POLYVER

Project number: *FP6 - 032967*

Project acronym: POLYVER

Project title: *Production of polyhydroxyalkanoates from olive oil mills wastewater*

Instrument: Cooperative Research

Thematic priority:

Publishable final activity report

Period covered: from M1 to M27	Date of preparation: 15/12/2008
Start date of the project 15/09/2006	Duration: 30 months
Project coordinator name Giorgio Recine	
Project coordinator organisation name LABOR Srl	Revision 0

SECTION 1 Project execution

Europe, in particular the Mediterranean countries, is the prime producer of olive oil, accounting for more than 95% of the worldwide production.

Olive oil benefits in human diet are nowadays well known to all the consumers but few people are aware that, despite of its beneficial effects, its production process has intrinsically a huge impact on the environment, since olive oil mills wastewater (OOMW) contains a high quantity of toxic organic and inorganic compounds. For hundreds of years the olive oils mills wastewater has been discharged into rivers and onto land causing serious damages to flora, fauna and fish; nowadays, legislation has prohibited uncontrolled discharge into soil and water, so at present oil producers dispose OOMW mainly according to 3 methods:

- chemical-physical treatments;
- evaporation in open tanks and subsequent incineration of solid residuals;
- limited release on the ground for fertilisation purposes.

1.1 Project objectives

The final objective of the POLYVER project is **to develop and implement a technology able to permit the conversion of olive oil mills wastewater from a dangerous waste to a renewable source for biopolymers production**.

In order to achieve these objectives, the research implied to involve expertise in many different scientific and technological fields such as microbiology, biochemistry, biotechnology, water purification technologies and biomaterials technology.

Two main scientific and technological objectives have been identified in the project:

1. **Formulation of a new process for olive oil mills wastewater (OOMW) treatment and production of Polyhydroxyalkanoates** (**PHA)**.

Purification of water is currently possible with different technologies. These technologies are often expensive and limit to separate the polluting compounds and give back purified water.

The POLYVER project conversely aims at developing a novel process which simultaneously reduce the polluting contents and leads to the production of a biopolymer with a commercial value. The organic compounds are, in the general idea of the project, a source of nutrients for bacteria strains able to live, grow up and produce the biopolymer in OOMW. The main technological steps to implement the process are:

- Pre-treatment of the OOMW for homogenization;
- First degradation step via biofermentation;
- Second degradation step via fermentation and biopolymer accumulation into bacteria cells;
- Biopolymer recovery;
- Post-treatments for water purification;
- Water recycling.

2. Up-scaling study of the process for on-site transfer of the results and widespread application of the POLYVER system to OOMW

A preliminary scale–up study was performed for the exploitation at the end of the project. An evaluation and estimation of the process and equipment scale-up (from demonstrative to industrial plant) on the efficiency and on process parameters was evaluated, for both pilot and industrial scale production capacities.

The POLYVER project was therefore trying to achieve the following *targets*:

- ***Preliminary bacteria strains isolation from alpechìn***

The major representative bacteria strains with widely reported PHA accumulation characteristics present in alpechìn and other residues obtained during the olive oil process such as water from olive washing step were isolated end tested for PHA production. At the end of the first year two different bacteria strains were chosen.

- ***Fermentation substrate development***

On the basis of the previous results, an OOMW based fermentation substrate was developed and then tested to grow selected strain and to obtain PHA production.

- ***Pre-treatment of OOMW***

Following, a screening (corse filtering, flocculation or other routine processes) of the solution in continuous mode the process effluent can be sent to a microfiltration batch process.. The wastewater will be treated by means of an ultrafiltration membrane process before the PHA production to facilitate biomass recovery . In fact some of the OOMW components are useless or inhibitory or toxic for the bacteria responsible of the fermentation process.

- ***Post-treatment and disposal of OOMW***

After PHA production the content of toxic or polluting components into OOMW can be quite similar to the starting one. The exiting process stream should be treated before disposal. For this reason it has been envisaged to implement and test some membrane technologies to remove these compounds. The resulting wastewater was collected in a reservoir and treated by means of a nanofiltration and reverse osmosis batch process in order to produce a purified outlet stream which respects the limits by Law to be disposed in the municipal sewage system. Membrane operations removed the COD content of the downstream water up to the values allow by the law. Alternative technologies were also considered taking into account wastewater residual characteristics but membrane processes resulted in the most promising one.

The main challenge to be tackled will be the reduction of fouling on the membranes which causes both a smaller productivity and a shortened life of the membranes thus reducing drastically the industrial application of such technology. Membrane processes have been increasingly used for a large number of applications in the wastewater treatment. Scientific studies a focused on wastewater management have demonstrated the possibility to drastically reduce these components up to the limits by Law for wastewater disposal.

- ***Plate scale experimentation and growth optimisation***

The main physical and chemical growth factor such as pH, aeration, temperature and nutrients were varied in order to determine the optimum conditions for growth and PHA accumulation. This work was performed mainly during the first year of the project after while the process operation conditions were establish. At this point the design of the pilot scale reactor started aimed to find the scale up characteristics of the process.

- ***Pilot scale bioreactor design and construction***

As said before, the design of the POLYVER bioreactor was preceded by the lab tests on micro-organisms. For the design were taken into account the critical variables, i.e. volume, dilution, aeration, agitation, etc. From lab scale was found also that a preliminary anaerobic fermentation was needed. The pilot plant development included also the implementation process control system for complete automation of the all the operations of the both reactors.

- ***Pilot-scale fermentative process experimentation and optimisation***

The fermentative process was tested and optimised in progress on the basis of the evaluated results.

The results was deeply evaluated by:

- *Polymer recovery and extraction*

  Several extraction methodologies were tested in order to decrease the environmental load of polymer production.

- *Polymer characterisation*

  Physical/chemical tests such as gas chromatography, mass spectroscopy and nuclear magnetic resonance tests to estimate the quality and quantity of the obtained polymer

- *Water quality assessment*

  Pollutants present in water before and after fermentation treatment were both assessed and compared.

