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Phenolic wastes valorization through bioenergy and bioactive compounds production

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PHENOLIC WASTES VALORIZATION THROUGH BIOENERGY AND BIOACTIVE COMPOUNDS PRODUCTION

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Wastewater and Biosolids Treatment and Reuse: Bridging Modeling and Experimental Studies.
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climate change & sustainable development

Keywords

energy

2030

World's energy needs: 50% higher than current

International Energy Agency-IEA

Global emissions of CO_2 (sector of energy): may rise 27 %

[Santos, F. D. e Miranda, P. *Alterações climáticas em Portugal cenários, impactos e medidas de adaptação.. Projecto SIAM II*, Gradiva, 2006]

wastes

The agricultural and industrial processing activities produce large amounts of waste that are only partially valorized at different value-added levels (spread on land, animal feed, composting), whereas the main volumes are managed as waste of environmental worry

Our interest is mainly focused on **liquid wastes** generated by agricultural and industrial processes.
Most of them have in common the high content of phenolic compounds.

1. Olive mill wastewaters (OMW)

2. Cork boiling wastewaters (CBW)

3. Chestnut wastewaters (CW)

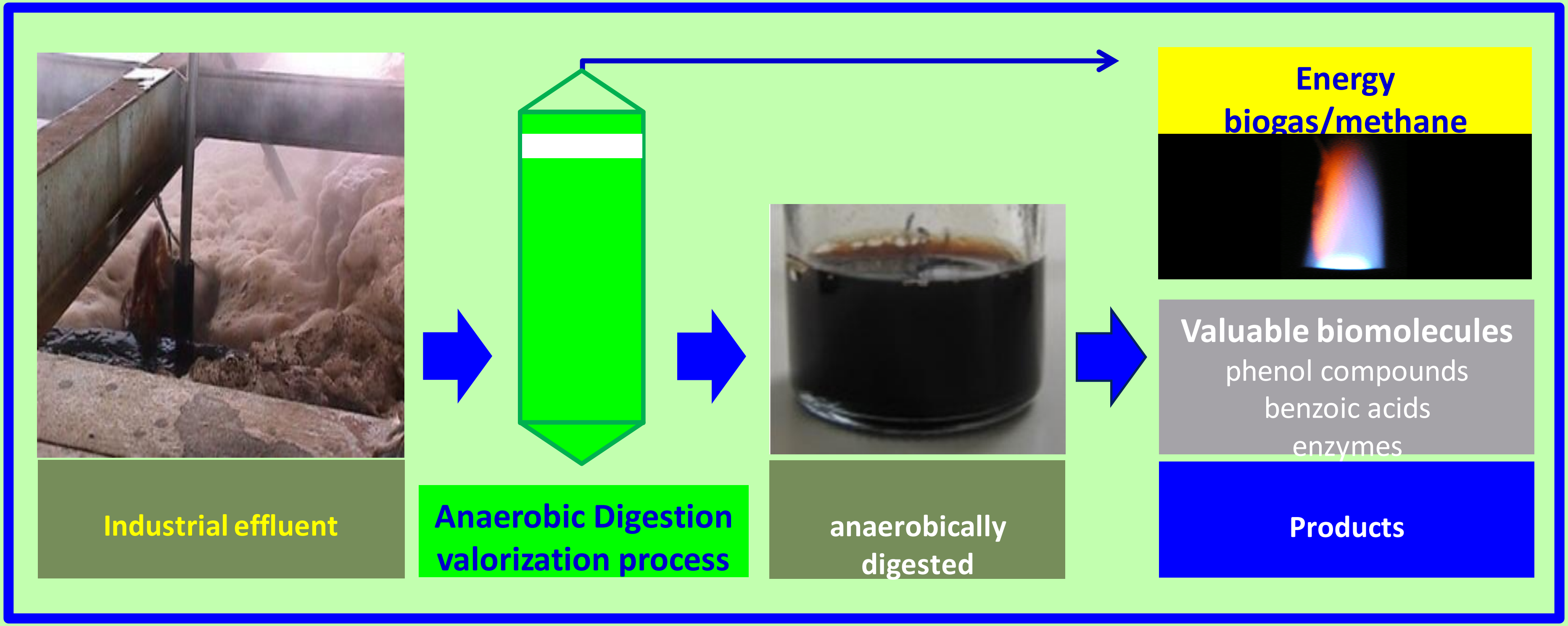
The western Mediterranean region is of high importance for typical cultivations leading to the production of food and other goods

