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Examining Water Diffusion Through Phospholipids by Molecular Dynamics

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Abstract: Biodiesel is a carbon neutral renewable source of energy which can be an alternative to fossil fuel. One potential source of biodiesel which is recently being studied is microalgae. Microalgae are composed of lipids which can be processed into biodiesel. However, one of the primary concerns regarding the commercialization and production of microalgal biofuel is the drying process. A search for a new drying method that is less energy intensive is needed to make microalgal biodiesel viable. On this regard, we opted to model the drying process of microalgae using molecular dynamics.

The objective of this study is to investigate the effects of varying the temperature and pressure on the mechanism of extracting water from a lipid bilayer composed of 32 dilauroylphosphatidylcholine (DLPC) lipids, 32 dioleolyphosphatidylcholine (DOPC) lipids, 32 dipalmitoylphosphatidylcholine (DPPC) lipids, 32 distearoylphosphatidylcholine (DSPC) lipids and 6000 water molecules in an all-atom force field. The lipid bilayer was modelled using GROMACS (GROningen MAchine for Chemical Simulations) and VMD (Visual Molecular Dynamics) software. The system was equilibrated at 300 K temperature and 1 bar pressure for 100 ns. The temperature was varied from 300 K to 370 K with 10 K interval. The pressure was varied from 7.50 MPa to 13.50 MPa with 2 MPa interval to investigate the effect of increasing the pressure on the migration of water through the lipid bilayer.

Results showed that increased temperature increased the bilayer thickness. Furthermore, an increase in pressure resulted to a decrease in the time it took for the water to migrate through the lipid bilayer.

Key Words: Molecular Dynamics; lipid bilayer; GROMACS

1. INTRODUCTION

Fossil fuels supply most of the world's energy needs (Dresselhaus and Thomas, 2001). Approximately 80% of the global energy demand for transportation, electricity and energy generation is being supplied by fossil fuels (Halim et al., 2012). However, the continuous reliance on fossil fuels is one of the causes of global warming because of its high carbon dioxide (CO_2) emission. Figure 1 show the projected increase in world energy consumption from 1965 to 2035 (Dudley et al., 2014). It is expected that the production of fuel by using renewable source of energy will grow.

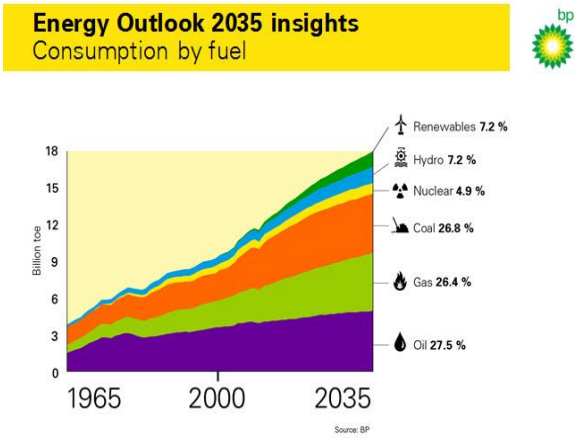

Fig. 1. BP's Energy Outlook 2035

Many researchers have engaged in assessing and determining which among the alternative sources of energy is scientifically achievable, environmentally suitable, and technologically promising. One relatively new source of renewable energy which has gained much attention is the production of biodiesel from microalgae (Christi, 2007, Halim et al., 2012). 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 are not part of human food consumption.

The processes in producing microalgal biodiesel are cultivation, harvesting, drying, oil extraction and transesterification. The challenge is to minimize the energy requirement in each part of the production process to make the commercialization of microalgal biodiesel feasible. The drying process has the highest energy input among other processes (Yanfen et al., 2012).

Microalgae cells undergo the drying process to remove all the water molecules and only lipids are left. Lipids have hydrocarbon which is the main component of petroleum and natural gas. A cell membrane (e.g. microalgae cells) is consists of three types of lipids. These are phospholipids, glycolipids, and sterols. The amount of each type of lipid varies depending on the type of cell. Generally, phospholipids are the most abundant (Lodish et al., 2005).

Karjiban et al. (2013) investigated the structural and dynamics properties of a bilayer comprising 128 molecules of different types of lipids in water using coarse-grained molecular dynamics simulation technique. They used different types of lipids to give a more accurate representation of a cell membrane. They found out that the area per lipid and bilayer thickness were different from when single lipid was used.

Manrique et al. (2014) investigated the migration of water in a lipid bilayer for microalgae drying using dioleolyphosphatidylcholine (DOPC) lipids since it is the most abundant in one type of microalgae called the *chlorella vulgaris*. They used molecular dynamics simulations in a coarse-grained bilayer model to show the effects of varying osmotic pressure and temperature on the migration of water across the lipid bilayer.

