Isolation and Screening of Oleaginous Marine Microalgae from Kendari Waters, Southeast Sulawesi Suitable for Outdoor Mass Cultivation in Hypersaline Media as Biodiesel Feedstocks

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Background

Fossil fuels run out vs fuels consumption increase – The need for renewable biofuels

Microalgae are potential biodiesel feedstock

Species/strain selection – the 1st and critical factors

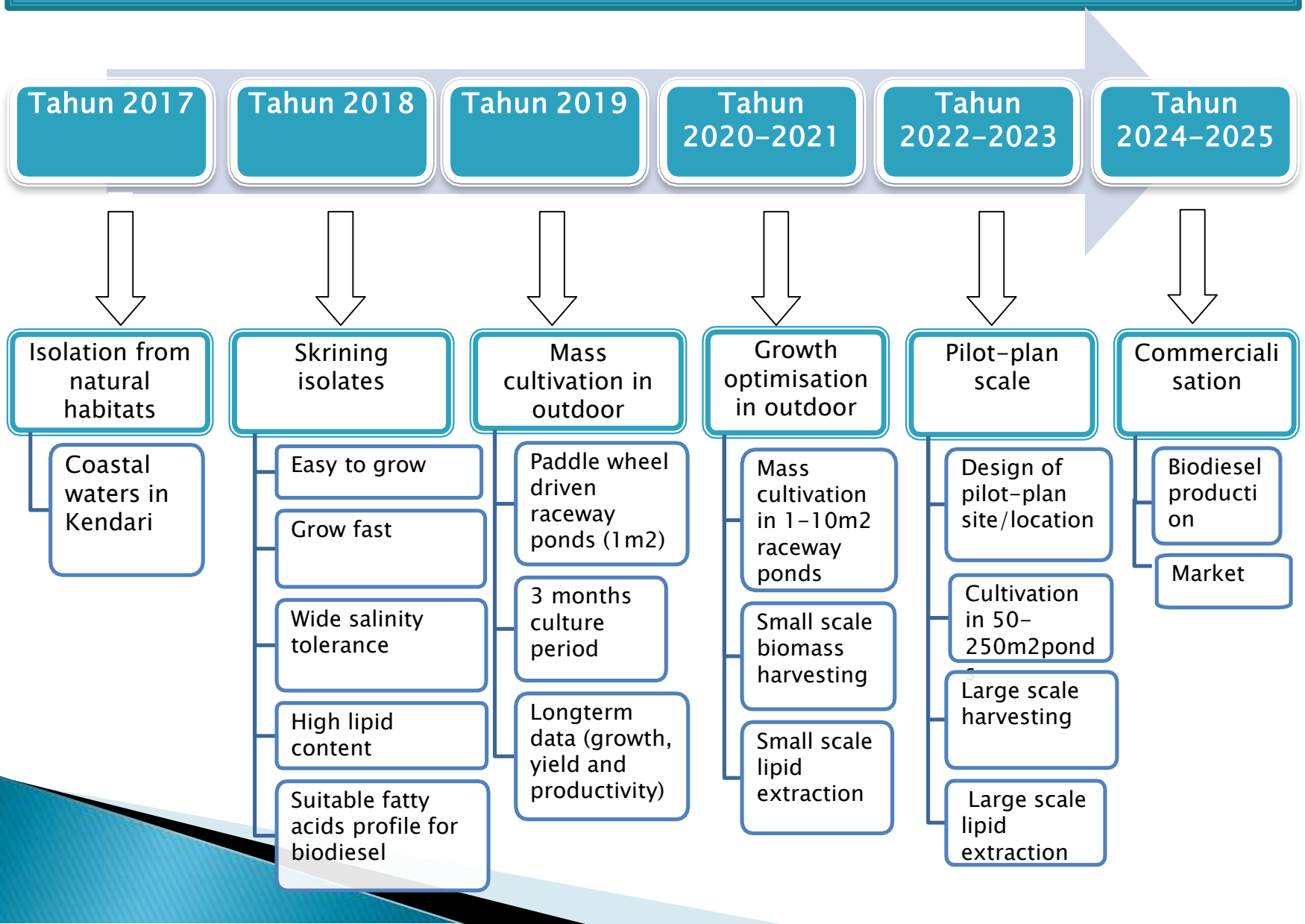
Aims of study :

1. To isolate local species marine microalgae from Kendari Waters

2. To screen the newly isolated microalgae for biodiesel feedstock

3. To cultivate the selected isolates in outdoor raceway ponds

Roadmap


Tahun 2017
Tahun 2018
Tahun 2019
Tahun 2020-2021
Tahun 2022-2023
Tahun 2024-2025
Isolation from natural habitats
Coastal waters in Kendari
Skrining isolates
Easy to grow
Grow fast
Wide salinity tolerance
High lipid content
Suitable fatty acids profile for biodiesel
Mass cultivation in outdoor
Paddle wheel driven raceway ponds (1m2)
3 months culture period
Longterm data (growth, yield and productivity)
Growth optimisation in outdoor
Mass cultivation in 1-10m2 raceway ponds
Small scale biomass harvesting
Small scale lipid extraction
Pilot-plan scale
Design of pilot-plan site/location
Cultivation in 50-250m2pond
Large scale harvesting
Large scale lipid extraction
Commerciali sation
Biodiesel producti on
Market

Materials and Methods

- Sampling sites : Batu Gong Beach, Toronipa Beach, Bokori Island, Nambo Beach and Tanjung Tiram Beach

