



Università degli Studi Mediterranea di Reggio Calabria
Archivio Istituzionale dei prodotti della ricerca

Integral valorization of orange peel waste through optimized ensiling: Lactic acid and bioethanol production

This is the peer reviewed version of the following article:

Original

Integral valorization of orange peel waste through optimized ensiling: Lactic acid and bioethanol production / Fazzino, F., Mauriello, F., Paone, E., Sidari, R., Calabro, P.S.. - In: CHEMOSPHERE. - ISSN 0045-6535. - 271:(2021), p. 129602. [10.1016/j.chemosphere.2021.129602]

Availability:

This version is available at: <https://hdl.handle.net/20.500.12318/81524> since: 2024-11-28T20:27:18Z

Published

DOI: <http://doi.org/10.1016/j.chemosphere.2021.129602>

The final published version is available online at: <https://www.sciencedirect>.

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website

Publisher copyright

This item was downloaded from IRIS Università Mediterranea di Reggio Calabria (<https://iris.unirc.it/>) When citing, please refer to the published version.

(Article begins on next page)

Integral valorisation of orange peel waste through optimized ensiling: lactic acid and bioethanol production

Filippo Fazzino¹, Francesco Mauriello¹, Emilia Paone², Rossana Sidari³, Paolo S. Calabrò^{1,*}

¹Università degli Studi Mediterranea di Reggio Calabria, Department of Civil, Energy, Environmental and Materials Engineering, Via Graziella, loc. Feo di Vito, 89122, Reggio Calabria, Italy.

²Università degli Studi di Firenze, Dipartimento di Ingegneria Industriale (DIEF), Via di S. Marta 3, I-50139 Firenze, Italy

³Università degli Studi Mediterranea di Reggio Calabria, Department Agraria, loc. Feo di Vito, 89122, Reggio Calabria, Italy.

*Corresponding author: paolo.calabro@unirc.it

Abstract

The management of the huge amount of orange peel waste (OPW) is a complex issue although it has a very high potential in terms of biorefining. One of the main problems in the valorisation of OPW is the seasonality of its production with the ensiling method being largely proposed as a possible solution. During the ensiling process, value added chemicals including lactic acid, acetic acid and ethanol are spontaneously produced together with a significant loss of volatile solids (VS). In this contribution, the stimulation of lactic acid bacteria by either a biological (inoculation with leachate coming from a previous ensiling process) or chemical (MnCl₂ supplementation) methods has been tested with the aim to increase the chemicals production preventing, at the same time, the VS loss. The inoculation with the leachate improves both the VS recovery (+7%) and the concentration of lactic acid (+113%) with respect to the uninoculated one (control). The overall yields of the process are noticeable, up to about 55 g·kg_{TS}⁻¹ of lactic acid, 26 g·kg_{TS}⁻¹ of acetic acid and 120 g·kg_{TS}⁻¹ of ethanol have been produced. On the other hand, the chemical stimulation enhances the production of liquid products together with a significant VS loss. The proposed preservation method, due to its

simplicity, can be easily implemented at full-scale allowing the production of added-value chemicals and the concurrent storage of the OPW that can be further valorised (e.g. animal feed, pectin or biomethane production).

Keywords: Acetic acid; ensiling; ethanol; lactic acid; microbial population; orange peel waste

Highlights

- Full valorisation of orange peel waste (OPW) is highly advantageous
- Ensiling is a suitable preservation method for OPW prior to valorisation
- Ensiling can be optimized for preserving as many volatile solids as possible
- During the ensiling process, lactic acid and other chemicals can be efficiently produced

1. Introduction

The management of the huge amount of orange peel waste (OPW), produced each year worldwide as a by-product of orange juice manufacturing (de la Torre et al., 2019; United States Department of Agriculture - Foreign Agricultural Service, 2020) is a very complex issue.

At the moment, most of the produced OPW is dumped near production sites or in landfills causing severe impacts on air, soil and water while its use as animal feed, although quite common, is limited by difficulties related also to transportation costs (Ahmadi et al., 2015; Ricci et al., 2019; Zema, 2017; Zema et al., 2012).

