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A *Leptolyngbya*-dominated consortium for the biological treatment of mixed agro-industrial effluents

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INTRODUCTION & AIM

✓ Wastewater streams are a low-cost nutrient source for cultivating microalgae and cyanobacteria. However, single streams often exhibit imbalanced nutrient profiles, insufficient to support optimal microbial growth. Mixing streams with complementary physicochemical properties can balance nutrients and eliminate the need for external supplementation. Optimizing the mixing ratio is essential to achieve favorable carbon-to-nitrogen (C:N) ratios, enhance biomass and biocompound production.

✓ This study aimed to identify the optimal mixing ratio of two agro-industrial wastewaters: second cheese whey (SCW) and poultry wastewater (PW), for the cultivation of a *Leptolyngbya*-dominated consortium. Different mixing ratios were examined to enhance key culture parameters, including nutrient stoichiometry, turbidity, and pH, thereby creating more favorable conditions for microbial growth and metabolite production.

METHOD

Experimental Set up:

✓ SCW originated from a local dairy facility in Western Greece.

✓ PW originated from small local farms in Western Greece. The extracts were obtained after sun drying (48 h), wet milling (15% w/v) and tulle netting the manure.

✓ The culture consisted of the cyanobacteria *Leptolyngbya* sp. (95% abundance) and the coccoid microalga *Choricystis* sp.. After the inoculation into the unsterilized wastewaters a mixed microalgal/cyanobacterial-bacterial consortium was established. Microscopic observations at the end of the bioprocesses revealed that *Leptolyngbya* sp. dominated over the microbial consortium.

✓ Experiments were conducted in 150 mL working volume and magnetically stirred (150 rpm) Erlenmeyer flasks. Treatment conditions: 24:0 h L:D illumination of 200 μmol $m^{-2} s^{-1}$ and T = 26 ± 2ºC.

✓ Formulation of mixtures: Four mixing ratios of SCW:PW (50:50%, 60:40%, 70:30%, and 85:15%) were evaluated based on an initial dissolved chemical oxygen demand (d-COD) concentration of 3000 mg L^{-1}. Due to SCW's composition, higher proportions increased C:N ratios, intensified N and P limitation (Table 1), reduced turbidity (Figure 1), and adjusted pH toward neutrality, as SCW is more acidic than PW.

Analytical measurements:

✓ Biomass was determined gravimetrically (APHA (1998), while biomass yields according to Patrinou et al., (2020). Pollutant removal rates were determined using APHA (2005). Finally, lipids were extracted from the microbial biomass following Folch's method (1957).

Table 1. Initial pollutant and biomass concentrations under the different mixing ratios.

% Mixing ratios (SCW: PW)	Initial concentrations (mg L^{-1})				N:P/ C:N
	d-COD	TN	PO_4^{3-}-P	Biomass	
50:50 %	3017.0 ± 186.0	141.7 ± 13.2	24.0 ± 1.5	303.0 ± 34.2	5.9/ 21.3
60:40 %	2904.0 ± 19.8	109.8 ± 3.6	19.5 ± 2.2	271.5 ± 42.1	5.63 / 26.5
70:30 %	2914.0 ± 56.0	86.5 ± 2.4	15.7 ± 2.9	248.5 ± 22.1	5.51 / 33.9
85:15 %	2732.0 ± 173.0	64.3 ± 3.1	8.5 ± 1.0	202.0 ± 32.8	6.28 / 42.5

RESULTS & DISCUSSION

✓ Biomass productivity ranged from 174.6 to 276.6 mg $L^{-1}d^{-1}$ (Table 2). The highest values were observed at 60:40% and 70:30% ratios (276.6 and 268.3 mg $L^{-1}d^{-1}$), while 50:50% showed the lowest (174.6 mg $L^{-1}d^{-1}$), likely due to elevated turbidity (Figure 2). Despite the lowest turbidity at 85:15%, biomass accumulation was not improved, possibly due to its slightly acidic pH (~6.5), suboptimal for the alkaliphilic *Leptolyngbya* sp. strain.

Figure 1. Gradual turbidity reduction in mixed effluents.

✓ Pollutant removal improved with increasing SCW content, peaking at a 70:30% ratio with removal rates of 89.2% (d-COD), 64% (TN), and 60% (PO_4^{3-}-P) (Table 2, Figure 3).

