A molecular dynamics investigation of water migration in a lipid bilayer for microalgae drying

Robby Manrique[1], Aristotle Ubando[1], Al Rey Villagracia[2], Jennifer Corpuz[2], Allan Abraham Padama[3], Melanie David[2], Nelson Arboleda Jr.[2]*, Alvin Culaba[1], and Hideaki Kasai[3]

[1]Mechanical Engineering Department, De La Salle University, 2401 Taft Avenue, Malate 1004, Manila, Philippines
[2]Physics Department, De La Salle University, 2401 Taft Avenue, Malate 1004, Manila, Philippines
[3]Department of Precision Science & Technology and Applied Physics, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, Japan

Microalgae are one of the best candidates for biofuel production owing to their numerous advantages over other oil crops. However, challenges still exist in the entire production chain due to the high energy input at every step of the process, particularly the drying stage. This study investigated the mechanism of water extraction at the molecular level of an algal cell under the influence of osmotic pressure using Molecular Dynamics simulations. The calculated area per lipid of 0.67 nm^2 of the cell membrane agrees well with other simulation and experimental results. Moreover, the membrane thickness and volume were seen to increase with rising temperature from 330 K. An osmotic pressure as low as 69.2 MPa showed a significant amount of water permeating across the lipid membrane. The occurrence of this water permeation is hastened at higher osmotic pressures. These results can lead to new techniques and suggest further research to provide information that may help realize an alternative and cheaper method of microalgae drying.

*Corresponding author
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INTRODUCTION

With the imminent depletion of fossil fuel and the increasing global carbon dioxide emission due to transportation, the demand to find alternative sources of energy with lesser discharge of greenhouse gases has been a prevalent topic in research, economics and politics, locally and globally (Shafiee and Topal 2009, Menten et al. 2013, de Vera 2011, Angara 2011), according to the International Energy Agency (2012). Biofuels, as the one that is derived from plant sources, lowers the need for fossil fuels through carbon fixation, and requires less energy in terms of production as compared to fossil fuels (Sobrino et al. 2011). Biodiesel, particularly that derived from microalgae, has been given much attention due to its great potential in producing oil even in a limited land area as compared to any other biofuel feedstock, as stated in the Philippines Republic Act 9367 (Congress of the Philippines 2006) and in an analysis of the Philippines situation by the Energy Information Administration (2008). The Philippines, being an archipelago, has a limited arable land area - about one-third of the total land area of the country (Corpuz and Wolf 2011). This limitation leads to a food-

KEYWORDS
microalgae drying, lipid bilayer, phospholipids, dioleoyl-glycero -phosphocholine, osmotic pressure, water, biodiesel, molecular dynamics calculations

