Executive summary:

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.

Project context and objectives:
The project objectives are:

(a) the production of enzymes from olive mills wastewater;
(b) a cost-effective process for ethanol production from olive mills residues;
(c) purification of the olive mills wastewater and valorisation of olive mills waste.

Specifically the project targets are:

(a) the development of the thermophilic fermentation process for ethanol production that produces as least 3.6 g / l of bioethanol;
(b) the development of the simultaneous saccharification and fermentation (SSF) process that produces as least 15 g / l of bioethanol;
(c) the 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 litre of ethanol produced. The achievement of this objective will be at month 20 when the milestone 2 will be reached;
(d) the 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 litre of bioethanol;
(e) the ethanol recovery process design and optimisation 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 %;
(f) 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.

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 EUR 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:

(a) an efficient, cost-effective and environmental friendly pre-treatment is needed to treat solid residues of the olive oil extraction;
(b) cellulase enzymes cost accounts for 40 % of the ethanol production costs;
(b) Cellulase enzymes cost accounts for 40% of the ethanol production costs;
(c) Olive mill wastewater is a highly polluted waste, a handicap to the development and growth of the olive oil industry;
(d) Low-cost feedstock is needed to lower ethanol production costs;
(e) Glucose is inhibitory in a saccharification process;
(f) Optimal temperature for enzymatic hydrolysis is supraoptimal for common yeasts;
(g) Highly ethanol producer yeast is not capable of fermenting hemicellulose;
(h) Hemicellulose sugars are discarded;
(i) Pentose (xylose) conversion rates are lower than hexose (glucose) conversion rates;
(j) Ethanol inhibitory effects on the microorganisms reduce the yield of ethanol production;
(k) Ethanol concentration and purification is often realised by means of a highly energy intensive unit operation;
(l) Ethanol is already produced at lab scale from olive oil residues. However, the process integration of the above stages is not immediate.

Project results:

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, the ETOILE process was designed and implemented at 30 l plant scale. The 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:

(a) Type of added chemical used;
(b) Concentration of chemical used, (v / v or w / v);
(c) Time of chemical treatment, (days);
(d) Time of thermal treatment, (min);
(e) Temperature of thermal treatment, (degrees of Celsius);
(f) Solids loading, (w dry biomass / v of liquid); and
(g) Mechanical treatment (milling of solids to = 1 mm).

The raw olive mill solid residues, after removing approximately 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:

(a) 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.
as well as relative increase of sugars compared to control.

(b) 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 1.5 L (30 FPU / g solids) and Novozyme 188 (40 FPU / 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 40 degrees of Celsius.

(c) Growth and ethanol production efficiency of selected yeast strains (Saccharomyces cerevisiae and Pachysolen tannophylus) 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 favourable for the viability of the overall process. The highest yield was 197 ± 7 mg sugars / g dry olive mill solid residues and was observed for H2SO4 1.5 %, at 130 degrees of Celsius and 45 min thermal treatment.

Regarding, the enzymatic saccharification of the remaining solids 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 remaining solids (1.5 %, NaOH, 45 min treatment, 130 degrees of Celsius), thus resulting to the highest total yield of 499 ± 2 mg sugars / g dry olive mill solid residues.

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 (1.17 g ethanol / g sugars). Fermentation of the total pre-treated biomass was conducted via simultaneous 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 0.39 g 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

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 olive oil mill wastewater (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 FPU h-1 gfungus-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 thermophylic fermentation process since the bacterium growth was severely inhibited.

4.4. Development of the Simultaneous SSF process

Commercial cellulase activity was tested by LABOR on olive pomace releasing 10 g / 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 approximately 14 g / l of ethanol when some released sugars of olive pomace were used.

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

Next SSF step test was carried in a 2 litres reactor reaching approximately 9 g / l of produced ethanol.
Next SSF step test was carried out in a 2 litres reactor reaching approximately 9 g/l of produced ethanol. 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. In conclusion ETOILE project has reached milestone 1: Ethanol fermentation assessment. SSF fermentations were optimised 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. Experimental data on ethanol absorption on different carriers was also obtained. 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 degrees of Celsius 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 48 hours and for other 24-hour successive steps. The ethanol detection measures detected that after 72 hours 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 piping and instrumentation diagram (PID). 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.

Ethanol absorption system

The Absorption column consists in a cylindrical polyvinyl chloride (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:
- Wg (gas flow rate) = 60 L/h
- WI (liquid flow rate) = 120 L/h
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 carbon dioxide (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. Also UNIRO develop a pilot plant for olive mills wastewater treatment.

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. The characteristics of process control system have been outlined:

(a) controlled variables;
(b) sensors;
(c) control system architecture and end-user's interface.