The biorefining potential of this residue is very high since the extraction of value-added products such as chemicals, bioactive compounds for the preparation of nutraceuticals, sorbents for water treatment and bio-combustibles has been fully demonstrated at industrial, pilot or laboratory scale (Mahato et al., 2020; Satari and Karimi, 2018; Sharma et al., 2017; Zema et

al., 2018). On the other hand, some issues still remain: the large amount produced, the seasonality and the presence of residual d-Limonene (toxic for microorganism and thus problematic in case of biologic valorisation processes) prevent the full exploitation of the OPW (Ángel Siles López et al., 2010; Benito et al., 2016; Moraci et al., 2011; Tian et al., 2014; Zema et al., 2018).

The ensiling process (a forage preservation method under anaerobic conditions) has proven to be a suitable option to overcome these limitations since it can preserve the biomass reducing, at the same time, d-limonene content thus allowing its use along the year (Ashbell and Lisker, 1987; Büyükkılıç Beyzi et al., 2018; Calabrò et al., 2020; P. S. Calabrò et al., 2018; Calabrò and Panzera, 2018). One of the key limits of the ensiling process is the considerable loss of volatile solids (VS) that can reach up to 40% of the initial mass.

During the ensiling, a spontaneous anaerobic fermentation process occurs without the need of any particular inoculation or management operation leading to the production of several chemical compounds. In particular, lactic acid bacteria (LAB) produce lactic acid (homofermentative) together with acetate, ethanol and carbon dioxide (heterofermentative). In particular, yeasts utilise sugars for the production of ethanol while acetic acid bacteria (AAB) oxidise sugars and ethanol using the residual air entrapped in the biomass during the silo loading (Liang et al., 2016; Mamlouk and Gullo, 2013). The valorisation of the OPW by spontaneous or inoculated ensiling is a research field in expansion (Calabrò et al., 2019; Ricci et al., 2019; Zerva et al., 2019). However, more research effort is needed to optimize the process without a significant increase of the overall complexity/cost of the ensiling (e.g. use of specialized microorganisms, complex management operations such as pH or temperature control). In this context, in order to improve both the VS preservation as well as the production of value-added chemicals (with particular attention to lactic acid), the stimulation of LAB is a possibility to be explored. This stimulation could be implemented by either the inoculation of already adapted

microorganisms contained in the leachate of a previous ensiling experiment or by the addition of manganese chloride due to its stimulating role on LAB (Archibald, 1986; Ohkouchi and Inoue, 2006; Racchach and Marshall, 1985; Terpstra et al., 2001).

Even if the catalytic valorisation of lignocellulosic waste and residues into energy, fuel and chemicals is a well consolidated field of research (Luque and Clark, 2013; Xu et al., 2020), research on valorisation of OPW through the ensiling procedure for the production of high added-value feedstocks (lactic acid especially) for modern biorefineries is lacking in the literature.

This research activity has two main objectives: (i) to reduce the VS loss occurring during the ensiling in view of the possible valorisation of OPW (e.g. use as animal feed, pectin production, anaerobic digestion) and (ii) to increase the production of high added-value compounds (e.g. ethanol and lactic acid).

A strong advantage of the proposed OPW valorisation process is the simplicity of the adopted approach, that avoids pH and temperature control and the use of specialized microbial strains.

2. Materials and Methods

2.1 Ensiling process

The OPW used for the ensiling tests was collected from an orange processing industry in Reggio Calabria – Italy (Agrumaria Reggina s.r.l.). After sampling, the OWP was frozen at – 20 °C (freezing is not expected to affect its biological activity (Pognani et al., 2012)). Samples were then thawed at room temperature and the OPW was characterized in terms of pH, TS and VS parameters, according to APHA et al. (2012), before the beginning of the experiment.

The experiment procedure (Figure 1) consisted of two stages. First, 750 g of OPW were equally divided and ensiled in three glass batches in order to produce a leachate that was used as inoculum for the subsequent experiment. Each batch was hermetically sealed by a stopper and

101 connected to an acidic trap ($\text{pH} < 5$) to allow the biogas venting. After 28 days, the ensiling was
102 stopped and the OPW was extracted and centrifuged at 9000 rpm for 3 min in order to separate
103 the liquid fraction.