Following this study, we simulated the migration of water across a bilayer with different phospholipids using molecular dynamics. It is important to understand the mechanism of extracting water from microalgae in order to come up with an alternative way to reduce the energy spent in drying. This study is an attempt to elucidate the diffusion of water across a microalgal cell to propose an alternative way of drying-up microalgae. Particularly, it aims to determine the effects of changing temperature and inducing pressure.

With the motivation to make biodiesel from microalgae marketable, we aim to simulate the mechanism of water transport on cell membranes, specifically by means of passive transport. This study aims to provide a theoretical explanation on the mechanism of water migration on cell membranes by using Molecular Dynamics (MD) simulations. MD is one of the primary tools used in analyzing and providing nano-scale picture of membrane structures. It facilitates in studying cell membranes in the molecular level which cannot be easily investigated through an experiment.

2. METHODOLOGY

Phosphatidylcholines (PCs) were chosen for this study since they are the most abundant class of phospholipids. The PCs used were the following: dilauroylphosphatidylcholine (DLPC), dioleolyphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), and distearoylphosphatidylcholine (DSPC). These PCs vary on their alkyl chain lengths which range from 12 carbon atoms in DLPC to 18 carbon atoms in DOPC and DSPC (Fig. 2).

The calculations were performed using the software GROMACS ver.4.6.5 (van der Spoel et al., 2005) which implements molecular dynamics. The systems were visualized using Visual Molecular Dynamics (VMD). The structure of the membrane model used was created by using GROMOS53a6 all-atom forcefield (Oostenbrink et al., 2004). The membrane model was composed of 32 DLPC, 32 DOPC, 32 DPPC, 32 DSPC, and 6000 water molecules (Fig. 3).

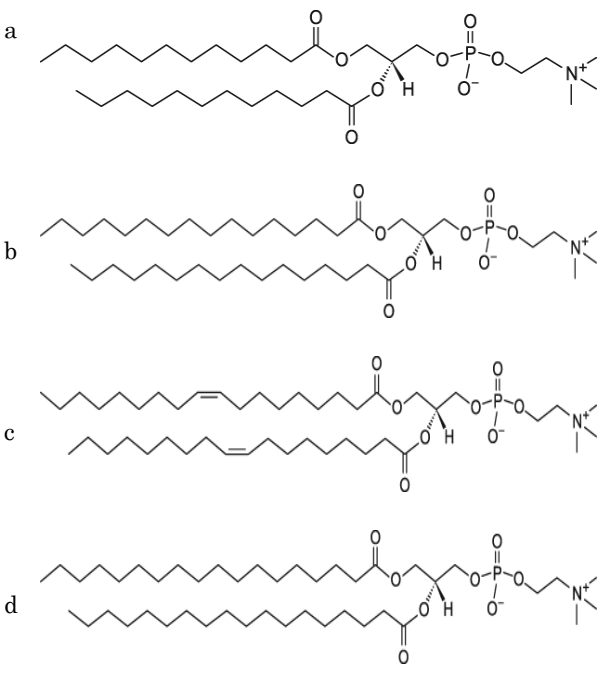

Fig. 2. Phosphatidylcholine lipids chemical structure (a) DLPC (b) DPPC (c) DOPC (d) DSPC (www.avantilipids.com)

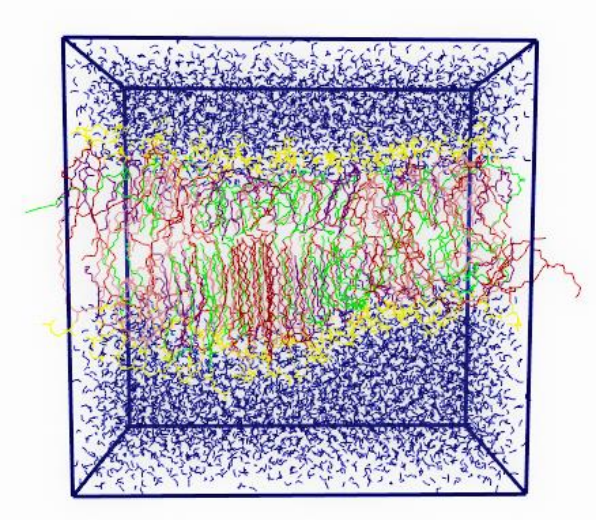

Fig. 3. Bilayer with 32 DLPC (purple), 32 DOPC (green), 32 DPPC (pink), and 32 DSPC (red) in dynamic bonds representation, and 6000 water molecules (blue) in VDW representation as visualized by VMD.