1.2 Contractors involved

The POLYVER project is implemented by a Consortium of **7 SMEs** operating in **5 European countries** (Italy, Greece, Spain, Germany and Czech Republic), and **4 RTD performers** (Italy, Greece and Spain). LABOR played the role of project Co-ordinator.

The partners identified own suitable competencies and skills to fulfil project objectives and were able to manage the development of project steps, including advanced phases of improvement, validation of the system and final products and adaptation to potential further applications. The trans-national character of the Consortium helped the diffusion of the developed technology in Europe and favour the start-up of a technology transfer process, increasing the benefits for the proposers

Participant role	Participant type	Participant No.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	RTD	**1**	Labor srl	**LABOR**	IT	Month 1	Month 27
CR	SMEP	**2**	Argus Unweltbiotechnologie GmbH	**ARGUS**	DE	Month 1	Month 27
CR	SMEP	**3**	Antico Frantoio Toscano Srl	**AFT**	IT	Month 2	Month 27
CR	SMEP	**4**	Sociedad Cooperativa Agricola Olivarera “Nuestra Senora de Desamparados”	**CAONSD**	ES	Month 1	Month 27
CR	SMEP	**5**	Biolea G. Dimitris & Co.	**BIOLEA**	HE	Month 1	Month 27
CR	SMEP	**7**	Dekonta a.s.	**DEKONTA**	CZ	Month 1	Month 27
CR	SMEP	**8**	Idroplax Srl	**IDROPLAX**	IT	Month 1	Month 24
CR	RTD	**9**	Universidad de Granada – Institute of water research	**UGR**	ES	Month1	Month 27
CR	RTD	**10**	Foundation for Research and Technology Hellas	**FORTH / ICE-HT**	HE	Month1	Month 27
CR	RTD	**11**	University of Pisa	**UNIPI**	IT	Month1	Month 27

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Project’s web site: http://www.polyver-project.eu/

1.3 Work performed

The work performed in the first year was carried out in the following Workpackages:

- ***WP1: Definition of the requirements and specifications***
  - The definition of the requirements and technical specifications has been performed taking into account three main aspects:
    1. OOMW chemical properties, mainly COD content, related to the olives characteristics and milling process;
    2. Characteristics of the biofermentation process on the basis of potential bacteria strains;
    3. Process operations and recovery of the biopolymer in order to reduce the environmental impact.
  - Process operating conditions were analysed in order to outline the critical parameters to be taken into account. SMEs provided their contributes regarding the production processes and technologies available for wastewater characterisation and treatments.
  - Chemical and physical properties of the biopolymer have been outlined with respect of the possible industrial applications.
  - A preliminary market analysis have been performed. This phase was essential to clearly identify the potential market of the POLYVER process and biopolymer and pave the way for the research activities. The market analysis allowed partners to preliminarily outline the economical impact of the POLYVER technology improving their competencies and providing new products for the world market (fermentation process, water purification and biopolymers)

On the basis of the results achieved, WP1 was concluded successfully.

- ***WP2: Plate scale PHA production***
  - The selection of bacteria and definition of the fermentation process has been recognised as the most important activity of the first year, the general process conditions a major representative bacteria strains. The selection of the microorganisms was based on technical specifications resulting from WP1 and on specific factors coming from WP2 investigations including the capacity to use several cheap substrates for the PHA production, growth rate, polymer synthesis rate and the maximum polymer accumulation. Among the 300 bacterial strains fitting these properties only few microorganisms were evaluated and selected for the production of PHA and/or PHB:
  - For the definition of bacteria strains able to produce PHA from olive oil mills wastewater (OOMW), the following activities have been performed:
    1. Testing the viability of the isolated and lyophilized bacterial strains
    2. Evaluation of the capacity for PHA forming by these strains under optimal growth conditions and substrates: i.e. glucose as carbon source

3 Evaluation the growth and PHAs production (as g/l) of the bacterial strains under several volatile fatty acids (VFAs) substrates
4 Evaluation of the PHAs produced (homo- or hetero-polymers)
5 Use of the VFAs yielded in an anaerobic process designed for different wastes for testing the PHA formation using the isolated strains

- By adopting the technological solutions foresee, the POLYVER process for PHA production has been modified from a one step process into a multi step process.
- Recovery of PHA was considered in relation to its affects on the post-processing and the economical aspect of a PHA production.

- ***WP3: Pilot scale plant development and process optimisation***
  - Based on the microbiological results obtained in WP2 the pilot plant, including the control system, for the POLYVER process was defined and constructed. The definition of the POLYVER process required to take into account different issues:
    1. Pre-treatments;
    2. Fermentation process for bacteria growth and PHA production;
    3. Wastewater post-treatment;
    4. Recovery of the biopolymer.
  - Pre and post-treatments, so as the recovery of the biopolymer, were preliminarily drafted during the first year and completely defined after the pilot scale tests.
  - The work performed demonstrated the possibility to produce PHA and/or PHB from olive oils wastewater via microbial fermentation but still with a low yield. Biopolymer production was possible in a two phases process:
    a) under anaerobic conditions sufficient amounts of VFAs were reached in short period of time by the indigenous microbiota. In parallel, moderate amounts of total water soluble sugars were released into media.
    b) under aerobic conditions, the sugar content was sufficient to support the growth of the selected microorganisms inoculated in the fermented waste. Both strains grew up using as a carbon source the VFAs generated during the anaerobic fermentation. However after pilot plant tests, it was found that anaerobic step is only needed when fresh OMWW is used, and it not necessary for a 3 phase Olive Oil production process wastewater.
  - Realisation of the pilot plant was performed according to decisions among partners concerning layout and equipment.
  - Experiments showed that during the aerobic phase the carbon source is used for PHA production and not for cells growth. In addition it was found that from the anaerobic phase and the following aerobic process there is not need for sterilisation of the media.
  - From industrial point of view it was concluded that one strain grows faster but more sterile conditions are needed. It present the advantage that air flow is not required in the fermentation meaning that less energy is consumed.
  - At pilot plant scale from the engineering point of view, was simpler to maintain the second strain culture, that resulted in higher net quantity of PHA.