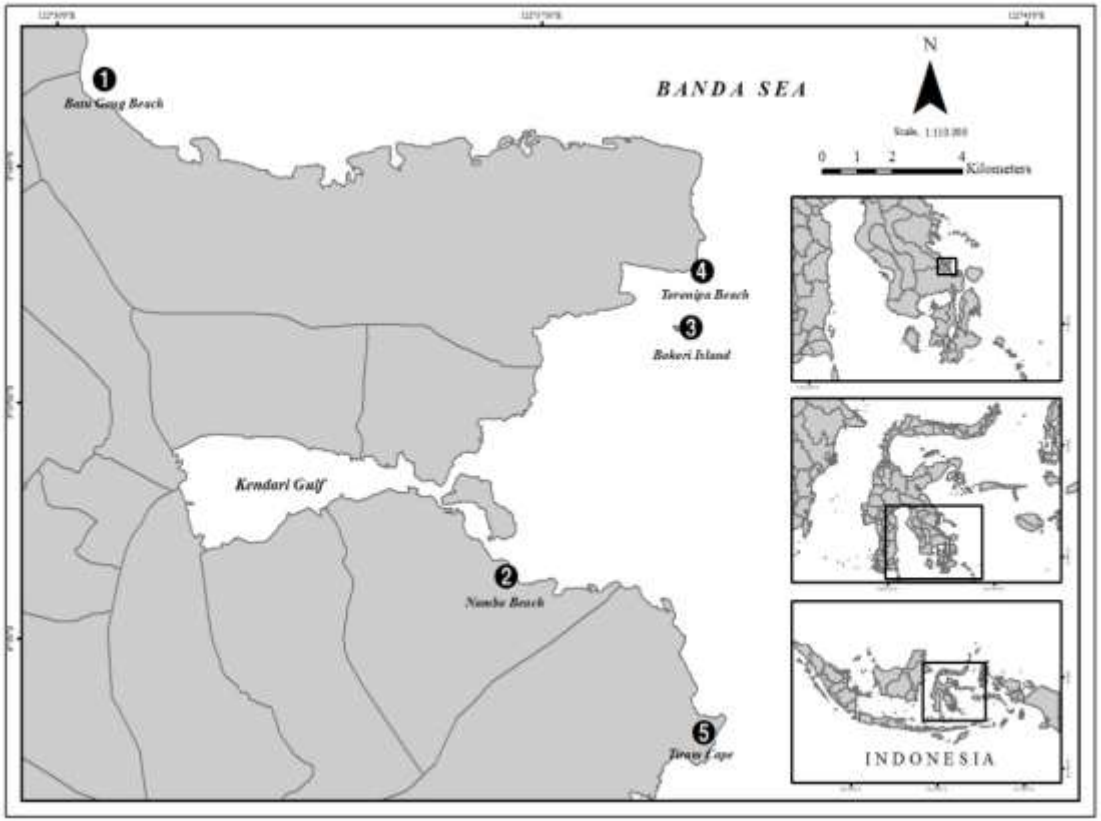


Fig 1. Sampling sites

Materials and Methods

Isolation

- Sampel collected manually and using a planktonet
- Isolation with agar plating and enrichment methods
- Repeated streaking on agar plates to obtaine pure isolates

Screening

- Scale-up isolates from colonies-2mL-10mL-100mL
- Preliminary screening
- Grow the selected isolates under different salinity (2-7% NaCl), ambient room temp, f/2 medium, 12 h LD cycles, 100 µmol.photon.m^{-2}.s^{-1} , bubling with air to facilitate mixing
- Determine growth, biomass and lipid yields, lipid content, biomass and lipid productivities, fatty acid compositions

Outdoor culture

- Inoculum preparation in the laboratory
- Inoculation in outdoor raceway ponds (2x0.8x0.4m) (PxLxT)
- Culture for 3 months under semi-continuous mode
- Sampling for cell counting every 2 days
- Sampling for biomass dan lipid every 4 days

Materials and Methods

Analytical Methods

- Cell numbers determined using Neubauer haemocytometer
- The specific growth rate (μ) was calculated using the following equation: μ=(Ln (N2/N1)/(t2-t1)
- Where *N1* and *N2* are the cell density at time 1 (*t1*) and 2 (*t2*).
- Biomass Productivity = μ x biomass yield
- Total lipid determination was conducted by the method of Bligh and Dyer (1959) as modified by Kates and Volcani (1966)
- Lipid Productivity = μ x lipid yield
- Fatty acids composition using GC-MS

Statistical Analysis

- Significant differences between treatments were analysed with a one-way analysis of variance (ANOVA).
- All statistical analysis was performed using Sigma-Plot 14 Systat Software Inc., USA.

Results and Discussion

- **Isolation of Microalgae**

a

b

c

Fig 2. Isolates grown on agar plate (a), 24 and 96 microtiter well-plates (b) and small containers and flasks (c)

- Hundreds of isolates generated
- 67 isolates can grow in liquid medium
- 8 isolates showed good growth and did not stick to the culture vessels
- The 8 isolates were then screened for their ability to grow at high salinity
- Two isolates (IND-UHO-029, IND-UHO-003) showed very good growth over a wide range of salinity tested (3-5% NaCl)

