Electronic Supplementary Material (ESI) for Green Chemistry

Supplementary Information

An effective, cost-efficient extraction method of biomass from wet microalgae with functional polymeric membrane

Gursong Yoo‡[a], Youngmin Yoo‡[a], Jong-Hee Kwon[a], Cornelius Darpito[a], Sanjiv K. Mishra[b], Kwanyong Pak[a], Min S. Park[ab], Sung Gap Im*[ac], Ji-Won Yang*[ab]

[a]Department of Chemical & Biomolecular Engineering, Korea Advanced Institute of Science & Technology, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea. E-mail: sgim@kaist.ac.kr, jiwonyang@kaist.ac.kr; Fax: +82 42-350-3910; Tel: +82 42-350-3924

[b]Advanced Biomass R&D Center, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

[c]KI for NanoCentury, 291 Daehak-ro, Yuseong-gu, Daejeon 305-701, Republic of Korea

‡ These authors contributed equally

1. Images of microalgae with contacting polymer coated membrane and pristine membrane (Supplemental Figure S1)

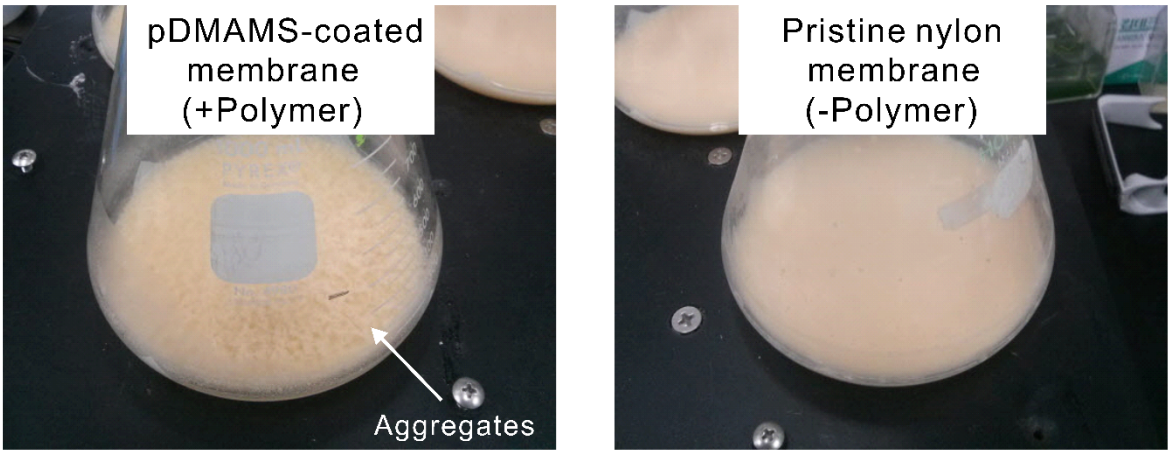
2. Optical microscope images of microalgae by increasing treatment time of polymer coated membrane (Supplemental Figure S2)