Automation and monitoring system were also implemented. Overall plant was controlled by this Pc.

5.4 Whole process calibration and validation

Pilot plant process control

ETOILE pilot plant principal experimental process
Experimental procedure
Thermal step: 130 degrees of Celsius, 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 degrees of Celsius
- Cellulase: NS50013 1.2 litres
- Cellulase: 18 FPU / g dry matter
- Beta-glucosidase: 120 ml NS50010
- Glucose: 120 grams
- Yeast K. marxianus: 1.5 litres
- Final volume: 24 litres
- Fermentation total: 5 days
- Start CO2 recycling: 24th hour
- End CO2 recycling: 5th 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 1 mm 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 degrees of Celsius. The pH was corrected until a pH of 5.8 was reached inside the stainless steel tank.

SSF protocol:

Simultaneous SSF is performed in a CSTR for 72 hours. 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 degrees of Celsius
- Yeast K. marxianus
- Fermentation total time: 72 hours.

Then it was transferred to the bioreactor and the pH was finally corrected to 6. Bioreactor temperature was raised until 46 degrees of Celsius. Enzyme was added and 2 hours were waited in continuous agitation at 100 RPM. Then bioreactor was cooled until about 43.5 degrees of Celsius (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 hours 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 ppmO2),
- processing by one coagulation-settling and three (nano-, ultra- and reverse-osmosis filtration) membrane processes.

OOMW membrane treatment

The flocculation / coagulation process is allotted up 24 hours 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, nanofiltration and reverse-osmosis processes permit the recovery of 80 - 85 %.
Each of the ultrafiltration, nanofiltration and reverse osmosis processes permit the recovery of, 90 - 95 % of its inlet water content.

**Ethanol absorption**

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 at 40 hours.  
- Up to 25 hours the removed EtOH was following the production curve.  
- From 25 hours to the end, something happened like a gas leakage.

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 hours. 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.

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 results**

**Coagulation**

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.

**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.

**Conclusions**

The ETOILE technology protocol was successfully tested at pilot scale, using a 30-litre bioreactor. Low energy ethanol removal from fermentation broth is achieved using CO2 as a carrier gas. The overall process energy cost and lifecycle assessment will confirm this.

6. Assessment of the ETOILE technology

6.1. Methane recovery

The methane potential tests were performed on the biomass residues that are effluent of SSF process after the removal of ethanol. Olive pomace residue was a suitable substrate for anaerobic digestion and
after the 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 inoculum 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 optimise the plant on the pilot scale.
- The processing condition investigated in the project envision quite substantial amount of enzyme utilisation. The available lifecycle 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. reseei fungi adapted to grow on high-polyphenol 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.

Potential Impact:

Main results of the project are:

(a) fungal delignification of olive cake;
(b) olive cake chemical treatment protocol for future fermentation;
(c) cellulase production and wastewater treatment;
(d) acidic pasteurisation and coagulation of wastewaters;
(e) SSF of olive wastes and ethanol production downstream valorisation;
As mentioned above 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. The project has impact on small and medium-sized enterprise (SME) olive oil producers as their own wastes are being valorised, on the ethanol producers as olive oil wastes are almost no cost feedstock and on general society as no-food feedstock for the production.

Dissemination of knowledge

1. Description of dissemination activities

1.1 Scientific publications

Scientific publications have been produced by research and technological development (RTD) performers about the results achieved for enzymatic treatment of olive pomace, cellulase production, fungal pretreatment of olive cake and the overall ETOILE technology.

For all RTDs and publications the Abstracts were sent to partners as soon as possible after approval of the scientific committee of the conferences.

UNIRO presented some of the ETOILE results to international conferences:

- 2nd international conference on Industrial Biotechnology on 11 - 14 April 2010, Padua, Italy. An oral presentation entitled 'Cellulase Production from Olive Processing Residues' was given. This resulted in a proceedings book. The abstract of this paper has been annexed to the present document.
- 14th International Biotechnology Symposium and Exhibition on 14 - 18 September 2010, Rimini, Italy. A poster presentation entitled 'Cellulase Production by T. reesei RUT - C30 Using Olive Pomace as Inducer: a Step Towards an Integral and Sustainable Olive Waste Energetic Valorisation' was held. UNIRO also disseminated the significance of the entire ETOILE project in a recognised mixed academic / industrial context by individual talks. After bringing their contribution in ETOILE work, Mr Gianluca Cassone and Miss Giorgia Vivona discussed their Bachelor thesis, respectively in chemical engineering and industrial and environmental biotechnology during December 2010.

UNIRO is also planning a publication during 2011 with prospective title: ‘Process Optimisation in Cellulase Production by T. reesei RUT-C30 and Olive Pomace Inducer’ and prospective abstract attached.