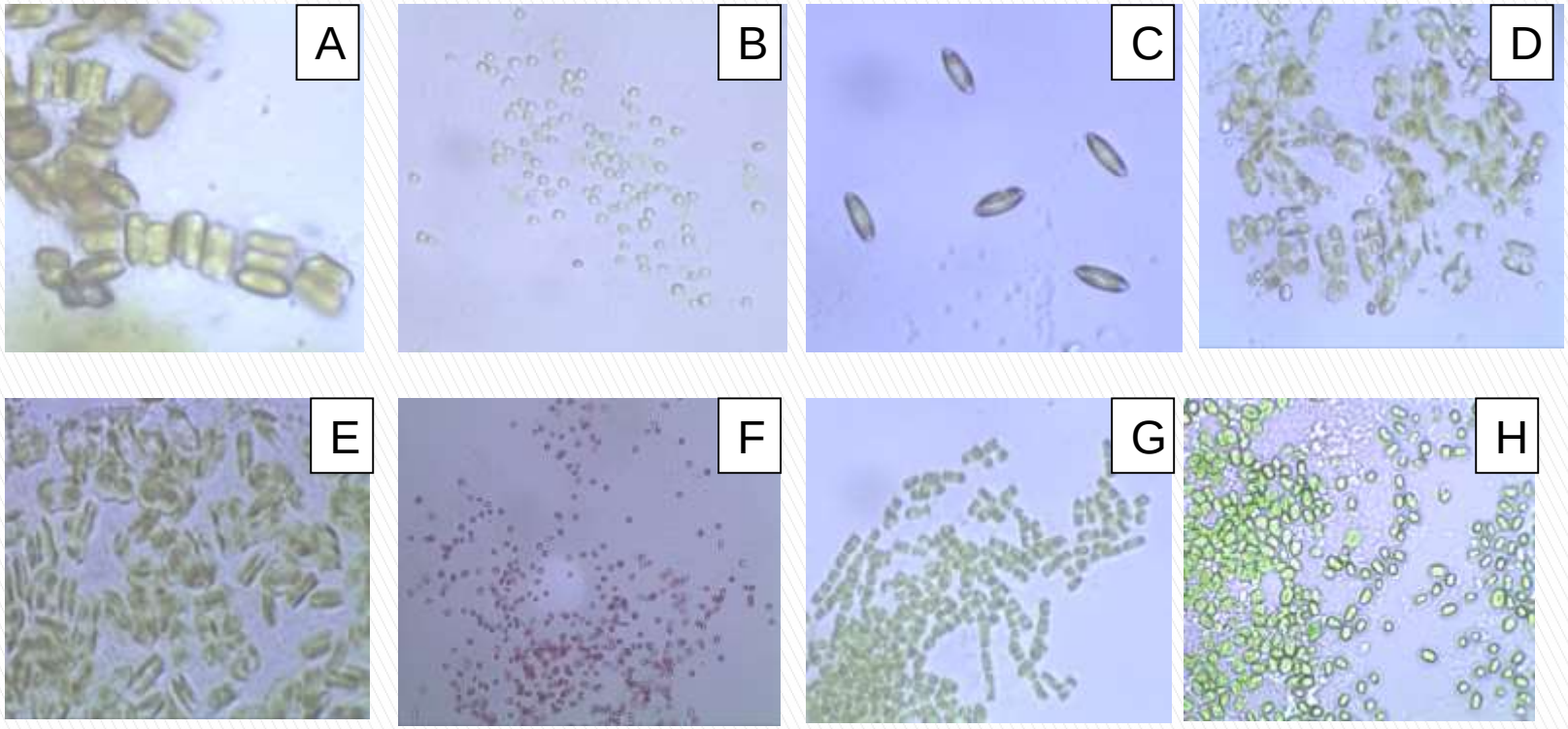
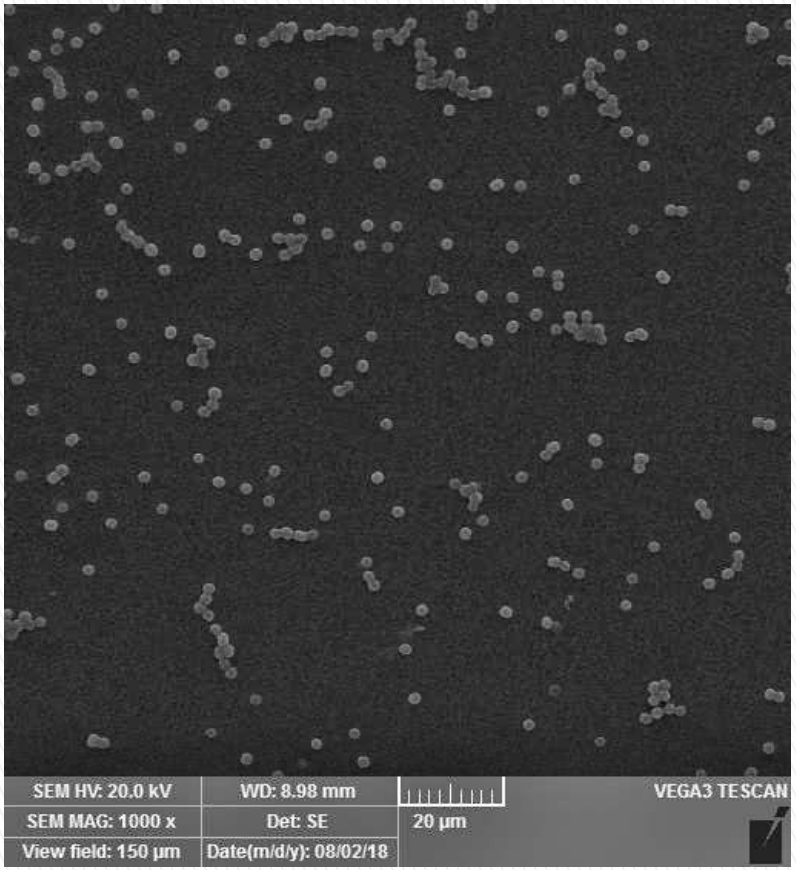
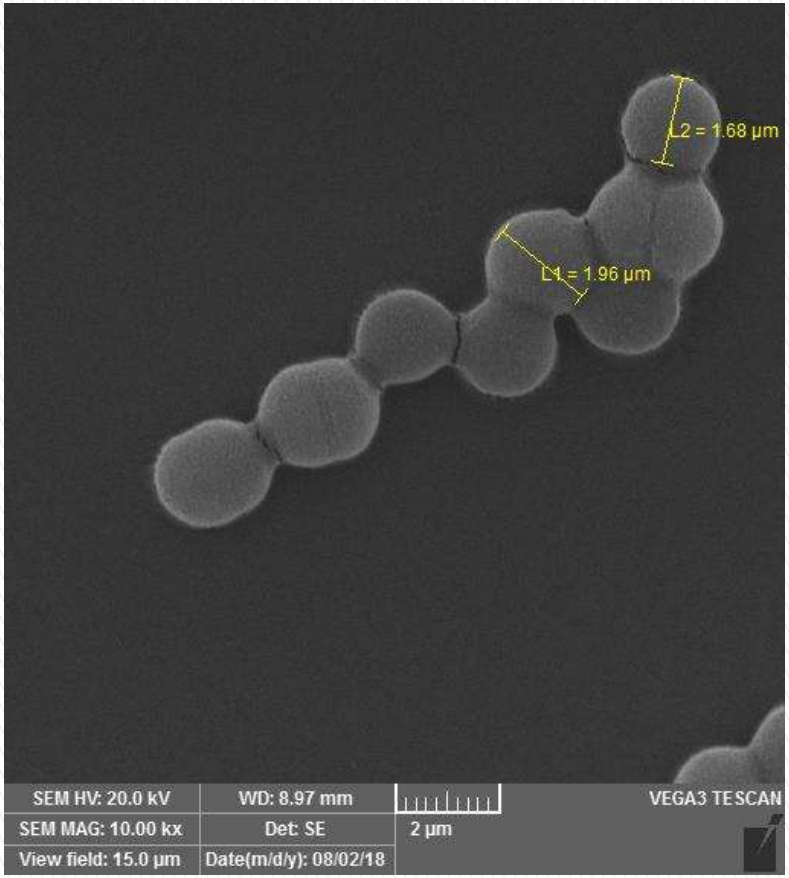


Figure 3. Photomicrograph (at 100x magnification) of Newly isolated microalgae potential for mass cultivation in outdoors: A.Isolate IND-UHO-002, B. Isolate IND-UHO-003, C. Isolate IND-UHO-017, D. Isolate IND-UHO-018, E. Isolate IND-UHO-019, F. Isolate IND-UHO-022, G. Isolate IND-UHO-029, H. Isolate IND-UHO-072