3. Enlargement of part of Figure 5.A (Supplemental Figure S3)

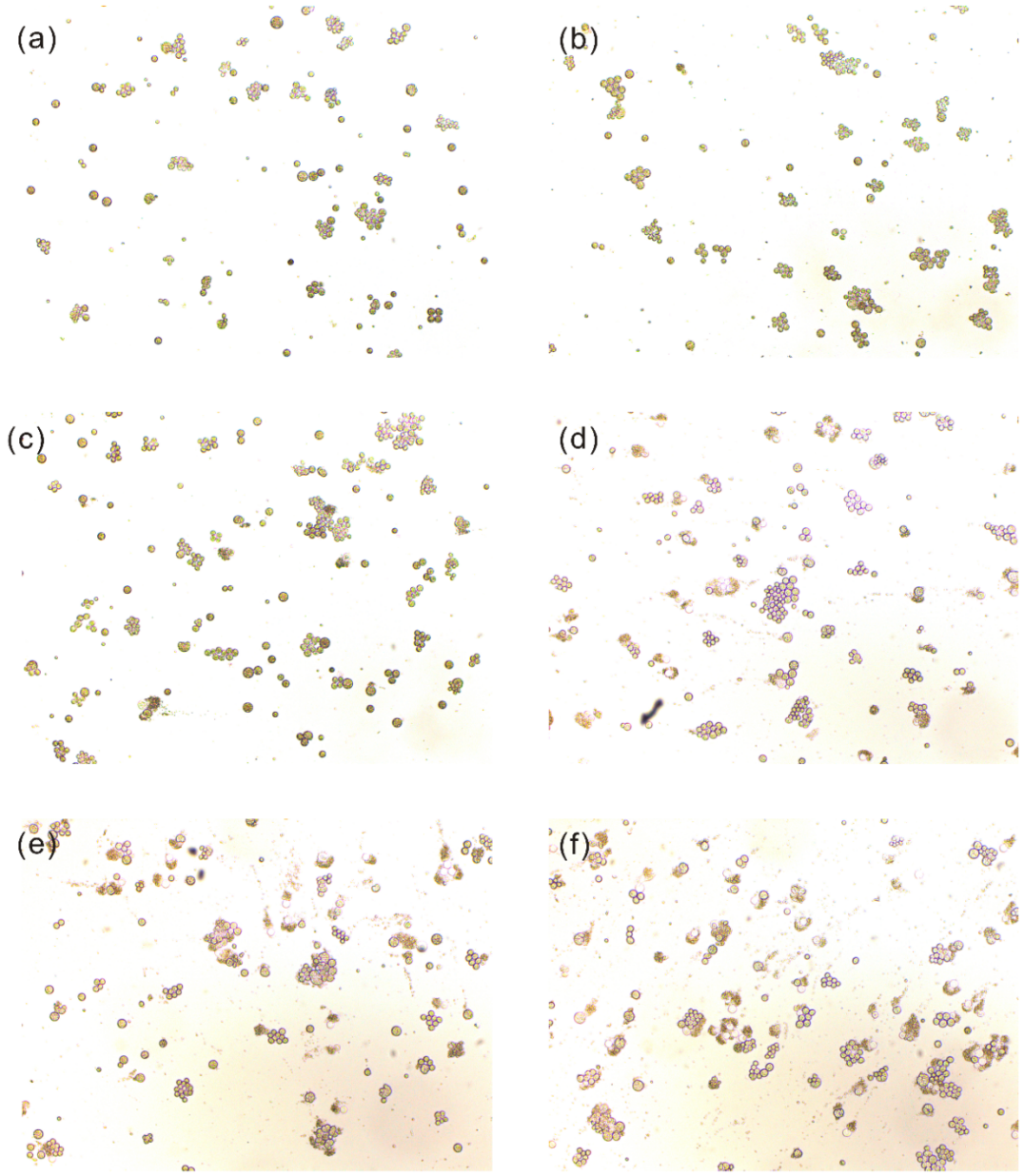
4. Increase of cell agglomeration yield by the contact of *Aurantiochytrium* culture with pDMAMS- coated membrane (Supplemental Figure S4)

5. Lipid extraction yield from wet biomass with hexane (Supplemental Figure S5)

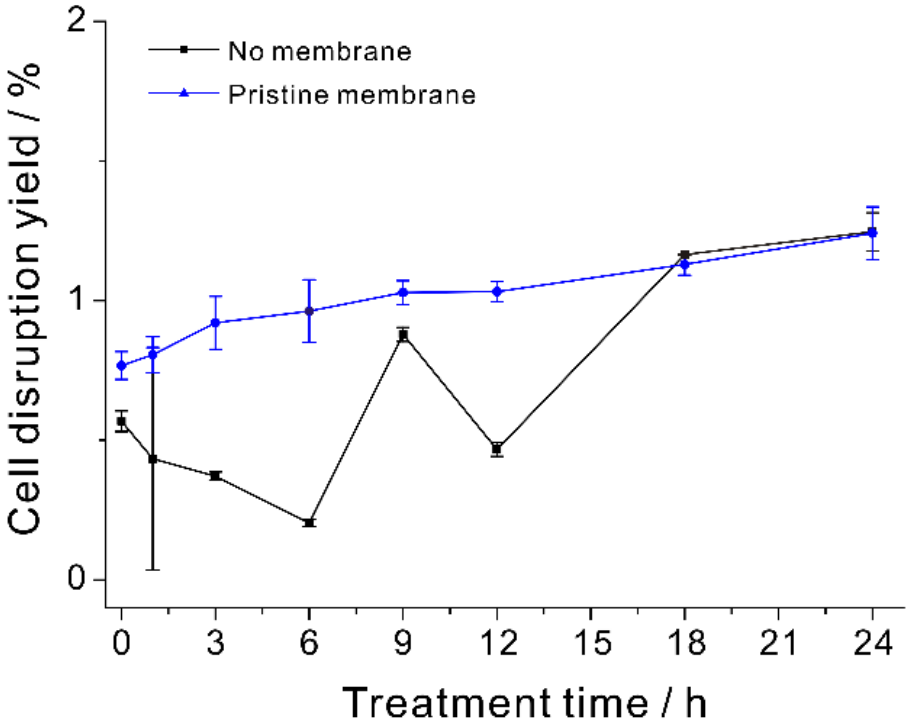
6. Extraction experiment was performed three times with same membrane. Rinsed membrane was used at second experiment and third experiment for checking the reusability of the membrane. (Supplemental Figure S6)



