Supplementary information

Breaking Dormancy: An energy-efficient means of recovering astaxanthin from microalgae

Ramasamy Praveenkumar[†], Kyubock Lee[†], Jiye Lee and You-Kwan Oh*

Biomass and Waste Energy Laboratory, Korea Institute of Energy Research (KIER), Daejeon 305-343, Republic of Korea

[†] These authors contributed equally to this work.

*** Corresponding author**

Tel.: +82 42 860 3697, Fax: +82 42 860 3495

E-mail address: ykoh@kier.re.kr (Y.-K. Oh)

Table S1: Cell-wall properties and compositions of *H. pluvialis* at different stages of germination process (Damiani et al., 2006; Hagen et al., 2002)

Cell stage	Cell-wall structure					Reaction to chemical treatments
	Primary wall	Trilaminar sheath	Secondary wall	Tertiary wall	Plasmalemma	
Mature red cysts (aplanospores)	O/×	O	O	O	O	Highly resistant
Dividing cysts	×	×	×	O	O	Susceptible
Just-released motile cells (zooids)	×	×	×	×	O	Highly susceptible

O, present; ×, absent; O/×, depending on culture age.

Primary wall: 150-200 nm thick.

Trilaminar sheath: 30-40 nm thick, composed of algaenan (acetolysis-resistant material).

Secondary wall: 400-700 nm thick, composed of mannose and cellulose with homogeneous arrangement;

Tertiary wall: composed of mannose and cellulose with heterogeneous arrangement;

Typical cell-wall composition: 70% carbohydrates (66% hexoses and 3% cellulose), 6% proteins and 3% acetolysis-resistant material

<References>

Damiani, M.C., Leonardi, P.I., Pieroni, O.I., Cáceres, E.J. 2006. Ultrastructure of the cyst wall of Haematococcus pluvialis (Chlorophyceae): wall development and behaviour during cyst germination. *Phycologia*, **45**(6), 616-623.

Hagen, C., Siegmund, S., Braune, W. 2002. Ultrastructural and chemical changes in the cell wall of Haematococcus pluvialis (Volvocales, Chlorophyta) during aplanospore formation. *European Journal of Phycology*, **37**(2), 217-226.

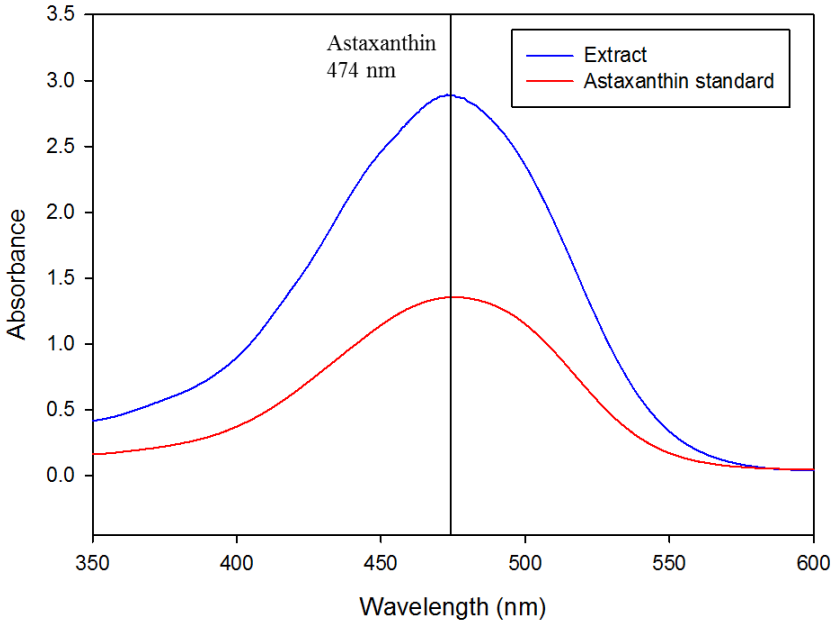

Fig. S1: UV-vis spectrum of astaxanthin extract from *H. pluvialis* (in blue) compared with the chemical AXT standard (in red). Astaxanthin gives peak at 474 nm, which could be a suitable wavelength for HPLC detection and quantification of astaxanthin in the extracts.

