

Template

PROJECT FINAL PUBLISHABLE REPORT

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Project title: BIOETHANOL PRODUCTION VIA LIGNOCELLULOSIC FERMENTATION OF OLIVE OIL RESIDUES

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4.1 Final publishable summary report

ETOILE *BIOETHANOL PRODUCTION VIA LIGNOCELLULOSIC* *FERMENTATION OF OLIVE OIL RESIDUES*

1. Project description

The ETOILE project intends to develop a new integrated process where olive mill waste water from traditional three-phase centrifugation process is purified and at the same time cellulase enzymes are obtained. These enzymes are then used onto the solid olive oil residues, such as olive pulp and husks, to obtain ethanol. The research envisaged will thus provide a viable alternative to the growing bioethanol industry seeking for new cost-effective production processes competitive with oil industry.



Figure 1 Traditional olive oil production process

The project objectives are:

- Production of enzymes from olive mills wastewater
- A cost-effective process for ethanol production from olive mills residues.
- Purification of the olive mills wastewater and valorization of olive mills waste.

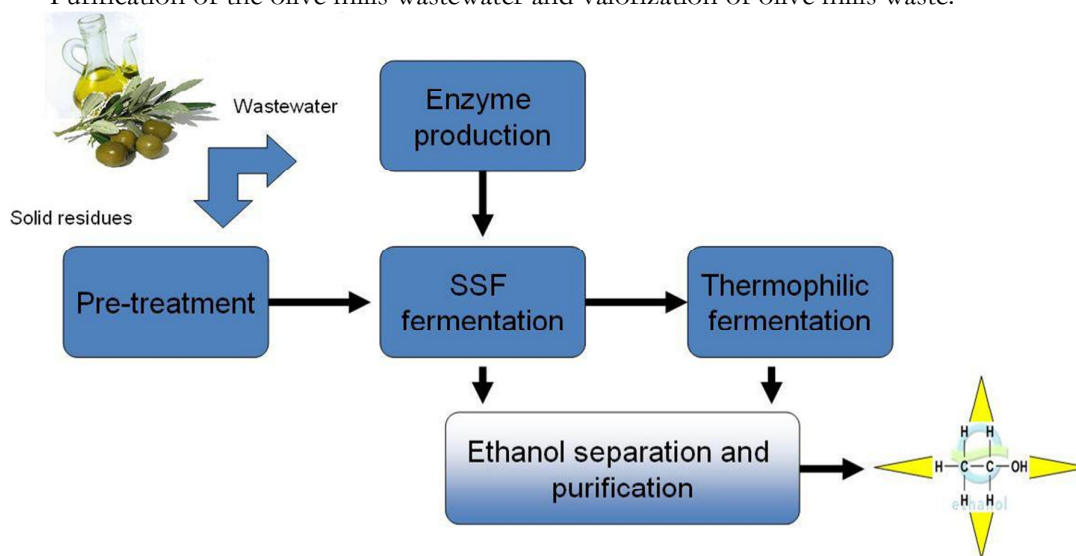


Figure 2 ETOILE technology approach

Specifically the project targets are:

- ***Development of the thermophilic fermentation process for ethanol production*** that produces as least 3.6 g/l of bioethanol
- ***Development of the Simultaneous Saccharification and Fermentation process*** that produces as least 15 g/l of bioethanol.
- ***Olive mill waste water treatment for cellulose enzyme production*** and/or recycle the enzymes so as to reduce enzyme costs less than 5 cents per liter of ethanol produced. The achievement of this objective will be at month 20 when the Milestone 2 will be reached.
- ***Development of a pre-treatment process of olive oil residues*** which is effective, does not require expensive chemicals and/or high pressure expensive equipment and whose cost constitutes less than 50% of the production cost of a liter of bioethanol.
- ***Ethanol recovery process design and optimization*** so as to keep fermentation rates high, and allow recycle of enzymes w/o thermal destruction to increase the production rate of ethanol by 20%. .
- ***Process integration*** by realisation of the ***pilot-scale bioethanol production plant*** that achieves 30-40% reduction of fuel consumption and energy costs. The achievement of this objective will be at month 24 when the final tests are performed on the pilot plant and Milestone 4 : ETOILE technology assessment will be reached.

2. Potential impact

Where is the idea coming from?

Olive oil residues are both a source of pollution and a waste of a valuable biomass resource that can constitute a low cost feedstock for cellulosic ethanol production.

Is there a real need?

In the ETOILE project three main challenges have been identified for cellulosic ethanol technology being a cost-effective industrial process:

1. Finding low cost feedstocks delivered to the plant (in the range of 15-20€ per ton).
2. Getting the cost of cellulose enzymes down.
3. Finding a robust microorganism that can use all types of sugars, is highly ethanol tolerant and can produce high levels of ethanol in a short time period.

Main challenges that ETOILE project will face are:

- An efficient, cost-effective and environmental friendly pre-treatment is needed to treat solid residues of the olive oil extraction
- Cellulase enzymes cost accounts for 40% of the ethanol production costs.
- Olive mill wastewater is a highly polluted waste, a handicap to the development and growth of the olive oil industry
- Low cost feedstock is needed to lower ethanol production costs.
- Glucose is inhibitory in a saccharification process.
- Optimal temperature for enzymatic hydrolysis is supraoptimal for common yeasts.
- Highly ethanol producer yeast is not capable of fermenting hemicellulose.
- Hemicellulose sugars are discarded.
- Pentose (xylose) conversion rates are lower than hexose (glucose) conversion rates.
- Ethanol inhibitory effects on the microorganisms reduce the yield of ethanol production.
- Ethanol concentration and purification is often realised by means of a highly energy intensive unit operation

- Ethanol is already produced at lab-scale from olive oil residues. However process integration of the above stages is not immediate

3. Consortium

Project Coordinator Labor Srl (Italy) <http://www.labor-eu.net/>

SME participants:

- Explora Biotech Srl (Italy)
- ARGUS Umweltbiotechnologie GmbH (Germany) <http://argus-umwelt.de/>
- Prisma Domi SA (Greece) <http://www.prismadomi.gr/>
- Tarikm: Tarimsal Kimya Teknolojileri San.ve Tic. A.S. (Turkey), exiting at month 7
- SEDNA Spa (Italy) www.sednagroup.it

RTD performers:

- Aalborg University (Denmark)
- Foundation for Research and Technology Hellas / ICE-HT (Greece) <http://www.chemeng.upatras.gr/>
- University of Rome “La Sapienza” (Italy)

4. Final Results

Feedstock availability and characteristics were determined during the first year. Ethanol producer was essential to assess the technical specifications of a bioethanol production plant.