Italy and Portugal are among the world-leading producers and exporters

http://www.lneg.pt

http://www.ibbr.cnr.it

Anaerobic digestion is reported as one of the most promising technologies for the disposal of LIQUID WASTES



AIM OF THE WORK

Maximize the LIQUID WASTES valorization through

1st Energetic valorization: Anaerobic Digestion (AD) - Biogas

2st Value-added chemicals before and after Anaerobic process

antioxidant molecules, enzymes, etc.

Olive oil production process

Olive mill wastewaters - OMW

olives olive oil OMW

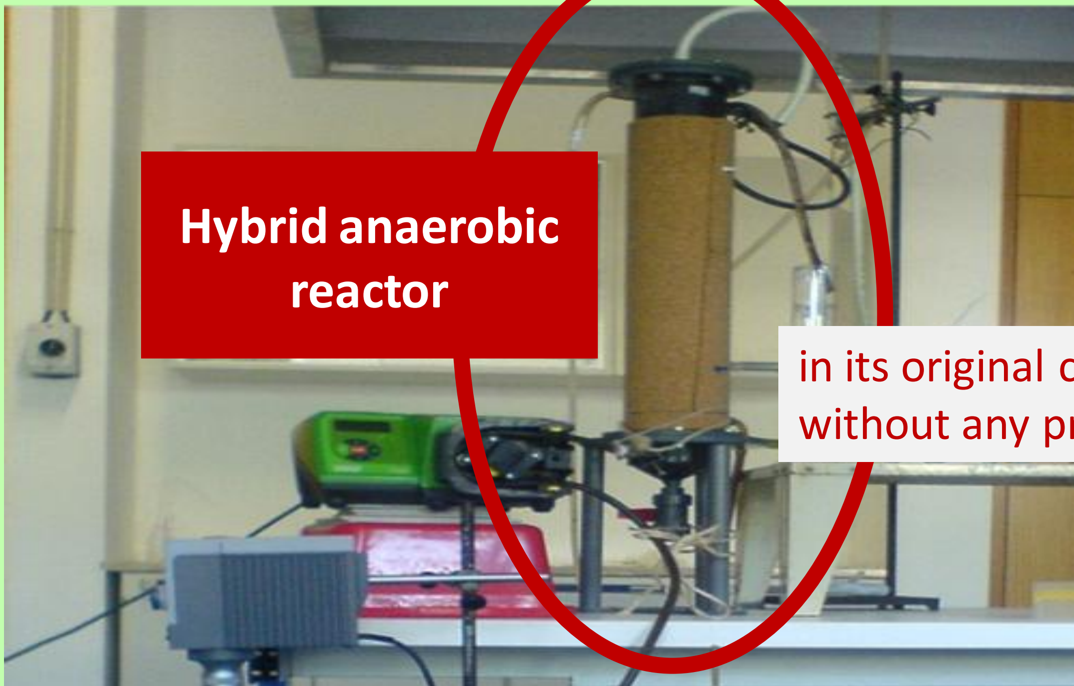
1L ----------- 5 L

The OMW present:

Acid pH (3-6),
High organics content (COD: 40-220 g L^{-1})
High electrical conductivity,
High content of ohenolic compounds (0.5–24 g L^{-1}),
Reducing sugars up to 60% of the dry substance,
potassium as the predominant inorganic material (~4 g L^{-1})

Olive mill wastewaters - OMW

AD- Anaerobic Digestion. Biogas



Load (kg COD m^{-3} d^{-1})	Influent (kg COD m^{-3})	Removal (% COD_r)	Biogas (m^3 m^{-3} d^{-1})	Methane (%)	Y (m^3 CH_4 kg^{-1} COD_r)
8	55.4	82	3.8	64	0.361