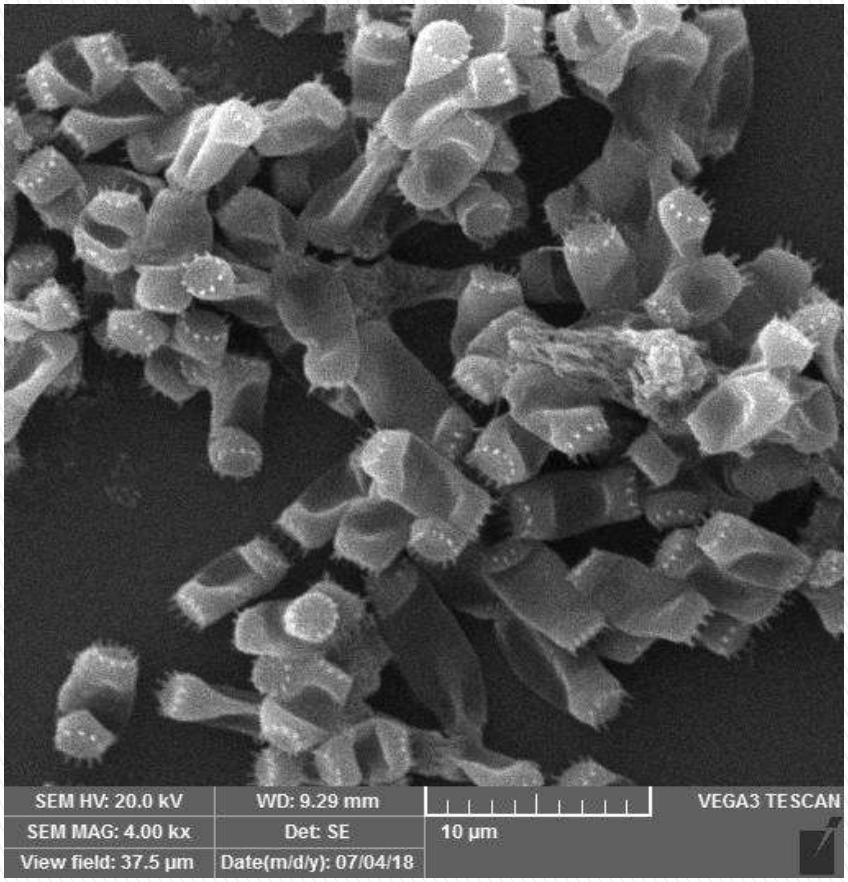


1000x magnification

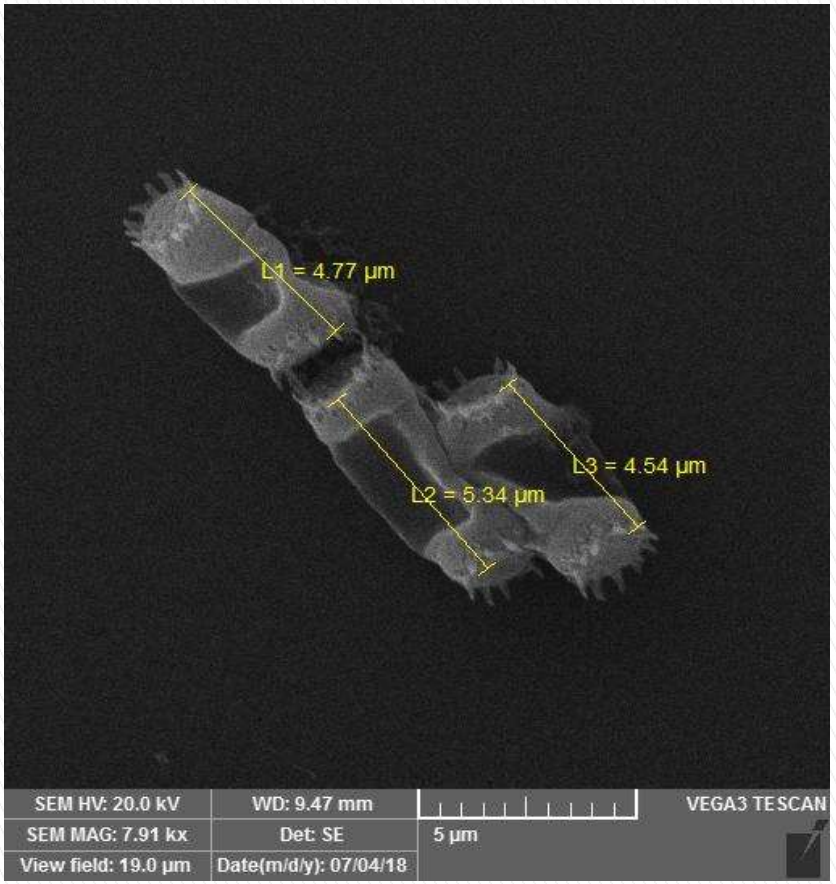


10000x magnification

Figure 4. SEM Images of Isolat *Nannochloropsis* sp.UHO 003



4000x magnification



8000x magnification

Fig 5. SEM images of *Skeletonema* sp.UHO 029

Growth, Lipid Productivity and Fatty Acids Composition of *Nannochloropsis* sp.UHO 003 and *Skeletonema* sp.UHO29 at increasing salinity

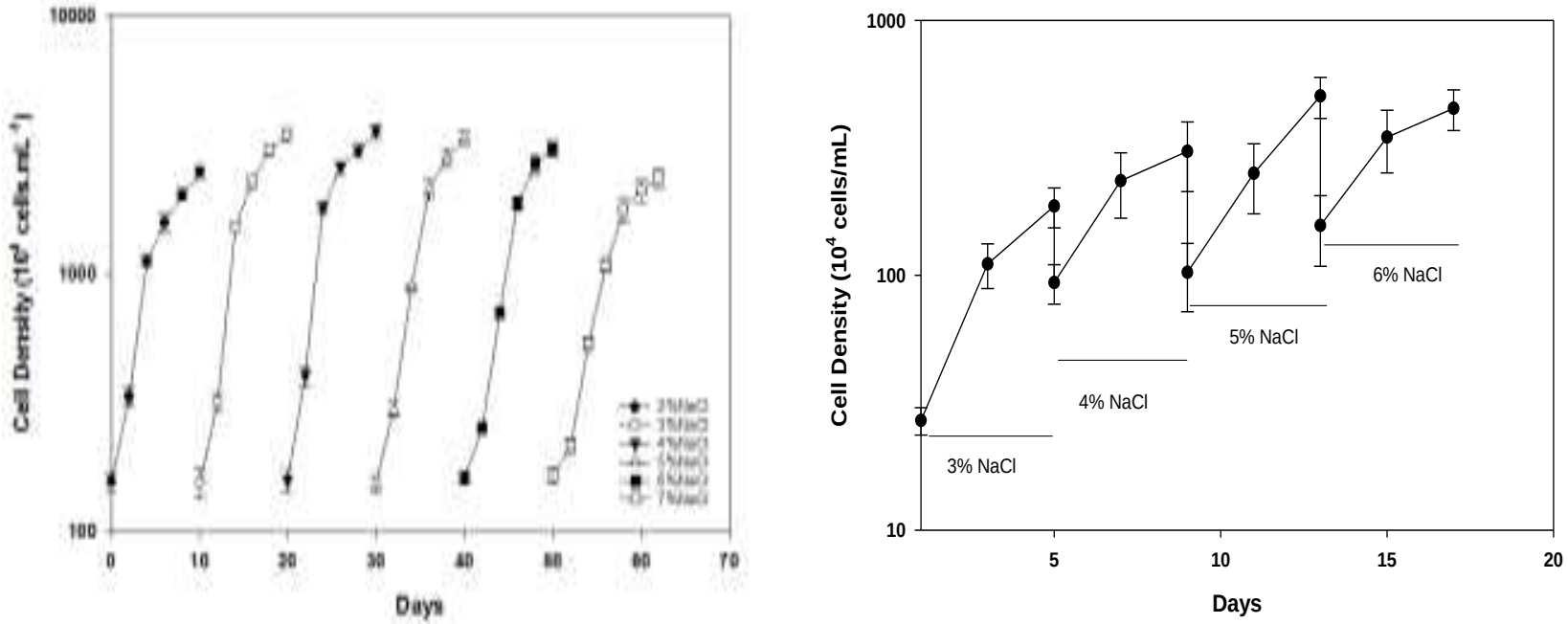


Figure 6. Growth curve of *Nannochloropsis* sp.UHO3 (left) and *Skeletonema* sp.UHO29 (right)