Lab scale results are already reached during the first year of the project. On the second year ETOILE process was designed and implemented at 30 l plant scale. Main technical achievements are :

4.1. Development of pretreatment process on olive oil residues

Regarding the optimisation of thermochemical pre-treatment, methods that would have minimum requirements in terms of added chemicals and equipment used, thus methods that would be mild and cost efficient were evaluated. The parameters that FORTH tested were 1. type of added chemical used, 2. concentration of chemical used, (v/v or w/v), 3. time of chemical treatment, (days), 4. time of thermal treatment, (min), 5. temperature of thermal treatment, (oC), 6. solids loading, (w dry biomass/v of liquid) and 7. mechanical treatment (milling of solids to $\leq 1\text{mm}$)

The raw olive mill solid residues (O.S.R.), after removing ~30% of the stones (corresponding to 15% biomass in terms of dry weigh), were subjected to simultaneous thermal and chemical treatment according to different combinations of conditions for the parameters tested. Subsequently, the hydrolysate was separated from the solids via filtration, and the efficiency of each method was evaluated against the following criteria: 1. saccharification yield due to pretreatment, estimated as mg of generated sugars per g initial biomass as well as relative increase of sugars compared to control, 2. effect of each pretreatment method on the enzymatic digestibility, estimated as mg of generated sugars per g pretreated biomass as well as relative increase of sugars compared to control. For this reason a mixture of commercial cellulolytic enzymes, i.e. Celluclast 1.5L (30FPU/g solids) and Novozyme 188 (40FPU/g solids), was added to the remaining solids after adjustment of pH to 4.8 and the liberated sugars were measured after 3 hours and 1,2 and 3 days of treatment at 40oC., 3. growth and ethanol production efficiency of selected yeast strains (*Saccharomyces cerevisiae* and *Pachysolen tannophilus*) from the hydrolysates and total pretreated biomass (without separation of solids).

It was shown that highest saccharification yields were observed for H₂SO₄ and H₃PO₄, leading to 4 fold higher yields compared to NaOH, whereas Ca(OH)₂ proved to be totally inefficient for that type of waste, since it did not lead to any liberation of sugars (parameter 1). Results of direct saccharification showed increasing tendency for higher concentrations (parameter 2) and temperatures (parameter 5). The time of chemical treatment (parameter 3) did not seem to further facilitate the

liberation of sugars, whereas the effect of time of thermal treatment (parameter 4), into the limits tested, enhanced only slightly the final yield in some cases. Due to the added energy needed for longer thermal treatment time, the shortest treatment time (45 min) were finally considered as most favorable for the viability of the overall process. The highest yield was 197 ± 7 mg sugars/g dry OSR and was observed for H₂SO₄ 1.5% , at 130 oC and 45 min thermal treatment.

Regarding, the enzymatic saccharification of the remaining solids (RS) after pre-treatment, in all cases higher concentration of chemicals and higher temperatures seemed to enhance the effectiveness of enzymes. However, contrary to direct saccharification results NaOH proved to most efficient chemical to facilitate enzymatic digestibility, leading to yields of 485 ± 13 mg sugars/g dry RS (1,5%, NaOH, 45 min treatment, 130 oC), thus resulting to the highest total yield of 499 ± 2 mg sugars/g dry OSR.

Solids loading (parameter 6) at 5% lead to the highest saccharification, whereas above that value seemed to inhibit the direct saccharification effect. Regarding mechanical treatment, acid pre-treatment was shown to be favoured when no milling was performed especially in terms of direct saccharification. However in alkali pre-treatment milling of the biomass had such substantial positive effect during enzymatic treatment, that milling was considered favourable for the overall process.

Fermentation test with hydrolysates showed that concentration of acid or alkali during pre-treatment did not affect the final yield, indicating that there is no inhibition due to by-products formation (furfurals, hydroxyl-methyl-furfurals) in to the range tested. However, hydrolysates from alkali pre-treatment leaded to higher ethanol yields compared to hydrolysates from acid pre-treatment (1.17g ethanol/g sugars). Fermentation of the total pre-treated biomass was conducted via simultaneous saccharification and fermentation (SSF). The results were similar to those during the fermentation of hydrolysates, i.e. the concentration of chemical did not inhibit ethanol production, and final yields were better for alkali pre-treated biomass. The highest observed ethanol yield was 0.39g ethanol/g sugars.

Besides biochemical pretreatment was also investigated. Fungal delignification by white rot fungi was tested to pre-treat the feedstock. Solid state fermentation was performed:



4.2. Enzyme production from olive processing wastes and wastewater treatment

– Enzyme production from olive processing wastes

UNIRO worked on improving with *T. reesei* Rut-C30 (the highest publicly available cellulase producer) the cellulase production that had been obtained with *T. viride* wild type (efficient OOMW biotreatment but modest cellulase production).

Main new results that UNIRO has obtained during the 2nd part of the Project or improved over those collected during the 1st part of the Project are:

- *T. reesei* Rut-C30 maximum activity attained during fermentations was increased from 1.2 FPU/ml to 3.5 FPU/ml (+190%). In the same conditions of the tests carried out in the first

term, using olive pomace (10 g/l) as the inducer, maximum activity is 3 FPU/ml (+150%), with an enzyme productivity exceeding $10 \text{ FPU h}^{-1} \text{ g}_{\text{fungus}}^{-1}$, that is, olive pomace is a better inducer than usually employed microcrystalline cellulose, with a negligible cost footprint. The produced enzymatic broth can be used without concentration allowing a hydrolytic load of 20% solids at 15 FPU ml^{-1} on dry pretreated biomass.