After the membrane model was created, the system was equilibrated for 100 ns at 300 K temperature and 1 bar pressure. The box size was increased to 25 ns along the z-direction to give enough space where water molecules could diffuse to the other side of the bilayer.

A Van der Waals cut-off of 1.4 nm was used and the electrostatic interactions were treated using PME with a real space cut-off of 1.0 nm. All bonds were constraint using the LINCS algorithm (Hess et al., 1997). Temperature coupling was set to v-rescale thermostat (Bussi et al., 2006). However, no pressure coupling was set to ensure that the box size will not change.

At a system pressure of 1 bar, temperature was varied from 300 K to 370 K with 10 K interval to see the effect of varying temperature on the bilayer thickness. The simulations for varying the temperature were performed for 100 ns.

The drying of microalgae was investigated by examining the movement of water molecules across the lipid bilayer. Pressure difference was induced across the cell membrane model by applying a constant force along the z-direction to all the water molecules in the system (Zhu et al., 2002).

At a system temperature of 300 K, a pressure difference was applied on the water molecule towards the z-direction to simulate the pressure that will cause the water to migrate across the lipid bilayer. Osmotic pressure was varied from 7.50 MPa to 13.50 MPa with 2 MPa interval. The simulations for varying the pressure were performed for 100 ns.

3. RESULTS AND DISCUSSION

3.1 Effect of varying temperature on the bilayer thickness

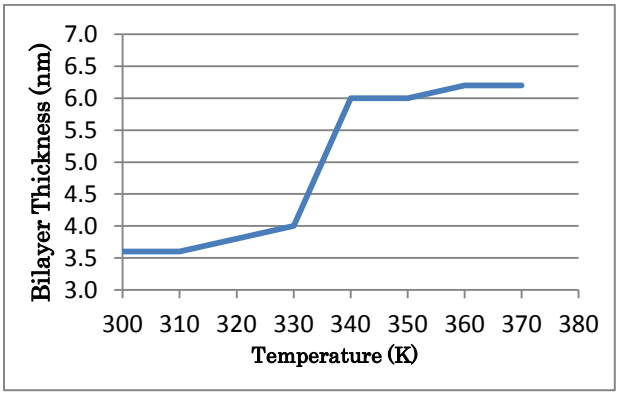

Fig. 4. Bilayer Thickness vs. Temperature

The bilayer thickness was computed from the plot of the phosphate group of the system against the periodic box. The distance between the two centers of mass of the phosphate head group corresponds to the thickness of the bilayer.

Results showed that as the temperature increased the bilayer thickness also increased (Fig. 4). This is in agreement with the finding of Manrique et. al. when they investigated the temperature dependence of DOPC bilayer. The molecules moved rapidly when the temperature was increased which caused the increase in the bilayer thickness of the system.

At 300 K, the bilayer thickness was 3.8 nm. At 310 K to 320 K, it became 3.8 nm. At 330 K, the bilayer thickness increased to 4.0 nm. At 340 K and 350 K, the bilayer thickness was approximately 6.0 nm. At 360 K to 370 K, the bilayer thickness became 6.2 nm.

3.2 Effect of varying pressure

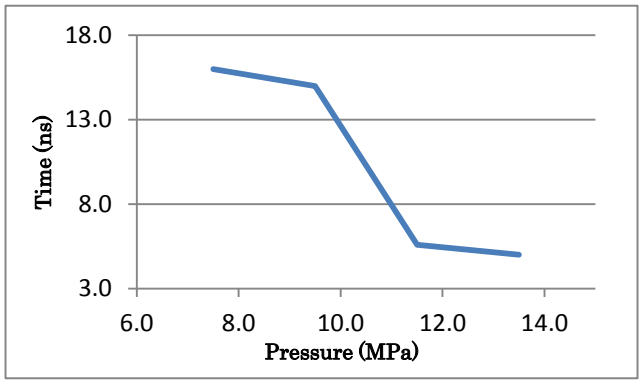

Fig. 5. Time vs. Pressure

The pressure on each water molecule was increased from 7.50 MPa to 13.50 MPa with 2 MPa interval. It was exerted by applying a uniform external force on all the water molecules along the +z direction. This force was computed by multiplying the atomic mass of all water molecules in the system with acceleration in nm/ps^2.

As seen in figure 5, the time needed for the water molecules to cross the bilayer decreased as pressure increased. At 7.50 MPa, it took approximately 16.0 ns before they reached the other side of the bilayer. At 9.50 MPa, the time was around 15.0 ns. At 11.50 MPa, 5.6 ns elapsed before the water molecules were able to cross the bilayer. Lastly, at 13.50 MPa, the time was 5.0 ns.