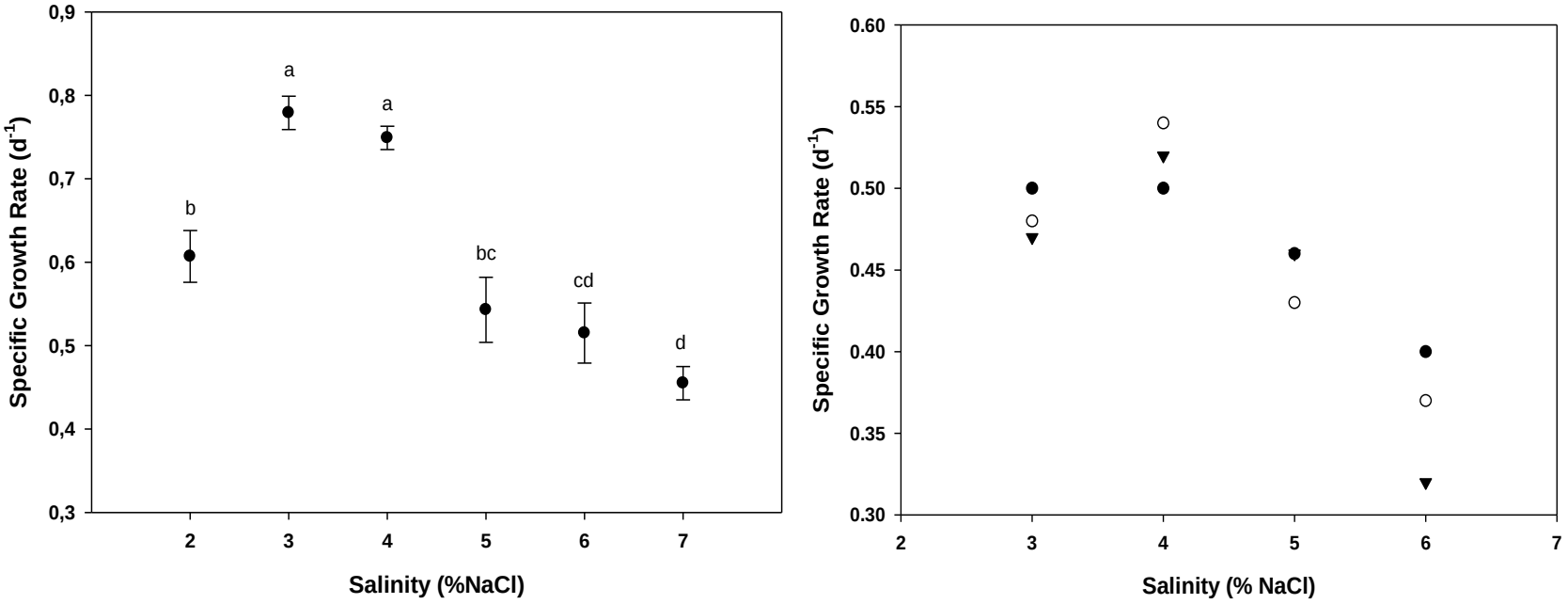


Figure 7. Specific growth rate (d^{-1}) of the *Nannochloropsis* sp.UHO 003 (left) *Skeletonema* sp.UHO29

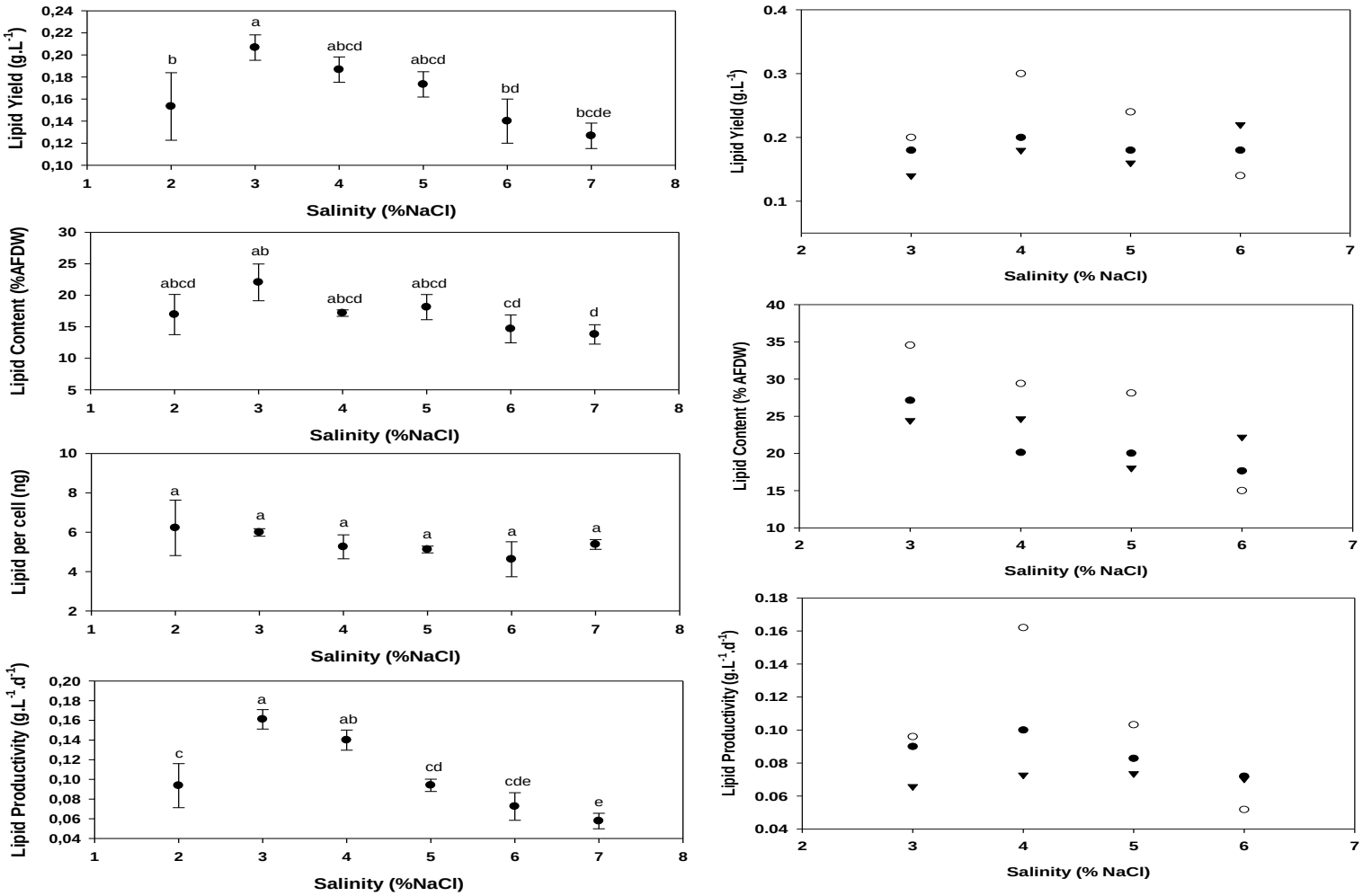


Figure 8. Lipid yield (g.L^{-1}) , lipid content (%AFDW) and lipid productivity (g.L^{-1}.d^{-1}) of *Nannochloropsis* sp.UHO 003 and *Skeletonema* sp.UHO29

