Flash hydrolysis of microalgal biomass to recycle nutrients towards a sustainable biofuels and bioproducts production

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Microalgae have the potential to provide a scalable source of renewable fuels. However, the supply of nutrients (mainly N and P), represents a major limitation to large-scale microalgae cultivation: based on the elemental composition of microalgal biomass, roughly 60-90 kg of N and 3-15 kg of P would be required to produce 1 ton of dry algae. When considering productions that aim at displacing significant amounts of fossil fuels, it appears clear that nutrients supply is not sustainable by exploiting natural available resources, particularly in the case of phosphorus, whose reserves are expected to be depleted in the near future. In order to develop a sustainable process, nitrogen and phosphorus should be recovered from the residual microalgal biomass (i.e., the non-oleaginous fraction) and recycled to the cultivation system, reducing the amounts of fresh fertilizers required.

Flash Hydrolysis (FH) is a hydrothermal process which consists in treating the wet biomass under subcritical water conditions and a very short residence time (less than 10 s), and has proved to be a viable way to fractionate lipids in a solid product (biofuels intermediate) and recover most of the N (up to 60%) and P (up to 80%), of the initial biomass, together with other micronutrients, in the aqueous phase (hydrolysate). In particular, P is available mainly in the form of orthophosphates, while most of nitrogen is present in simple organic forms (amino acids and soluble oligopeptides), with only a small percentage of inorganic ammonium.

The liquid hydrolysate produced by FH carried out at 280°C and 9 s of residence time on *Scenedesmus* sp. was used to cultivate a microalga of the same genus (*S. obliquus*) in order to assess the growth potential in this substrate as well as the capability of up-taking the organic nitrogen from the medium. Batch experiments showed that *S. obliquus* could grow well in the hydrolysate, and in particular the specific growth rate resulted to be enhanced compared to that obtained in standard cultivation medium, thanks to mixotrophic growth. Continuous cultivation experiments were also carried out, with the aim of optimizing the nutrients utilization, allowing a stable steady-state biomass production to be reached.

As an alternative to direct recycling of the liquid hydrolysate, the possibility of precipitating the inorganic N and P from the substrate in the form of struvite crystals ($NH_4MgPO_4 \cdot 6H_2O$), to be used as a source of nutrients for further cultivation, was also evaluated: this would allow to recover other high-value biomolecules preserved in the hydrolysate (e.g., arginine), potentially increasing the revenues of the process. Struvite was successfully precipitated from the hydrolysate, and it proved to be a good source of nutrients for microalgal cultivation, allowing growth rates and productivities equal to those obtained with standard media.

In summary, flash hydrolysis appears as a promising technology to improve the sustainability of algae-derived biofuels production.