Supplemental Figure S1: Direct response of cell culture to the immersed pDMAMS-coated membrane. Aggregates were observed on short contact of pDMAMS-coated membrane. Pristine nylon membrane did not give any morphologic difference in the cell culture.

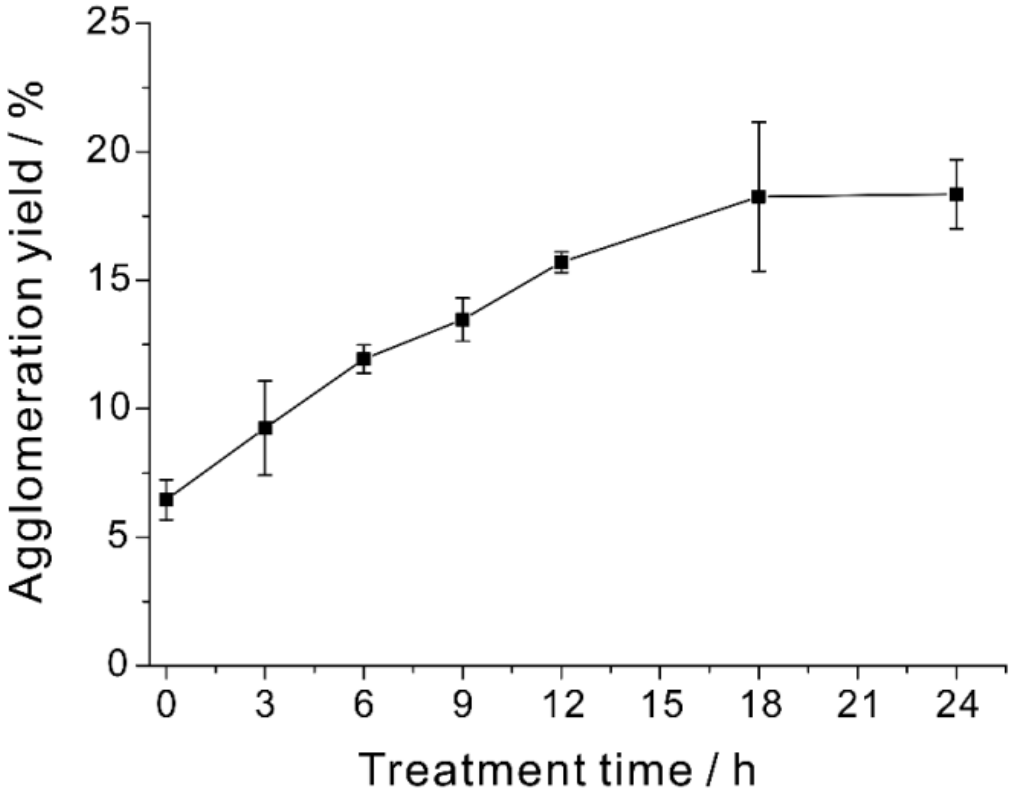


Supplemental Figure S2: Optical microscope observation (a-f) of *Aurantiochytrium* cells with the increasing treatment time (0, 3, 6, 12, 18, and 24 hrs) of the pDMAMS-coated nylon membrane.



