water. These fatty acids

are also known as triglycerides which are the main component in producing biofuels particularly biodiesel.

Movement of Materials across Plasma Membranes

The two kinds of movement/process of materials across the plasma membranes are called passive and active. In active processes, the cell uses energy to traverse substance from an area of low concentration to an area of high concentration. In passive process, substances traverse to an area of high concentration to an area of low concentration without using the energy of the cell. Passive diffusion includes simple diffusion, facilitated diffusion and osmosis.

1. Simple diffusion

   Figure 3a shows a simple diffusion which refers to as the overall movement of molecules or ions from an area of high concentration to an area of low concentration. The movement will carry on until the molecules or ions are evenly distributed or until it reaches equilibrium. Cells rely on simple diffusion to transport small molecules such as oxygen and CO_2.

2. Facilitated diffusion

   Figure 3b and 3c shows the facilitated diffusion wherein transporter (membrane protein) functions as channels or a carrier that aids the movements of molecules or ions across the plasma membrane. This movement is similar to simple diffusion that cells do not spend energy simply because the substance moves from high to low concentration. These transporters which are common in eukaryotic cells permit large molecules such as sugars and vitamins.

3. Osmosis

   Referring to figure 3d shows the movement of solvent molecules from an area with high concentration to an area of with low concentration through an integral part called aquaporins.

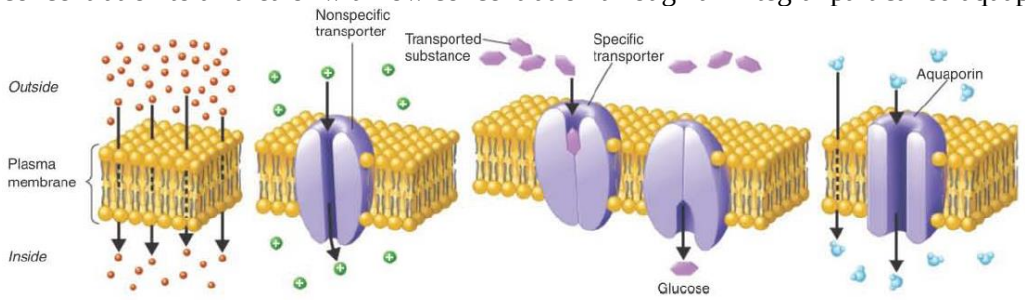


Figure 3a. Simple diffusion | Figure 3b. Facilitated diffusion through a nonspecific transporter | Figure 3c. Facilitated diffusion through a specific transporter | Figure 3d. Osmosis

Figure 3. Types of Passive processes (Tortora, 2010)

Three types of osmotic solution

a. Isotonic solution

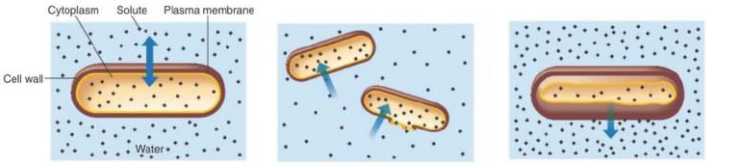
   Referring to figure 4a, isotonic solution is defined as a medium wherein the concentration inside the cell and outside the cell is in equilibrium where water leaves and enters the cell at the same rate (*iso means equal*).

b. Hypotonic solution

Figure 4b shows a hypotonic solution and defined as a medium wherein the concentration of the solution outside the cell is lower than compared to the inside of the cell (*hypo means under or less*).

c. Hypertonic solution

Figure 4c shows a hypertonic solution wherein the solution outside the cell is higher to the inside of the cell.



Fgure 4a. Isotonic solution. No net movement of water

Figure 4b. Hypotonic solution. Water moves into the cell. If the cell wall is strong it contains the swelling. If the cell wall is weak or damage, the cell burst (Osmotic lysis).

Figure 4c. Hypertonic solution. Water moves out of the cell, causing cytoplasm to shrink (Plasmolysis)

Figure 4. Types of Osmotic solution (Tortora, 2010)

Aquaporin (AQP)

Aquaporin (Figure 5) is a unit of membrane channel which is responsible for the transport of water thru cellular membranes. A related study conducted by the Biochemistry Department at the University of Illinois with AQP which results to the molecular understanding of the mechanism and selectivity of AQP.

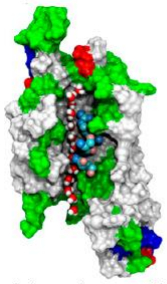


Figure 5. Aquaporin structure (Wang, 2007)

2. METHODOLOGY

1. The Topology

The topology contains all the necessary information to define a particular molecule within a simulation including bonded (bonds, angles and dihedrals) and non-bonded parameters (atom types and charges). The Topology (PDB txt) of the structure (lipid bilayer) that was used and downloaded at the RCSB website (protein bank). The structure can be visualized using a viewing program like VMD. The structure will be inputted into GROMACS tool (pdb2gmx) to generate three files (Topology of the molecule, a position restrained file and a post – processed structure file) which are needed for the succeeding simulations.

2. Defining the Boundary and adding Solvent

As the topology of the structure is created, the box (boundary) of the system should be established next using *editconf* command. Likewise, the structure should be placed in the center of the box with a clearance of 1.0nm from the box edge. This distance is an important parameter as the system uses periodic boundary conditions and may cause false computation if neglected. Solvation (filling of water) of the system will be generated using *genbox* command. The genbox command tracks all the water molecules that have been added to the entire system.

