



FROM SEWAGE SCUM UP TO LIPIDS PRODUCTION USING OLEAGINOUS YEASTS AS BIOCATALYSTS

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Significance and Relevance

For the first time, the fermentation of undetoxified sugar-rich sewage scum hydrolysate, a special waste separated during municipal wastewater treatment, was successfully achieved using a promising commercial yeast to produce microbial oil. One of the most critical factors affecting its cost production is the feedstock; therefore, using low-cost or even negative-value waste resources, such as sewage scum, could significantly reduce its manufacturing price and, consequently, that of the obtained biodiesel and fine oleochemicals. Moreover, the valorization of the sewage scum would lower the disposal expenses of this waste, also reducing its environmental concerns, in agreement with Green Chemistry principles.

Preferred and 2nd choice for the topic: 1. Green chemistry and biomass transformation, renewable resources conversion, 2. Circular economy; Preferred presentation: Oral preferred or Short Oral.

Introduction and Motivations

Recently microbial oils, namely single cell oils (SCOs), have gained significant attention as third-generation raw materials for producing biodiesel and fine oleochemicals. Among oleaginous microorganisms able to produce SCOs, yeasts are the most promising lipid-accumulating microbes, due to their ability to grow fast, independently from environmental conditions, and to utilize a wide range of carbon sources, including low-cost substrates, such as wastes, thus enhancing the economic and ecological feasibility of the industrial implementation of oil production¹. Among the various types of wastes generated globally, sludge from municipal wastewater treatment plants (WWTPs) is receiving significant attention due to its high carbon content and nutrient composition that oleaginous yeasts can use for cell growth and lipid accumulation, including cellulose, proteins and organic acids². Sewage scum (SS) is a special waste easily recoverable from WWTPs through preliminary flotation of urban sewage, and, at the moment, it is disposed of in landfills as a wet material. SS contains a polysaccharide fraction (mainly made up of cellulose, sourced from toilet paper, and hemicellulose), in addition to the more substantial lipid fraction³. Therefore, a promising alternative for valorizing this kind of wastewater sludge is its use as a carbon source for the growth of selected oleaginous yeasts in order to produce a new generation oil, suitable for biofuels and fine oleochemicals production. On this basis, the aim of this work, carried out in line with the goals of the national ReFil PRIN 2022 Project, was the fermentation of the hydrolysate obtained through the enzymatic hydrolysis of SS, containing mainly glucose and xylose, in the presence of oleaginous yeasts in order to valorize the SS waste. This innovative approach, which has not been explored so far, not only represents a novel contribution to the field, but also addresses two critical challenges simultaneously: the reduction of the cost of lipid production and the decrease of environmental and economic impacts associated with SS disposal.

Materials and Methods

The chemical composition of SS was initially determined, focusing on total solid content, easily hydrolysable sugars, cellulose, proteins, lignin, humic compounds and trans-esterifiable lipids. The analysis revealed that cellulose was the predominant component, accounting for 52.9 wt%. Therefore, in order to produce fermentable sugars, the enzymatic hydrolysis of SS was carried out in water in the presence of the commercial enzyme mixture Cellic[®] CTec 2 (dry sludge loading of 10 wt%, 24 FPU/g substrate, 50 °C, 72 h). The enzymatic hydrolysate of SS (SSH) resulted in a suspension, which was

centrifugated (4000 rpm, 10 min). The liquid phase of SSH was characterized through HPLC analysis and selected as the substrate for the growth of oleaginous yeasts, due to its high sugars concentration (44.1 g/L glucose, 9.3 g/L xylose). Different commercial oleaginous yeasts, *Cryptococcus curvatus*, *Trichosporon oleaginosus* and *Rhodospiridium toruloides*, were grown on a synthetic culture medium (SM), used as a model, and the most effective one was selected as the biocatalyst for fermenting SSH, using the same optimized process conditions adopted during the previous screening on SM. SM was characterized by a sugar composition (glucose and xylose) identical to that of the SSH, while all batch-mode fermentative processes were carried out by adapting the operating conditions described in our previous work¹. To assess the impact of the C/N ratio on lipid accumulation, three different C/N ratios (80, 100, and 120 g/g) were tested on SSH. Finally, the SCOs obtained from all the fermentations were characterized by GC-MS analysis. Based on the obtained FAMEs profile, several chemical and physical parameters of the produced biodiesel were calculated to predict its quality.

Results and Discussion

The preliminary experimental results on SM confirmed that the best oleaginous yeast among those tested was *T. oleaginosus* due to its very high lipid cell content (66.7 wt%), productivity ($168.0 \text{ mg L}^{-1}\text{h}^{-1}$) and lipid yield (22.6 wt%, equal to 68.5 wt% of the theoretical lipid yield). On this basis, this yeast was selected as the biocatalyst for the fermentative process of the undetoxified SSH (Figure 1). *T. oleaginosus* was able to grow on SSH and completely consume glucose and xylose in a slightly longer reaction time (96 hours) compared to the fermentation on the SM (72 hours). This difference can be explained taking into account that the microorganism needs to adapt itself to the particularly complex substrate, as evidenced by the starting lag phase in the growth curve. Adopting the optimal C/N ratio (80 g/g), this bioprocess enabled us to achieve 45.6 wt% of lipid accumulation, together with a lipids productivity and yield of $83.3 \text{ mg L}^{-1}\text{h}^{-1}$ and 15.0 wt%, respectively. These results are quite similar to those achieved by the fermentation of SM by the same biocatalyst, confirming the versatility and adaptability of *T. oleaginosus* in complex and unconventional substrates, such as SS, creating as a consequence, new opportunities for the valorization of those underutilized waste streams. Finally, the lipid profile characterization of all SCOs obtained in the present investigation highlighted that the composition of these oils were very similar to those of common vegetable oils, employed at the industrial scale for commercial applications, including the production of traditional biodiesel.

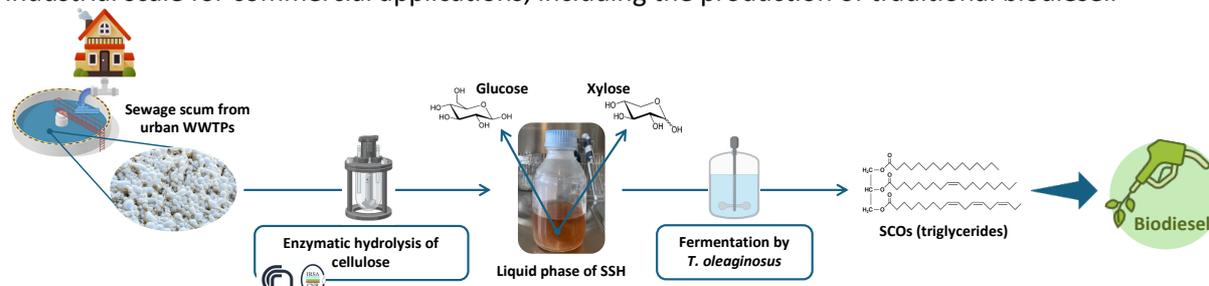


Figure 1. Process scheme of the production of SCOs from sewage scum through separated enzymatic hydrolysis and fermentation in the presence of the yeast *Trichosporon oleaginosus* as biocatalyst.

References

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