- While at the end of the first term our results indicated that fungal biomass and the olive pomace had to be renewed at every new cellulase producing fermentation, during the second term we ascertained that the fungal biomass and the olive pomace can be reused up to two times with limited productivity and activity reduction if fresh olive pomace is added after the first production run.
- A limited (up to 5%) entrainment of OOMW with pomace can be tolerated with a marginal enzyme productivity drop.
- OOMW cannot be used to support *T. reesei* Rut-C30 growth.
- Given the above, a 4-step (coagulation/settling, plus ultrafiltration, nanofiltration, and reverse osmosis) membrane process has been devised and tested which is able to treat OOMW and reduce its COD below 150 mg l^{-1} and its polyphenol content below 10 ppm.

4.3. Development of thermophilic fermentation process

Experiments in pure cultures with synthetic substrates as well as with substrates based on raw and pretreated olive pomace were performed by AAU.

Ethanol efficiency with raw olive pomace reached satisfactorily high levels but the ethanol productivity remained at very low levels mainly due to the low bioavailability of lignocellulosic sugars in raw olive pomace. Physicochemically pretreated olive pulp was not a suitable substrate for a thermophilic fermentation process since the bacterium growth was severely inhibited.

4.4. Development of the Simultaneous Saccharification and Fermentation process

Commercial cellulase activity was tested by LABOR on olive pomace releasing 10g/l of soluble sugars. Yeast was selected based on experimental trials for (a) temperature influence on yeast growth and ethanol production (b) Medium composition influence on ethanol production. Reaching c.a. **14 g/l of ethanol when some released sugars of olive pomace were used**

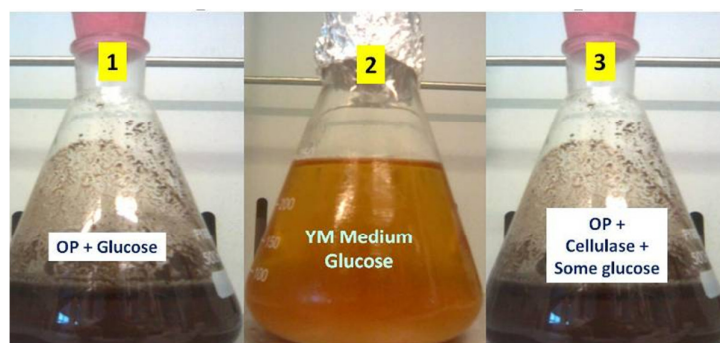


Figure 3 Medium composition influence on ethanol production tests

SSF test were carried at lab scale using only olive pomace as carbon source resulting in lower ethanol production due to lower sugars content in the media.



Figure 4 Experimental set up

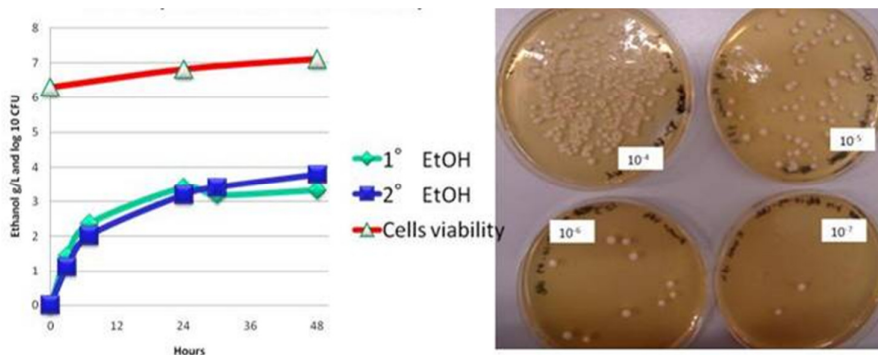


Figure 5 Ethanol production and cells viability

Next SSF step test was carried in a 2 liters reactor reaching c.a. **9 g/l** of produced ethanol.

Table 1 2L Experimental data protocol

| Technical procedures | |
|----------------------|-------------------------------|
| feedstock | Olive Pomace |
| treatment | Alkali 1,5% w/v NaOH |
| yeast | K. marxianus |
| enzymes | Cellulase 18 FPU |
| enzymes | Beta-glucosidase 5 ml NS50010 |
| temperature | 42° C |
| volume | 1,7 L |
| agitation | 200 rpm |
| gas | carbon dioxide |
| water column volume | 450 ml |

The whole process was carried out in a closed system that employs a reactor and a water filled absorption column in which CO₂ is the carrier gas of the fermented ethanol.



Figure 6 2L reactor setup

In conclusion ETOILE project has reached **Milestone 1: Ethanol fermentation assessment**. SSF fermentations was optimized for ethanol production from lignocellulosic olive oil residues.

4.5. Development of the ethanol recovery process

Preliminary design of the ethanol recovery process was achieved.

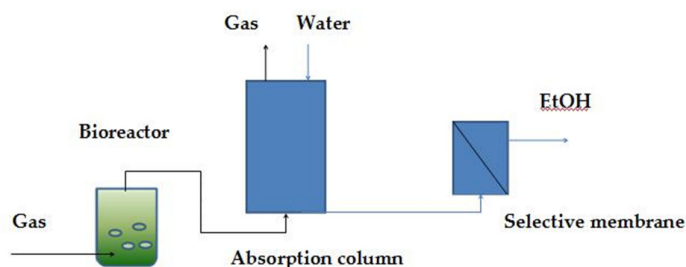


Figure 7 Ethanol recovery process

Experimental data on ethanol absorption on different carriers was also obtained.

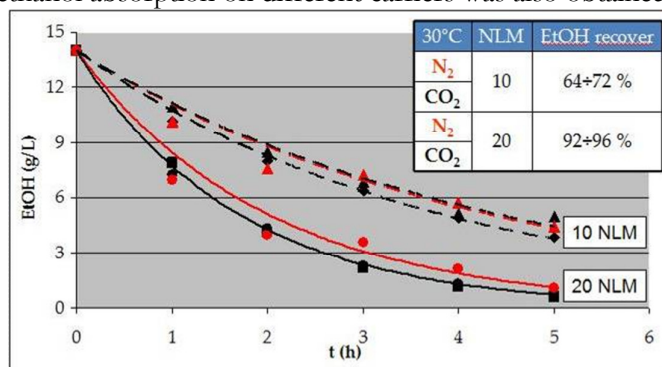


Figure 8 Experimental data on ethanol absorption

The measurement of ethanol removal ability of the absorption column was tested. The reactor's ethanol concentration was about 9 g/l, temperature inside the reactor was stable at 42°C during all experiment as well as the agitation and the gas flow. The column was charged with 1,2 litres of water to absorb the ethanol.

