Presented at the DLSU Research Congress 2017
De La Salle University, Manila, Philippines
June 20 to 22, 2017

An Investigation on the Effects of Time-Independent Electric Field on Periodic DPPC Lipid Bilayer using Molecular Dynamics for Microalgae Drying

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Abstract: The bottleneck in the extraction of commercially viable products from microalgae is the drying process. Solar drying has been the most popular method in the industry, nevertheless, it is a known fact that solar drying has fundamental limitations, which include weather dependency and process controllability. For this reason, an alternative method of extracting microalgae products without the need for drying process is currently being developed. Constant electric field application for a short time period can also be used to extract the nutrients from the microalgae. This may also be used to control contaminants in microalgae cultivation, that could lower costs and higher productivity of microalgae products. In order to optimize the application of constant electric field to microalgae experimentally, visualization and real time analysis of electroporation and permeabilization can be performed nanometer and nanosecond scale through computational simulations. In this study, the effect of uniform electric field (E field) on dipalmitoylphosphatidylcholine (DPPC) lipid bilayer was investigated using course grained molecular dynamics simulation. The direction of the applied electric field is normal to the surface of the bilayer. Results showed that by applying a 0.272-V/nm E field for 3.6 ns create pores which increased the water movement across the bilayer. Exposing the bilayer at a longer period increases the size of the pores until the bilayer collapses and aligns its cross section on the direction of the E field (t ~ 5 ns). The results of this study may contribute to the improvement of current technology for microalgae drying or cultivation.

Keywords: constant electric field, microalgae, drying, DPPC, molecular dynamics

1. INTRODUCTION

The most energy intensive step in the extraction of commercially viable products from microalgae is the drying process. Different methods have been employed to dry the microalgae, but the most common method of microalgae drying is solar drying [1, 27]. Recent improvements on solar dryer designs [2-4] and mathematical study on optimization of solar drying process [5] show lots of potential. Nevertheless, it is a known fact that solar drying has fundamental limitations, which include weather dependency and process controllability [6]. For this reason, an alternative method of extracting microalgae products without the need for drying process is currently being developed.

Pulse electric field (PEF) treatment has been shown by multiple researchers to lyse a variety of microalgae species through electroporation [7]. This eliminates the energy intensive drying step because it allows wet extraction of commercially viable products from microalgae [8]. PEF treatment may also be used to control predators in microalgae cultivation, such as bacteria, amoeba, and multicellular animals [9-11]. The electricity cost of such treatment would be inconsequential compared to the cost of a single pond crash [8]. Hence, PEF treatment shows a lot of promise to provide lower costs and higher productivity of microalgae products.

At present, this technology is still in its infancy and its applicability on industrial scale has yet to be demonstrated. Most researchers are focused on determining the specific pulse type and electric field strength that will enable wet extraction of commercially viable products. However, visualization and real time analysis of electroporation and permeability can only be done at nanometer and nanosecond scale. These are challenging, if not impossible to do in most experimental methods. Molecular dynamics (MD) simulations of lipid bilayers representing cell membranes provide a means to study these processes in the absence of direct observation. Tieleman pioneered this approach when he investigated the mechanism of pore formation by performing MD simulations on dioleoyl-phosphatidylcholine (DOPC) lipid bilayer [12]. His results suggest that the pore formation is driven by local electric field gradients at the water/lipid interface.

Although MD simulations have elucidated the electroporation process in molecular detail, current models are highly simplistic. Real cell membranes are composed of various types of lipids and the amount of each type of lipid varies depending on the type of the cell [13,14]. However, simulations that better resemble physical systems will require very high computational cost using atomistic molecular dynamics. An alternative approach is to use course-grained (CG) MD. Nonetheless, this is seldom done because course-grained water molecules are reduced to van der Waals particle, thus the effect of polarization is absent. Recently, polarizable water model for course-grained force field has been developed [15] but whether it can produce electroporation simulation results comparable to atomistic molecular dynamics has yet to be demonstrated

2. METHODOLOGY

In this study, the effect of uniform electric field (E field) on dipalmitoylphosphatidylcholine (DPPC) lipid bilayer (dominant lipid of Spirulina) was investigated using CG MD simulation. The objective is to simulate pore formation using CG MD simulation and the effects of E field on the bilayer membrane.

The DPPC lipids shown in Figure 1 are represented by 12 CG beads. The choline (NC) and phosphate (PO) headgroups are represented by a single bead. Two glycerol (GL) beads, each of which has attached four carbon (C) beads, represent the tails of the lipid. Four water molecules are represented by a single water bead, which are grouped in threes to form polarized water beads. The system is composed of 128 lipids and 19,536 water molecules which are initially generated from random positions.

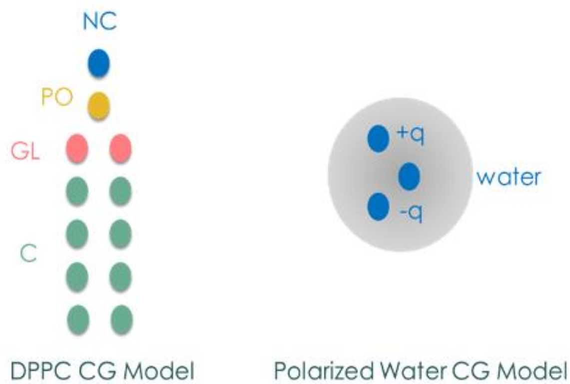

Figure 1 Coarse Grain model of DPPC Lipid

All simulations are performed using GROningen MAchine for Chemical Simulation (GROMACS) 5.1 package with MARTINI forcefield [16,22-26] equilibrated at 320 K and 1 atm as shown in Figure 2. The effect of electric field on microalgae membrane was investigated by performing 10 ns MD simulations with constant electric field (0.050 V/nm increment, 0.400 V/nm max) along the direction perpendicular to the surface of lipid bilayer (z-axis).