- PHA separation and extraction process should be further develop. On the tests performed $A_{l2}(SO4)_3$ coagulation and/or microfiltration were used prior aerobic fermentation with positive results in biomass physical recovery.
- Once biomass is produced easiest and cheaper way of separation is sedimentation. For this purpose aluminium sulphate addition is used for one strain. On the contrary the other strain forms flocs that sedimentate after 30 min.
- For process industrialization, bioreactor cycling and operation modes should be defined. On the tests performed N-C cycling guaranteed sterile operation of one strain. Although some biofilm is produced.

- ***WP4: Life Cycle Assessment***
  - LCA methodology was defined on the basis of a bibliographic search performed by UNIPI and data available.
  - A questionnaire for LCA data collection from olive oil mills was prepared and distributed.
  - Results of the pilot plant experiments and of the PHA analysis produced were used as input for the POLYVER LCA.
  - Taking gross energy use (GEU) and global warming potential (GWP) as relevant parameters, PHAs production by POLYVER technology was compared with the values found in literature for PHAs produced with other methodologies and for conventional petrochemical plastics such as PE, PP and PS.
  - In particular, it was found that PHAs produced with POLYVER technology have an environmental load dramatically superior to both PHAs produced with other methodologies and to PE, PP and PS. This result was mainly attributed to the low amount of polymer recovered (11.2 grams for 30 lit. Reactor).
  - The main responsible of the final burdens are: i) from the 2 phases (fermentation and extraction) point of view the extraction phase accounts for ca. 75% and ii) from the inputs point of view the electricity production accounts for ca. 85%.
  - However, OMW COD reduction after fermentation (ca. 75%) is largely comparable with the values reported in literature for the other conventional techniques.
  - The extraction methodology should be further refined in order to obtain competitive yields and reduce the electricity and solvents environmental costs of the POLYVER technology.

- ***WP5: Technology assessment***

  Polymer characterisation
  - Activity related to WP5 were contemporaneously to the pilot plant tests. The work was focused on characterisation of PHA and PHB produced taking into account process yield and biopolymer quality.
  - *PHAs Produced by FORTH isolated strain*: From TGA analysis it was found that all samples polymer are most likely composed by ca. 90% of P(3HB) copolymerized with other PHA units. This hypothesis seems to be confirmed, from the double melting peak

detected by DSC in one pilot scale sample. However, the molecular weight is lower compared with PHAs produced at lower scale .

- *PHAs Produced by UGR isolated strain pilot scale*: Among these samples, one showed a relatively high value of molecular weight (more than 1000 KDa). From TGA, this also was the sample containing less impurities (ca. 7 wt-%). The residual weight of other samples measured at 500°C ranged between 72 and 91 wt-%.
- After the definition of the most suitable method for biopolymer recovery the performances concerning water purification were taken into account. The tests outlined the need of post-treatment, mainly using membranes, to remove completely the polluting compounds and cut down the COD content before water disposal or re-use. Membrane process in sequence, nanofiltration (NF) and reverse osmosis (RO) were studied for water treatment. A portion of the purified water is then used for the dilution step of the PHA production process.

Water quality assessment

- By using spiral wounded membranes it is possible to recover 90% of purified water at a COD value lower than 500 mg/l, as required by the European law for the sewage discharge.
- For better performances, the SC based system appears to be more reliable. In this case, an increase of 12% of the RO membrane area and an increase of 62% of the RO operating pressure are necessary to compensate the flux losses, but again 90% of purified water at a COD value reduced by another 28,5% is produced, meeting the municipal sewer system quality grade target.

Industrial scale feasibility study

- Industrial feasibility of the POLYVER process was also delineated. The proposed industrial POLYVER process consists of a series of operations working in batch mode. The main operation is the bioreactor, which by a two stage process leads to the production of the biopolymer inside the bacteria cell mass.
- The capacity of the plant was fixed equal to 45 m3 of OVWW to be treated. This choice was done with the reference to a medium olive mill factory located in the Mediterranean area working around 2000 t of olive during a 4 months yearly campaign.
- From the industrial feasibility analysis it could be said that the total costs of PHA production and treatment of 1 m3 of OMWW are smaller than the costs usually considered for its disposal, that is 35 €/m3. However for being a feasible industrial process that justify the bioreactor investment, the PHA production yield should be greater. It could be said that the minimum PHA production yield to cover the plant costs should be at least 11 g/l if the polymer is sold at 3€/kg. This yield is technically feasible if more research is performed in PHA extraction and purification.

WP6: ***Exploitation and dissemination***

- Dissemination activities were partially started in the first year
- Three international papers and one master thesis were presented during the project duration.
- Project information and publishable results are available through the web site
- Patent feasibility analysis has been performed.
- Plan for using and disseminating knowledge has been also prepared

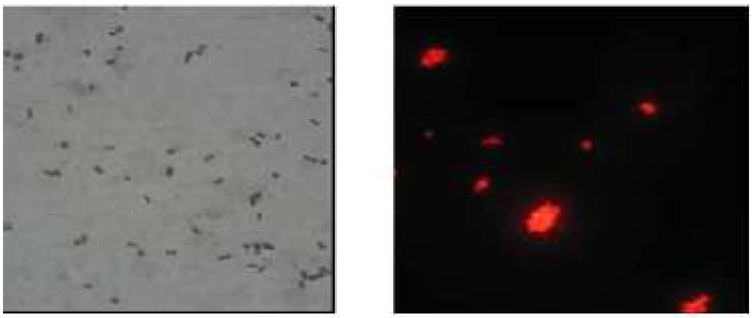
1.4 Final Results

Market analysis on biopolymers

Olive oil production presents specific features depending on the region and producer. Main differences are due to climate but also the production process strongly affect the quality of the final product and the pollutants content of wastes. This situation strongly affected the definition of the fermentation process. The characteristics of the biopolymer were outlined. The work carried out allowed also partners to prepare a market analysis concerning biopolymers and bioplastics future scenario (cost, market share, etc.).