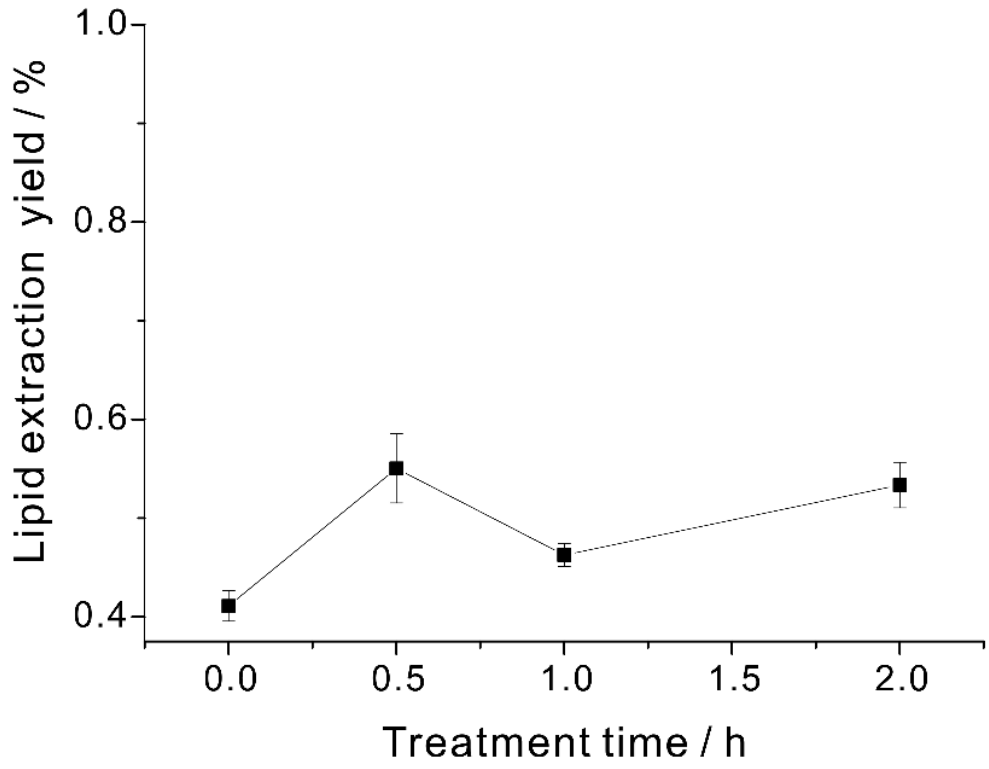
Supplemental Figure S3 : Enlargement of part of Figure 5.A

While the significant increase of cell disruption yield was observed in the flask containing pDMAMS-coated membrane, the flasks with pristine membrane without the functional polymer coating, and with only cell culture without any membrane showed only slight increase of cell disruption yield after 24 hours, 0.47 % and 0.67 %, respectively, which was almost ignorable compared with that from the cell culture with the functional membrane treatment. In our experiment setup, the cell disruption due to autolysis and/or mechanical damage was much lower than we expected.



Supplemental Figure S4 : Increase of cell agglomeration yield by the contact *of* *Aurantiochytrium* culture with pDMAMS- coated membrane.

We added “Supplemental Fig. 4 for the statistical analysis showing increase of degree of agglomeration after treatment of pDMAMS- coated membrane. An evaluation of the degree of cell agglomeration could be approached by counting number of cell agglomerate crowded with over 9 cells under microscopic observation. Agglomeration yield was presented as the ratio of number of over-9-cell agglomerates to total number of cell agglomerates including one cell population under same microscopic view. Three different microscopic regions were chosen for the evaluation of cell agglomeration induced by treatment of pDMAMS-coated membrane.



