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A Computational Study on the effects of Temperature and Osmotic Pressure on Water Extraction on Microalgae using Big Multipole Water – Martini Forcefield on GROMACS Simulation

Darl Vincent Perez [1,*], Al Rey Villagracia[1], Melanie David[1], Nelson Arboleda Jr.[1,2], Robby Manrique[3], Aristotle Ubando[3], Alvin Culaba[3], Hideaki Kasai [4]

[1] *Physics Department, De La Salle University*
[2] *HPCL, CENSER, De La Salle University,*
[3] *Mechanical Engineering Department, De La Salle University*
[4] *Department of Precision Science & Technology and Applied Physics, Osaka University, Japan*
**Corresponding Author: darl_perez@dlsu.edu.ph*

Abstract: Microalgae as a biofuel has been the interest of researchers because it is one of the sources of renewable biodiesel that is capable of meeting the global demand for transport fuel without competition on human food consumption. Drying the microalgae down to ten percent (10%) of its weight in large scale production that is cost and energy efficient has been the challenge for researchers in order to fully utilize its oil content and without producing soap. In this study, GROningen MAchine for Chemical Simulations (GROMACS) is utilized to provide a theoretical explanation on the mechanism of water extraction from the microalgae using the latest Big Multipole Water (BMW) – MARTINI Coarse-grained forcefield which gives more fundamental membrane properties and improved energetics when compared to the original MARTINI forcefield for the interactions at the membrane-water interface. To represent the *Chlorella Vulgaris* microalgae in molecular dynamics, Dioleoyl-sn-glycero-3-phosphocholine (DOPC) was used which is the dominant lipid in a microalgae excluding membrane proteins. In this study, the temperature was varied from 300 K to 410 K with 10 K increment to verify the effects of temperature on the thickness and layer structure of the DOPC which provided a deeper explanation on the traditional method of drying by introducing thermal energy. Moreover, a minimum osmotic pressure of 150 MPa was determined that exhibits extraction of water from the microalgae by applying acceleration on water in our simulation on non-walled periodic system.

Key Words: Microalgae; GROMACS; DOPC; Osmotic pressure; BMW- Martini

1. INTRODUCTION

Modern lifestyles, like gadgets, vehicles, technologies for daily comfort, require a steady, reliable supply of energy (Dresselhaus and Thomas, 2001). Table 1.1 demonstrates the increase in world energy consumption from 2008 to 2035 which implies that an alternative source of energy is needed that can meet the global needs. This source should not compete with human food consumption and can be cultivated in a large-scale system (Patrick Buckwalter et al., 2012). With these requirements, microalgae are the only source

capable of providing the demand of people globally and can possibly replace conventional fossil fuel which can be cultivated easily and not part of human food consumption (Chisti, 1980).

Microalgae are sunlight-driven cell factories that convert light, sugars, carbon dioxide, nitrogen and phosphorus (Chisti, 2007). They are capable of doubling their biomass in 24 hours and can produce carbohydrates, lipids, and proteins which can be processed into biofuels and other valuable co – products (Brennan and Owende, 2010). Its photosynthetic efficiency can reach up to 10-20% as compared to the fastest growing plants with 0.5% - Cannabis Ruderalis (Huntley, et al., 2007).

Table 1.1 World energy consumption (IEO, 2011)

	2008				2020	2035
Energy consumption (EJ)	OECD	America	129.7	532.6	653	812
		Europe	86.7			
		Asia	41.4			
		Europe and Eurasia	53.3			
	NON-OECD	Asia	145.5			
		Middle East	27			
		Africa	19.8			
		Central & South America	29.2			

The drying stage is the main focus of this study since it requires the highest energy input to ensure efficient oil extraction (Yanfen et al., 2012). In order to fully utilize microalgae's oil content, it needs to dry it down to ten percent (10%) water content (Prakash et al., 1997). Also, drying algal biomass prolongs its shelf life (Viswanathan et al., 2011), and inhibits growth of bacteria and fungi (Becker, 1994).

Computational tools and simulations can reduce the cost in testing and experiments. For this reason, this method of computation prior to experimentation is very ideal for countries like Philippines where funds for research and education are limited. This opens a new perspective and discusses deeper understanding of microalgae drying process on a molecular level that uses computational tools for simulations to study the mechanism of water transport under induced osmotic pressure concept. With the use of simulations, extraction of information or data which cannot be easily produced through experiments due to certain limitations like maintaining a constant temperature or pressure is possible.