- Predominant fatty acids of both microalgal species :
  - Methyl Palmitate (C16:0)
  - Methyl Stearate (C18:0)
  - Methyl Oleate (C18:1)
  - Methyl Linoleate (C18:2)

Mass cultivation in outdoor raceway ponds

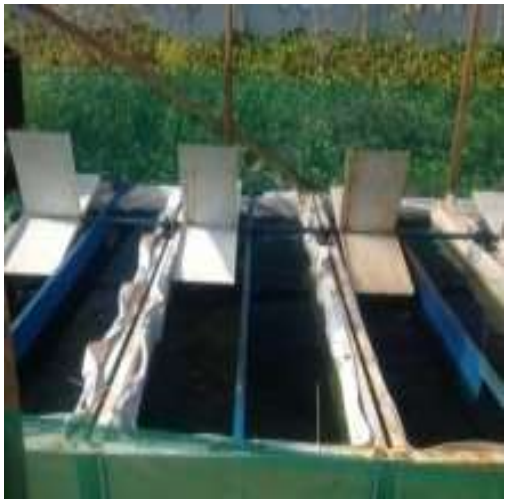




Fig. Mass Cultivation of *Nannochloropsis* sp.UHO3 dan *Skeletonema* sp.UHO29 in outdoor raceway ponds

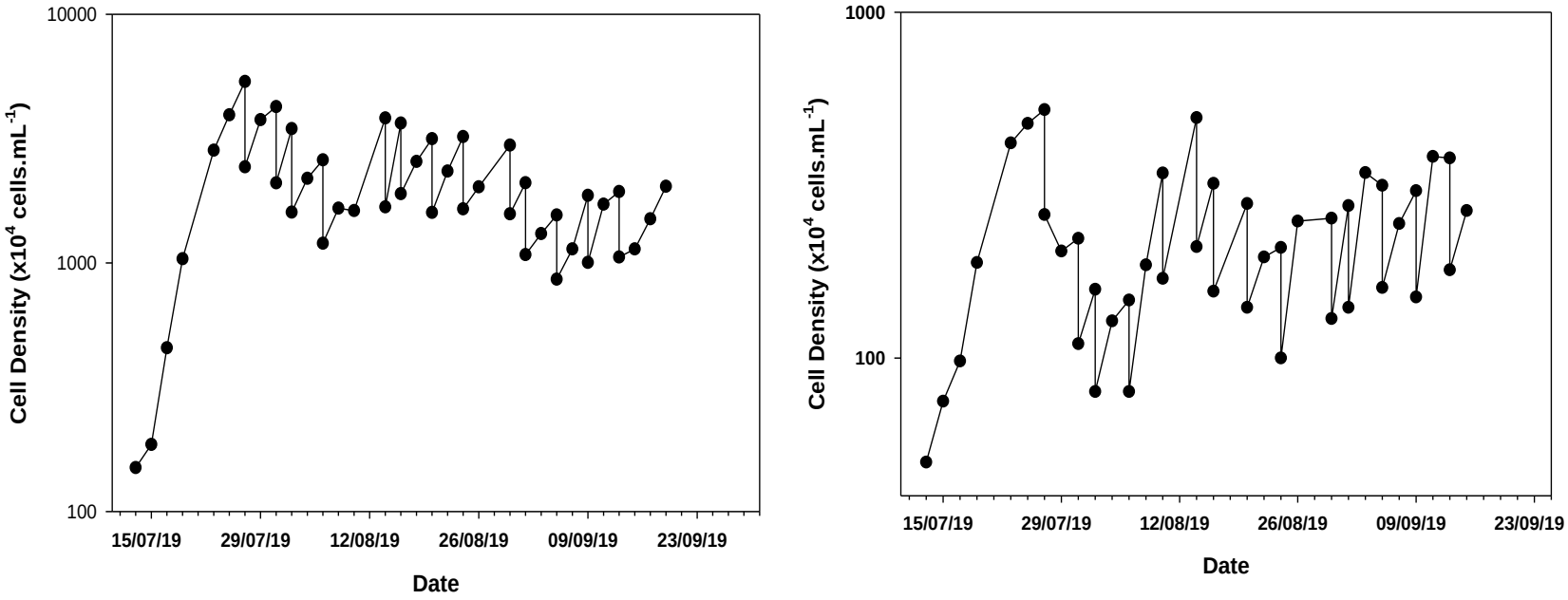


Fig. Growth curve of *Nannochloropsis* sp.UHO3 (left) dan *Skeletonema* sp.UHO29 (right) in outdoor raceway ponds

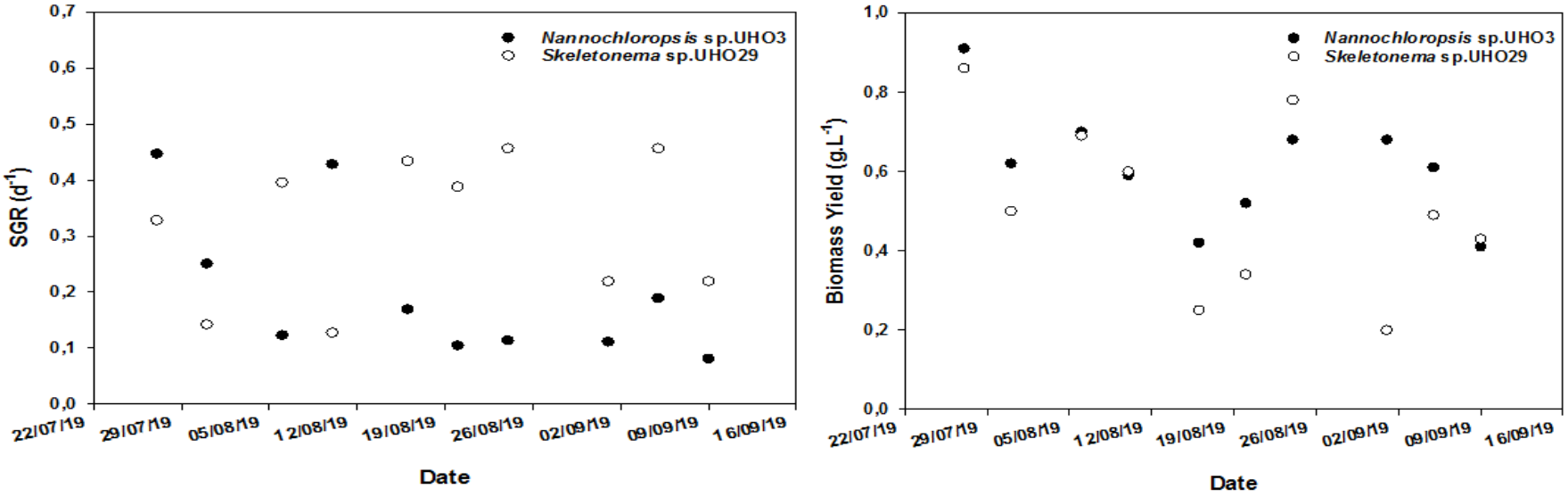
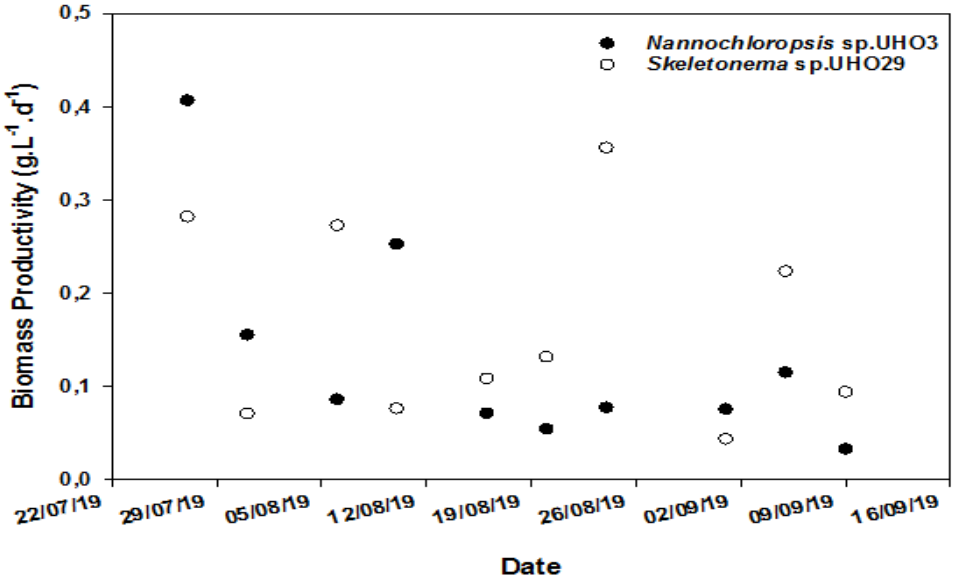