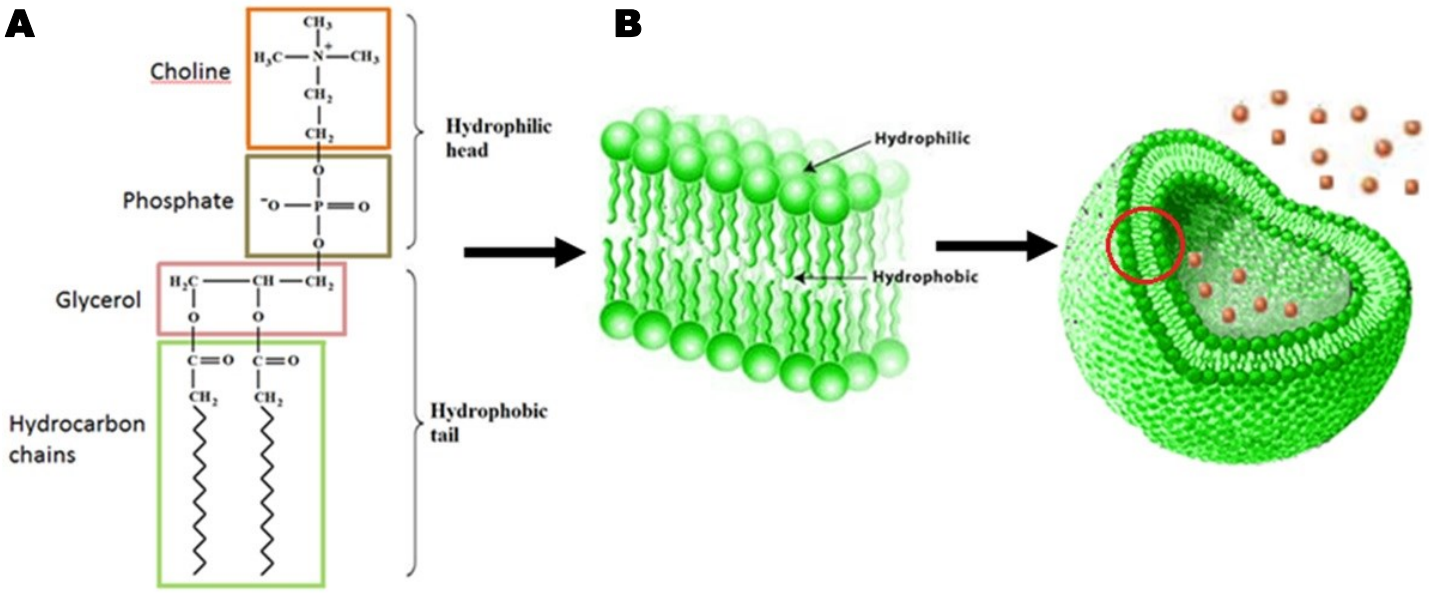

Figure 1. Lipid bilayer structure. A, Molecular composition of phospholipid and **B**, cell membrane with water molecules.

versus-fuel dilemma. Thus, the Department of Agriculture of the Philippines seeks an advanced biodiesel feedstock that significantly reduces the impact of land constraints while simultaneously supporting the demand for biodiesel in the country. The study by Chisti (2007) compared various biodiesel feedstocks in terms of their oil yield per hectare and it was shown that microalgae have by far a higher yield compared to corn, jatropha, and coconut.

One of the strains of microalgae that has high lipid content is *Chlorella vulgaris*. This specific strain has high oleic acid ($C_{18:1}$) ester (Yoo et al. 2010), which enhances the quality of biodiesel in terms of the balance between oxidative stability and low-temperature properties (Knothe 2009). In addition, lipids from microalgal biomass are suitable for biodiesel production due to the inherent neutral lipids, particularly the triacylglycerols which predominate among the polar lipids (phospholipids and glycolipids) for biodiesel (Knothe 2009). *Chlorella vulgaris* is commonly seen in the Philippines particularly in the West Bay of Laguna de Bay where it is being tested for its ability to remove and resist metals (Nacorda et al. 2007, Nacorda et al. 2010).

The five major processes in producing biodiesel from microalgae are cultivation, harvesting, drying, oil extraction, and transesterification. Each of the processes has its own concerns in terms of improving the technology and procedures to make them more efficient. This study mainly focuses on the drying process since the commercialization of microalgae to biodiesel is set back by the high energy requirements of conventional drying processes (Richmond 2000, Yanfen et al. 2012, Grierson et al. 2013, Prakash et al. 1997, Viswanathan et al. 2011). The drying of microalgae biomass to less than 10% of its original mass is required to produce high yields of biodiesel (Becker 1994). Moreover, drying the microalgal biomass improves its shelf life (Viswanathan et al. 2011) and also inhibits the growth of other microorganisms such as bacteria, mould, and fungi which contaminate the biomass (Becker 1994). Efforts in sustainably minimizing the energy consumption of microalgal drying are done using solar energy (Prakash et al. 1997, Culaba et al. 2013). Nevertheless, solar drying of microalgae presents physical limitations such as weather dependency, yield, and process controllability. Other traditional methods such as drum drying, spray drying, and vacuum drying are all highly energy intensive (Becker 1994). The fundamental problem in drying can be best understood by looking at the molecular movement of water from the cellular level of microalgae.

