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Biotreatment of Second Cheese Whey in Pilot-Scale Photobioreactors Using Cyanobacteria-based Consortia for Lipid Production

Patrinou V.[1]*, Aggelis, G[2], Tekerlekopoulou A.G.[1]

[1] Department of Sustainable Agriculture, University of Patras, G. Seferi 2, 30100, Agrinio, Greece
[2] Department of Biology, University of Patras, 26500, Rio-Patras, Greece

INTRODUCTION & AIM

- Second cheese whey (SCW), is a highly polluted by-product generated during cottage cheese production. While the biotreatment of cheese whey and dairy wastewaters, often containing wash waters, has been extensively investigated using microalgae/cyanobacteria, the specific treatment of SCW remains insufficiently explored. Moreover, studies involving pilot-scale photobioreactors for SCW remediation are particularly limited.
- This study aimed to evaluate the most effective treatment configuration for potential full-scale application, while simultaneously assessing the lipid-producing potential of the resulting microbial biomass. Unsterilized SCW was treated employing a cyanobacteria-based culture in pilot-scale suspended (SCW-S) and attached (SCW-A) growth system. Additionally, to isolate the influence of SCW's native microbial community, a synthetic chemical medium was tested under attached-growth conditions (CM-A), enabling direct evaluation of the culture's intrinsic biomass and lipid productivity.

METHOD

Experimental Set up:

- ✓ SCW originated from a local dairy facility in Western Greece.
- ✓ The cyanobacteria-based culture consisted of the filamentous strain *Leptolyngbya* sp. (95% abundance) and the green coccoid microalga *Choricystis* sp.. After the inoculation into the raw, unsterilized SCW, a mixed microalgal/cyanobacterial-bacterial consortium was established, operating under mixotrophic conditions.
- ✓ Photobioreactor: 9L vertically oriented tubular photobioreactor made of plexiglass (inner Ø 9 cm). Treatment conditions: continuous illumination of 200 μmol m^{-2} s^{-1}, substrate recirculation rate of 1000 mL min^{-1}, T = 26 ± 2ºC, pH 8 ± 1 and constant aeration.
- ✓ Support material: 55 cubic plastic biofilm carriers (Type BWT-X, protected surface 650 m^2/m^3) and 54 plastic air tubes (Rislan PA11) were fixed successively in a fishing line. Totally, 6 fishing lines were placed inside the photobioreactor (Figure 1).
- ✓ The SCW was evaluated under a fixed C:N ratio of ≈ 45 (corresponding to an initial d-COD concentration of 2000 mg L^{-1}, Table 1), based on literature indicating enhanced lipid accumulation by *Leptolyngbya* sp. under such conditions.

Analytical measurements:

- ✓ Biomass was determined gravimetrically (APHA (1998), while biomass yields according to Patrinou et al., (2020). Attached biomass was measured after removing and scrapping all the materials from one line. Total biomass combined suspended and attached fractions. Pollutants were determined using APHA (2005). Lipids were extracted (Folch's method, Figure 2), while fatty acids analyzed by gas chromatography system (Agilent 7890A).

Table 1. Initial pollutant and biomass concentrations in the pilot-scale photobioreactors.

Experimental Substrate	Initial Concentrations (mg L^{-1})							
	d-COD	NO_3^--N	Total Nitrogen	PO_4^{3-}	Total Sugars	Total Biomass	N:P	C:N
SCW-S	2100.0 ± 55.0	20.1 ± 0.4	49.1 ± 2.5	22.5 ± 0.1	1797.4 ±26.9	278.0 ± 56.6	2.2	42.8
SCW-A	2315.0 ± 32.2	19.3 ± 1.1	53.3 ± 1.1	18.4 ± 0.4	1753.1 ± 18.9	280.0 ± 14.0	2.9	43.4
CM-A	-	51.4 ± 0.1	52.5 ± 0.1	20.0 ± 2.0	18.8 ± 0.1	93 ± 36.3	2.6	-

RESULTS & DISCUSSION

- ✓ Both growth systems achieved high pollutant removal rates (Table 2). However, SCW-A system proved to be the most efficient achieving the highest biomass yield (346.7 mg $L^{-1}d^{-1}$, μ: 0.677 d^{-1}, Figure 3) and lipid content up to 15.1% d.w.
- ✓ The proposed SCW-A facilitates easier biomass recovery, potentially reducing operational costs and improving scalability.

Figure 1. Pilot-scale attached photobioreactor.