The ethanol detection measures are carried out in the reactor daily while in the column after 48h and for other 24 hours successive steps.

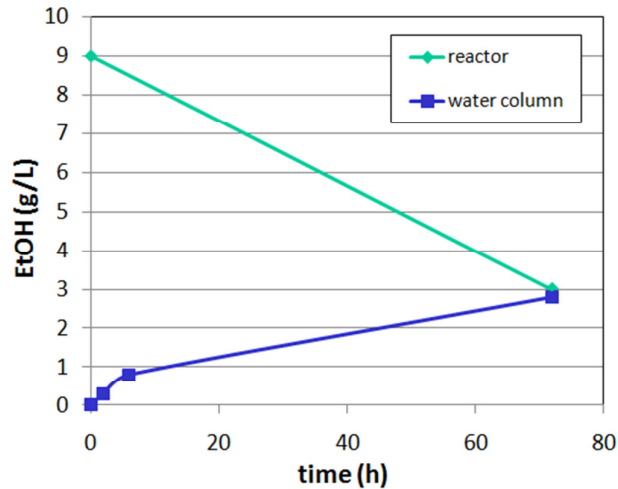


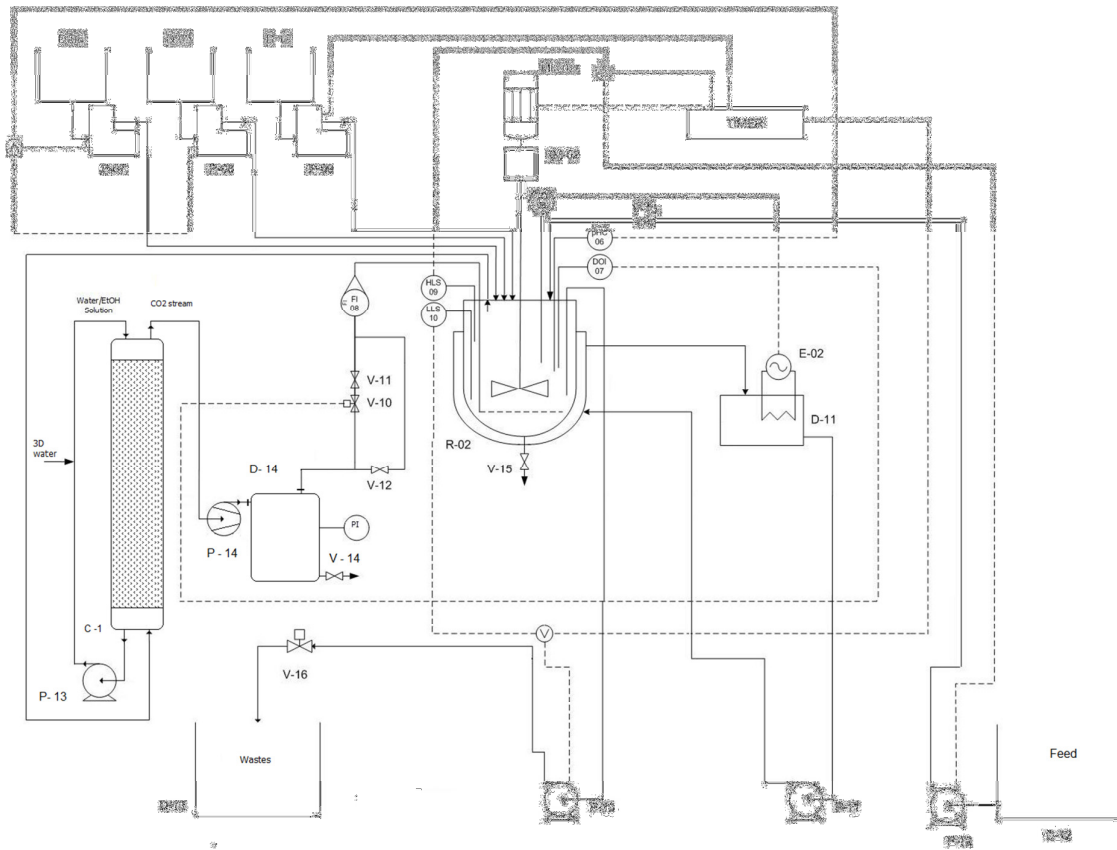
Figure 9 ethanol removal test

The ethanol detection measures detected that after 72h the reactor and the column reached the equilibrium concentration of the ethanol of 3 g/L.

5. Process Integration Design and Development

5.1. Design of the Integrated Process

Based on the results of the lab scale tests an overall pilot plant was designed. It had integrated the SSF and ethanol removal system as presented in next PID diagram. OP pretreatment was performed previously in a big autoclave.



In order to speed up the pilot plant construction and arrive on time to the project pilot scale results, an existing 30 l bioreactor, located at LABOR premises was adapted in order to test the ETOILE lignocellulosic ethanol production technology.

