Optimization of Wet Milling and Protein Extraction from *Ulva* sp. Dry Biomass Cultivated with IMTA Nutrients

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Motivation

Ulva cultivated with IMTA effluents contributes to bioremediation of nutrient-rich waters and produces protein-rich macroalgae biomass[1]. However, protein extraction from macroalgae requires intensive cell wall disruption and optimized solubilization conditions[2].

Cell Rupture

Parameters

Milling time (min)
Dilution Ratio (w/v)
Rotational Speed (rpm)

Ulva Cultivation with IMTA nutrients

↓

Drying

↓

Size Reduction

↓

Cell Rupture

↓

Protein Extraction

↓

Centrifugation

↓

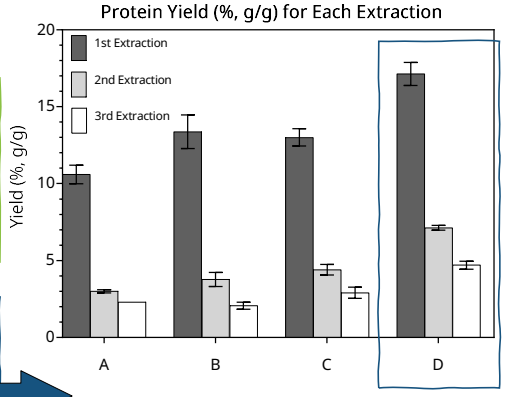
Lyophilization

Protein Extraction

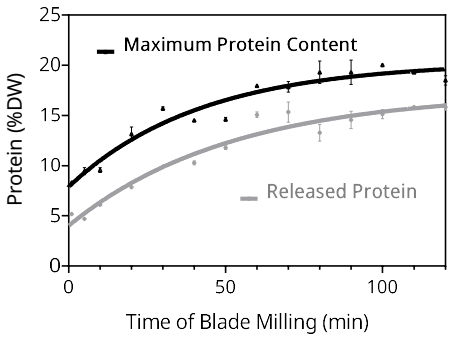
Conditions

A	Acidic pH
B	Slightly Alkaline pH & High Conductivity
C	Slightly Alkaline pH
D	Alkaline pH

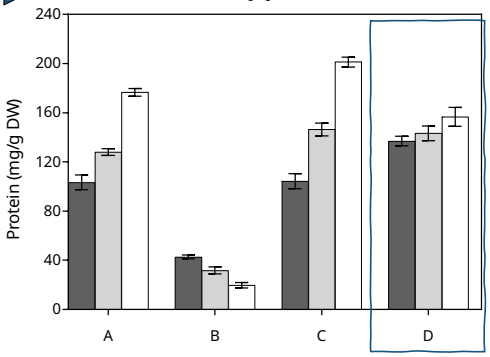
Protein Yield (%, g/g) for Each Extraction



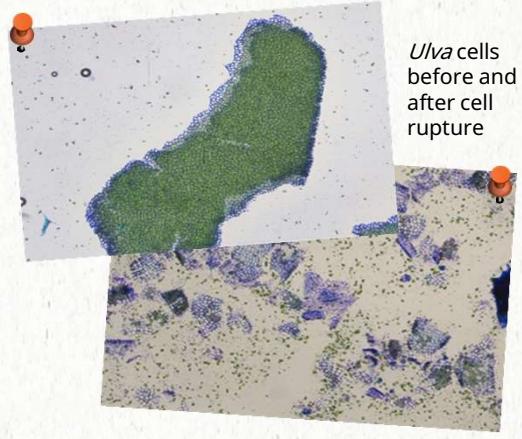
Protein Release as a Function of Cell Rupture



Solubilized Protein (mg/g DW) for Each Extraction



Optimized wet milling conditions achieved ~70% cell rupture.



Ulva cells before and after cell rupture

Blade Milling + **Alkaline pH** = Highest protein concentration: 141.3 ± 1.5 mg/g extract DW and highest protein yield: 29.0 ± 1.1%

Conclusions

- *Ulva* cultivated with IMTA-derived nutrients shows high protein content (20–30% DW).
- Optimized blade milling achieved ~70% cell rupture, as indicated by protein release.
- Prolonging milling time beyond optimal point increases disruption up to ~85% but leads to biomass (protein) degradation and energy costs.
- Blade milling + alkaline extraction conditions achieved 29% protein recovery, matching the upper range (5–29%) reported in literature[3].

References

[1] Nardelli, A.E. et al. (2018). Integrated Multi-Trophic Farming with *Ulva lactuca*, Mussels & Fish: Production & Bioremediation. J. Appl. Phycol. 31, 847–856.
[2] O'Brien, R. et al. (2022). Macroalgal Proteins: A Review. Foods, 11(4), 571.
[3] Nissen, S.H. et al. (2024). Protein Extraction from Green Seaweed (*Ulva* spp.) & Digestibility Study. Food Bioprod. Process. 148, 353–364.

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