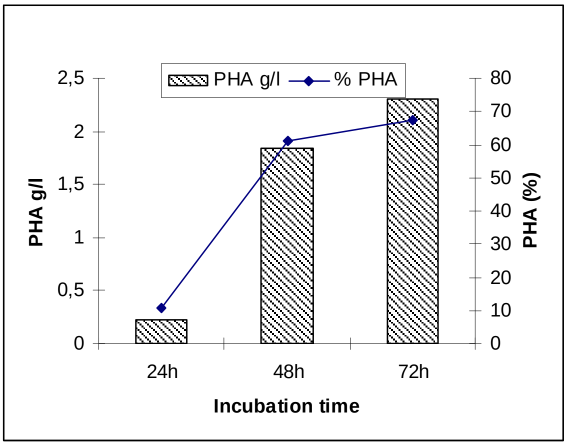
Bacteria identification and Isolation

The major representative bacteria strains, showing the highest PHA accumulation potential were selected obtained from olive mill wastes like alpeorujo and alpechin or by enrichment of mixed cultures derived by active sludge from a wastewater treatment plant.



Bacteria with PHA accumulation capacity

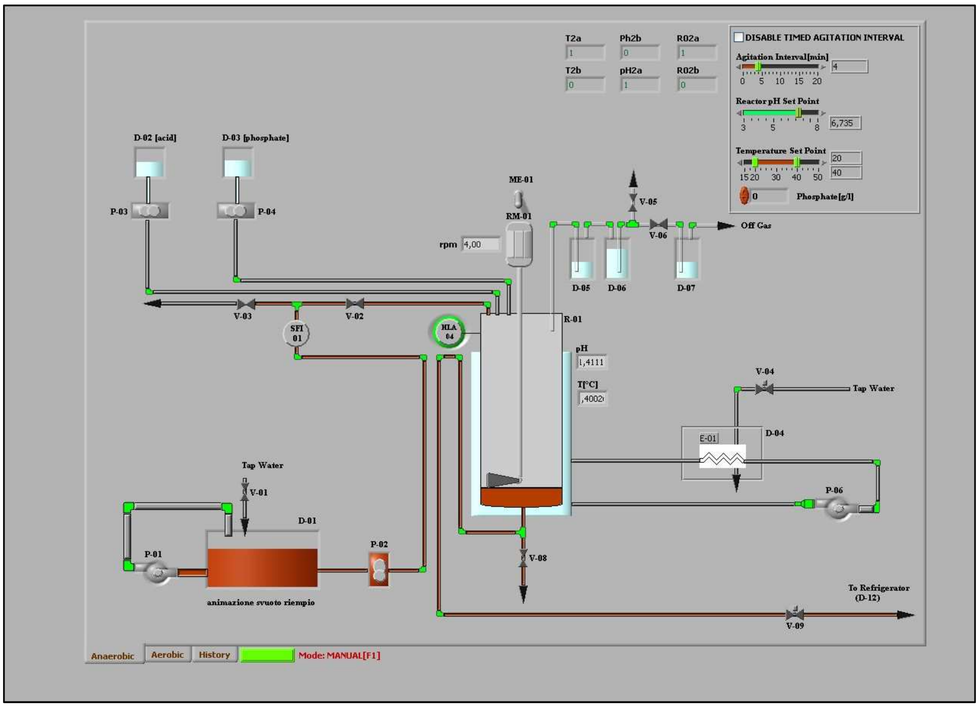
The selected bacteria showed high PHA accumulation potential at lab scale