2. METHODOLOGY

2.1 GROMACS software

This study utilizes Groningen Machines for Chemical Simulations (GROMACS) to simulate and provide a theoretical explanation on how to extract water from the microalgae. GROMACS was developed in the University of Groningen in Netherlands. GROMACS is a fast molecular dynamics (MD) simulations program that is compatible with other forcefield like GROMOS, OPLS, AMBER and ENCAD (Spoel et al., 2005).

In experimental and computational studies, particularly for biological systems, water is the most utilized solvent. However, compared to other solvents, water has many exceptional properties, such as increased diffusivity upon compression and, a density maximum at 277 K which complicates its dynamics and physics. (Hadley et al., 2012). In order to provide better representation, this study uses the latest BMW – MARTINI Coarse-grained forcefield which gives more basic membrane properties and improved energetics in terms of the interactions at the membrane-water interface showed in Figure 2.1. Its geometry and charges were optimized to best reproduce both the dipole and the quadrupole moment of a four-water cluster in a center-of-mass (COM) mapping style.

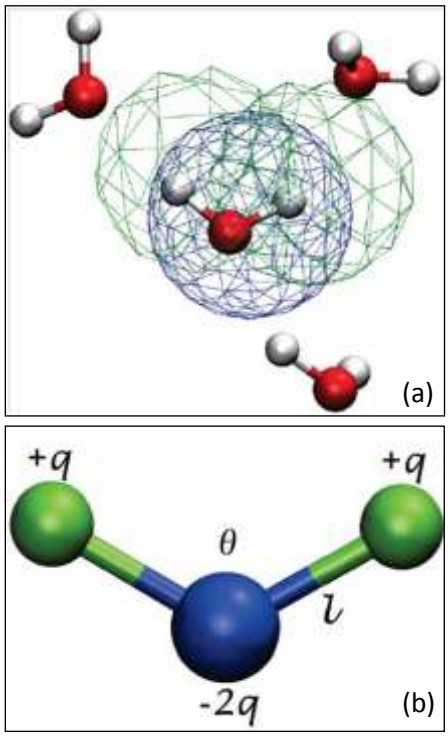


Fig. 2.1 The 4-water clustering (a) described by the big multipole water model (b). The green beads (oxygen) interact through coloumbic forces, while the center bead (blue) interacts through both coloumbic and van der Waals forces. (Wu et al., 2011)

In this study, Figure 2.2 shows the flowchart of the methodology.

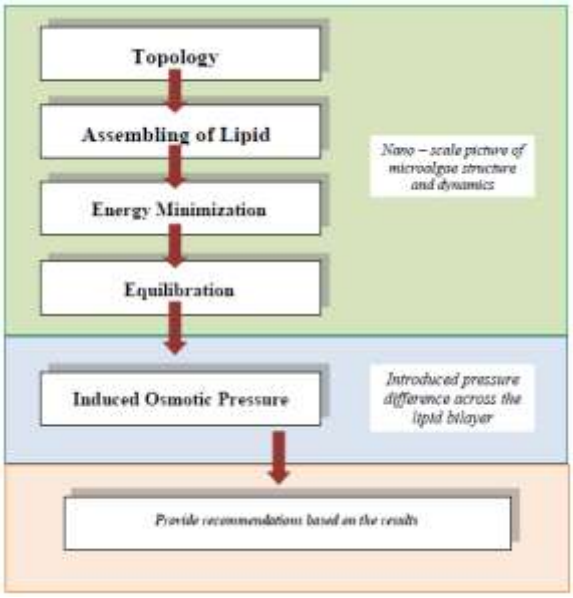


Fig. 2.2 Flow Chart of Methodology

2.2 The Topology

The topology contains all the necessary information to define a particular molecule or the entire system within a simulation which includes bonded (bonds, angles and dihedrals) and non bonded parameters (atom types and charges).

The topology structure can be visualized using a viewing program like Visual Molecular Dynamics (VMD). Figure 2.3 shows the molecular composition of a DOPC lipid and its corresponding representation on GROMACS software which was used in succeeding simulations.