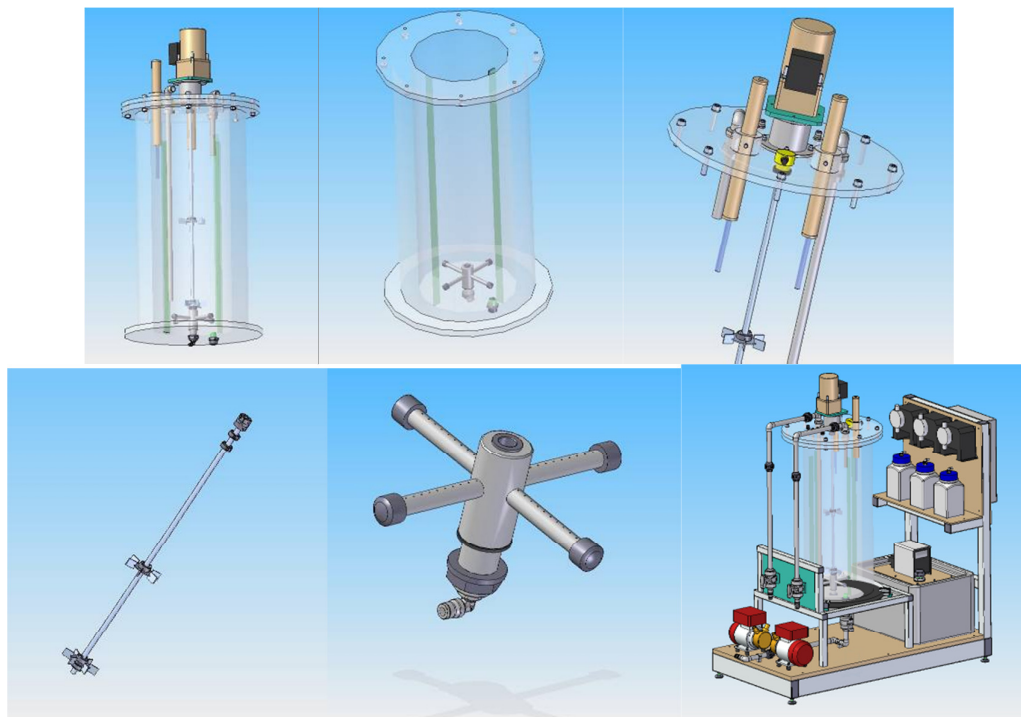


Figure 10 30L pilot plant reactor

Ethanol absorption system

The Absorption Column consists in a cylindrical PVC tube sealed on the bottom and on the top. The closed H₂O-circuit operates by a water pump while the closed gas-circuit, connected with the reactor, operates by an air pump. The Raschig rings are obtained also cutting the cylindrical PVC tubes.

The dimensional data of the column are:

- Z (height of the column) = 200 cm
- D (diameter of the column) = 11 cm
- d (diameter of the raschig rings) = 1,6 cm
- l (height of the raschig rings) = 2 cm
- h (height of the packing) = 150 cm
-

The volumetric flow rates of the pumps are:

- W_g (gas flow rate) = 60 L/h
- W_l (liquid flow rate) = 120 L/h

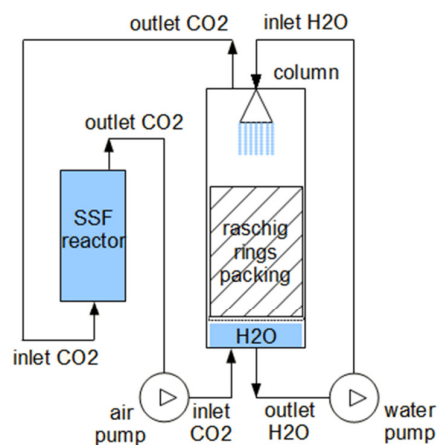


Figure 11 Pilot Plant flow scheme

5.2. Construction of the Pilot Plant

The pilot plant was built based on project needs. The anaerobic reactor of 30 litres of capacity was built to reach the specific features needed to carry out the SSF experiments to produce bioethanol from OP as feedstock, to remove the ethanol from reactor in a more purity solution and recycle the same CO₂ during many days of work without the addition of external carbon dioxide. All these features are necessary for a cost and energy efficiency of the process and to carry out the anaerobic fermentation of ethanol. Some preliminary tests were performed to validate the pilot plant both mechanically and hydraulically.

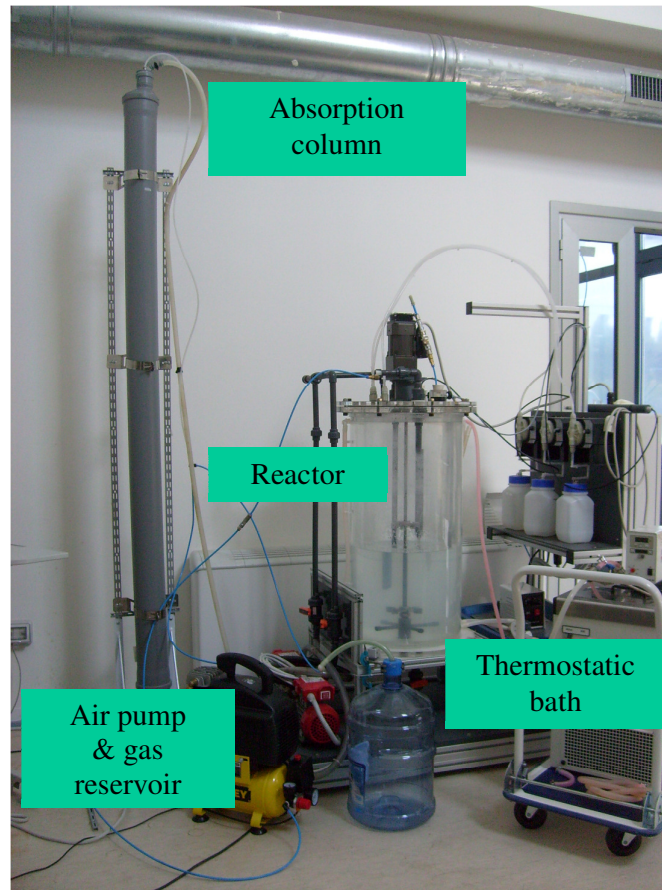


Figure 12 Pilot Plant Experimental Setup

Also UNIRO develop a pilot plant for olive mills wastewater treatment.



Figure 13 Pilot Plant Experimental OOMW Setup

5.3.Process Control System Development

A software that manage several key parameters like the air flux, the speed of agitation, the balance of pH, the volume and temperature of liquid inside the reactor was develop

Characteristics of process control system have been outlined:

1. Controlled variables
2. Sensors
3. Control system architecture and end-user's interface

Automation and monitoring system were also implemented. Overall plant was controlled by this Pc
Next figure presents a screenshot of the software:

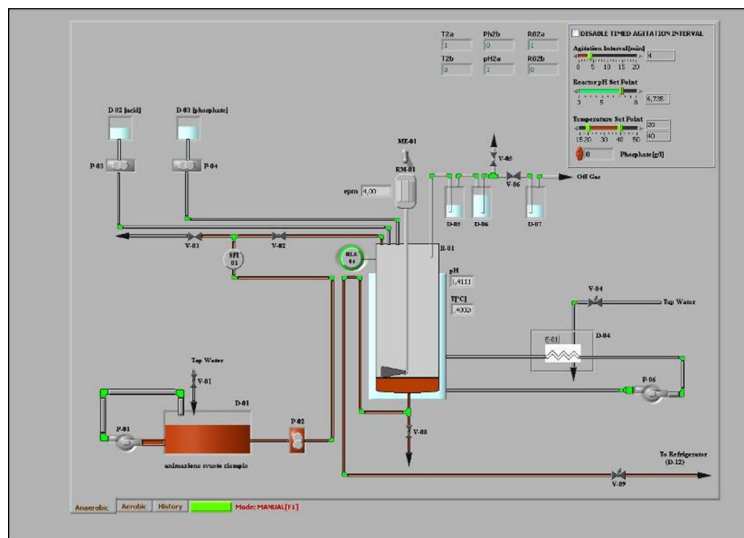


Figure 14 Screenshot of the Software Control System

5.4. Whole Process Calibration and Validation

Pilot Plant Process protocol

Table 2 ETOILE pilot plant principal experimental process

| Experimental procedure | |
|------------------------|---|
| Thermal step | 130°C 45 minutes |
| NaOH | 1,5 % w/v |
| OP concentration | 200 g/l |
| OP total | 4,2 kg dry matter (1,7 Kg powder; 2,5 kg gross) |
| Temperature | 43,5- 44°C |
| Cellulase | NS50013 1,2 Liters |
| Cellulase | 18 FPU/g dry matter |
| Beta-glucosidase | 120 ml NS50010 |
| Glucose | 120 gramms |
| Yeast | K. marxianus 1,5 liters |
| Final volume | 24 liters |
| Fermentation total | 5 days |
| Start CO2 recycling | 24° th hour |
| End CO2 recycling | 5° th day |
| sample ethanol | reactor and column |
| sample sugars | reactor at beginning and at finish |

Pilot Plant Pretreatment protocol

Olive pomace was dried and milled. For this test 1.7 kg of grinded Olive Pomace, less than 1mm sieve grid was taken. In order to complete the necessary concentration of 200 g/L, 2.5 kg of the olive pomace that remains after the sieving, including the olive stones was used. The olive pomace was first treated with NaOH in a 30 l stainless steel tank, and then in autoclave for 45 min at 130°C.

The pH was corrected until a pH of 5.8 was reached inside the stainless steel tank.

SSF protocol:

Simultaneous saccharification and fermentation is performed in a CSTR for 72 h. Simultaneous ethanol removal is achieved by bubbling CO₂ into the fermentation broth. The concentration of the reagents and the characteristics of the SSF are:

- 15 FPU/g OP Novozyme's NS50013
- 0,5 ml Betaglucosidase/100 ml substrate
- Operation temperature 46°C
- Yeast *K. marxianus*
- Fermentation total time 72 h

Then it was transferred to the bioreactor and the pH was finally corrected to 6. Bioreactor temperature was raised until 46 °C. Enzyme was added and 2 h were waited in continuous agitation at 100 RPM. Then bioreactor was cooled until about 43.5°C (fermentation temperature). At this point 1.5 l of yeast

was added together with 120 g of glucose. Total volume of the bioreactor was reached up to 24 l. Bioreactor was sealed and for 24 h the fermentation was carried out in order to increase the yeast biomass. At the 20th hour CO₂ flux was started for ethanol recovery. Absorption column was filled with 1.5 l of 3D water.

OOMW Treatment:

An OOMW treatment only using chemical & physical processing stages was set-up. The specifications of the devised process were:

- Processing capacity: 1 m³/day of wastewater;
- Processing until compliance with the law (COD = 500 ppm_{O₂})
- Processing by one coagulation-settling and three (nano-, ultra- and reverse-osmos filtration) membrane processes.

An abstract of the process conditions used for the pilot plant test are presented in the following table.

OOMW Membrane Treatment

The flocculation/coagulation process is allotted up 24 h for the complete sedimentation of the formed sludge. A clarified stream is obtained, which includes 80-85% of the inlet OOMW. An aeration process (labelled as AIR), aimed at oxidating any readily oxidable dissolved matter which cannot be flocculated. Each of the ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) processes permit the recovery of, 90-95% of its inlet water content.

The process was continuously monitored. On Figure 15 are presented the path of the main variables. Constant process variables are not shown for clarity.