104 In the second part of the experiment (see Figure 1), five ensiling modes were tested:

- 105 1. the blank (control) sample (OPW supplemented with distilled water - 10% w/w);
- 106 2. the OPW inoculated with the leachate (10% w/w) produced in the previous experiment;
- 107 3. the OPW supplemented with MnCl_2 , at a dosage of 0.05 g/kg_{OPW} and dissolved in
108 distilled water (10% w/w);
- 109 4. the OPW supplemented with MnCl_2 , at a dosage of 0.01 g/kg_{OPW} and dissolved in
110 distilled water (10% w/w);
- 111 5. the OPW supplemented with MnCl_2 , at a dosage of 0.005 g/kg_{OPW} and dissolved in
112 distilled water (10% w/w).

113 Each ensiling test was carried out in duplicate using 400 g of OPW per batch and lasted 28 days
114 (Calabrò et al., 2020; P. S. Calabrò et al., 2018; Calabrò and Panzera, 2017). The biogas volume
115 ($> 95\% \text{CO}_2$ - Calabrò et al., 2020) was measured by the water displacement method using an
116 acidic trap. The biogas produced during the ensiling process flowed into another reactor filled
117 with an acidic solution (the trap), the pressure increase displaced a volume of solution equal to
118 that of the biogas produced that was then collected into a graduated cylinder where it was
119 measured (Gou et al., 2014).

120 The determination of pH, TS, VS (APHA et al., 2012) was done on the ensiled samples
121 collected both after 10 and 28 days. Moreover, in addition to the analyses mentioned above, the
122 quantitative analysis of lactic acid, acetic acid and ethanol products in the liquid phase separated
123 from those samples by centrifugation was also carried out. In particular, the analysis of VS was
124 used for mass balances in order to calculate the recovery respectively after ensiling (the amount

present in the sample respect to that fed at the beginning of the process) and in the solid phase separated by centrifugation.

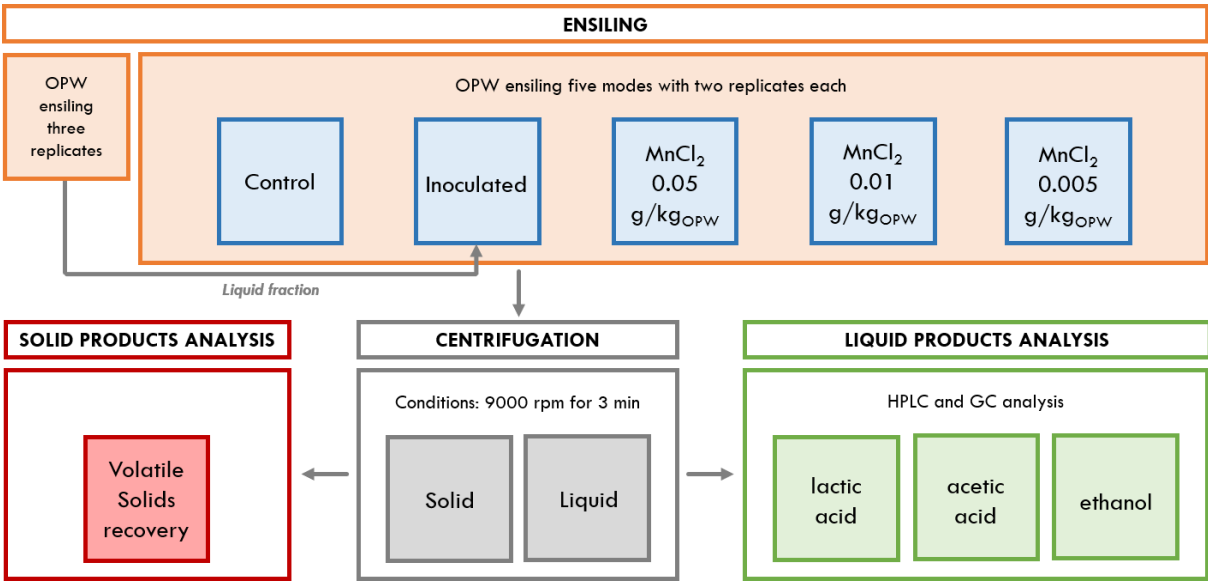


Figure 1. Experimental design for the valorisation of orange peel waste through optimized ensiling

2.2 Analytical methods

The quantitative determination of lactic acid, acetic acid and ethanol was carried out following an analytical procedure reported in the reported in the last years by some of the authors (Gumina et al., 2019; Malara et al., 2018). Products present in the aqueous liquid phase were quantified through an off-line High Performance Liquid Chromatography (HPLC) analysis (Agilent 1290 infinity HPLC) equipped with an Aminex HPX-87-H column by using RID as detector, following these parameters: mobile phase 5mM H₂SO₄ at a speed flow of 0,6 ml/min and the oven heated at 70°C (every measurement was performed for 30 min).