Influent - Unfavorable OMW
[High concentrations, acid pH (5.0) and lack of alkalinity and nitrogen]
was converted
Effluent with basic pH (8.1) and high alkalinity

Olive mill wastewaters - OMW

AD- Anaerobic Digestion. Biogas

Hybrid digester

Reactor operation OMW % (v/v)	Total polyphenols mg CAE mL^{-1}	Antiradical Activity EC_{50}*
8 - in 8 - out	0.25 0.18	2.32 1.84
12 - in 12 - out	0.27 0.12	2.54 1.19
69 - in 69 - out	1.07 0.30	6.65 2.73
83 – in 83 - out	1.32 0.38	8.53 3.62
Piggery effluent	0.20	2.02

*antiradical activity was defined as the amount of antioxidant (expressed as μg of total polyphenols) necessary to decrease the initial DPPH concentration by 50 % (EC_{50} = Efficient Concentration)

Olive mill wastewaters - OMW

AD- Anaerobic Digestion. Biogas

Phenolic compounds (µg mL^{-1}): HPLC analysis

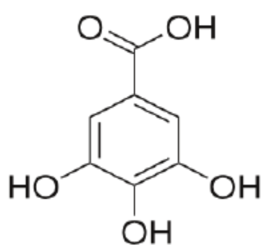
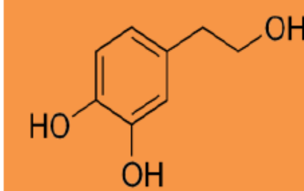
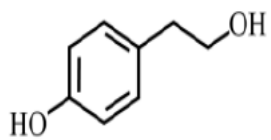
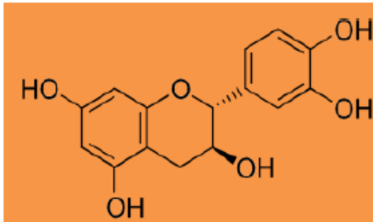
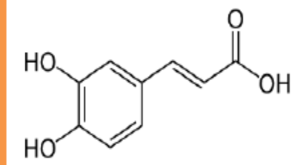
OMW % (v/v)	Gallic acid	Hydroxy -tyrosol	Tyrosol	Catechin	Caffeic acid	Ferulic acid	Rutin	Oleuropein	Quercetin
8-in	2.30	nd	38.48	38.50	13.85	1.38	0.19	179.5	3.63
8-out	**0.75**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**194.0**	**23.63**
12-in	nd	nd	nd	13.0	7.63	9.00	0.30	93.55	21.15
12-out	**4.56**	**nd**	**8.05**	**23.80**	**1.33**	**nd**	**nd**	**22.50**	**6.95**
69-in	25.02	87.50	35.95	13.0	23.58	55.98	nd	300.0	4.88
69-out	**30.65**	**nd**	**nd**	**nd**	**nd**	**nd**	**nd**	**27.20**	**10.50**
83-in	5.85	52.50	55.15	16.25	8.65	21.25	0.14	1125.0	4.05
83-out	**1.38**	**nd**	**26.38**	**nd**	**3.18**	**nd**	**nd**	**115.8**	**23.70**
Pig. effl.	1.38	8.13	21.05	16.08	5.55	14.10	nd	8.18	nd