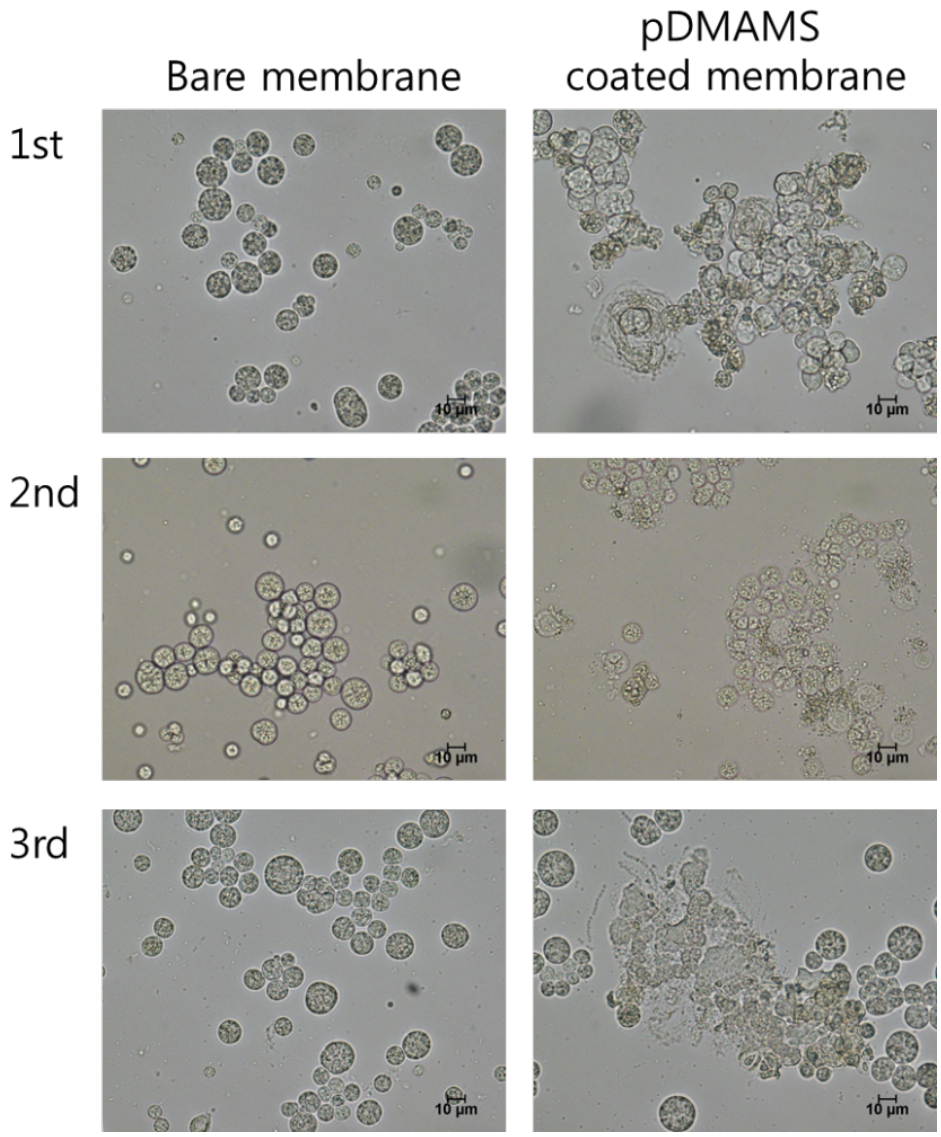
Supplemental Figure S5: Lipid extraction from wet biomass with hexane.

Traditional lipid extraction methods, such as those developed by Folch (Folch et al., 1957) and Bligh & Dyer (Bligh and Dyer, 1959), used a mixture of organic solvents, to extract the lipids from dry biological material. When these techniques are directly applied to a wet microalgal sample, the microalgal cells tend to remain in the water phase due to their surface charges that prevents the direct contact with the organic solvent phase, which resulting in their low extraction yields.

Hexane is an immiscible solvent with water and polar lipid but is a good solvent only for lipids of low polarity. However, only hexane, as mono-solvent system, is hard to extract neutral lipid from the intact wet biomass. Hexane solvent extraction, however, is generally

effective to the dry lipid-rich biomass (Jones et al., J Am Oil Chem Soc (2012) 89:1371–1381).

In this work, cell disruption yield was evaluated by the ratio of the amount of lipids released only from the disrupted cell to total cellular lipids. Therefore, it was mandatory to quantify the amount of lipids released only from the disrupted microalgal cells exclusively. We confirmed no reaction between hexane and wet, intact cell broth culture in terms of inter-lipid extraction. For the comparison, same volume of hexane was added to intact wet algal culture and shaken for 2 hours. No remarkable lipid release from the microalgae was observed after 2 hours hexane treatment.



Supplemental Figure S6. Optical microscopic images of microalgae treated with bare membrane (left) and pDMAMS coated membrane (right). Extraction experiment was performed three times with same membrane. Rinsed membrane was used at second experiment and third experiment for checking the reusability of the membrane.

After the first extraction experiment with the functional membrane, both bare membrane and pDMAMS-coated membrane were taken out from the flask, then washed the used

membrane with 0.01N HCl solution for 5 minutes, followed by rinsing with DI water moderately. With rinsed membrane, the second extraction test was performed. We repeated rinsing process and extraction experiment for third reusable extraction test. Optical microscopic images of microalgae in each test batch are shown in figure S6 above. Most of microalgae treated with bare membrane were still alive and the shape of microalgae was intact in all repeated tests. On the other hand, many of microalgae treated with pDMAMS-coated membrane are disrupted and coagulated with debris and extracted lipid.