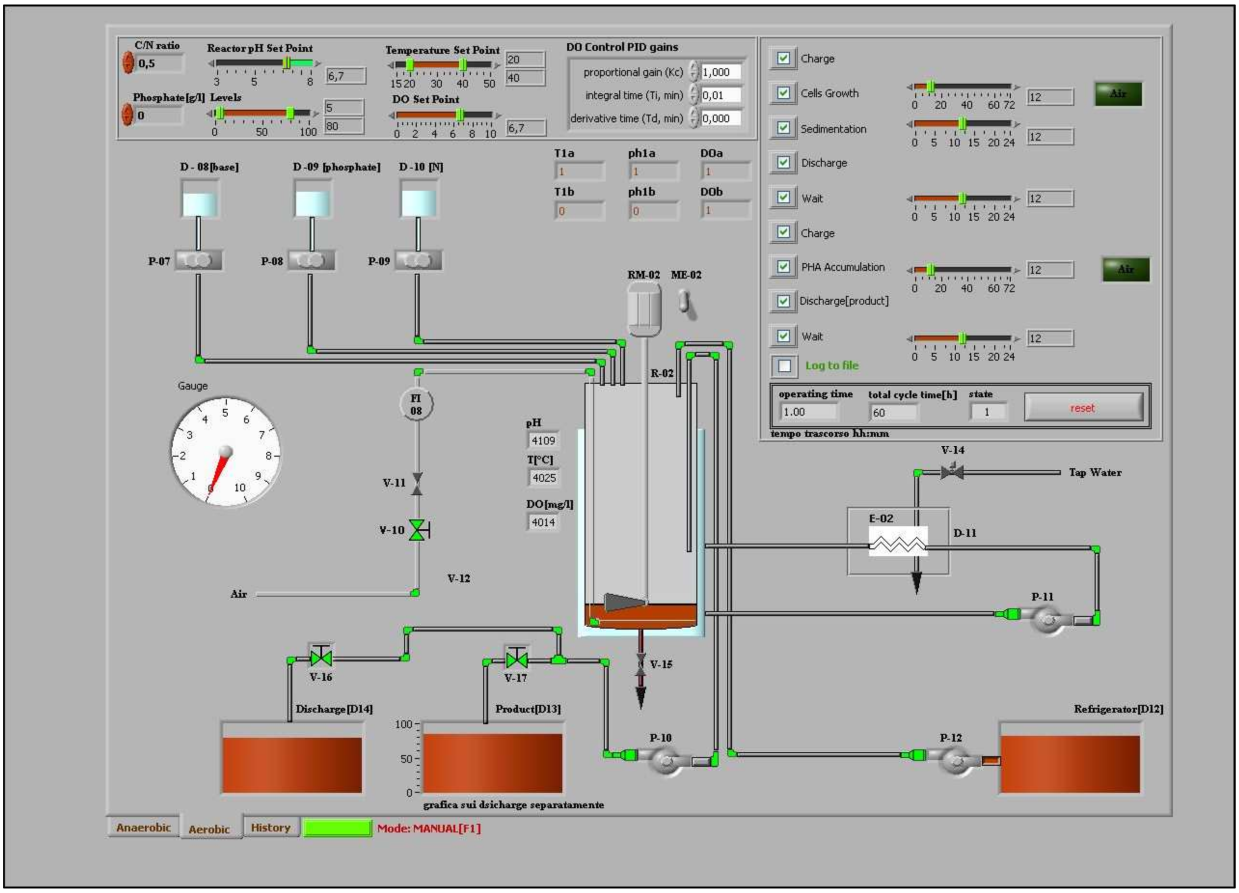


Pilot plant design and construction

A pilot plant was designed accordingly to the results of the lab scale experimentation. A 30 l automatic production plant was constructed.

Automation of both bioreactor cycles was also achieved though personalized user interface.





Pilot scale VFA production

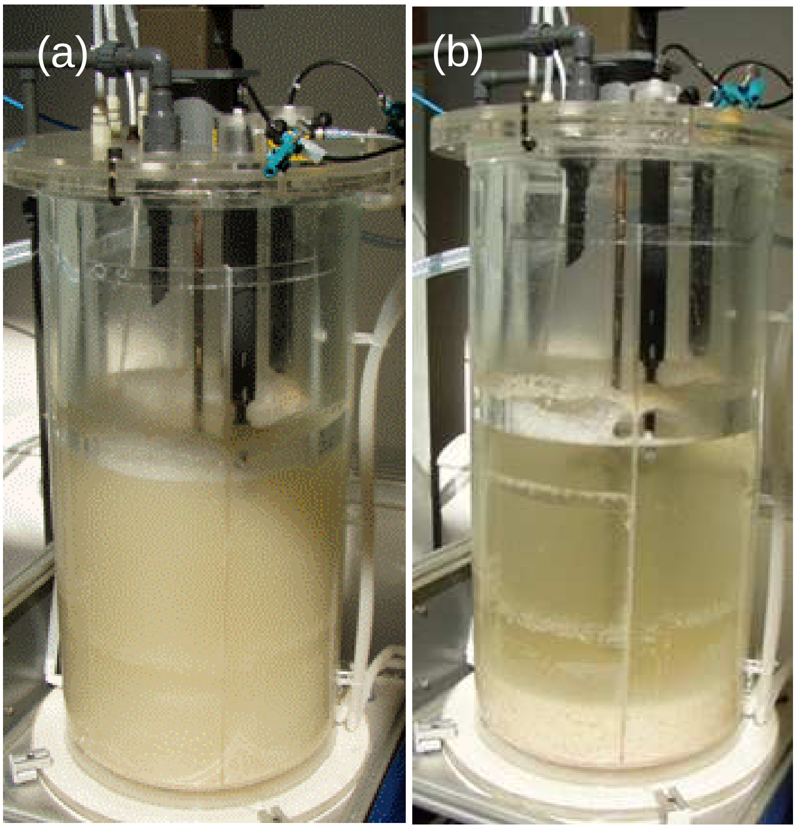
OMWW was used for anaerobic fermentation and Volatile Fatty Acids production. 30 days steady state operation was reached.

The following table presents the VFA results obtained in the pilot plant:

(mg/L)	Acetate	Buryrate	Propionate	Others	TOTAL
Reactor	751.7 ± 109.2	903.6 ± 134.1	94.7 ± 14.6	44.4 ± 3.9	1794.5 ± 154.4
Feed	636.3 ± 100.3	158.7 ± 11.1	66.9 ± 25.3	28.8 ± 6.7	907.7 ± 89.4
Produced	121.0 ± 33.8	751.0 ± 96.1	30.3 ± 1.5	22.6 ± 3.2	**917.0 ± 131.3**