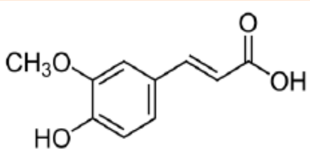
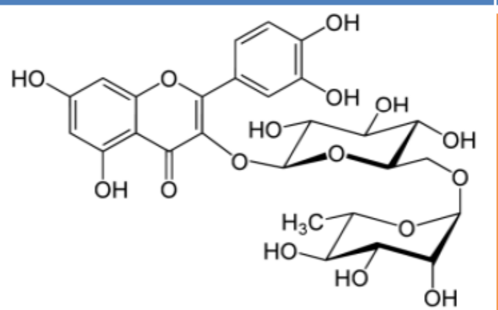
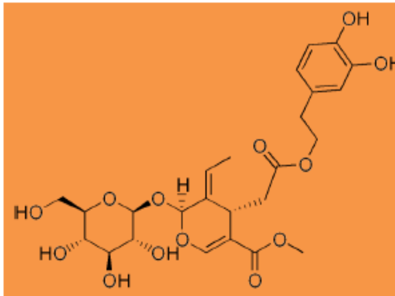
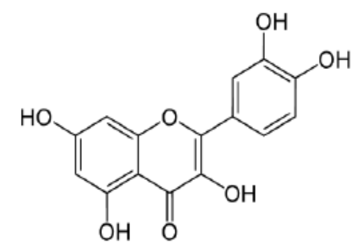
Oleuropein: main phenolic compound present in the substrate before and after AD
After the anaerobic treatment, several phenolic compounds remained

Quercetin concentration increases during anaerobic process

OMW anaerobically digested: phenolic compounds with antiradical activity

HPLC analysis: phenolic compounds

Gallic acid	Hydroxy-tyrosol	Tyrosol	Catechin	Caffeic acid
				

Ferulic acid	Rutin	Oleuropein	Quercetin
			

Enzymatic activities in anaerobic hybrid bioreactor

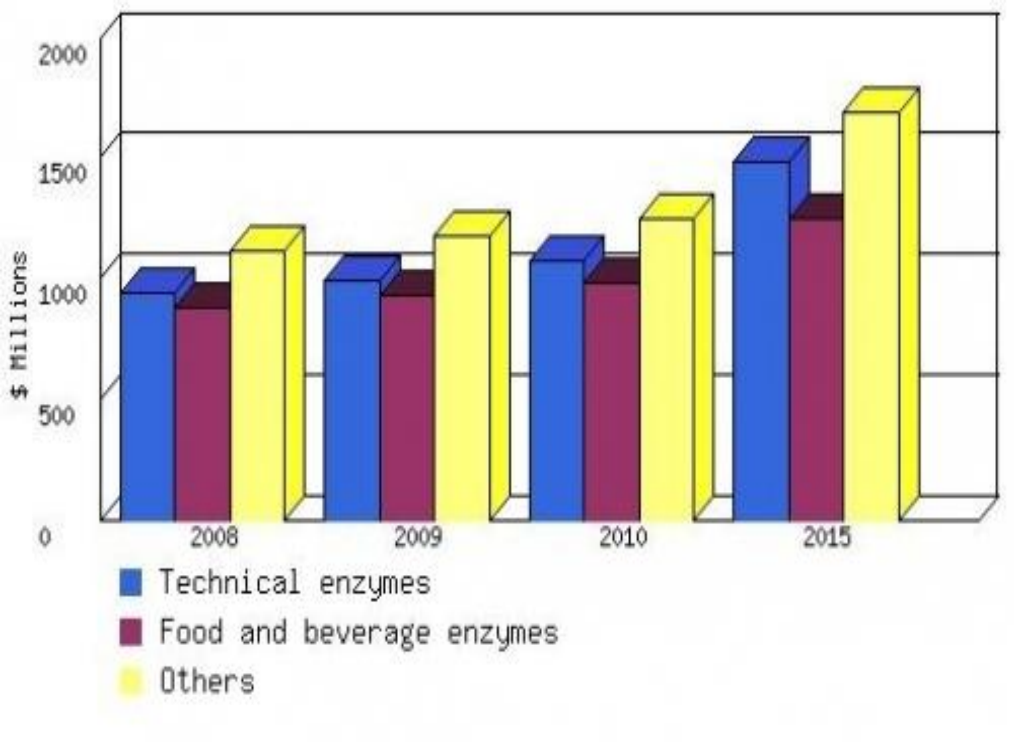
Sample	Hybrid reactor (OMW % v/v)	Lipase (U/mL)	β-Glucosidase (U/mL)	Laccase (U/mL)	Cellulase (mU/mL)	Xylanase (mU/mL)
1	69 in	4.80	2.88	5.17	3.20	1.10
2	69 out	6.90	3.08	4.97	n.d.	n.d.
3	83 in	4.49	2.57	1.44	30.30	5.70
4	83 out	6.17	0.82	0.92	3.90	2.28
5	100 in	2.97	n.d.	0.51	tr.	tr.
6	100 out	3.55	n.d.	tr.	tr.	tr.