With the motivation to establish a new perspective of alternative drying with less energy consumption and which does not require a complex mechanical system, this study proposes a new approach to drying by means of induced osmotic pressure. This phenomenon is defined as the transmission of solvent (usually water) through a semi-permeable membrane into a solution of higher concentration. In other words, drying is executed by means of extracting the water molecules from the inside to the surface of an algal cell by applying a pressure difference across the bilayer. The study provides a theoretical explanation on the mechanism of water extraction with the use of Molecular Dynamics (MD) simulations. MD is one of the primary tools used in analyzing biomolecules and it provides an atomic-level picture of membrane structure and dynamics which cannot be easily investigated through experiment. MD, together with other theoretical and computational procedures (e.g., density functional theory), provides atomic and molecular mechanisms that give a deeper understanding of the interaction of different materials (Abanador et al. 2013, David et al. 2006a, David et al. 2006b, David et al. 2007a, David et al. 2007b, Diño et al. 2004; Arboleda et al. 2004, Arboleda et al. 2006, Arboleda et al. 2007, Tsuda et al. 2006, Son et al. 2007, Ozawa et al. 2008, Sarhan et al.

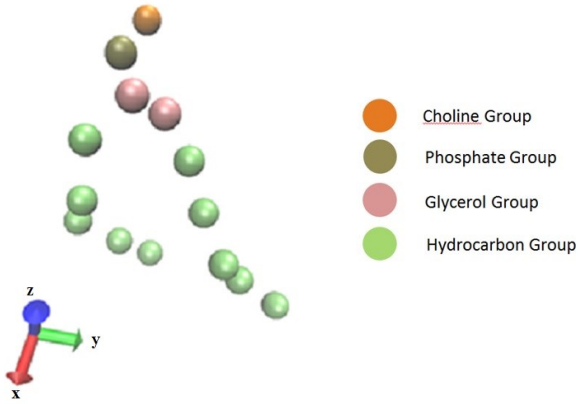

Figure 2. DOPC lipid model using Martini force field.

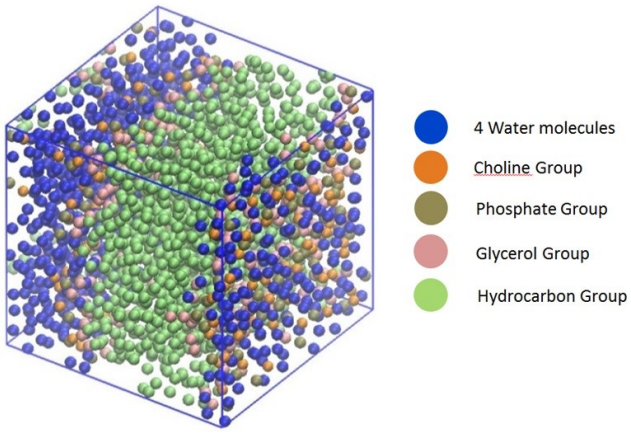

Figure 3. DOPC lipid bilayer as visualized by VMD.

2009, Padama et al. 2012, Villagracia et al. 2012a, Villagracia et al. 2012b). MD simulations adopt the principle of classical mechanics (Newton's equation of motion) that explains the behaviour of atomic particles including their positions, velocities, and the forces acting on them. The simulations depend on empirical approximations (called force field) that contain a set of equations and parameters that will calculate the potential energy and forces based on the coordinates of the particles. The force field that was used for this study is Martini (Marrink et al. 2007) Coarse-grained type which has been proven to significantly shorten the simulation time as compared to traditional all-atom force field like AMBER (Case et al. 2005), CHARMM (Brooks et al. 1983), GROMOS96 (van Gunsteren et al. 1996), and OPLS (Jorgensen et al. 1996).