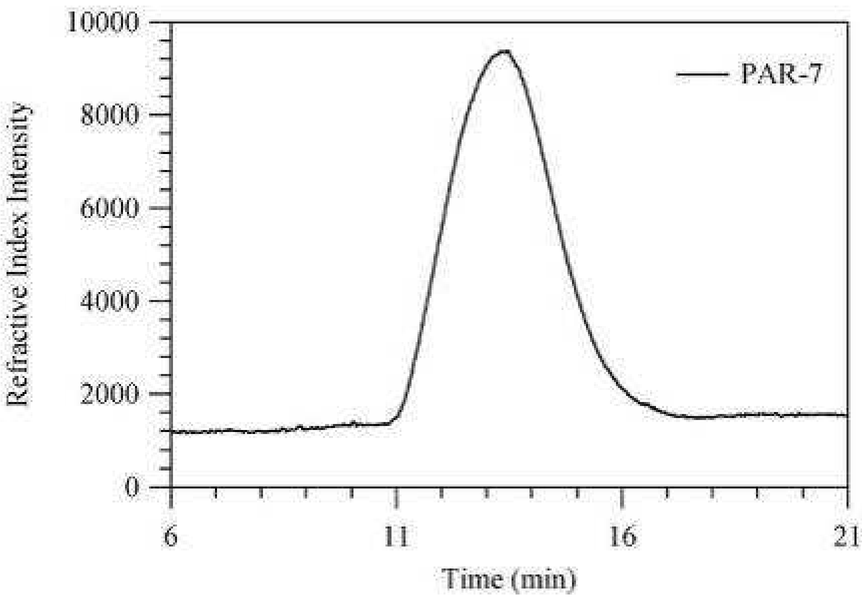
Pilot scale PHA production

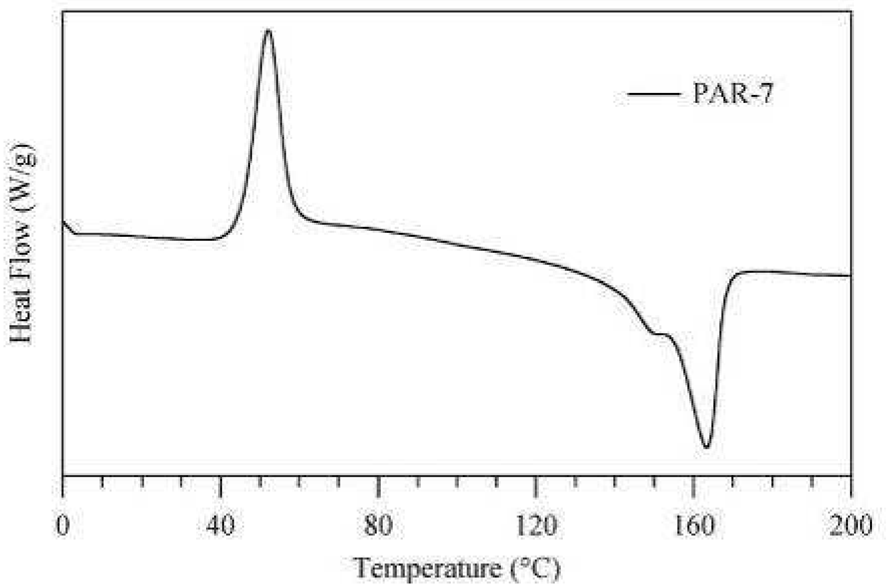
Both selected strains were scale up to 30 l. Several batches were obtained during the last part of the project. Initially synthetic media was used, then OMWW digested or not was employed as carbon source biopolymer production.



Operation of the PHAs producing reactor at full working volume loading (30L) with synthetic feed (a) during accumulation phase, (b) during sedimentation phase of the biomass.

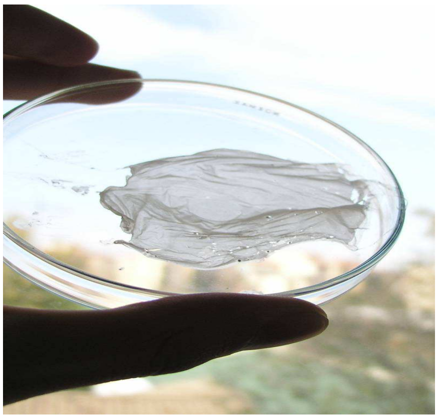
The obtained polymer presents a weight average molecular weight (Mw) of around 490 KDa with a polydispersity (Mw/Mn) of 5.15. The copolymeric nature of the sample, as suggested by NMR analysis was confirmed by the DCS analysis obtaining typical values of 3(HB) copolymerized with 3-hydroxyvalerate 3(HV) units





PHA recovery method

Different methodologies for PHA extraction and purification were tested. Lab scale purification was reached successfully, however more research is needed in this area aimed lower the environmental impact of the overall process.



Lab scale sample of the purified biopolymer


(a) (b)

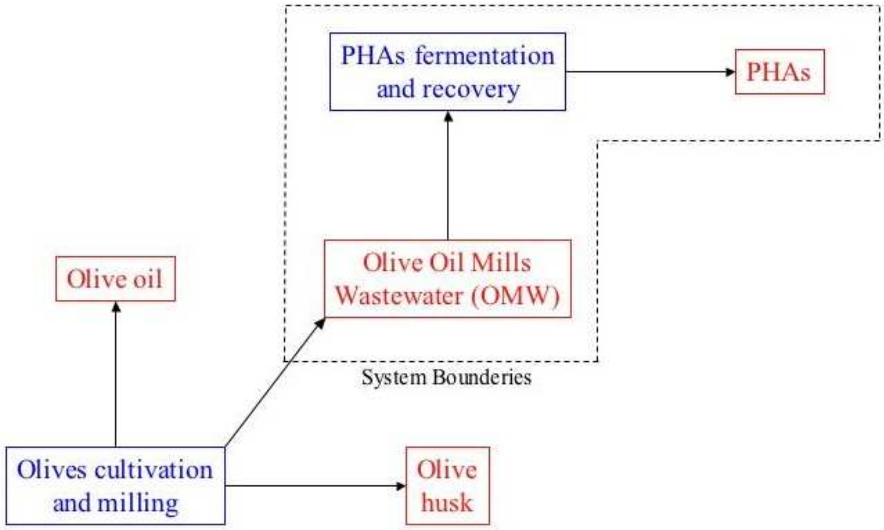
Pilot scale biomass before (a) and after (b) the treatment at LABOR premises


(a) (b)

Example of Non Purified (a) and Purified (b) Biomass obtained at pilot scale

Life cycle assessment

System boundaries for the POLYVER technology were defined. The LCA takes gross energy use (GEU) and global warming potential (GWP) as relevant parameters.



