Lactobacilli consortium and *C.glutamicum* sequential cultivation to produce single cell protein (SCP) from seaweed by-products

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1 Background and Motivation

- Growing global population demands sustainable protein sources, making single-cell proteins (SCP) a promising option due to their essential amino acids content.
- Marine residues, rich in nutrients like carbohydrates and proteins, are underexplored as substrates for SCP production.
- Utilizing these marine by-products as fermentation raw-materials could enhance SCP yields and valorize residual biomasses.
- Sequential fermentation with *Lactobacilli* (LAB) and *Corynebacterium glutamicum* offers a novel approach to optimize biomass and protein production from marine substrates.

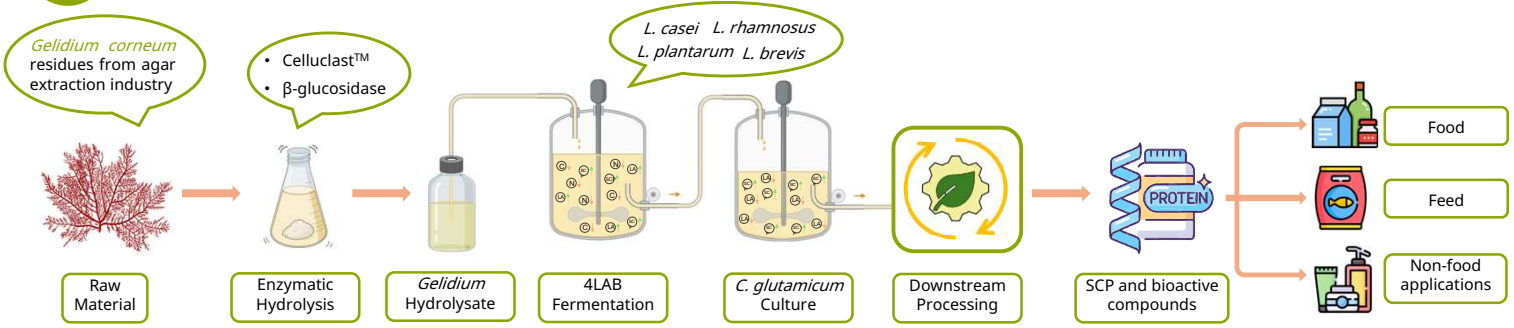
50% of the earth habitable land is already used for agriculture.

37.4 million tons of aquafeeds will be required by 2025.

Marine sources remain an underexploited type of biomass to be used as substrates for SCP production.

SCP production by fermentation offer sustainable solutions that leave land use for food and deliver proteins as food and aquaculture feed.

2 Methodology



3 Results and discussion

• Sequential Fermentation

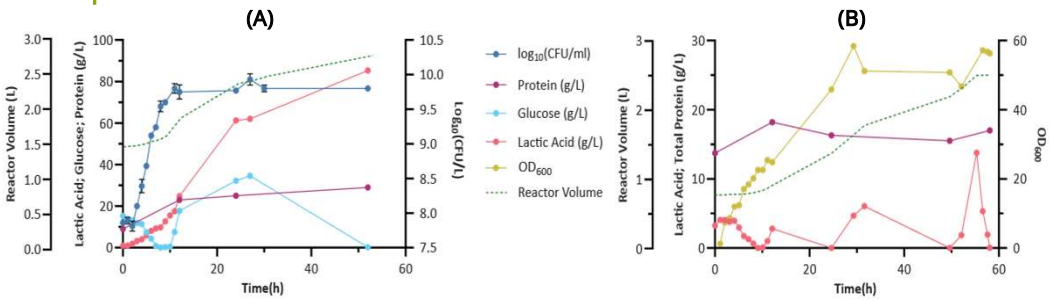


Figure 1. Bench-scale 2L bioreactor fermentation results for metabolite concentration and cell growth in 4LAB reactor (A) and *C. glutamicum* reactor (B) using fed-batch sequential fermentation by 4LAB with the addition of a 600 g/L glucose feed throughout 56 hours followed by centrifugation of reactor contents for supernatant recovery and feeding into a second reactor with *C. glutamicum* culture for SCP production.

- LAB fermentation yielded a supernatant with 85 g/L of lactic acid (LA) and a protein rich pellet with 37.4 ± 0.7 $g_{protein}/100\ g_{DW}$.
- *C. glutamicum* culture was able to consume all the available LA feed from LAB fermentation and presented high growth rate and stable SCP production.
- Final lyophilized *C. glutamicum* broth presented a protein content of 32.8 ± 0.8 $g_{protein}/g_{DW}$.

• Protein Quality Assessment

Table 1. Protein quality assessment of *Gelidium corneum* residues and of products from 4LAB + *C. glutamicum* sequential fermentation.

Material	Protein Content	Protein Bioaccessibility	∑EAA[1]	∑NEAA[1]	Protein Score[2]
	($g/100\ g_{DW}$)	(%)	($g/100\ g_{protein}$)		(%)
Gelidium Residues	36.0 ± 1.2	28.2 ± 4.6	53.0 ± 1.4	62.4 ± 1.4	37 (Val)
4LAB Pellet	37.4 ± 0.7	62.9 ± 6.0	53.4 ± 0.9	62.1 ± 0.9	77 (Met+Cys)
C. glutamicum Whole broth	32.8 ± 0.8	71.7 ± 8.0	47.9 ± 1.6	67.4 ± 1.7	88 (Lys)

[1] Sum of essential amino acids (EAA) and non-essential amino acids (NEAA).
[2] Protein Digestibility Corrected Amino Acid Score (PDCAAS) calculated in accordance with 1991 FAO report entitled "The State of Food and Agriculture", with limiting essential amino acid presented in brackets. PDCAAS is expressed on a scale from 0 to 100, where a score of 100 represents an excellent protein source and scores above 75 indicate a good protein source.

• Antioxidant Properties

Table 2. Measured total phenolic content (TPC), ferric reducing antioxidant power (FRAP), DPPH and ABTS scavenging of products from 4LAB + *C. glutamicum* sequential fermentation.

Material	TPC	FRAP	DPPH	ABTS
	$E_{galic\ acid}$ (mg/g)	E_{trolox} (µmol/g)	IC_{50} (mg/mL)	
Gelidium Residues	0.7 ± 0.0	3.1 ± 0.1	> 25.0	3.9 ± 0.1
4LAB Pellet	1.3 ± 0.03	5.5 ± 0.0	8.4 ± 0.2	2.7 ± 0.03
C. glutamicum Whole broth	4.7 ± 0.02	11.5 ± 0.1	1.8 ± 0.02	1.1 ± 0.01

4 Conclusions

- Sequential fermentation of *Gelidium* corneum industrial residues successfully produced SCP ingredients, demonstrating an effective strategy for marine biomass valorization.
- LAB fermentation generated a protein-rich pellet with enhanced protein bioaccessibility (62.9%), achieving good protein quality, and improved antioxidant potential.
- The lactic acid-rich supernatant was successfully reused as a substrate for *Corynebacterium glutamicum*, producing an SCP ingredient with 32.8 g/100 g DW protein, good protein score, and superior antioxidant activity, maximizing resource utilization.

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