(n.d.) – not detected; tr. traces; in – influent; out - effluent

Lipase activity was produced during the hybrid digester anaerobic process

The production of enzymes involved in the hydrolysis of complex carbohydrates (cellulase and xylanase) is higher when the feed mixture contains 83 % v/v.

GLOBAL INDUSTRIAL ENZYMES MARKET 2008-2015 ($ MILLIONS)



Source: BCC Research

Technical enzymes are valued at just over $1 billion in 2010. This sector will increase at a 6.6% compound annual growth rate to reach $1.5 billion in 2015. The highest sales of technical enzymes occurred in the leather market, followed by the bioethanol market.

The food and beverage enzymes segment is expected to reach about $1.3 billion by 2015, from a value of $975 million in 2010, rising at a compound annual growth rate (CAGR) of 5.1%. Within the food and beverage enzymes segment, the milk and dairy market had the highest sales, with about $400 million in 2009.

Cork manufacture residues

Harvested Cork

CBW

Gives rise to high volumes (140-1200 L ton^{-1} cork) of an organic effluent: the cork boiling wastewater (CBW).

This residue has no utility and its potential pollution charge makes is a serious environmental hazard.

CBW characterization

Parameter	CBW
pH	5.8 ± 0.0
COD (kg m^{-3})	6.5 ± 0.1
BOD (kg m^{-3})	-
TOC (kg m^{-3})	-
TS (kg m^{-3})	5.13 ± 0.08
VS (kg m^{-3})	4.05 ± 0.04
TSS (kg m^{-3})	0.58 ± 0.11
VSS (kg m^{-3})	0.15 ± 0.07
Conductivity (mS cm^{-1})	1.5 ± 0.1
Total phenols [a] (kg m^{-3})	1.20 ± 0.00
Total Nitrogen (kg m^{-3})	0.04 ± 0.00

COD-Chemical Oxygen Demand; BOD-Biochemical Oxygen Demand; TS, VS (total and volatile solids); TSS, VSS (total and volatile suspended solids) [a]phenolic content as gallic acid; *values are expressed as an average ± standard deviation of three replicates

Methane yield and pH at the end of the experiments performed with 3 and 6 kg COD m^{-3} of cork boiling wastewater (CBW)

CBW (kg m-3)	pH	CH_4 Yield (m^3 CH_4 kg^{-1} COD_{added})
3	7.67 ± 0.02	0.142 ± 0.014
6	7.52 ± 0.12	0.126 ± 0.016

CBW characterization

Parameter	CBW	AD0	AD3	AD6
pH	7.2	8.23 ± 0.08	7.67 ± 0.02	7.52 ± 0.06
VFA (kg m^{-3})	0.215	0.18 ± 0.04	0.18 ± 0.05	0.14 ± 0.01
Total phenols (mg mL^{-1})	1.2	0.05 ± 0.01	0.90 ± 0.01	1.41 ± 0.02
Optical density (254 nm)	43.5	8.41 ± 0.64	29.08 ± 0.66	43.96 ± 0.02
DPPH inhibition (%)	74.0	78.90 ± 0.42	76.07 ± 1.56	80.80 ± 1.06
EC_{50}* (antiradical activity)	7.2	2.22 ± 0.08	5.90 ± 0.08	8.74 ± 0.06
Conductivity (mS cm^{-1})	1.5	4.00 ± 0.11	4.60 ± 0.01	5.4 ± 0.11

CBW: Cork boiling wastewater, reactor influent; Anaerobic digestion assay: blank (AD0), at 3 kg COD m^{-3} (AD3) and 6 kg COD m^{-3} of CBW (AD6); *antiradical activity was defined as the amount of antioxidant (expressed as μg of total polyphenols) necessary to decrease the initial DPPH concentration by 50 % (EC_{50} = Efficient Concentration)