Particularly for this study, the molecular movement of water across the lipid bilayer of an algal cell can be best described by MD which was also used by Zhu et al. (2002) in the analysis of the permeation rate of water molecules across a membrane protein called aquaporin. The lipid bilayer of an algal cell is basically composed of two layers of phospholipids (Figure 1) with their hydrophobic tails facing each other. It encloses the algal cell and protects the internal organelles from the environment. Also, lipid bilayers are semi-permeable which only allows small molecules like water and gas to pass through them. The lipid bilayer structure has been well studied using existing experimental methods like neutron and x-ray diffraction (McIntosh and Simon 1986, McIntosh 1990, Wiener and White 1992a, Wiener and White 1992b, Tristram-Nagle et al. 1998), and has been simulated using MD (Benz et al. 2005). Problems encountered were due to the differences in the space and time scales, that is, experimental methods are observed at macro level and over long periods of time, whereas MD simulations are analysed at nano level and in nano seconds.

The general objective of the study is to investigate the mechanism for the extraction of water molecules from the inside to the surface of an algae cell using MD simulation. Specific objectives are to obtain a nano-scale picture of microalgae structure and dynamics, to determine the temperature dependence of the membrane thickness and volume, and to evaluate the effects of inducing osmotic pressure on the water extraction from an algal cell. This study does not cover the processes and methods of producing biofuels from microalgae, but focuses on analyzing, at the molecular level, the components which include the structure and composition of lipids. Due to the high oleic acid content of microalgae *Chlorella vulgaris*, the phospholipid used in this study is dioleoyl-phosphatidylcholine (DOPC).

The simulations are limited to room temperature (300 K) and pressure (1 atm) conditions to best represent the actual microalgae harvest conditions. The osmotic pressure applied is varied to determine the minimum value at which water migration occurs. The study also examines the mechanism of water transport across the lipid bilayer.

MODEL AND METHODOLOGY

The drying of microalgae was analysed by observing the movement of water molecules across the lipid bilayer. In MD simulations, the pressure difference across a cell membrane is produced by applying a constant force on the z-direction to all the water molecules in the system (Zhu et al. 2002). It is assumed that the lipid cell membrane consists of 128 DOPC lipids as shown in Figure 3. The calculations on the lipid membrane system were performed using the open source software Gromacs (ver.4.6.1) (Spoel et al. 2005) which implements molecular dynamics and were visualized using Visual Molecular Dynamics (VMD). The chosen force field was Martini which characterizes the interaction of the molecules in the DOPC lipids as shown in Figure 2.

The structure of the lipid membrane was generated from random positions of 128 DOPC lipids solvated with 768 beads of water molecules, and was optimized by energy minimization at 300 K and 1 atm pressure. The resulting unit cell size is 6.55585 nm x 6.55585 nm x 6.55585 nm as shown in Figure 3. To study the effect of temperature on cell membrane thickness and vol-

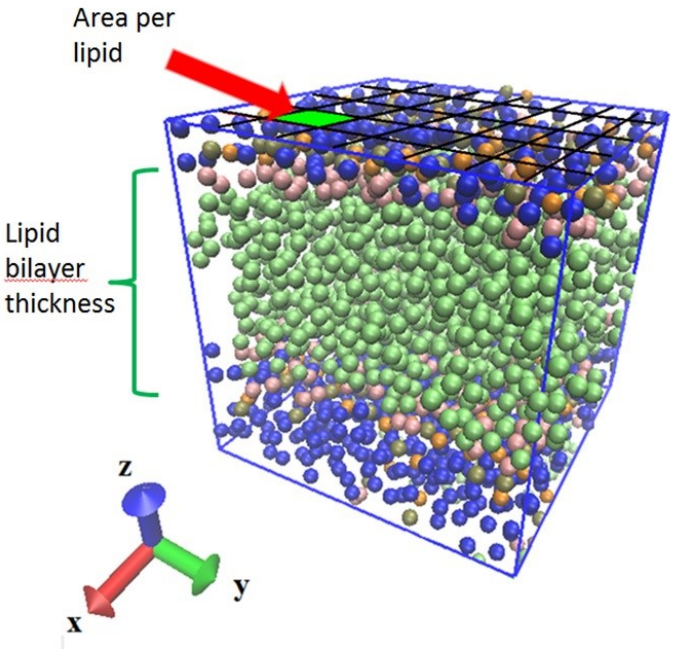

Figure 4. Representation of the area per lipid (green square) and the thickness of the lipid bilayer.