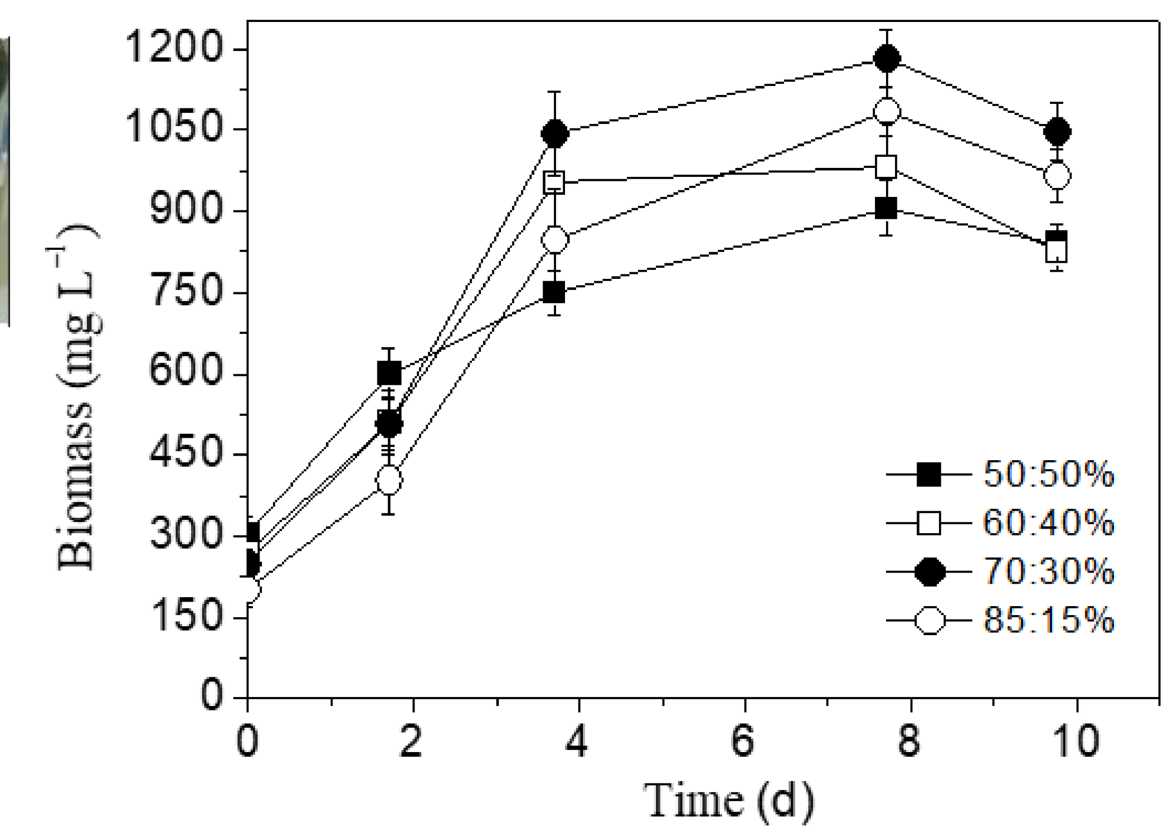

Figure 2. Biomass production at varying mixing ratios.

Table 2. Effect of mixing ratios on pollutant removal, biomass, and lipid production.

% Mixing ratios (SCW: PW)	% Removal rates			Total biomass productivity (mg L^{-1} d^{-1})	Maximum specific growth rate (d^{-1})	% Total lipid content
	d-COD	TN	PO_4^{3-}-P			
50:50	78.4 ± 1.7	54.5 ± 2.2	52.4 ± 0.1	174.6 ± 21.8	0.341 ± 0.05	10.3 ± 0.9
60:40	77.3 ± 0.9	56.1 ± 3.6	52.7 ± 0.1	276.6 ± 10.4	0.385 ± 0.02	11.7 ± 1.6
70:30	89.2 ± 1.4	64.0 ± 4.0	60.0 ± 0.3	268.3 ± 9.0	0.360 ± 0.02	14.0 ± 0.5
85:15	82.8 ± 2.4	63.0 ± 0.6	58.8 ± 0.1	221.8 ± 4.3	0.346 ± 0.01	11.9 ± 1.3

✓ The lipid-producing potential of the resulting biomass was also evaluated, with lipid contents ranging from 10.3% to 14.0% dry weight (d.w.). The darkest mixture, at a 50:50% ratio, showed reduced lipid accumulation.