Fig. SGR (d^{-1}) and biomass yield ($g.L^{-1}$) of *Nannochloropsis* sp.UHO3 and *Skeletonema* sp.UHO29 in outdoor raceway ponds

Fig. Biomass productivity per volume ($g.L^{-1}d^{-1}$) and per area ($g.m^{-2}d^{-1}$) of *Nannochloropsis* sp.UHO3 and *Skeletonema* sp.UHO29 in outdoor raceway ponds



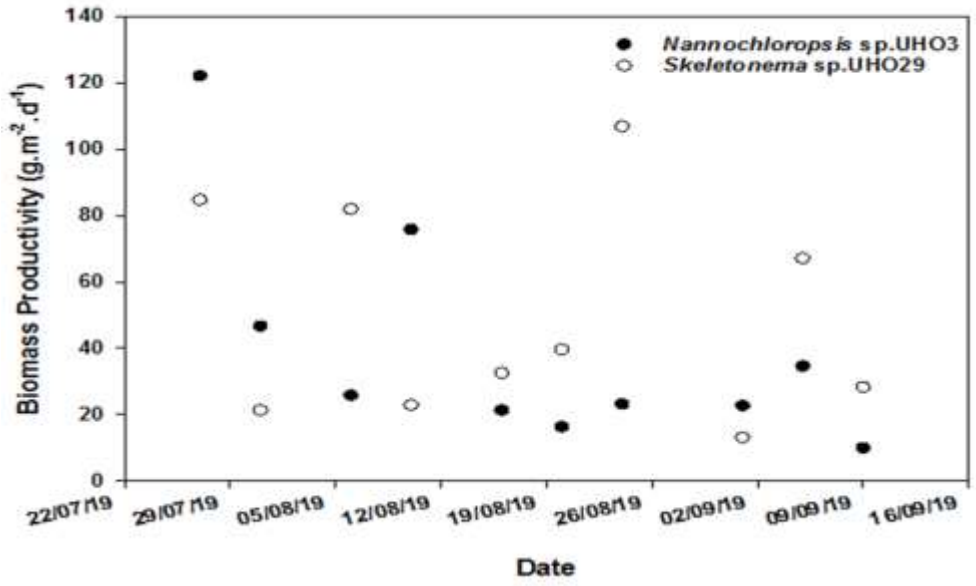
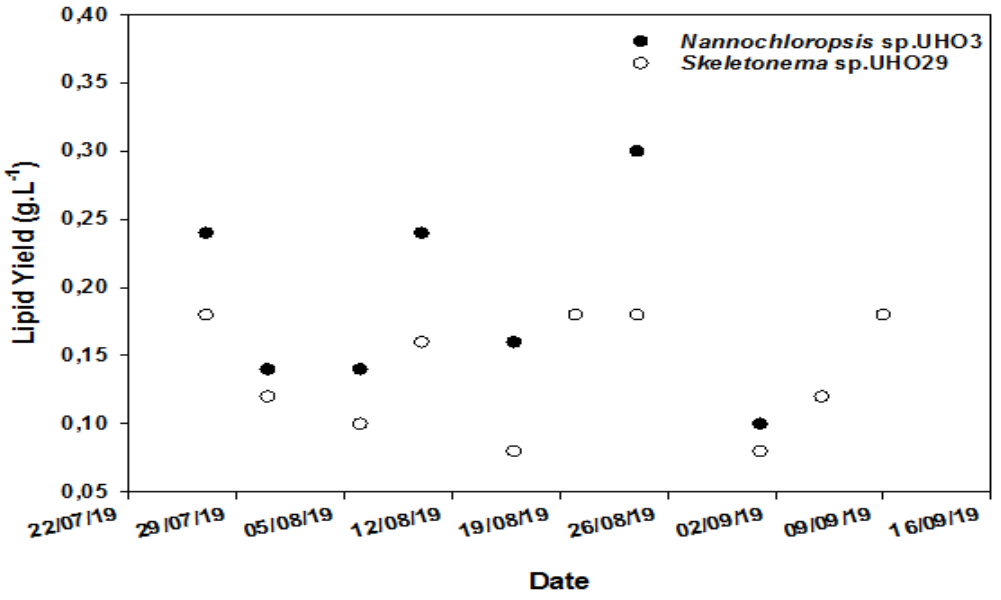


Fig. Lipid yield ($g.L^{-1}$) and lipid content (% AFDW) of *Nannochloropsis* sp.UHO3 and *Skeletonema* sp.UHO29 in outdoor raceway ponds