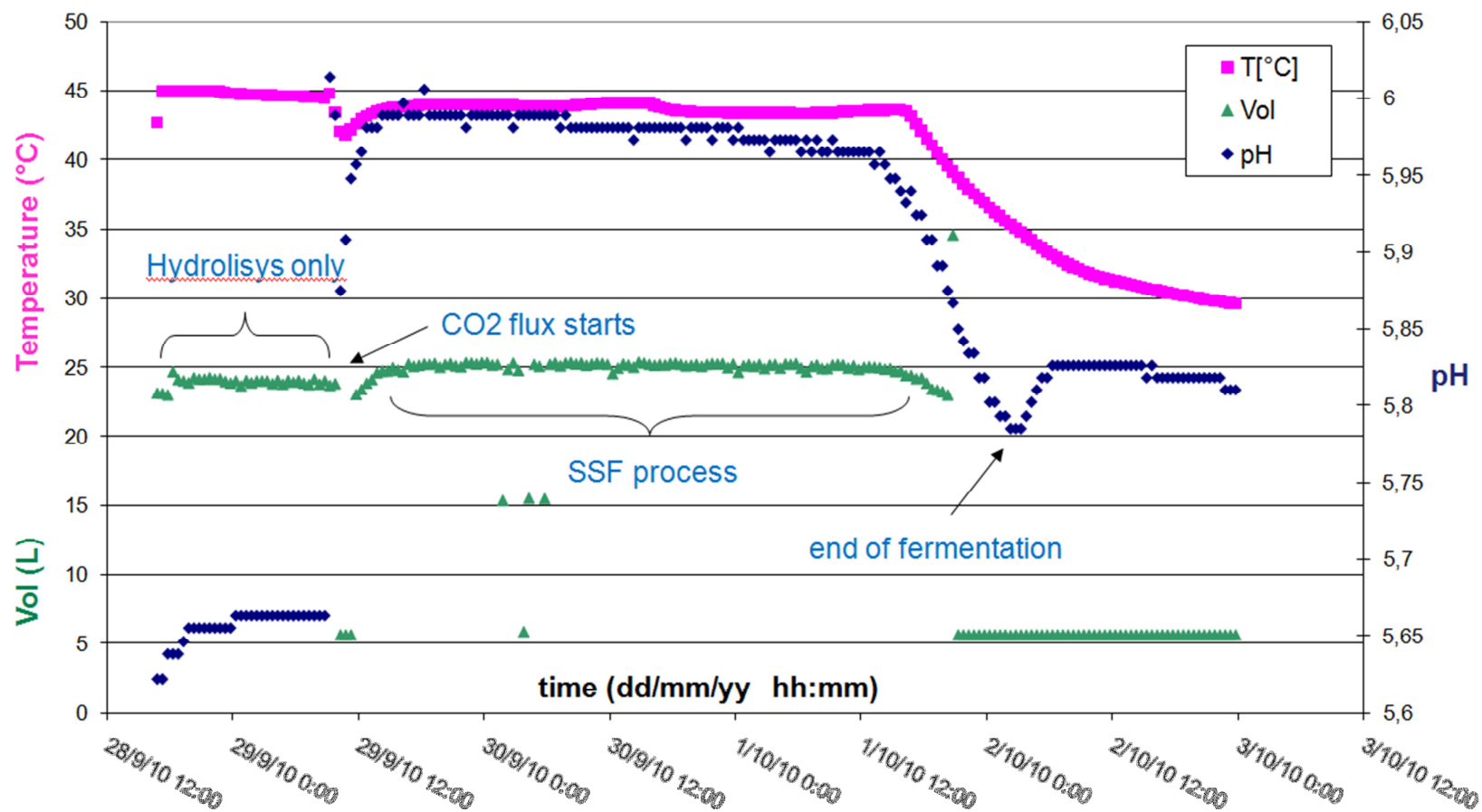


Figure 15 ETOILE pilot plant principal process variables control

Ethanol Absorption

Figure 16 presents the ethanol concentration behavior in the absorption column. The EtOH Removal was not effective like in the pilot plant blank test:

- we can suppose that the production of the EtOH (blue continue line) was at most completed @ 40h;
- up to 25h the removed EtOH was following the production curve;
- from 25h to the end, something happened like a gas leakage;

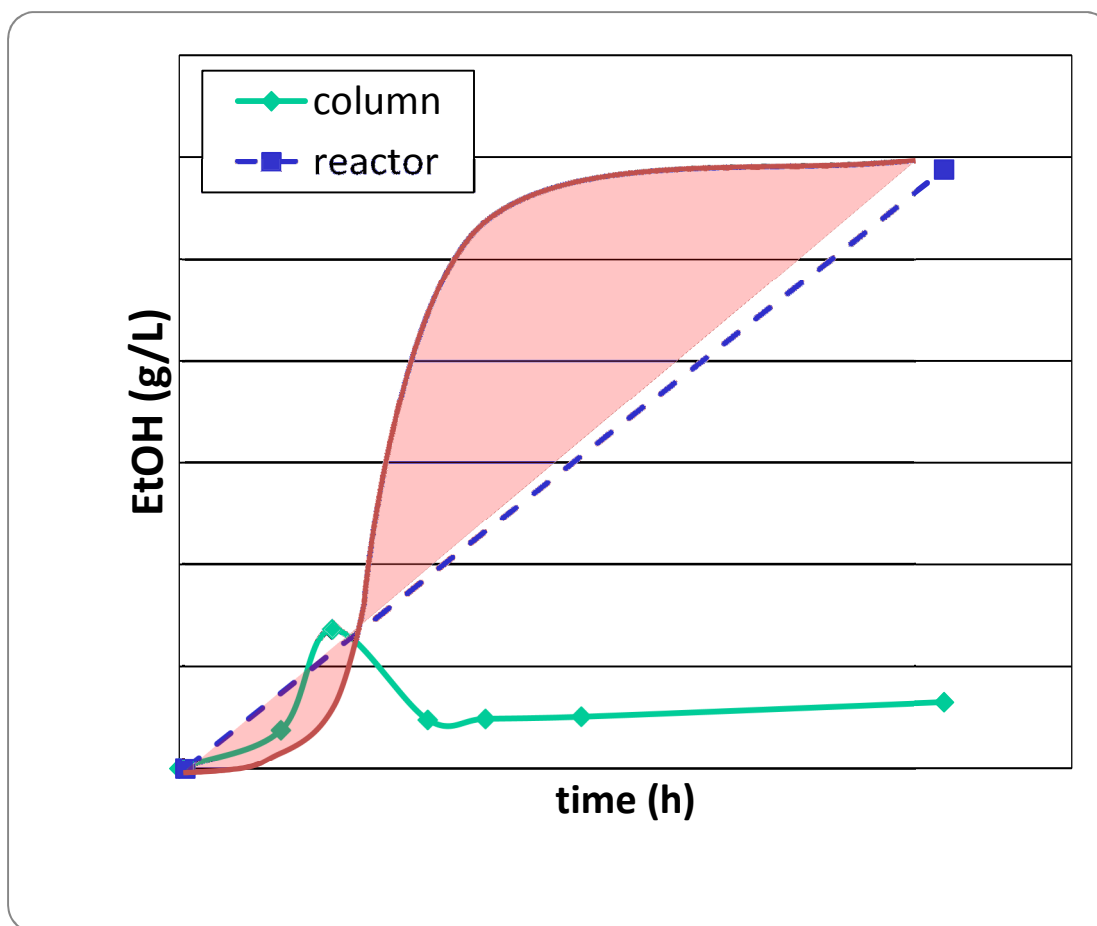


Figure 16 Ethanol removal on the SSF fermentation on the ETOILE pilot plant

Ethanol concentration in the water was low and it didn't reach the expected 3 g/l, however final ethanol concentration in the bioreactor was high as **5,88 g/l** at 120 h. This means that the CO₂ flow rate should be increased in order to facilitate the ethanol mass transfer from the bioreactor to the absorption column.