2.3 Assembling of Lipid

The *Chlorella Vulgaris* microalga was chosen because of its high lipid content which is good for oil extraction. The phospholipid Dioleoylphosphatidyl -choline (DOPC) was used in the simulation since the *Chlorella Vulgaris* microalgae has a high content of oleic acid and it is the dominant lipid in the microalgae excluding membrane protein. A total of 128 DOPC lipids were randomly inserted on a box with an initial dimension of 7.50000 nm x 7.50000 nm x 7.50000

nm. It was then solvated with 768 water bead molecules.

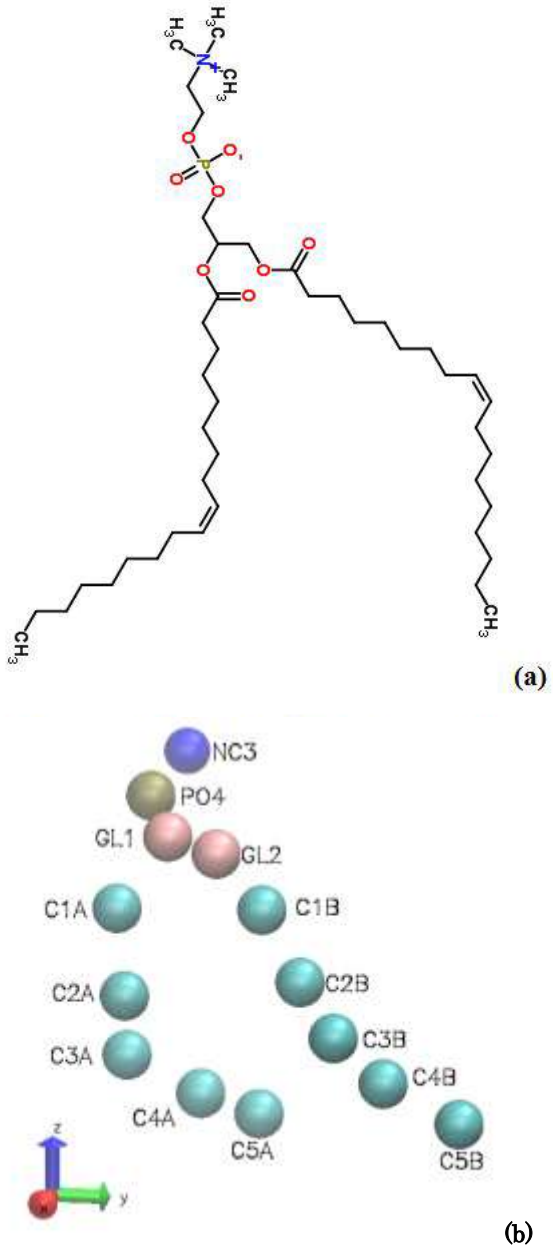


Fig. 2.3 Phospholipid structure (a) Molecular composition (b) Coarse grained model.

2.4 Energy Minimization

Energy minimization was executed to ensure that the system has no improper geometry and steric clashes before performing dynamics. The structure is relaxed through energy minimization in such a way that the movement of all atoms are stable making its net forces almost negligible. Moreover, energy minimization is done to ensure that the starting structure is reasonable in terms of geometry and orientation. The system was minimized at 300K and 1 atm with flexible water with harmonic bonding interactions with other three water molecules. The system was also optimized with rigid water. This optimization led to a periodic box of lipid bilayer with dimensions of 6.66195 nm x 6.66195 nm x 6.66195 nm.

2.5 Equilibration

Equilibration is performed in two phases. The first phase is under isothermal–isochoric or canonical ensemble where the number of particles, volume and temperature are constant. The second phase is under the isothermal–isobaric ensemble where the number of particles, pressure and temperature are constant.

The self–assembly of lipids is a spontaneous process wherein a chaotic system composed of molecules that comes together to form a well–organized microstructures without a guide from an external source. The MD simulation for the spontaneous self–assembly is carried out in GROMACS.

3. RESULTS AND DISCUSSION

3.1 Area per Lipid

The area per lipid is a significant factor that needs to be analyzed as it describes the distribution of lipids in the membrane structure. The area per lipid is 0.69346 nm^2 which is demonstrated in Figure 3.1.