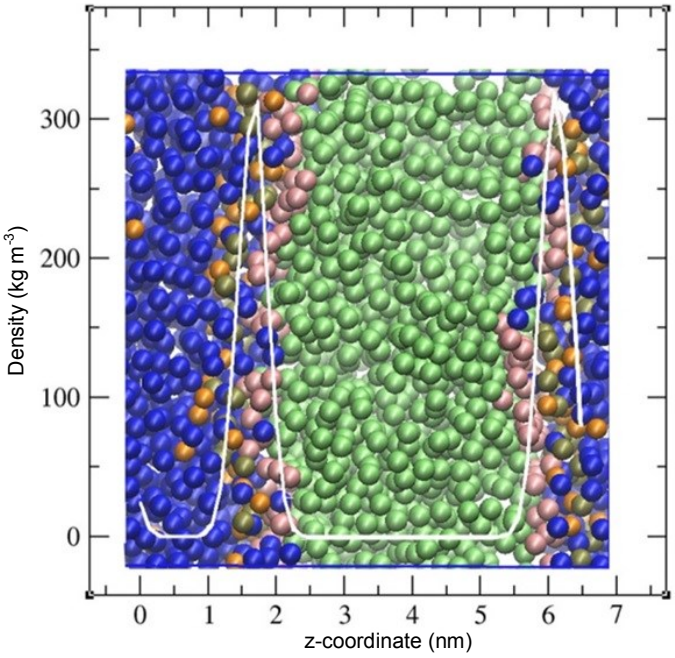

Figure 5. Density (*kg/m*^{3}) profile of the phosphate group of the DOPC lipid bilayer along the *+z-axis* (*nm*), at 300 K and 1 atm, superimposed on a 2D structural image that cuts parallel to the *yz-plane*.

ume, the box dimensions were allowed to relax during the formation of the DOPC bilayer at different temperatures from 300 K to 400 K, in increments of 10 K, at 1 atm pressure.

At a system temperature of 300 K and pressure at 1 atm, a pressure difference was applied on the water molecule components of the system to simulate the osmotic pressure that would cause the water transmission across the membrane, as well as the segregation of the water molecules on the membrane surface. Several values of pressure difference were used in order to determine the minimum amount of osmotic pressure that can lead to the segregation of the water molecules. This quantity is the minimal pressure difference between the outer surface and the inner surface of the cell membrane. Finally, the time elapsed before a cluster of water molecules first segregates from the membrane and the amount of water molecules in the cluster were determined for different values of pressure difference. This is to identify the effect of osmotic pressure on water permeation at 300 K.

RESULTS AND DISCUSSION

The area per lipid and the temperature-dependence of the lipid bilayer thickness and volume

The area per lipid factor is significant as it describes the asymmetric distribution of lipids in the membrane structure. This