Products in the liquid phase were also cross-checked by GC analysis (organic compounds were previously extracted with ethyl acetate and eventual water traces were removed by adding anhydrous sodium sulphate to the organic phase) by using an off-line gas chromatograph

(Agilent 6890 N) equipped with a wide-bore capillary column (CP-WAX 52CB, 60 m, i.d. 0.53 mm) and a flame ionization detector (FID). The injector was settled at 250 °C and the temperature program started from 50 °C (held for 5 min) up to 240 °C with a 10 °C/min rate (held for 10 min) and finally at 240 °C (held for 5 min) during the post run (Paone et al., 2020).

2.3 Statistical analysis

A one-way ANalysis Of VAriance (ANOVA) with repeated measures at the sampling dates of the batch experiments was carried out to check the significance of differences in biogas production, VS loss and production of chemicals during the ensiling process (the batch type was assumed as an independent factor). Dunnett's test was used to check if the statistical differences ($p < 0.05$) between control and the other treatments in terms of yield of ethanol, lactic and acetic acid are significant.

ANOVA assumes the normal distribution of residuals. This assumption was checked using the Adersen-Darling method, based on the function of the empirical distribution. All residuals of data resulted normally distributed ($p < 0.05$).

The XLSTAT software release 1.2019 was used for all statistical analysis.

3. Results

Table 1 shows the characteristics of the OPW used as feedstock for the experiments.

Table 1. Main characteristic of raw OPW

Parameter	Average Value	St. Dev.
pH	4,10	-
TS [%]	18,5%	0,66%
VS [%TS]	95,9%	0,39%