Area per lipid

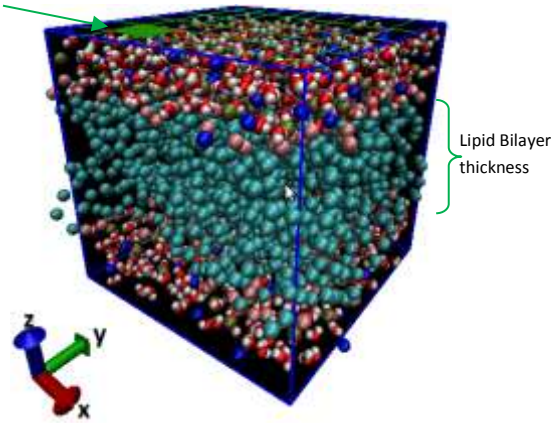


Fig. 3.1 Lipid Bilayer Properties

The resulting projected area per lipid agrees well with the other MD studies as well as the values obtained thru experiments as shown in table 3.1. Experimental value of Nagle et al., were obtained using neutron and x – ray diffraction. In neutron diffraction, samples were placed in a beam of either thermal or cold neutrons to obtain a diffraction pattern; this pattern gives information of the structure or a particular material. X – ray diffraction or crystallography is one of the principal methods used in characterizing atomic structure of materials. It can produce atomic and molecular structure of a particular crystal. Also, it is capable of producing three-dimensional picture of the density of electrons within the crystal. From this electron density, the average positions of atoms in the crystal can be determined as well as the chemical bonds and their disorder.

Table 3.1 Area per lipid of DOPC

	Simulated Area (nm^2)	Simulated Area (nm^2)*	Experimental Area (nm^2)**
DOPC	0.69 (300K)	0.67 (300K)	0.721 (300K)

* Marrink et al., 2004 **Nagle et al., 2000

3.2 Thickness of the lipid bilayer

The bilayer thickness was computed from the plot of the phosphate group of the system against the periodic box. The distance between the two center of mass of the phosphate head group peaks corresponds to the thickness of the bilayer as shown in figure 3.2. The bilayer thickness for 300 K is 4.578 nm.

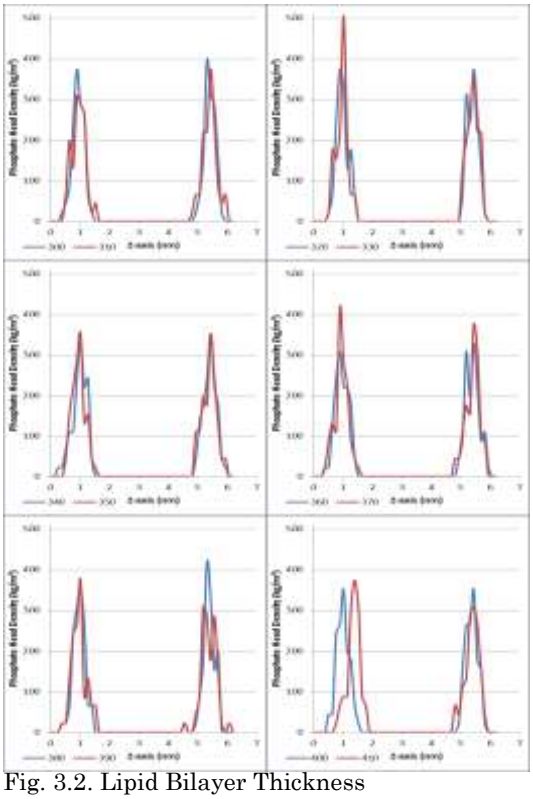


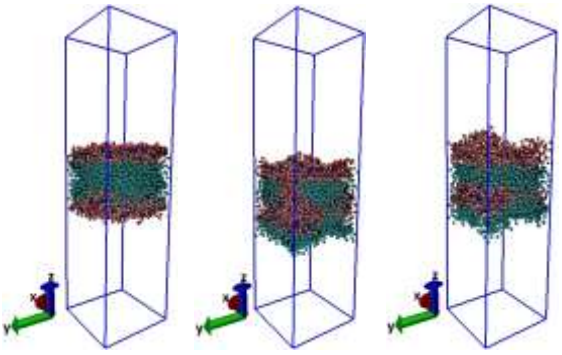
Fig. 3.2. Lipid Bilayer Thickness

Another set of simulations were performed using the same lipid bilayer that is equilibrated at 300K and 1 atm. The bilayer was then subjected to an increasing temperature rise with 10 K increment starting from 300 K up to 410 K. Results in figure 3.2 shows that the thickness of the bilayer varies with temperature. When the temperature was increased, the phosphate heads began to disperse and were no longer concentrated to a certain level of position. It is noticeable that the peaks of the phosphate heads were approaching to each other. This variation can be explained by an increase in kinetic energy of the system allowing the phosphate heads to move around the system. Moreover, as the temperature was increased, the carbon chains began to curl allowing the phosphate heads to move closer to each other as supported in the experimental study of Nagle and Tristram-Nagle in 2000 for dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC).