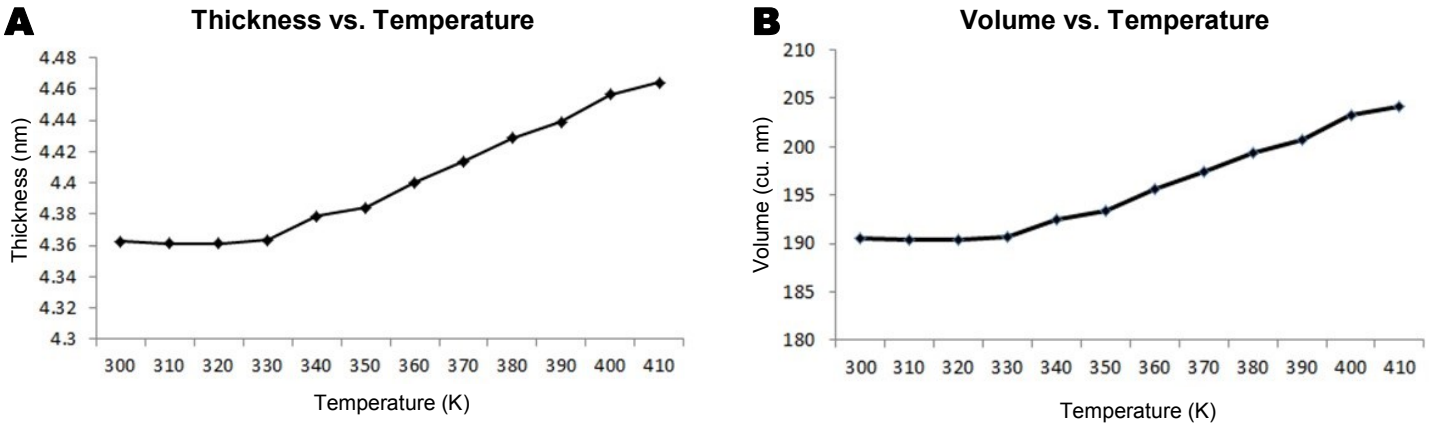

Figure 6. Plots of the thickness (nm) (A) and volume (cu. nm) (B) of the lipid bilayer as functions of temperature (K) at 1 atm.

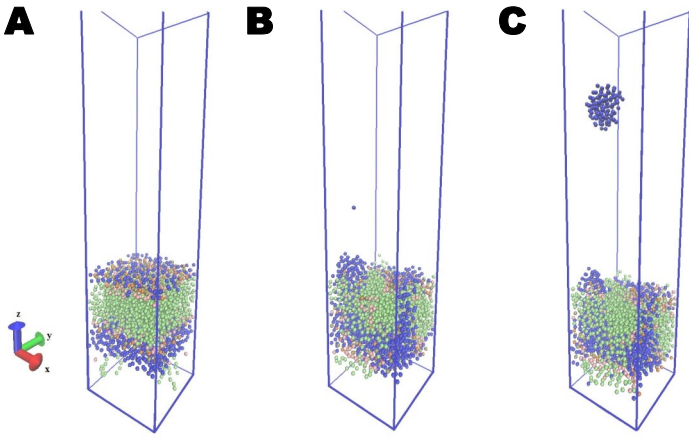

Figure 7. Images of the DOPC lipids (lime-green spheres) and the water molecules (blue spheres), under an osmotic pressure of 69.2 MPa (upward) at 300 K and 1 atm, observed at t = 0ns (**A**), t = 4.20ns (**B**), and t = 4.35ns (**C**). The box is 6.56 nm (L) X 6.56 nm (W) X 25 nm (H).

is computed using the area in the simulation box parallel to the xy-plane (Figure 4) divided by half of the number of lipids in the system. The dimension of this area is 6.55585 nm X 6.55585 nm at 300 K and 1 atm. Hence, the area per lipid was computed as *ca.* 0.67 nm^2. This result agrees well with other MD studies (0.67 nm^2) (Marrink et al. 2004) and to the values obtained thru neutron and x-ray diffraction experiments (0.72 +/- 1 % nm^2) (Nagle et al. 1996).

To determine the effects of temperature on cell membrane thickness at 1 atm, the density profiles of the phosphate head group of the DOPC lipid bilayer along the membrane's normal surface (*+z-axis*) at temperatures 300 K to 400 K were obtained. This is shown in Figure 5, superimposed on a 2D structural image that cuts parallel to the *yz-plane* (see also Figure 4). The two peaks centered at z = 1.7185 nm and z = 6.081 nm indicate that the membrane is roughly 4.3625 nm thick. The thickness remained almost unchanged as the temperature is increased to 330 K, but kept rising until 410 K as shown in Figure 6A. The same pattern is observed when the membrane volume is plotted against temperature (Figure 6B). These results suggest that both the thickness and volume of the lipid bilayer remained almost unchanged below 330 K, while they both increased with temperature above 330 K. The latter is due to the enlargement of the inter-lipid spaces which facilitates the passage of water molecules across the cell membrane.