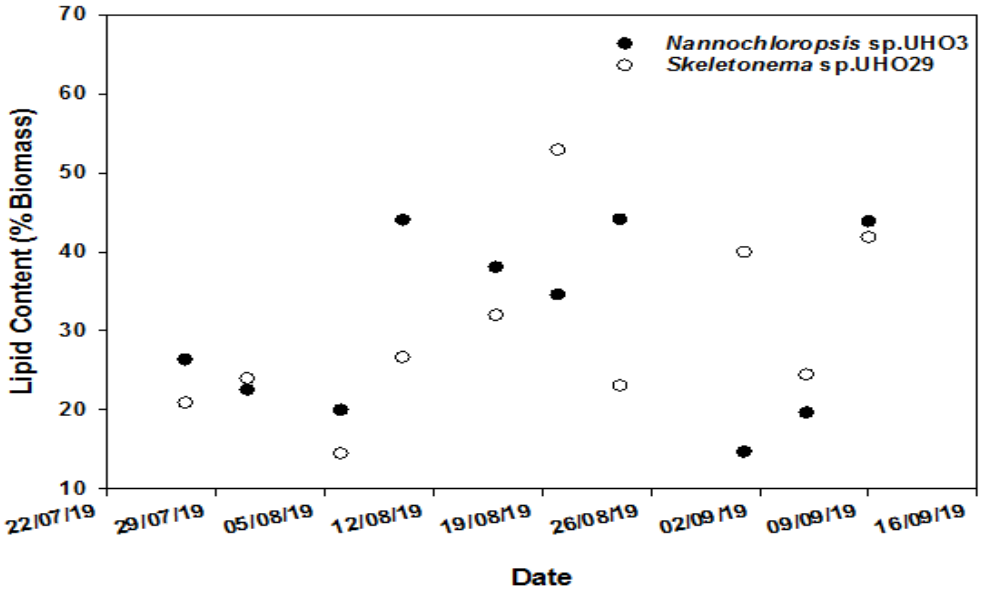
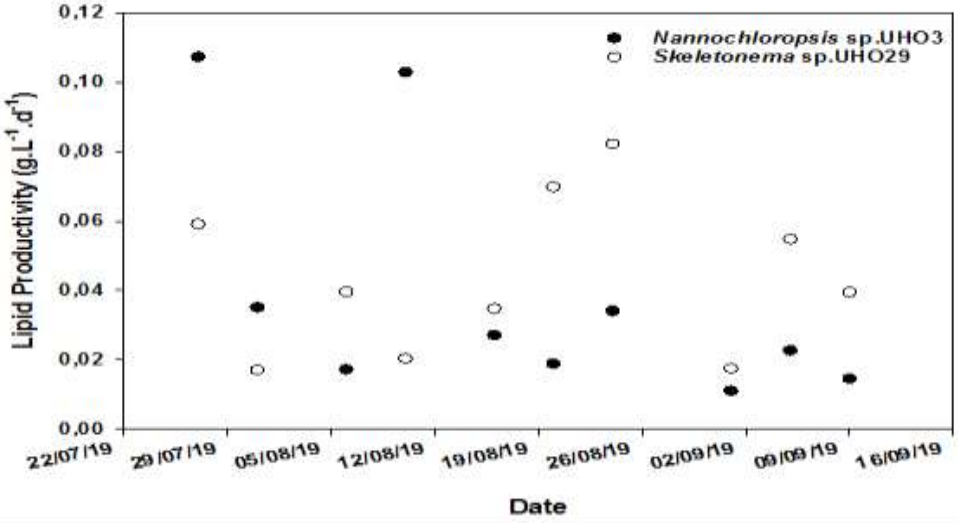
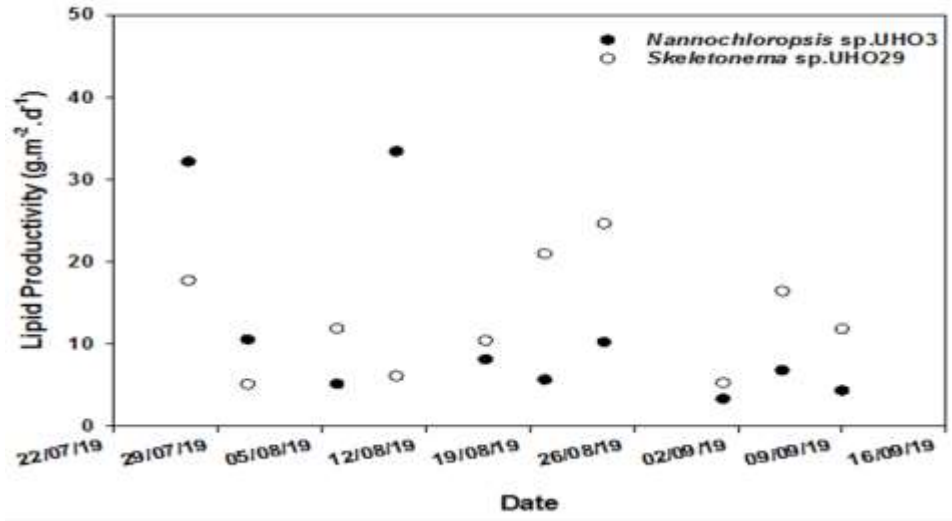


Fig. Lipid productivity per volume ($g.L^{-1}d^{-1}$) and per area ($g.m^{-2}d^{-1}$) of *Nannochloropsis* sp.UHO3 and *Skeletonema* sp.UHO29 in outdoor raceway ponds





Conclusion and Recomendation

Mikroalgae *Nannochloropsis* sp.UHO3 and *Skeletonema* sp.UHO029 are promising strains for biodiesel feedstock due to their high growth rates, wide salinity tolerance, high lipid content and lipid productivity, desirable fatty acid composition for biodiesel and ability to grow well in outdoor raceway ponds.

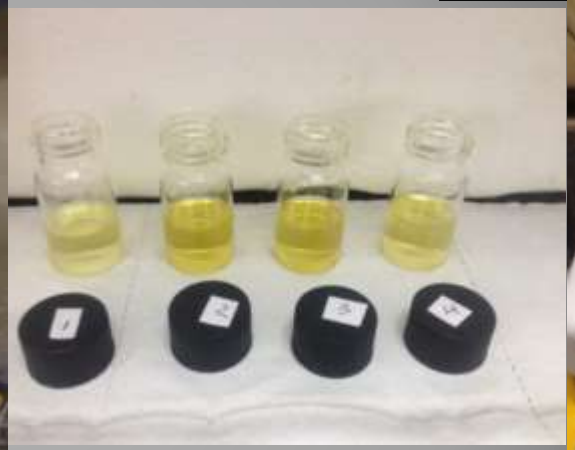
Outputs

- Isolat murni lokal strain *Nannochloropsis* sp.UHO3 dan *Skeletonema* sp.UHO29
- Paten sederhana "HALOPHILIC MIKROALGA *Skeletonema* sp.UHO29 SEBAGAI BIODIESEL FEEDSTOCK " telah Terdaftar.
- Artikel "Isolation and screening of marine microalgae from Kendari waters, Southeast Sulawesi, Indonesia suitable for outdoor mass cultivation in hypersaline media' (Published in AACL Bioflux, 2018, Volume 11, Issue 5,Scopus, Q3)
- Artikel berjudul ".Growth and lipid production of a newly isolated microalga *Nannochloropsis* sp.UHO3 at increasing salinity telah disubmit pada Hayati Journal of Biosciences (Scopus, Q2).
- Artikel berjudul "Growth, biomass and lipid productivities of a newly isolated tropical marine diatom, *Skeletonema* sp.UHO29, under different light intensity" akan disubmit pada Jurnal "International Microbiology" (Scopus, Q3)
- Draft Patent Sederhana berjudul "Oleaginous Mikroalga Laut *Nannochloropsis* sp.UHO3 Sebagai Biodiesel Feedstok"
- Buku ajar "Bioteknologi Perairan" sedang dalam proses editing











Thank You