The data gathered from the simulations were as expected. Pressure is directly proportional to force which means that acceleration is also proportional to pressure. The molecules moved faster as the pressure was increased. This is in good agreement with previous studies conducted by Parsegian et al. (2000).

3.3 Snapshots of the membrane model at 7.50 MPa

Figure 6 shows the movement of the water molecules when the membrane model was subjected into 7.50 MPa pressure.

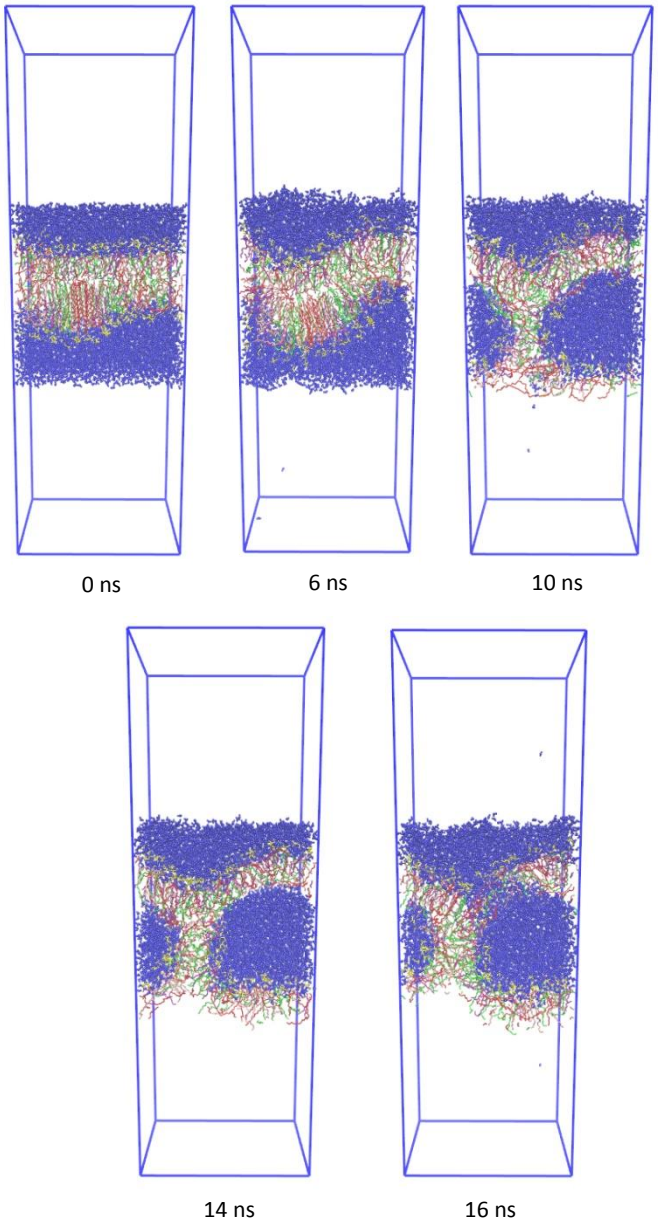

Fig. 6. Snapshots of the membrane model subjected to 7.50 MPa pressure at different timeframe. The phospholipids are represented using dynamic bonds representation while all the water molecules (blue) are in vdw representation

It is apparent from Figure 6 that the water molecules moved across the bilayer. At 6 ns, the water started to move across the bilayer. At 10 ns and 14 ns, water molecules moved continuously across the bilayer. At 16 ns, the water molecules reached the other side of the bilayer.

4. CONCLUSIONS

In this study, we were able to investigate the movement of water molecules across a cell membrane of microalgae. The membrane model used was properly equilibrated at 300 K temperature and 1 bar pressure for 100 ns.

Results from the molecular dynamics calculations showed that bilayer thickness increased when the temperature was increased. Furthermore, at increasing pressure, the movement of water across the bilayer became faster. These results are in close agreement with previous studies (Parsegian et al., 2000, and Manrique et al., 2014).

Information gathered from the simulations, such as the effects of varying pressure and temperature, helps us understand the mechanism of water extraction on cell membranes. This can be implemented in drying microalgae by inducing osmotic pressure using salt or other substances which can cause the spontaneous movement of water from higher concentration to lower concentration. This drying process can be an alternative method which does not require complex mechanical systems and high energy input. This, in turn, will minimize the cost of the drying process. Further simulations and actual experiments are needed to realize the effectiveness of this approach in microalgae drying.

5. ACKNOWLEDGMENTS

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