Water permeation versus the osmotic pressure

To understand how osmotic pressure affects the water permeation across the cell membrane, several simulations of the water extraction process at different osmotic pressures were performed. The approximate time in which the first cluster of water molecules successfully segregates from the membrane and the amount of water molecules in the cluster were determined. In all simulations performed, the system was kept at room temperature (300 K) and at 1 atm pressure.

Figure 7 shows unit cell images of the water/lipid bilayer system at a pressure difference (PD) of 69.2 MPa (directed upward) observed within a total time frame of 4.35 ns at 0.03 ps (Marrink et al. 2010) time interval. In the figure, the water molecules and the DOPC lipids are represented by blue spheres and lime-green spheres, respectively, with the simulation box kept at 6.55585 nm (L) X 6.55585 nm (W) X 25 nm (H). Here, the box height was increased to clearly observe and measure the segregated water molecules. At time t = 0 ns (Figure 7A), water molecules reside on both the upper and lower surfaces of the membrane. In the first 4 ns, a single blue sphere corresponding to a

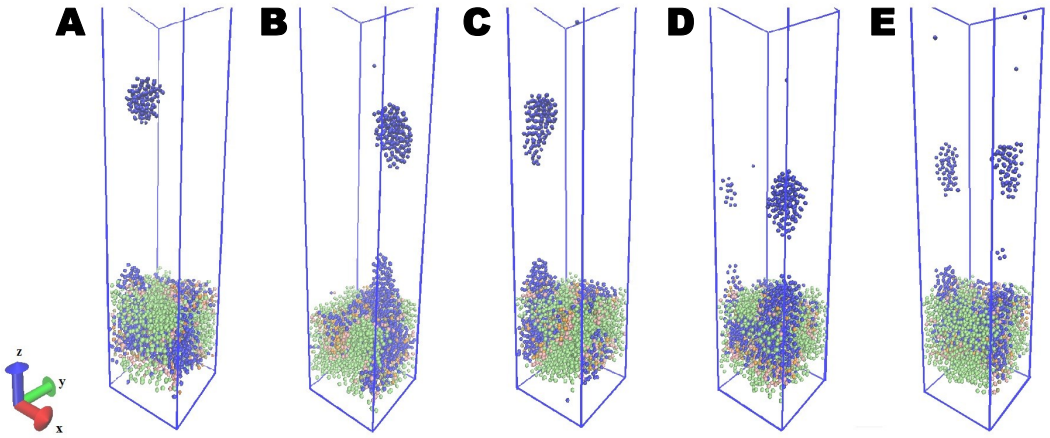

Figure 8. Images of the DOPC lipids (lime-green spheres) and the water molecules (blue spheres) at 300 K and 1 atm, with PD = 69.2 MPa, t = 4.35 ns (**A**), PD = 71.2 MPa, t = 4.14 ns (**B**), PD = 73.2 MPa, t = 3.48 ns (**C**), PD = 75.2 MPa, t = 2.94 ns (**D**), and PD = 77.2 MPa, t = 2.85 ns (**E**). PD is the applied osmotic pressure or pressure difference (upward) and t is the elapsed time before the first cluster of water molecules desorbs from the outer (upper) side of the membrane. The box is 6.56 nm (L) X 6.56 nm (W) X 25 nm (H). The cluster sizes are 113, 151, 133, 90, and 133 beads of water molecules, respectively.