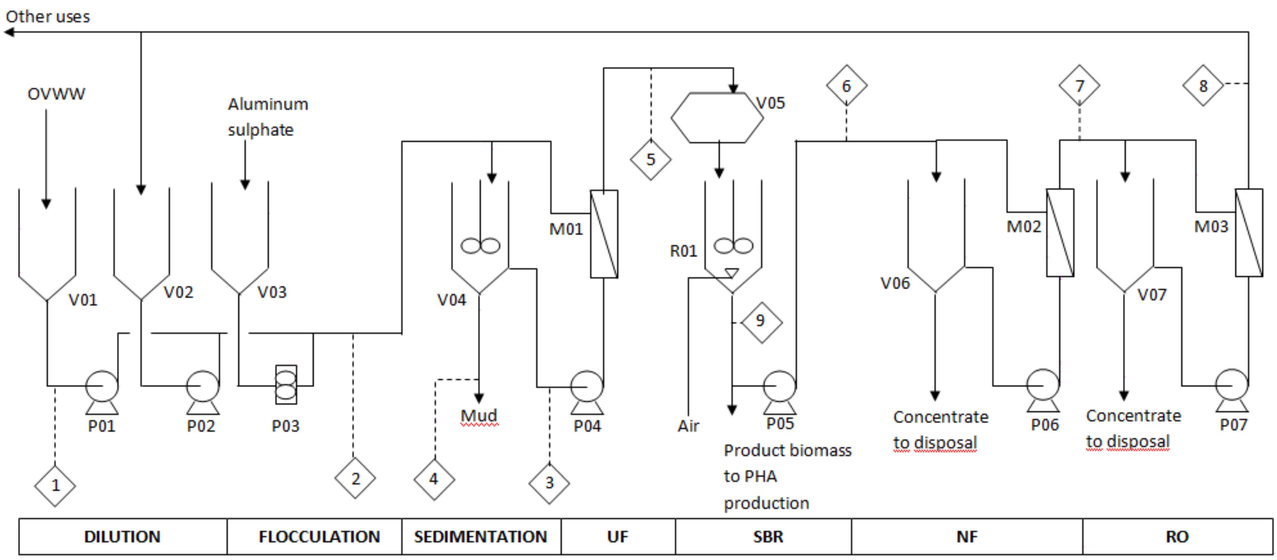
GEU and GWP associated to the production of 1 kg of PHAs according to POLYVER technology.

		Input	GEU MJ/kg PHA	GWP kg CO2 eq./kg PHA
Fermentation	Nitrogen Growth Phase	Tap water	6.8	0.4
		Inoculum	3.4	0.2
		Electricity	30804.7	1887.7
	Sedimentation	Electricity	2.3	0.1
	Carbon Growth Phase	Electricity	15403.5	943.9
	Biomass Recovery	Tap water	3.4	0.2
		Electricity	5.1	0.3
OMW Pre-treatment	Dosing	Tap water	6.8	0.4
		Electricity	18.0	1.1
PHAs Recovery	Heat and centrifuge	Electricity	271.1	16.6
SUBTOTAL FOR FERMENTATION		46525.0	2850.9	
PHAs Pretreatment	Liofilization	Electricity	54229.7	3323.2
PHAs Purification	Stirring	Electricity	32537.8	1993.9
		Acetone	14801.9	489.8
PHAs Extraction	Kumagawa	Electricity	15817.0	969.3
		Chloroform	10434.1	919.4
	Concentration	Electricity	1536.5	94.2
	Precipitation	Electricity	1355.7	83.1
		Diethyl ether	3476.4	169.2
SUBTOTAL FOR EXTRACTION		134189.2	8042.1	
OMW Spreading		Diesel production	2.54	0.03
		Diesel combustion	—	0.16
SUBTOTAL FOR OMW SPREADING		2.5	0.2	
TOTAL FOR THE PRODUCTION OF 1 KG OF PHAS		180712	10893	

These data are dramatically high if compared with values reported in literature for PHAs production. It is shown clearly that the main part of the environmental load is due to the extraction step, which accounts for ca. 75% for both GEU and GWP. In particular, electricity generation for liofilization is the most critical phase (ca. 30% of the total share), followed by the stirring step (ca. 18%). The extraction methodology should be further refined in order to obtain competitive yields and reduce the electricity and solvents environmental costs of the POLYVER technology.

Industrial production feasibility analysis

Based on pilot scale results a 45 m3 industrial scale plant for PHA production was sketched.



Economical analysis for the industrial plant was also performed.

Costs and income referred to 1 m3 of OMWW

	Costs per m^3 of OVWW
EXPENSES	
Depreciation (20 years at 6% annual interest rate)	€ 20,97
Operational costs	€ 4,96
Personnel	€ 4,44
INCOME	
PHA Income (3 €/kg)	€ 1,6
TOTAL	**€ 28,78**

It was found that the total costs of PHA production and treatment of 1 m3 of OMWW are smaller than the costs usually considered for its disposal, that is 35 €/m3. However for being a feasible industrial process that justify the bioreactor investment, the PHA production yield should be greater. It could be said that the minimum PHA production yield to cover the plant costs should be at least 11 g/l if the polymer is sold at 3€/kg. This yield is technically feasible if more research is performed in PHA extraction and purification.

Water quality assessment

Pilot scale tests allowed to perform the water quality assessment of the POLYVER technology. OMWW pre-treatment consists in:

- Coarse gridding
- Dilution with tap water at a ratio 1:4
- UF filtering

Effluent of the aerobic fermentation was analyzed and further investigation for its post treatment and disposal was performed. Results are presented in the following table. The target of the waste water treatment was a value of COD lower than 500 mg/l in order to permit the legal discharge of the wastewater into the municipal sewer system.

Parameter	Value
COD OMWW (before dilution)	79000 mg/l
COD Effluent	23504 mg/l

Comparison between the obtained and bibliography results of the best membranes system SYS2 is presented in the following table.

<table>
<tr><th rowspan="2"></th><th colspan="2">SYS2</th><th colspan="2">BIBLIOGRAPHY</th></tr>
<tr><th>COD [mg/l]</th><th>COD reduction [%]</th><th>COD [mg/l]</th><th>COD reduction [%]</th></tr>
<tr><td>Feedstock</td><td>79000</td><td></td><td>55000</td><td></td></tr>
<tr><td>After coagulation</td><td rowspan="2">23504</td><td rowspan="2">70,2</td><td>26015</td><td>52,7</td></tr>
<tr><td>UF Permeate</td><td>20344</td><td>21,8</td></tr>
<tr><td>NF Permeate</td><td>9986</td><td>57,5</td><td>8463</td><td>58,4</td></tr>
<tr><td>RO Permeate with type SC</td><td>498</td><td>95,0</td><td>423</td><td>95,0</td></tr>
</table>

SECTION 2 Dissemination and use

The main publishable results and their possible exploitation are described in this section.