AAU has being accepted for publication for the:

- 3rd International Conference on Engineering for Waste and Biomass Valorisation, Beijing, China, 17 - 19 May 2010. The abstract of this paper have been annexed to the present document. AAU is also planning to present a paper entitled 'Growth and metabolic products of Thermoanaerobacter ethanolicus on synthetic substrates and agricultural residues'.
LABOR presented also:

- 2nd International Conference on industrial Biotechnology on 11 - 14 April 2010, Padua, Italy. An oral presentation entitled 'Optimisation of the enzymatic treatment of olive oil pomace for lignocellulosic ethanol production'. This resulted in a proceedings book. The abstract of this paper has been annexed to the present document.
- 14th International Biotechnology Symposium and Exhibition on 14 - 18 September, 2010 - Rimini, Italy. A poster presentation entitled 'Yeast viability for second generation ethanol production from olive oil wastes' was presented. This resulted also in a journal publication.

Besides the student David Cannella discussed his Bachelor thesis on Industrial and Environmental Biotechnology on 27 January 2009.

FORTH has presented:

- 9th International Conference on 'Sacharov Readings 2008: Environmental Problems of the 21th Century Minsk', Republic of Belarus, 21 - 22 May 2009 entitled 'Comparative ethanol production from solid olive mill residues after pretreatment with dilute acid and lime'. The abstract of this presentation is included in the annex.
- They have sent a paper to CEST conference (see http://www.gnest.org/cest/ online for further details) with the fermentation results entitled 'Bioethanol production from thermochemically pre-treated olive mill solid residues using the yeast pachysolen tannophylus' to be held next September.

Also is planning to present a paper entitled 'Effect of thermochemical pretreatment and enzymatic hydrolysis on the saccharification of lignocellulosic olive oil mill solid residues' on the Biochemical Engineering Journal or Waste Management journal.

1.2 Other conferences and workshops

As described above, the partners of ETOILE project participated to international conferences and workshops presenting the research work performed during the project. One of this is the EBTC workshop on 'Sustainable Transport Solutions for India - Opportunities for European Clean Technologies' Bangalore, India 26 - 27 November 2009, where the project was presented to an international group. The presentation is annexed to the present document.

The ETOILE project was also presented by LABOR at CPAC workshop at Rome on a conference entitled 'Second Generation Bio-Ethanol Production from Olive Oil Wastes' on 22 - 26 March 2010. Besides these publications, LABOR has also participated in the conference 'Zero Emission Rome 2009 - Biofuel Expo', on 1 October 2009.

The research results already published are:
(a) experimental study with commercial enzymes on olive pomace for sugars release;
(b) experimental study with K. marxianus on artificial media;
(c) experimental study with S. cerevisiae on artificial media;
(d) experimental study with T. reesei Rut-C30 on synthetic medium added with olive pomace;
(e) experimental study with membrane processes on OOMW remediation.

2. Description of publishable results

The results of the ETOILE project are extremely innovative with respect of the state of the art. This would mean the possibility to patent the results achieved for industrial applications such bio-fermentation of OOMW and olive pomace, lignocellulosic ethanol production, cellulase production from wastewater, technological solutions for water purification. Thus the project partners have decided to limit as much as possible any publication related to sensible information.

2.1 Experimental study with commercial enzymes on olive pomace for sugars release

2.1.1 Result description
The result consists in the optimisation of commercial cellulase enzymes for fermentable sugars production. Experimental results report information on olive pomace concentration, cellulase dose, reaction temperature and time curves of only enzyme treatment of the olive pomace, under different operating conditions. These data were published.

2.1.2 Possible market applications
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production. The process developed during the research could be applied to different wastewaters and by products of food and agriculture industry.

2.1.3 Stage of development
Lab-scale tests have been performed at LABOR to investigate the enzymatic treatment of the olive pomace under different operating conditions. The work was continued into include the yeast and produce ethanol. Results have been used and will be transferred to the pilot plant which will be designed and built.

2.1.4 Collaboration sought or offered
The work performed and the results achieved did not require or imply any collaboration or agreement with external companies or research institutes. All the activities were performed by the project partners by using their own resources.

2.1.5 Intellectual property rights (IPRs) granted or published
A paper regarding the enzymatic tests and characterisation sugars has been presented in an international conference. In order to protect results and guarantee the IPRs to SMEs involved in the project, any dissemination action has been and will be carefully discussed among partners in due time.

2.1.6 Contact details:
Ms Catalina Valencia Poroni, PhD
2.2 Experimental study with K. marxianus on artificial media

2.2.1 Result description
The result consists in the optimisation of yeast growth using artificial media. Experimental results report information on yeast behaviour (growth, conversion rate, process conditions, etc.) under different operating conditions using artificial media. These data with the theoretical explanation of fermentation process will be published.