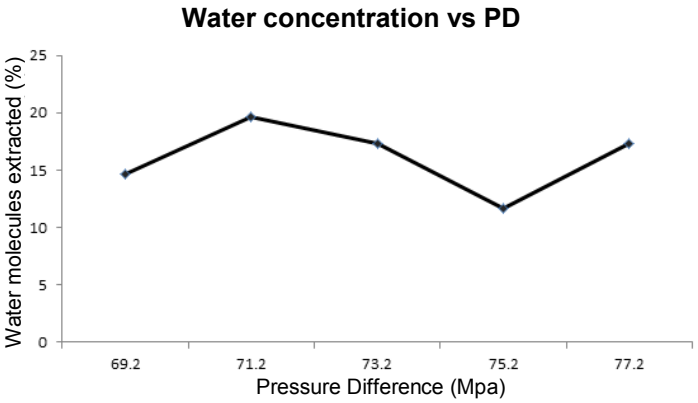

Figure 9. Graph representing the water concentration in the cluster as a function of pressure difference.

group of four molecules of water occasionally (at t = 0.6, 2.10, 3.15 and 3.6 ns) gets freed from the upper side of the membrane while the rest of the water molecules together with the DOPC lipid components are seen to constantly rearrange themselves. At t = 4.20 ns (Figure 7B), a cluster of water molecules (shown as a large group of blue spheres) begins to segregate from the membrane, accompanied by a transmission of some water molecules from the lower side to the upper side. Finally, at t = 4.35 ns (Figure 7C), the cluster comprising 14.7% of the total water molecules present in the system (113 out of 768 beads) is completely isolated from the membrane.

Moreover, the elapsed times, *t*, for each case of the PD when the first cluster segregates, as well as the amount of water molecules in the cluster, were compared. Figure 8 shows the lipid membrane system at PD = 69.2 MPa (Figure 8A), 71.2 MPa (Figure 8B), 73.2 MPa (Figure 8C), 75.2 MPa (Figure 8D), and 77.2 MPa (Figure 8E), t = 4.35 ns, 4.14 ns, 3.48 ns, 2.94 ns, and 2.85 ns, respectively. Thus, it can be said that the first cluster segregation takes less time as the osmotic pressure is increased. On the other hand, the amount of water molecules present in the cluster varied for each osmotic pressure applied as shown in Figure 9. The largest amount of water in a cluster is determined to be 151 water molecules at PD = 71.2 MPa (Figure 8C), while the smallest is 90 at PD = 75.2 MPa (Figure 8D), corresponding to 20% and 12% of the total water molecules, respectively. Simulations below 69.2 MPa did not show any cluster segregation, nor was water transmission through the lipid observed. In general, since water transmission always precedes the first cluster segregation in Figures 8A-E, it may be stated that the former process activates the latter.

CONCLUSION AND RECOMMENDATIONS

Results obtained for the area per lipid and the bilayer thickness were in close agreement with previous theoretical and experimental studies. The membrane thickness and volume started to increase with rising temperature from 330 K, and is attributed to an increase in the inter-lipid spaces that is favourable to water migration across the membrane. Meanwhile, a minimum osmotic pressure of 69.2 MPa would lead to a significant amount of water to permeate across the membrane. The occurrence of this water permeation is hastened at higher osmotic pressures.

Experiments can be performed to verify the above results on the effects of osmotic pressure on the rate of water extraction from the cell membrane. This could be done by adopting the procedures of Parsegian et al. (1986) who studied DPPC liposomes subjected to various osmotic stresses using polyvinylpyrolidone. Further study on the effect of temperature on the rate of water extraction is also recommended. Heterogeneity in the lipid composition can also be investigated as a better representation of many types of lipid membranes.

Information extracted from the simulations, such as the effects of osmotic pressure, temperature, and time, provides deeper understanding of the mechanism of water extraction from cell membranes. Adopting the principle of osmosis for microalgae drying offers an alternative method, which does not require complex mechanical systems and high energy input. Thus, it will minimize the cost of the drying process. Further simulations and actual experiments (such as the use of different solutes) are needed to realize the effectiveness of this approach specific for microalgae drying.

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CONFLICTS OF INTEREST

None

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