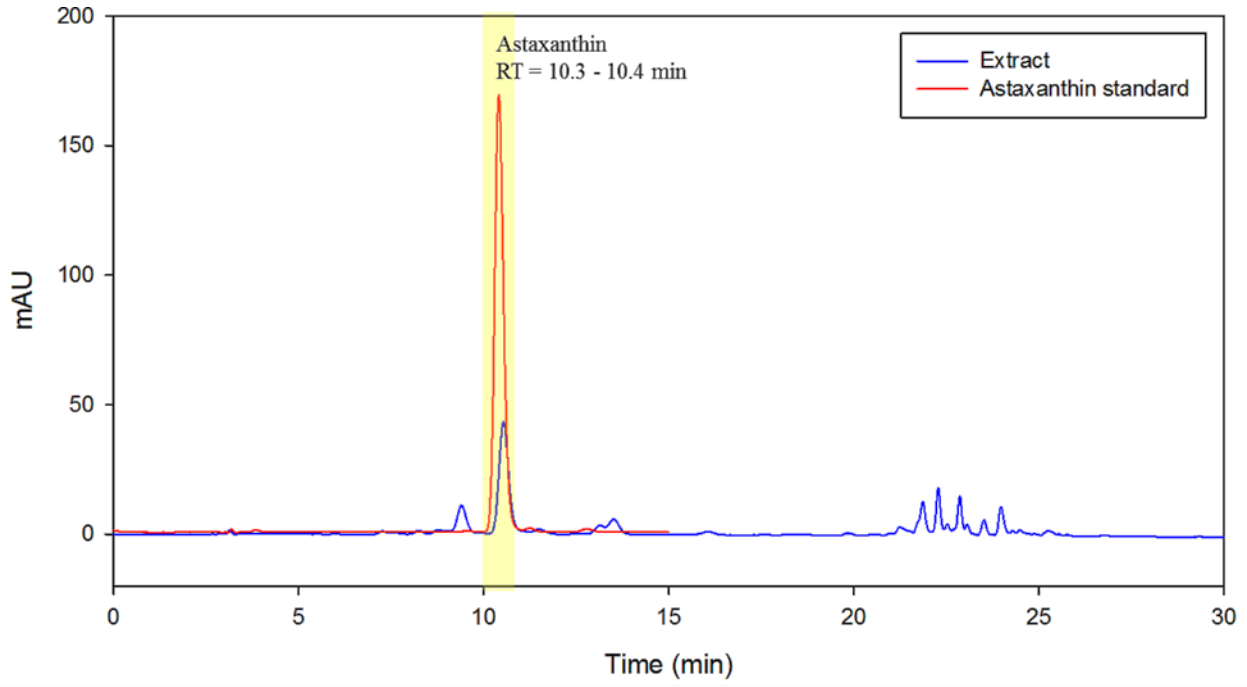

Fig. S2: HPLC chromatograms of astaxanthin from germinated *H. pluvialis* extracted with [Emim] $EtSO_4$ (in blue) compared with the chemical astaxanthin standard (in red).