CBW anaerobically digested: phenolic compounds with antiradical activity

Phenolic compounds (µg/mL)	CBW	AD0	AD3	AD6
Gallic acid, GA	19.50± 3.71	4.17 ± 0.26	15.47 ± 4.08	22.43 ± 3.97
Protocatechuic acid, PCA	8.50± 1.02	0.64 ± 0.09	5.07 ± 1.59	9.82 ± 1.33
Caffeic acid, CA	2.14± 0.15	0.59 ± 0.44	1.15 ± 0.21	1.77 ± 0.36
Vanillic acid, VA	2.00± 0.19	tr	1.15 ± 0.37	1.91 ± 0.36
Syringic acid, SA	tr	nd	tr	1.10 ± 0.99
Ellagic acid, EA	**96.50±11.50**	**2.49 ± 0.39**	**22.3 ± 3.48**	**41.80 ± 8.49**
p-coumaric acid, p-CA	4.10± 0.26	1.10 ± 0.18	1.77 ± 0.28	2.47 ± 0.40
Ferulic acid, FA	6.50± 0.78	1.02 ± 0.08	1.55 ± 0.37	5.60 ± 0.98
o-coumaric acid, o-CA	3.80± 0.29	tr	1.17 ± 0.32	4.51 ± 0.33
Trans-cinnamic acid, t-CA	3.70± 0.37	2.16 ± 0.26	2.10 ± 0.29	3.13 ± 0.63

CBW-Cork boiling wastewater, reactor influent; AD - Anaerobic digestion assay: blank (AD0), at 3 kg COD m^{-3} (AD3) and 6 kg COD m^{-3} of CBW (AD6); nd: not detected, tr: traces; ± standard deviation.

The main bioactive compound identified in CBW was ellagic acid, with the highest amount detected in the input stream.

Enzymatic activities in CBW anaerobically digested

Enzymes (U/mL)	CBW	AD0	AD3	AD6
Lipase	3.63	0.83 ± 0.0	0.66 ± 0.0	0.84 ± 0.0
β-Glucosidase	tr	0.16 ± 0.0	nd	tr
Laccase	0.79	0.99 ± 0.0	1.97 ± 0.0	2.10 ± 0.0
Cellulase	0.21	tr	0.08 ± 0.0	0.12 ± 0.0
Xylanase	tr	nd	0.10 ± 0.0	0.15 ± 0.0

CBW-Cork boiling wastewater, reactor influent; AD - Anaerobic digestion assay: blank (AD0), at 3 kg COD m^{-3} (AD3) and 6 kg COD m^{-3} of CBW (AD6); nd: not detected; tr: traces

An interesting result is obtained for the laccase activity that increases in both treated effluents. An implement of about 2.5 fold were registered at AD6.

Marques *et al. Biotechnology for Biofuels* 2014, **7**:67
http://www.biotechnologyforbiofuels.com/content/7/1/67

Biotechnology for Biofuels

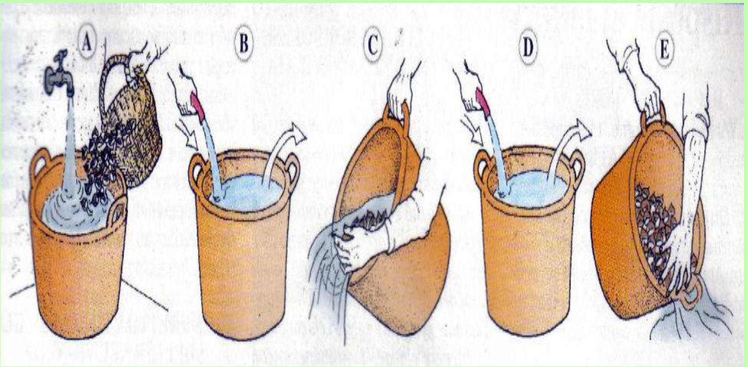
RESEARCH **Open Access**

Energetic and biochemical valorization of cork boiling wastewater by anaerobic digestion

Isabel Paula Marques[1*], Luís Gil[1] and Francesco La Cara[2]

The results of the study allowed the valorization of CBW in terms of energy and valuable biomolecules. By exploiting the anaerobic digestion process potential, a novel methodology to toxic and recalcitrant cork processing wastewater was developed.