Total Sugar Analysis

| 250 ml SSF | Initial sugars content g/L | Final sugars content g/L | Ethanol produced g/L |
|---------------|-------------------------------|-----------------------------|-------------------------|
| 1% 5FPU | 10,2 | 12,7 | 4,2 |
| 1% 10 FPU | 10,9 | 14,9 | 7,5 |
| 1,5% 18 FPU | 14,8 | 15,7 | 9 |
| 2% 15 FPU | 19,6 | 16,1 | 8,1 |

| 30 L SSF | Initial sugars content g/L | Final sugars content g/L | Ethanol produced g/L |
|-------------|-------------------------------|-----------------------------|-------------------------|
| 1,5% 18 FPU | 12,6 | 26,7 | 6 |

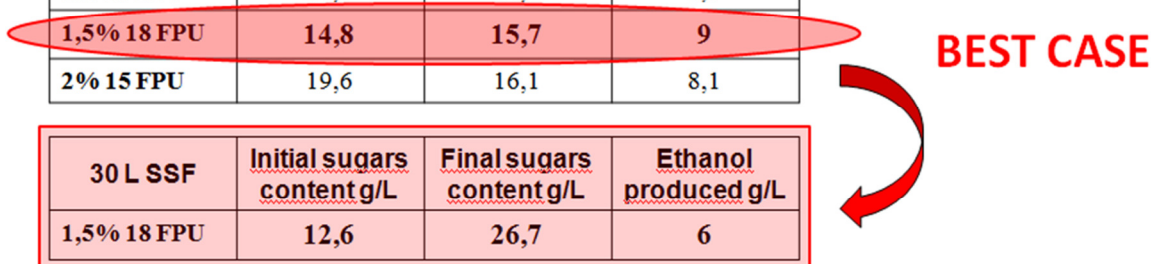


Figure 17 Total sugars concentration of the SSF fermentation on the ETOILE pilot plant

Unlucky due to the viscosity of the olive pomace that makes the substrate of the fermentation very heavy, it was very difficult to take a sample in the middle of the fermentation and saccharification process. However from these results it could be said that some sugars were still available for fermentation. The produced EtOH is lower in the 30L pilot plant:

The total sugar analysis shows a greater amount of final sugar content, which can explain the lower rate of ethanol, not all the possible sugars have been converted

OOMW treatment analysis resultsCoagulation

Coagulation was carried out with 6 g/l of coagulant, and a comparable efficiency to the previous determinations was obtained.

Lime was added to increase the pH value to a corrosion-safe value for zinc-plated surfaces.

| Coagulation | | | |
|-------------------|---------------|---------------|---------------|
| Sample Origin | COD [mg/l] | TPh [mg/l] | COD Reduction |
| After coagulation | 23200 | 520 | - |
| After coagulation | 21430 | - | - |

Ultrafiltration

The ultrafiltration membrane used is seemingly very permeable to polyphenols, especially when their concentration is high, i.e. toward the end of the operation.

| Ultrafiltration | | | |
|--------------------------|---------------|---------------|---------------|
| Sample Origin | COD [mg/l] | TPh [mg/l] | COD Reduction |
| UF Feed Tank, t = 0min | 20240 | 314 | - |
| UF Permeate, t = 440min | 17110 | 216 | 15.46% |
| UF Feed Tank, t = 880min | 20020 | 484 | - |
| UF Permeate, t = 880min | 15700 | 480 | 21.57% |

Nanofiltration

| Nanofiltration | | | |
|--------------------------|------------|------------|---------------|
| Sample Origin | COD [mg/l] | TPh [mg/l] | COD Reduction |
| NF Feed Tank, t = 0min | 19730 | 274 | - |
| NF Feed Tank, t = 500min | 29270 | 820 | - |
| NF Permeate, t = 500min | 13890 | 356 | 52.55% |

Reverse Osmosis

| Reverse Osmosis | | | |
|-------------------------|------------|------------|---------------|
| Sample Origin | COD [mg/l] | TPh [mg/l] | COD Reduction |
| Feed Tank, t = 120 min | 11140 | 341 | - |
| RO Permeate, t = 120min | 108 | 9 | 99.03% |

Conclusions

The ETOILE technology protocol was successfully tested at pilot scale, using a 30 l bioreactor. Low energy ethanol removal from fermentation broth is achieved using CO₂ as a carrier gas. Overall process energy cost and Life Cycle Assessment will confirm this.

6. Assessment of the ETOILE technology**6.1. Methane recovery**

Methane potential tests were performed on the biomass residues that is effluent of SSF process after removal of ethanol. Olive pomace residue was a suitable substrate for anaerobic digestion and methane production. The methane potential reached a relatively high value of 400 ml per g-TS. The methanogenesis was not inhibited but an inoculums adaptation was required.

6.2. LCA

From the sustainability analysis of ETOILE process we can outdraw the following conclusions:

- We have performed a gate-to-gate (comparison of different processing options, not taking into account the agricultural part of the system) comparison SSF appears to have an environmental advantage with respect to a process envisioning a separate hydrolysis followed by fermentation. This is evidently due to the energy requirement for an additional reaction step.
- The introduction of a the separation step envisioning stripping with CO₂ presents significant advantages due to the lower energy requirement deriving from the elimination of the first distillation step. This represents a significant innovation in the process and would deserve further development to better optimize the plant on the pilot scale.
- The processing condition investigated in the project envision quite substantial amount of enzyme utilization. Available Life Cycle Inventory data for cellulase production are not easily transferred to the present case, and are still subject to consistent uncertainty due to the rapid

technological changes and the difficulty to retrieve data from the producers. In this perspective, the in-situ production of the enzyme would surely imply a reduction in the environmental burdens of the ethanol process production. This technological objective was unfortunately not met by the project, but it would be important to devote further research to address this issue (for example by developing *T. reesei* fungi adapted to grow on high polyphenols concentration substrates).

From the economic point of view, obtaining ethanol from olive oil waste present some advantages:

- almost zero costs of the feedstock (here we assumed to take into account only transportation costs)
- assuming a plant design and related capital costs similar to those employed in existing studies related to lignocellulosic ethanol production, costs related to enzyme purchase still represent an important contribution to the overall process.
- The economic advantage of using low-cost feedstock is in many cases not sufficient of offset the high costs incurred in purchasing the enzyme from external producers
- As already investigated in other literature studies, on-site cellulose production does not necessarily implies an economic advantage compared to purchasing it externally: the cellulose production reduce the ethanol yield; the economic advantage is achieved only by introducing particular process configurations.

7. Contact

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