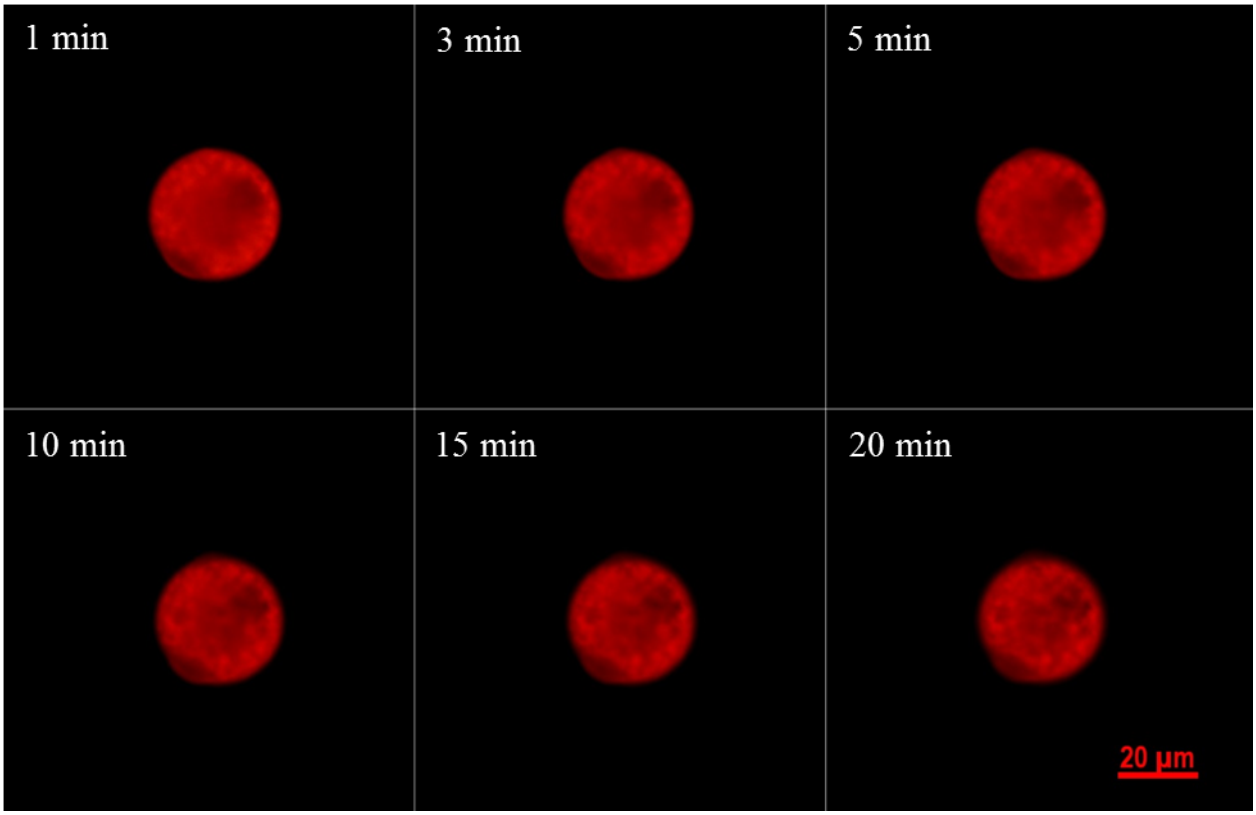

Fig. S3: Chlorophyll auto-fluorescence microscopic images of mature red cyst treated with [Bmim] $MeSO_4$ at 60ºC for 20 min

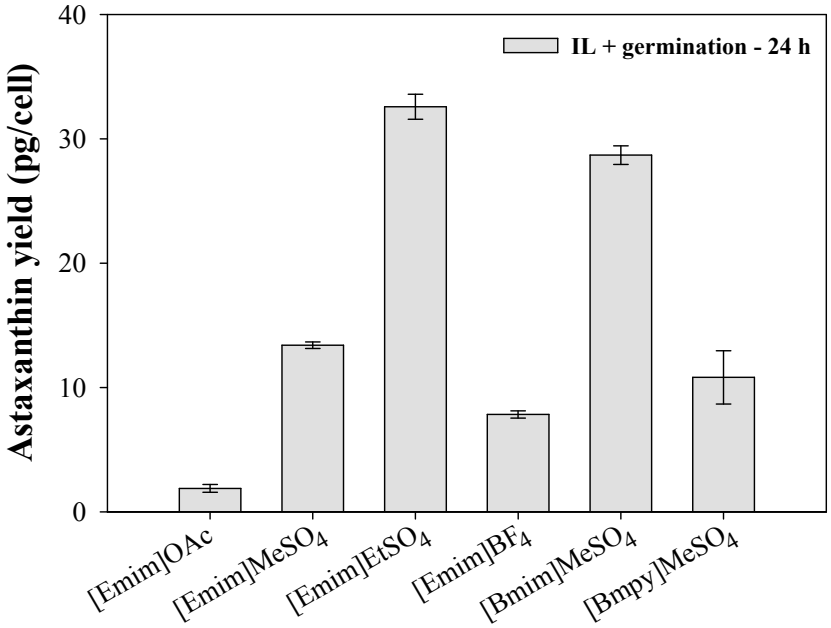

Fig. S4: Astaxanthin extraction yields from *H. pluvialis* cells germinated for 12 h using various ILs. Germinated cells (1 mL) were harvested and mixed with ILs (0.5 mL) and then incubated for 24 h at room temperature.