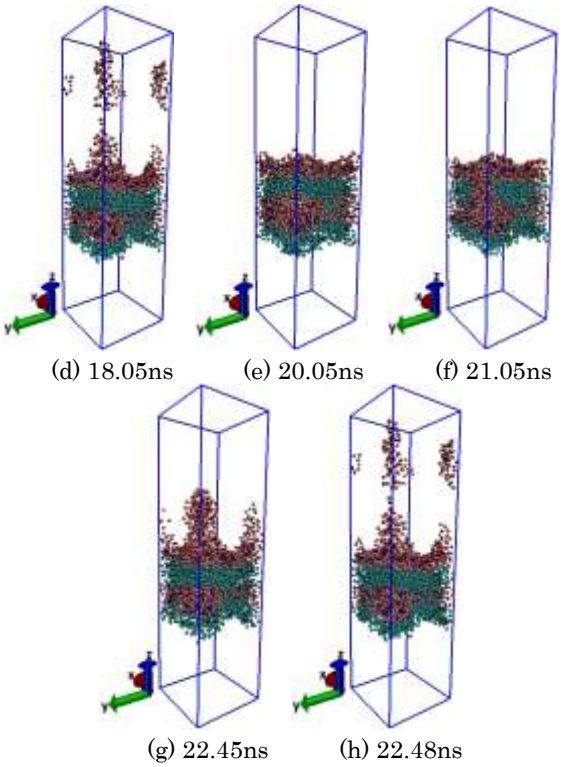
3.3 Osmotic Pressure

When there is a pressure difference across the bilayer, water moves from inside to outside. It was produced by applying a uniform external force on all the water molecules on the +z direction. This force is computed by multiplying the atomic mass of all water molecules (768 water bead molecules) in the system with acceleration in nm/ps^2. The acceleration was adjusted in order to obtain the desired pressure at which water molecules started to traverse on the other side of the bilayer. The unit cell was adjusted to 6.66195 nm x 6.66195 nm x 25 nm to avoid interaction with the next lipid layers along the z-axis.

Figure 3.3 shows the lipid bilayer subjected to a pressure difference across the bilayer of 150 MPa (under 40ns of simulation). It is noticeable that water from below slowly traverses the lipid bilayer as shown in figures 3.3 (a) to (c). However, the figure also clearly shows that the lipids were ruptured due to the forces exerted by the water molecules as it moves across the bilayer. In figure 3.3 (d) 18.05 ns, it was observed that a few number of water molecules were able to reach the other surface of the lipid bilayer before the water the succeeding flow of water up beyond the lipid bilayer. Succeeding snapshots show the gradual movement of water molecules as it leaves beyond the perimeter of the bilayer assembly at (h) 22.48 ns.



(a) 10.50ns (b) 12.05ns (c) 15.05ns



(d) 18.05ns (e) 20.05ns (f) 21.05ns

(g) 22.45ns (h) 22.48ns

Fig. 3.3 Snapshots of the phospholipids subjected to a pressure difference of 150 MPa at different timeframe. Headgroups are represented by orange spheres while tail atoms are blue-green and violet spheres for water molecules.

4. CONCLUSIONS

This study was able to calculate similar properties of a DOPC lipid bilayer compared with the experimental and other computational studies (Nagle et al., 2000; Marrink et al, 2004). The calculated area per lipid 0.69346 nm^2 is comparably near the values of other previous studies. The thickness of bilayer varies with temperature in terms of the spread of the phosphate heads. Results on variation of osmotic pressure showed that a pressure difference of 150 MPa will exhibit water transport across the lipid bilayer.

5. RECOMMENDATION

The minimum osmotic pressure was determined in this study. To determine the effect of osmotic pressure, it is recommended to perform simulations to determine the effect of pressure on the number of water molecules on the first cluster of water that traverses the lipid bilayer.

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