Combined remediation and protein production using microalgae growth on waste bakery products.

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Microalgae are a highly diverse and specialized group of microorganisms. The flexibility to switch their nutritional mode based on substrate availability and light condition is one of their multiple advantages (Hidalgo, 2015). *Chlorella sorokiniana* is a good example. This microalga has a great biotechnological potential due to its ability to synthetize products of industrial interest, rapid growth and the ability to adapt to differing nutrient sources in autotrophic, mixotrophic, and even heterotrophic regimens.

The sources of nitrogen and carbon as well as their availability affect algae growth and the amount of protein, carbohydrate and lipid composition of the algae biomass (Hu et al., 2008). Recently three strains of *C. sorokiriana* demonstrated higher biomass production when cultured in digested cattle manure. The harvested algae yielded high levels of protein (24% w/w) and starch (22% w/w) and low levels of lipids (12%w/w), revealing a high potential for animal feed application (Kobayashi et al., 2013).

The consideration of microalgae in waste treatment processes offers significant commercial opportunities. However, key aspects such as effluents (source of carbon and nutrients) availability, cultivation system design, productivity of algal culture, nutrient uptake, strain improvement, biomass harvesting and extraction, refining and residual biomass utilization needs further attention (Hidalgo, 2015).

In this work, a concept of utilizing bakery industry waste as a nutrient source for the production of proteins by microalgae growth has been developed. The proposed ideas provide an innovative approach to establish a system to valorise bakery industry waste, more specifically, the so-called waste ´biscuits flour´, and to produce rich protein algal biomass, which could potentially be applied in feed industry

Biscuit flour (Figure 1) is based on wheat flour, due to the raw materials from which it comes: a mixture of biscuit, bread, pastry, pasta and, to a lesser extent, snacks. Its composition is shown in Table 1. This sub-product, collected from a Spanish bakery company located in Venta de Baños (Palencia), was used as carbon and nutrients source in mixotrophic growth of *Chlorella sorikiniana,* pure culture supplied by the Spanish Bank of Algae (BEA) (Figure 2), and in mixotrophic growth of a mixed culture of microalgae, taken from a natural environment (Figure 3). The feasibility of utilizing bakery waste, raw and hydrolysate, for algal biomass production was investigated and compared with the growth of the microalgae in WARIS-H medium, prepared according McFadden and Melkonian (1986).

Table 1. Biscuit flour composition (dry weight calculations).

	Water (%)	Grease (%)	Ashes (%)	Nitrogen (%)	Protein (%)	Starch (%)	Fibre (%)	Sugars (%)
Annual average[1] (SD*)	9,51 (0.87)	11,86 (1.24)	2,42 (0.20)	1,82 (0.06)	11,33 (0.37)	49,17 (3.81)	1,11 (0.12)	13,10 (2.81)
Test sample[2]	8.06	-	-	1.72	10.74	-	-	-

*Standard deviation; Source: [1] Subproductos Tuero, S.L.; [2]CARTIF (sample used for experimentation).

Figure 1. ´Biscuits´flour.

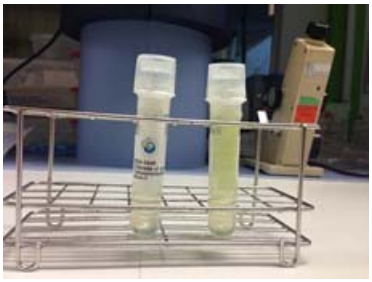

Figure 2. Pure culture of *C. sorokiniana.*

Figure 3. Microalgae mix-culture growing in natural media.

The assay consisted in the preparation of 5 batch cultures using 0.5 L sterilised bottles (Figure 4): 2 for the *C. sorokiniana* and 3 for the mixed culture. Then, WARIS-H medium, raw biscuits flour or hydrolysed biscuits flour were added to the microalgae samples according Table 2. The amount of flour added to the test B2, B4 and B5 was enough to reach the same Nitrogen concentration than in the test fed with WARIS-H medium. The evolution of the cultures was followed by turbidimetry (HACH 2100N) during 60 days. The turbidity represents a measure of the incident light scattered at right angles from the sample and is positively correlated with the algae growth.

All experiments and analyses were carried out in a laboratory where temperature was maintained at 20±2 ºC and the 0.5 L reactors were moderately illuminated by natural light (not ideal conditions for algae growth).

Figure 4. Culture reactors.

Table 2. Culture conditions

Test	B1	B2	B3	B4	B5
Microalgae	*C. sorokiniana*	*C. sorokiniana*	Mixed culture	Mixed culture	Mixed culture
Substrate	WARIS-H	Hidrolysed flour	WARIS-H	Raw flour	Hydrolised flour
Total N (medium)			35 mg/L		

Preliminary data reveal a good adaptation of the two groups of microalgae under analysis (pure and mix cultures) to the new substrate, although a slightly lower growth rate compared to the samples growing in WARIS-H medium is observed for the mixed culture mainly. Quantification of the protein content in the algal biomass generated is still pending.

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