2.2.2 Possible market applications
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production. The process developed during the research could be applied to different wastewaters and by products of food and agriculture industry.

2.2.3 Stage of development
Lab-scale tests have been performed at LABOR to investigate the optimal growth and production conditions of the yeast under different operating conditions and compare it to a traditional yeast. The work was continued to use olive pomace as a carbon source. Results have been used and were transferred to the pilot plant which was designed and built.

2.2.4 Collaboration sought or offered
The work performed and the results achieved did not require or imply any collaboration or agreement with external companies or Research Institutes. All the activities were performed by the project partners by using their own resources.

2.2.5 IPRs granted or published
A Bachelor thesis regarding the enzymatic tests and the yeast selection was discussed by January 2010. Also a paper was presented on the international conference IBS 2010 and on Journal of Biotechnology. In order to protect results and guarantee the IPRs to SMEs involved in the project, any dissemination action has been and will be carefully discussed among partners in due time.

2.2.6 Contact details
Ms Catalina Valencia Peroni, PhD
LABOR SRL
Via Giacomo Peroni 386
00131 Roma
Italy
2.1 Experimental study with S. cerevisiae on artificial media

2.1.1 Result description
The result consists in the optimisation of yeast growth using artificial media. Experimental results report information on yeast behaviour (growth, conversion rate, process conditions, etc.) under different operating conditions using artificial media. These data with the theoretical explanation of fermentation process will be published.

2.1.2 Possible market applications
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production. The process developed during the research could be applied to different wastewaters and by products of food and agriculture industry.

2.1.3 Stage of development
Lab-scale tests have been performed at LABOR to investigate the optimal growth and production conditions of the yeast under different operating conditions. The work was continued to use olive pomace as a carbon source. Results have been used and were transferred to the pilot plant which was designed and built.

2.1.4 Collaboration sought or offered
The work performed and the results achieved did not require or imply any collaboration or agreement with external companies or Research Institutes. All the activities were performed by the project partners by using their own resources.

2.1.5 IPRs granted or published
A Bachelor thesis regarding the enzymatic tests and the yeast selection was discussed by January 2010. Also a paper was presented on the international conference IBS 2010 and on Journal of Biotechnology. In order to protect results and guarantee the IPRs to SMEs involved in the project, any dissemination action has been and will be carefully discussed among partners in due time.

2.2 Experimental study with T. reesei Rut-C30 on synthetic medium added with olive pomace

2.2.1 Result description
The result consists in the optimisation of cellulase production using a synthetic medium supplemented with olive pomace. Experimental results report cellulase production under different operating conditions and discusses the existing opportunities for process optimisation. These data with the theoretical explanation of fermentation process will be published.

2.2.2 Possible market applications
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production.
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production. The process developed during the research could be applied to different solid by products of food and agriculture industry.

2.2.3 Stage of development
Shake-flask and small-scale pneumatically-agitated reactor fermentations have been performed during the project.

2.2.4 Collaboration sought or offered
The work performed and the results achieved did not require or imply any collaboration or agreement with external companies or research institutes. All the activities were performed by the project partners by using their own resources.

2.2.5 IPRs granted or published
Two Bachelor thesis regarding enzyme production tests were discussed in December 2010. Both authors had co-authored a (notified in due time) Congress presentation where the relevant data were disclosed.

2.2.6 Contact details
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2.1 Experimental study with membrane processes on OOMW remediation

2.1.1 Result description
The result consists in the optimisation of OOMW remediation by using physical processes including coagulation and membrane processes. Experimental results report the residual organic reduction as a function of the processing stage.

2.1.2 Possible market applications
The possible exploitation of the ETOILE technology is related to water remediation processes, cellulase production and bioethanol production. The process developed during the research could be applied to different wastewaters.

2.1.3 Stage of development
Pilot-plant OOMW treatment runs have been performed during the project.

2.1.4 Collaboration sought or offered
The work performed and the results achieved did not require or imply any collaboration or agreement with external companies or research institutes. All the activities were performed by the project partners by...
external companies or research institutes. All the activities were performed by the project partners by using their own resources.

2.1.5 IPRs granted or published
No IPR were granted or published.

List of websites: http://www.etoile-project.eu

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. AS (Turkey), exiting at month 7
- SEDNA Spa (Italy) http://www.sednagroup.it

RTD performers:
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- Foundation for Research and Technology Hellas / ICE-HT (Greece) http://www.chemeng.upatras.gr/
- University of Rome La Sapienza (Italy)

Related documents

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