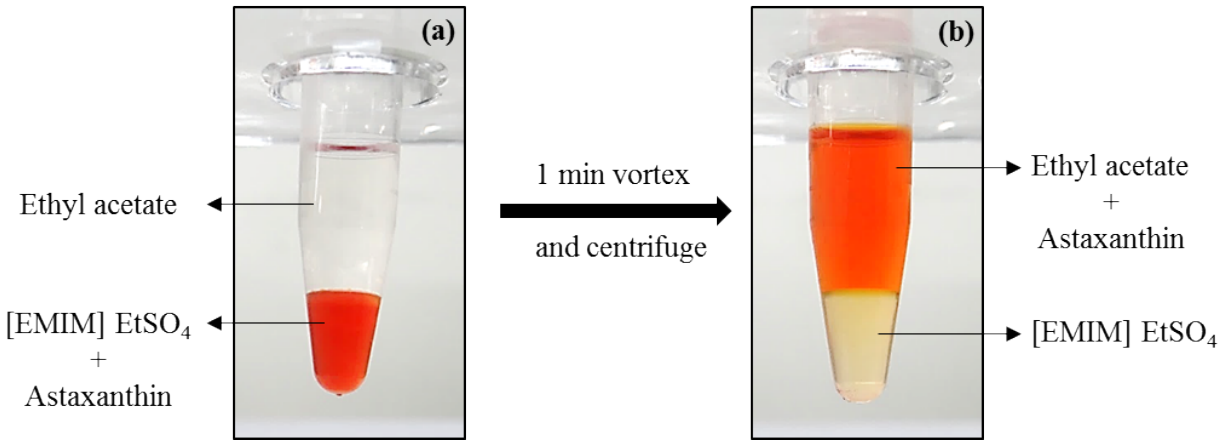

Fig. S5: Recovery of astaxanthin from [Emim] $EtSO_4$ through biphasic separation with ethyl acetate. (a) Biphase of astaxanthin extract in [Emim] $EtSO_4$ with ethyl acetate (b) recovery of astaxanthin in ethyl acetate layer from [Emim] $EtSO_4$ through simple mixing.

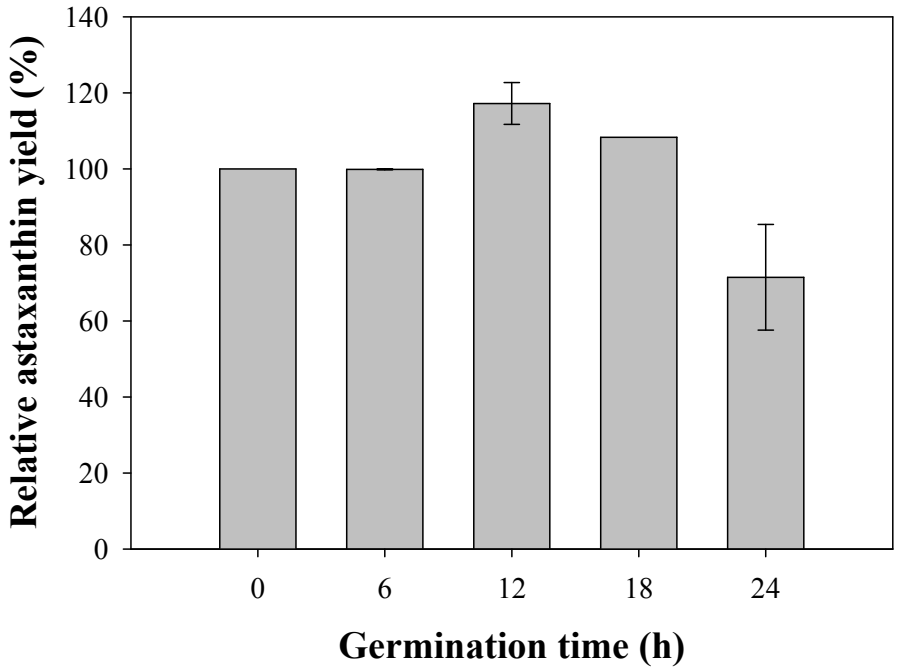

Fig. S6: Time-course changes of astaxanthin extraction yield during 24 h germination process of mature cyst cells. To ensure complete disruption of *H. pluvialis* cells, high-pressure (30,000 psi) homogenization was applied (see also Fig. S7), after which astaxanthin was extracted by ethyl acetate. The astaxanthin yield was quantified as pg/cell based on the absolute cell count value at each sampling point, and the 0 h yield was taken as 100%. Mature red cysts (~2 x 10^5 cells/mL) were transferred to 100 mL NIES-C medium and incubated (25^{o}C) in a shaking incubator (150 rpm). Light was continuously supplied (40 μmol/m^2•s).