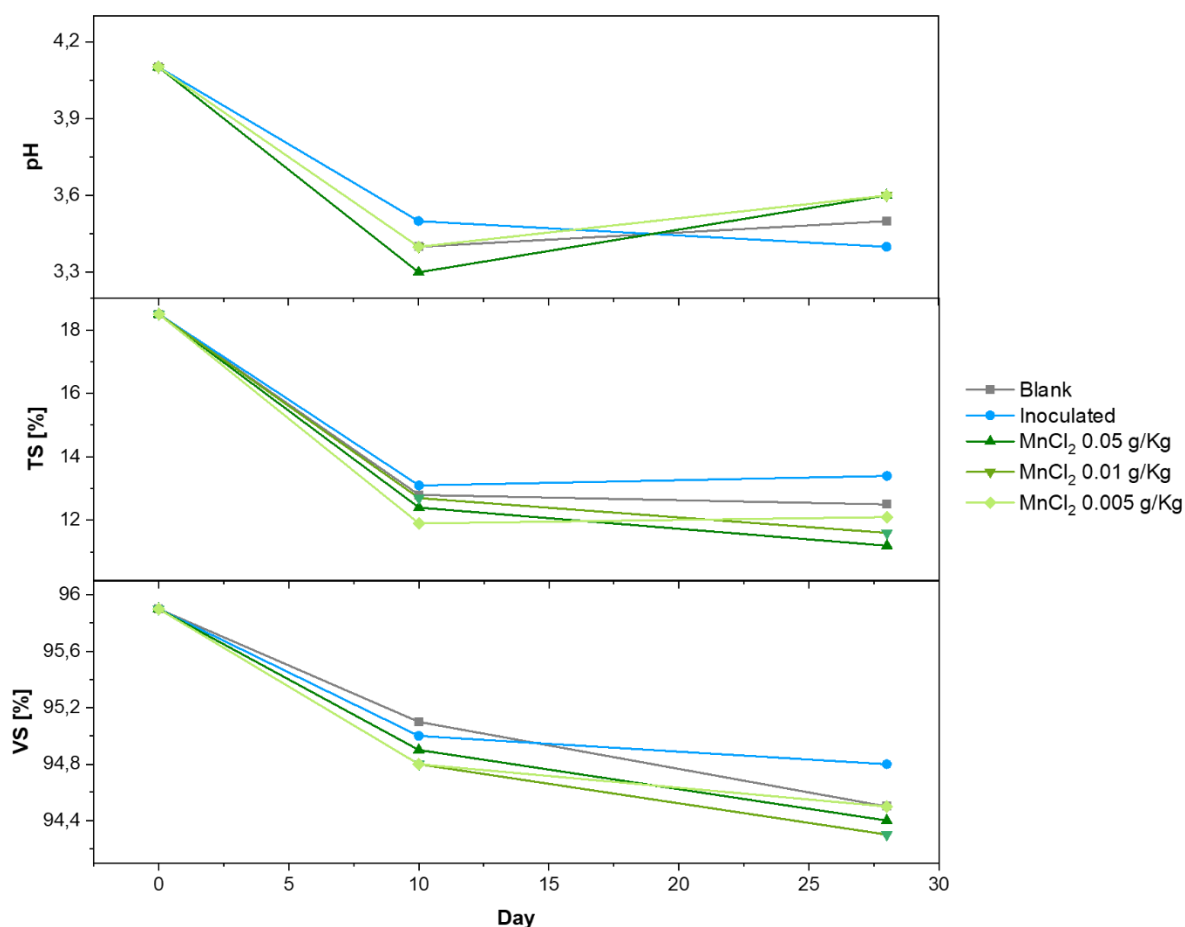


Figure 2. pH, Total Solids (TS) and Volatile Solids (VS) trends during the ensiling processes

During the ensiling process, pH values show a rapid decrease from the initial value of 4.1 to about 3.4 followed by a slight increase for all samples excepted those coming from inoculated batches (Figure 2). At the same time, total solids (TS) significantly decrease in the first 10 days (Figure 2) while a certain stability is evidenced for all the treatments in the final part of the experiment excepted those supplemented with the highest amounts of MnCl₂. Accordingly, VS trend is continuously decreasing but the reduction is larger in the first 10 days (Figure 2). In days 10-28, in the inoculated reactor, VS consumption is slower than that observed in the others while at the end of the process the reactor exhibited the highest VS value.

The biogas production (Figure 3) seems to follow two different patterns. The amount of biogas produced during the experiment is more regular in blank and inoculated batches: it is

concentrated in the first 10 days similarly to those of the two replicates of the same ensiling mode (differences from the average of the cumulated production from the single batch are limited to less than 20%). However, in the inoculated batch, the cumulated biogas production is 30% higher than that observed in blank samples (where biogas production is completed in 13-16 days).

In manganese chloride supplemented batches, the production of biogas is lower and irregular (differences between the replicates are larger). In these experiments, biogas production lasts 4-9 days and it seems to be inversely correlated to the amount of MnCl_2 supplemented: by increasing the total addition of MnCl_2 a lower biogas production is observed.

Noticeably, ANOVA ($p < 0.05$) did not show any statistically significant difference among treatments.