✓ The 70:30% ratio demonstrated superior biomass production, lipid content and treatment efficiency, and was therefore selected as optimal.

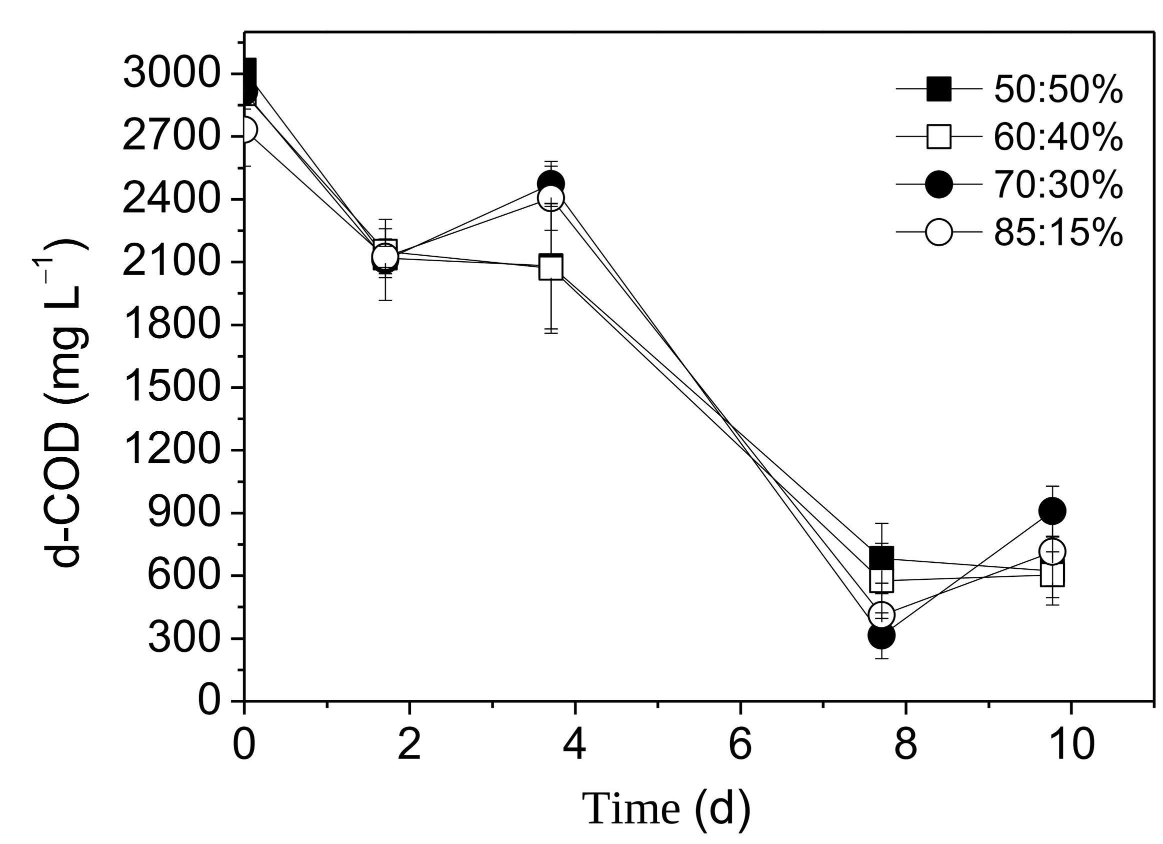

Figure 3. d-COD removal profiles in various mixture ratios.

CONCLUSIONS

✓ The reduced turbidity observed at the 70:30% mixing ratio enhanced the growth of the lipid-producing autotrophic microorganisms within the consortium, resulting in increased biomass and lipid accumulation.

✓ The elevated addition of SCW positively impacted lipid content by increasing the C:N ratio and concurrently limiting N and P availability. Under such nutrient-limited conditions, microalgae/cyanobacteria tend to favor lipid accumulation.

✓ The acidic conditions associated with the 85:15% ratio hindered further improvements in biomass and lipid production.

FUTURE WORK

The scalability potential of the optimized 70:30% ratio could be further evaluated in pilot-scale photobioreactors operating under suspended or attached growth systems.

REFERENCES

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