3. Adding Ions

The next part after filling the box with solvent is to add ions (figure 6). The tool for adding ions in GROMACS is called *genion*. The genion command reads through the Topology and replaces the water molecules with ions. An input file will then be created using GROMACS tool grompp that needs an additional extension file (ions.tpr) that contains all the parameters of the atoms within the system. However, to create the extension file, another additional input file (ions.mdp) is needed which is used to generate an atomic description of the system and that can be downloaded also at the RCSB site. Figure below shows the resulting structure with ions.

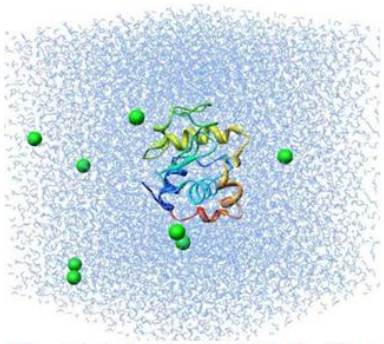


Figure 6. Ionized structure. (2008). Retrieved from http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/index.html

4. Energy Minimization

Energy minimization is executed to ensure that the system has no improper geometry before performing dynamics using the *mdrun* command. There are two ways to determine if the energy minimization is successful: The resulting potential energy (E_{POT}) should be negative and on the order of 10^5–10^6. Likewise, the maximum force (F_{MAX}) should be not greater than 1000kJ mol^{-1} nm^{-1}. Figure 7 shows that the convergence of potential energy of the system.

5. Equilibration

Equilibration is perform in two phases. The first phase is under NVT ensemble (constant number of particles, volume and temperature) using the grompp command and the *nvt.mdp* extension file. This is also called "isothermal – isochoric" or canonical. The second phase is under the NPT ensemble (constant number of particles, pressure and temperature) also by using the *grompp* command and the *npt.mdp* extension file which is also known as the "isothermal-isobaric" ensemble. Figure 8a, 8b and 8c shows the system is now stable with respect to temperature, pressure and density.

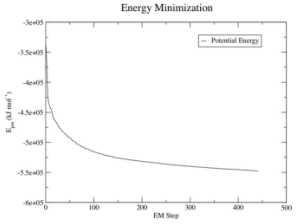


Figure 7. Energy Minimization curve. (2008). Retrieved from http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/index.html

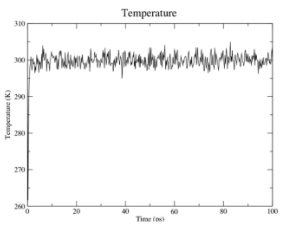


Figure 8a. Temperature progression

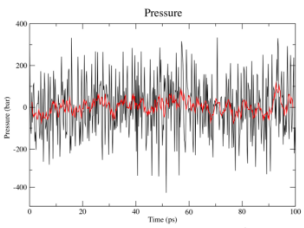


Figure 8b. Pressure progression

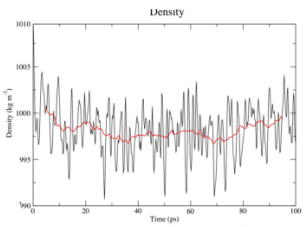


Figure 8c. Density progression

Figure 8. Equilibration results. (2008). Retrieved from www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/index.html

6. Production MD

Proceeding with the production of MD for data collection is only possible when the system is well-equilibrated at the desired temperature and pressure. The *mdrun* command will be used for the data gathering of the entire system.

3. RESULTS AND DISCUSSION

Figure 9a and 9b (crystal reference) shows the RMSD calculation relative to the structure present in the minimized equilibrated system. The RMSD levels off to ~0.1 nm which indicates that the structure is in stable condition.

The radius of gyration of the structure (Figure 9c) determines its compactness. As shown in the graph, the structure remains very stable in its compact form.

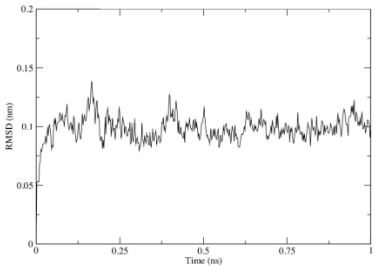


Figure 9a. RMSD Backbone

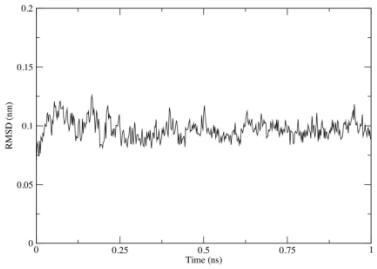


Figure 9b. RMSD Backbone (Crystal reference)

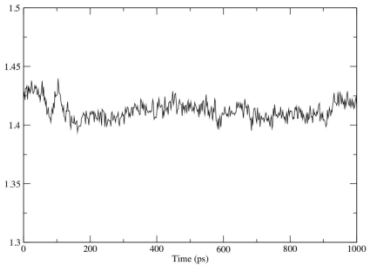


Figure 9c. Radius of Gyration

Figure 9. Energy Minimization results. (2008). Retrieved from http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/lysozyme/index.html

4. CONCLUSION

A methodology in modelling microalgae drying in the molecular level was developed using GROMACS. Energy minimization and equilibration are the most important steps in preparing the topology (structure) of the system before performing molecular dynamics as this will give erroneous results on the potential energy of the system. Future studies involve analysing of microalgae lipid bilayer with suspended water through pressure difference as well as varying the concentration from the outside of the cell.

5. ACKNOWLEDGEMENT

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