Chestnut manufacture residues



In the Mediterranean basin are produced and processed about 150,000 tons of chestnuts/year, with an incidence therefore of about 50,000 tons of solid residues, nowadays managed as wastes. The wastewaters from chestnut processing are estimated around 900,000 cubic meters that requiring a specific treatment (depuration) before being carried out in public managed waste treatment plants.

Chestnut processing wastewater, like other Mediterranean region effluents (e.g. olive mill wastewater,cork boiling wastewater), can be considered for bioenergy production purpose due to their organic materials content.

Chestnut manufacture residues

Chestnut curing wastewaters

Chestnut peeling wastewaters

Water curing of chestnuts, a commonly used postharvest method, is based on soaking fresh fruit in water (1). The cure normally is carried out in a 1 : 3 ÷ 5 chestnuts/water ratio, at 18 °C for 3 ÷ 9 d. Other chestnut phenolic wastewater were produced during the fine screen preliminary treatment (peeling) (2)

CW characterization: phenolic compounds with antiradical activity

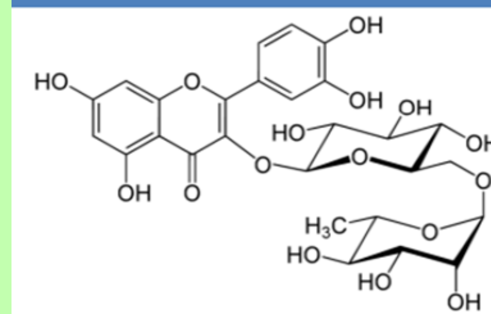
Chestnut Wastewater	Total polyphenols (mg /ml)	Ortho-diphenols (mg/ml)	Flavonoids (mg/ml)	Antiradical Activity (EC_{50})
Raw (curing) wastewater	0,046	0,040	0,009	**16.2**
Wastewater after peeling	0,058	0,048	0,012	**8.5**

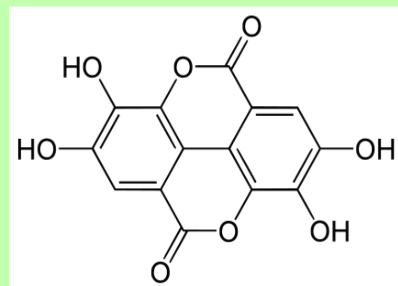
The *ortho*-diphenol fraction, provided with the highest antioxidant power among the phenolic molecules, corresponded to 83% of the total phenols.

CW characterization: phenolic compounds with antiradical activity

Phenolic compounds (µg/ml)	1	2
Gallic acid	2.78	3.21
Rutin	4.31	15.88
Ellagic acid	1.45	9.32
p-Coumaric acid	21.14	5.23
Ferulic acid	4.52	7.89

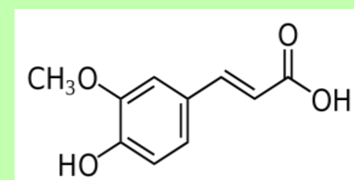
Rutin





ellagic acid

ferulic acid



(1) raw wastewater and (2) wastewater after fine screen preliminary treatment (mainly waste peel and skin solid removal).

Conclusions

- ✓ The phenolic effluents considered - olive mill, cork boiling and chestnut wastewaters – are interesting sources for energy purpose (biogas), through anaerobic digestion.
- ✓ A further valorization can be done to extract valuable biomolecules (bioactive compounds and enzymes) in a biorefinery approach.
- ✓ It was observed that bioactive molecules such the quercetin were produced inside the digester.
- ✓ A new reactor – anaerobic hybrid – and an operational methodology were developed. It makes possible to degrade these toxic effluents without any previous treatment or correction.

Thank you for the attention