2.1 *Overview table*

Publishable Knowledge (description)	**Publishable product(s) or measure(s)**	**Sector(s) of application**	**Time for commercial use**	**Patents or other IPR protection**	**Owner & Other Partners involved**
Experimental study with *Pseudomonas* on artificial media and OOMW	Bacteria strains for wastewater treatments: purification and production of biopolymers	Biotech processes and wastewater valorisation	2009	-	FORTH
Experimental study with Azotobacter on artificial media	Bacteria strains for wastewater treatments: purification and production of biopolymers	Biotech processes and wastewater valorisation	2009	-	UGR
PHA characterization via bio-fermentation of OOMW	Bacteria strains for production of biopolymers using wastewaters and by-products	Biotech processes and wastewater valorisation	2009 / 2010	-	UNIPI / IDROPLAX
Post-treatment with membranes	Membrane processes for water purification	Wastewater purification	2008 / 2009	-	ARGUS, LABOR and FORTH

2.2 *Description of publishable results*

The results of the POLYVER 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, VFA production, PHA/PHB 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.

2.2.1 Experimental study with *Pseudomonas* on artificial media

2.2.1.1 Result description

The result consists in the characterization of the Pseudonomas strains for production of VFA and then PHA from OOMW under aerobic/anaerobic conditions. Experimental results report information on bacteria behaviour (growth, conversion rate, process conditions, etc.) under different operating conditions using artificial media. These data with the theoretical explanation of fermentation process were published.

2.2.1.2 Possible market applications

The possible exploitation of the POLYVER technology is related to water remediation processes and biopolymer production. The process developed during the research could be applied to different wastewaters and by products of Food Industry.

2.2.1.3 Stage of development

A lab scale prototype has been developed at FORTH to perform several tests and investigate fermentation process under different operating conditions. The work is continuing to model the bacteria growth process. The biopolymer produced has been already characterized.

Results have been used and transferred to the POLYVER pilot plant which has been designed and built.

2.2.1.4 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.

2.2.1.5 Intellectual property rights granted or published

Papers and posters regarding the fermentation tests and characterization of the bacteria activity has been presented in an international conference. Other paper on pilot plant results with *Pseudomonas* strains is expected to be publish. 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.

2.2.1.6 Contact details

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2.2.2 Experimental study with Azotobacter on artificial media and OOMW

2.2.2.1 Result description

The result consists in the characterization of the Azotobacter for production PHA from OOMW under aerobic/anaerobic conditions. Experimental results report information on bacteria behaviour (growth, conversion rate, process conditions, etc.) under different operating conditions using artificial media. Part of the data with the theoretical explanation of fermentation process has been published. More complete information are present in the master thesis of the UGR Ph.D student.
Significant data produced using real OOMW from partners will be published only after a careful analysis and patent filing if possible.

2.2.2.2 Possible market applications

The possible exploitation of the POLYVER technology is related to water remediation processes and biopolymer production. The process developed during the research could be applied to different wastewaters and by products of Food Industry.

2.2.2.3 Stage of development

A lab scale bioreactor has been performed at UGR to perform several tests and investigate fermentation process under different operating conditions. The work is continuing to model the bacteria growth process and above all characterize the biopolymer produced and evaluate water purification.
Results have been used and transferred to the POLYVER pilot plant.

2.2.2.4 Collaboration sought or offered

Currently the work performed and the results achieved did not require or imply any collaboration or agreement with external companies or Research Institutes.

2.2.2.5 Intellectual property rights granted or published

A poster regarding the fermentation tests and characterization of the bacteria activity has been presented in an international conference. Also a master thesis has been issued as described above.

2.2.2.6 Contact details

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2.2.3 PHA production via bio-fermentation of OOMW

2.2.3.1 Result description

The result consists in the characterization of the PHA produced from OOMW. Experimental results report information on biopolymer chemical-physical properties and a preliminary evaluation of production process to improve the polymer properties. These data with the theoretical explanation of fermentation process have been partially presented in a poster at Biofoams 2007. Further details on the activity carried out and results will be published in the second year keeping sensitive data secret in order to allow the patent filing if possible.

2.2.3.2 Possible market applications

The possible exploitation of the POLYVER technology is related to biopolymer production. The process developed during the research could be applied to different wastewaters and by products.

2.2.3.3 Stage of development

The work performed so far allowed to characterize the biopolymer produced at lab scale. Main properties were indentified and the bio-fermentation process revised accordingly to improve yield and quality of the final product. This activity required additional effort in the second year.
Results and mainly the technological assessment arisen was used in the economical feasibility of an industrial plant.

2.2.3.4 Collaboration sought or offered

The work performed and the results achieved did not required or implied any collaboration or agreement with external companies or Research Institutes.

2.2.3.5 Intellectual property rights granted or published

A poster regarding PHA and bio-treatments of wastewaters has been presented in an international conference, Biofoams 2007.

2.2.3.6 Contact details

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2.2.4 Post-treatments with membranes

2.2.4.1 Result description

The final results will be the definition of membrane processes applied to agrofood wastewater.

2.2.4.2 Possible market applications

The POLYVER membrane processes could be applied to any industrial wastewater. By the way the technology and process conditions need to be investigated and defined case by case.

2.2.4.3 Stage of development

A preliminary experimentation has been performed by FORTH. The work will be continued according to what decide among partners in the mid-term meeting. Membranes process were applied to the POLYVER process and pilot plant for wastewater pretreatment and water purification. LABOR will cooperate actively in this activity.

Results on membranes and overall purification process efficiency could be extremely important for future industrial exploitation of the POLYVERY technology and plant. Any result will be published only after the patent filing will be evaluated and in case pursued.

2.2.4.4 Collaboration sought or offered

Currently the work performed and the results achieved did not require or imply any collaboration or agreement with external companies or Research Institutes.

2.2.4.5 Intellectual property rights granted or published

So far partners did neither publish any information regarding the membranes processes nor granted any intellectual property right to third parties.

2.2.4.6 Contact details

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