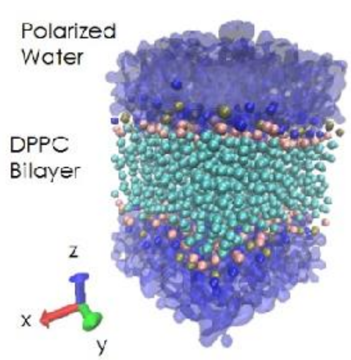

Figure 2 Equilibrated DPPC lipid membranem

3. RESULTS AND DISCUSSION

Spontaneous self-assembly of lipid membrane was observed by performing energy minimization and equilibration. The energy plot during structural minimization is shown in Figure 3.

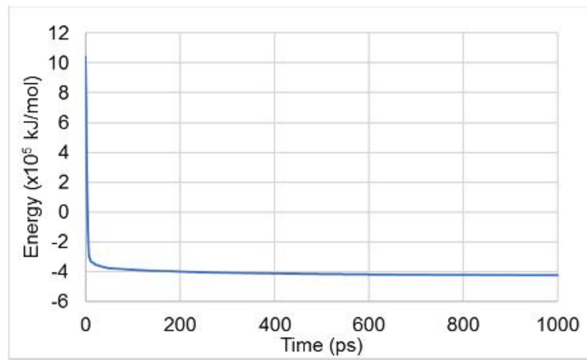

Figure 3 Energy Minimization

Figure 4 shows the thickness plot of the DPPC bilayer during the 100 ns equilibration.

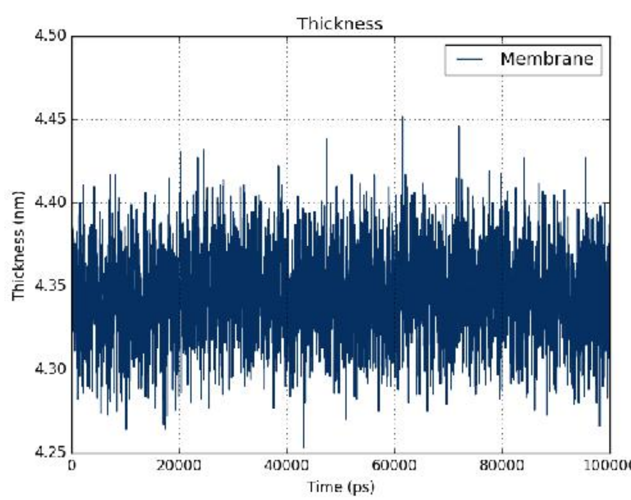

Figure 4 Bilayer Thickness during equilibration for 100 ns

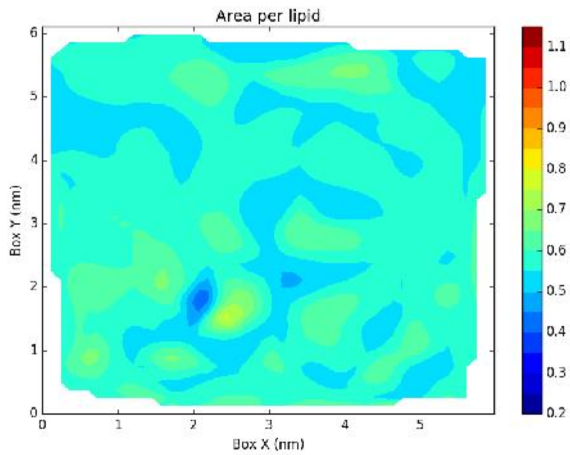

Figure 5 Area per lipid distribution at the end of 100 ns equilibration (color bar in nm^2)

The calculated average area per lipid and average bilayer thickness are 63.0 $Å^2$ as indicated in Figure 5 and 4.33 nm based on Figure 4, respectively. This agrees very well with experimental values [17]. Exposing the bilayer on 0.272-V/nm E field for 3.6 ns create pores which facilitate water movement across the bilayer as shown in Figure 6. Higher exposure times increase the size of the pores until the bilayer collapses and aligns its cross section on the direction of the E field. The electroporating field predicted in this study is comparable to the ones found in the computational literature [18-21]. This demonstrates that CG MD using polarizable water beads can handle electroporation simulations very well.

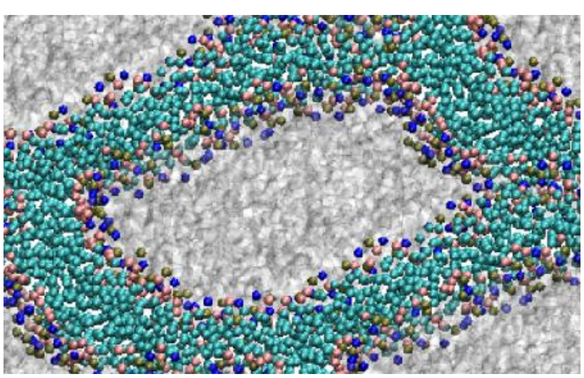

Figure 6 Nanopore formation on DPPC lipids (the white cloudy color are water beads)

More complex membrane systems can be studied using this method in the future. The results of this study may contribute to the improvement of current PEF technology for microalgae drying or cultivation.

5. ACKNOWLEDGMENTS

We would like to thank the United States Agency for International Development (USAID) - Science, Technology, Research, and Innovation for Development (STRIDE) Program, and University Research Coordination Office (URCO) of De La Salle University for the financial support on this project.

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