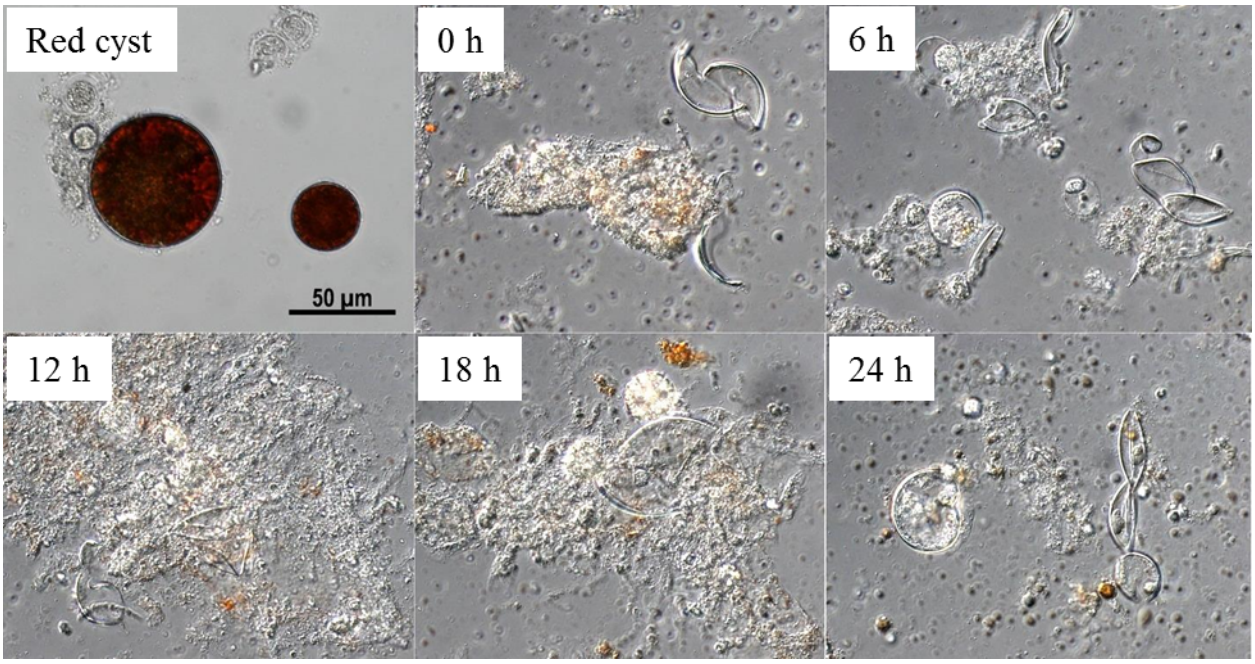

Fig. S7: Microscopic images after extraction of astaxanthin from germinating *H. pluvialis* cells using high-pressure homogenization plus ethyl acetate treatment. See Fig. S6 for the overall process and astaxanthin yields.

Video S1: Time-lapse video showing 24 h germination of mature red cysts of *H. pluvialis*. The mature cysts were introduced in NIES-C medium and incubated in a sealed gene frame under light microscopy. The images were captured by an AxioCam HRc CCD camera and compressed to 12 fps video using AxioVision software. The length of the video corresponds to 24 h.

Video S2: Time-lapse video showing efficiency of [Bmim] $MeSO_4$ in extracting astaxanthin from dividing cyst of *H. pluvialis*. The dividing cyst was collected after 12 h of germination of mature cyst cells in NIES-C medium and treated with 50 μL [Bmim] $MeSO_4$. The images were captured by an AxioCam HRc CCD camera and compressed to 6 fps video using AxioVision software. The length of the video corresponds to 5 min.