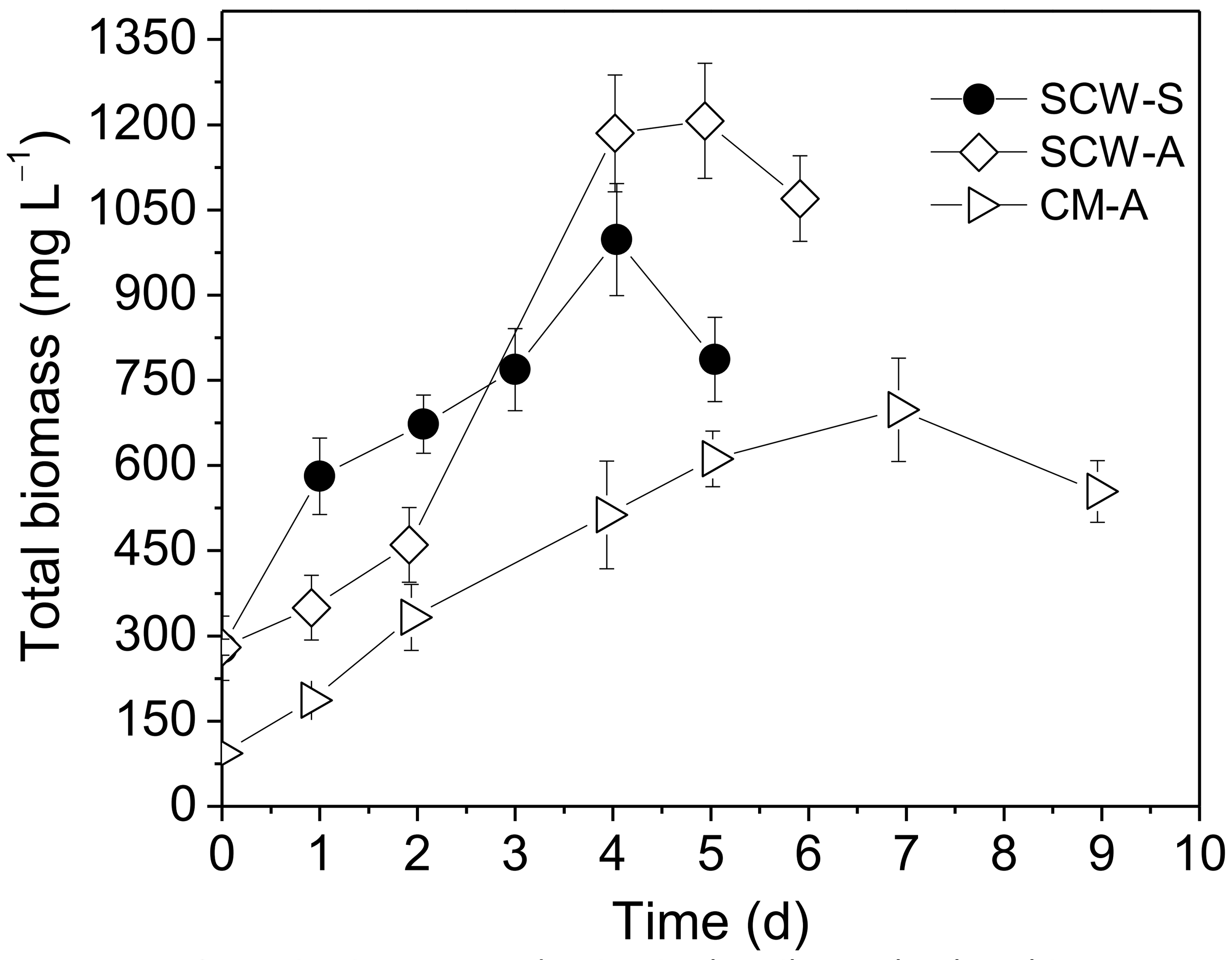

Figure 3. Biomass production in the pilot-scale photobioreactors.

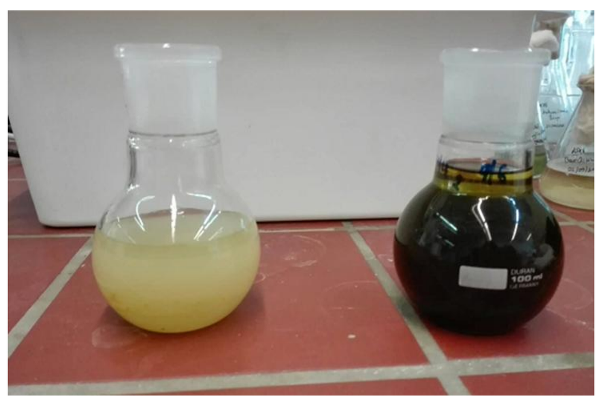

Figure 2. Microbial lipids.

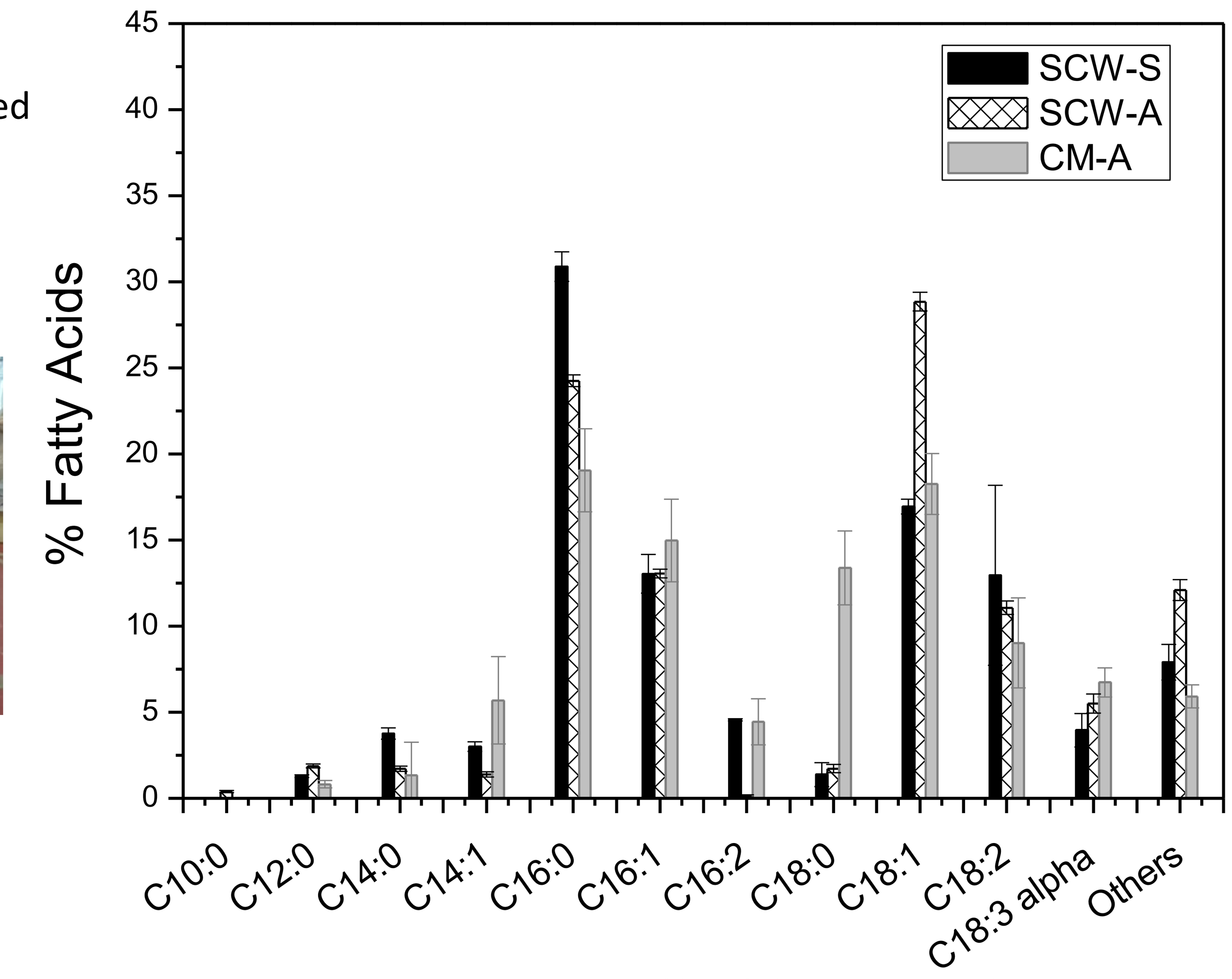

Figure 3. Fatty acid profile of the produced lipids.

Table 2. Pollutant removal rates, biomass yields and lipid contents achieved in the pilot-scale photobioreactors.

Experimental Substrate	% Removal Rate					Maximum Biomass Productivity		Specific Growth Rate (d^{-1})	% d.w Maximum Lipid Content	
	d-COD	NO_3^--N	Total Nitrogen	PO_4^{3-}	Total Sugars	Total mg L^{-1} d^{-1}	Attached g m^{-2} d^{-1}		Total	Attached
SCW-S	94.5 ± 0.5	87.0 ± 0.7	89.0 ± 1.0	98.8 ± 0.2	99.3 ± 3.3	303.0 ± 33.6	-	0.635 ± 0.01	16.1 ± 0.8	-
SCW-A	99.9 ± 0.1	93.3 ± 0.8	96.5 ± 0.1	93.4 ± 1.3	97.7 ± 2.6	346.7 ± 22.8	3.1 ± 0.3	0.677 ± 0.02	15.1 ± 0.7	14.0 ± 0.3
CM-A	-	63.2 ± 1.2	64.1 ± 0.7	93.2 ± 0.8	-	142.8 ± 18.7	0.5 ± 0.1	0.330 ± 0.01	12.0 ± 0.9	11.2 ± 0.6

CONCLUSIONS

- ✓ SCW's indigenous microbial load significantly enhanced biomass production and lipid content compared to the CM-A growth system. This indicated the establishment of a metabolically active and synergistically interacting culture which improved the bioremediation of SCW.
- ✓ Fatty acid analysis showed that the produced biomass contained mainly saturated and monounsaturated fatty acids (SCW-S 70.1%, SCW-A 74.6%, CM-A 67.0%).
- ✓ The composition of the microbial lipids demonstrated that they can provide sufficient oxidative stability rendering them suitable candidates for biofuel production.

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