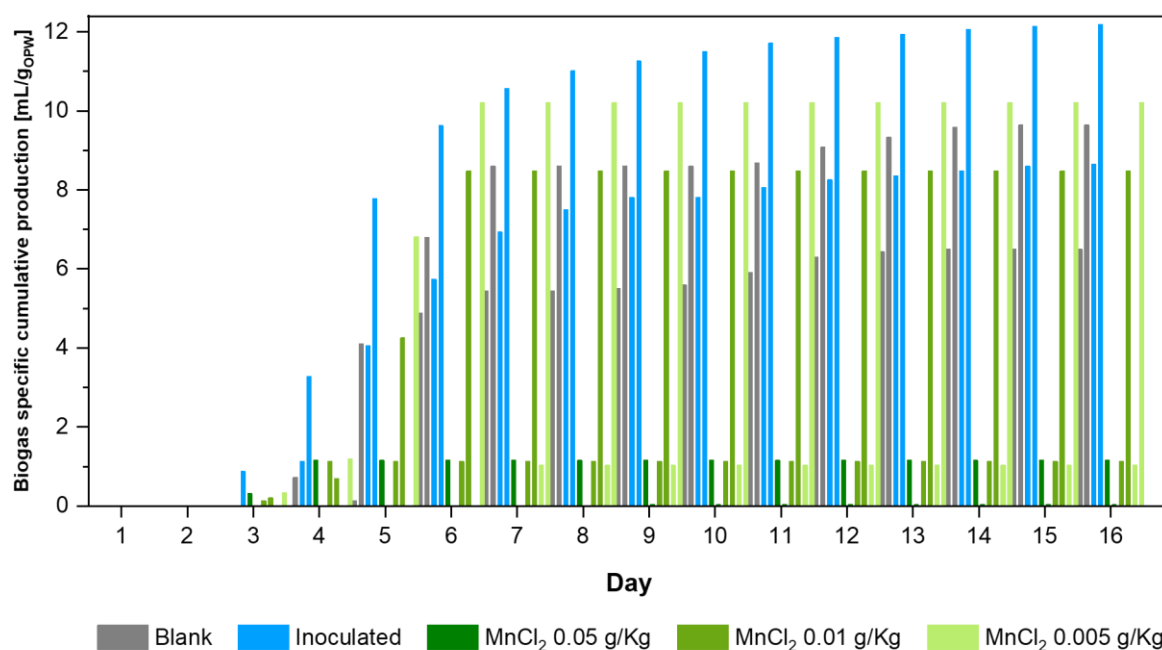


Figure 3. Biogas specific cumulative production during the ensiling processes.

The mass balance depicted in Figure 4 demonstrates that the VS loss during the ensiling occurs mainly (69 – 88% of the total) in the first 10 days when , the biological activity is more intense

as witnessed also by TS, VS and biogas production trends. The total loss accounts for 35 – 47% of the VS initially present in the batch. In the reactors supplemented with MnCl_2 , the VS loss is higher and continues in the second part of the ensiling experiment (days 10-28).

Also in this case, ANOVA ($p < 0.05$) does not show any statistically significant difference among treatments.

Figure 5 further confirms these trends: the liquid fraction - separated by centrifugation - is larger in manganese chloride supplemented batches most probably as a consequence of the longer and more intense biological activity.

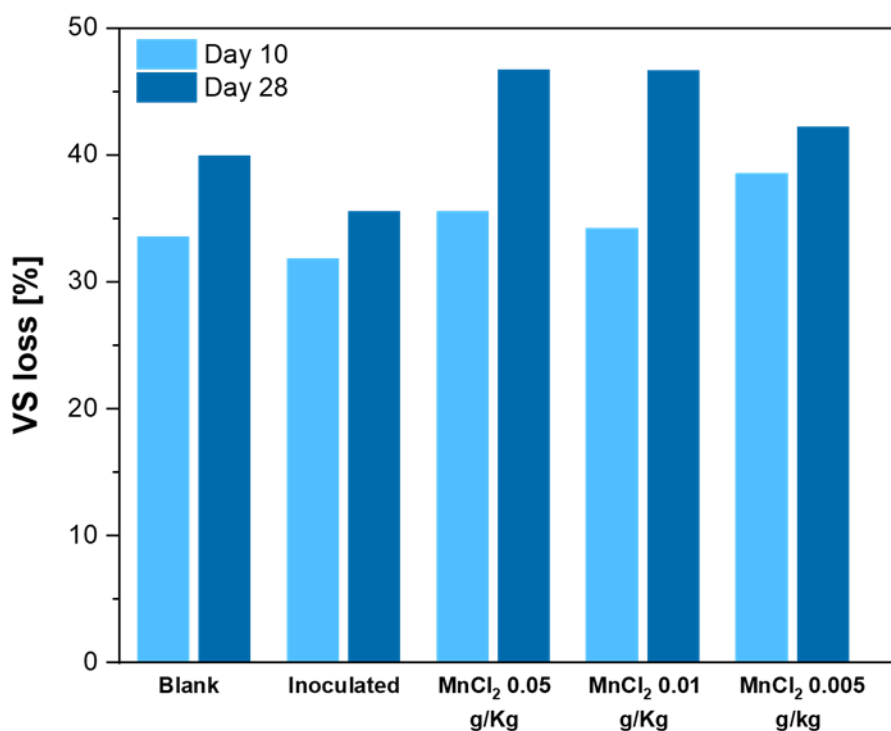


Figure 4. Volatile solids loss during the ensiling processes.

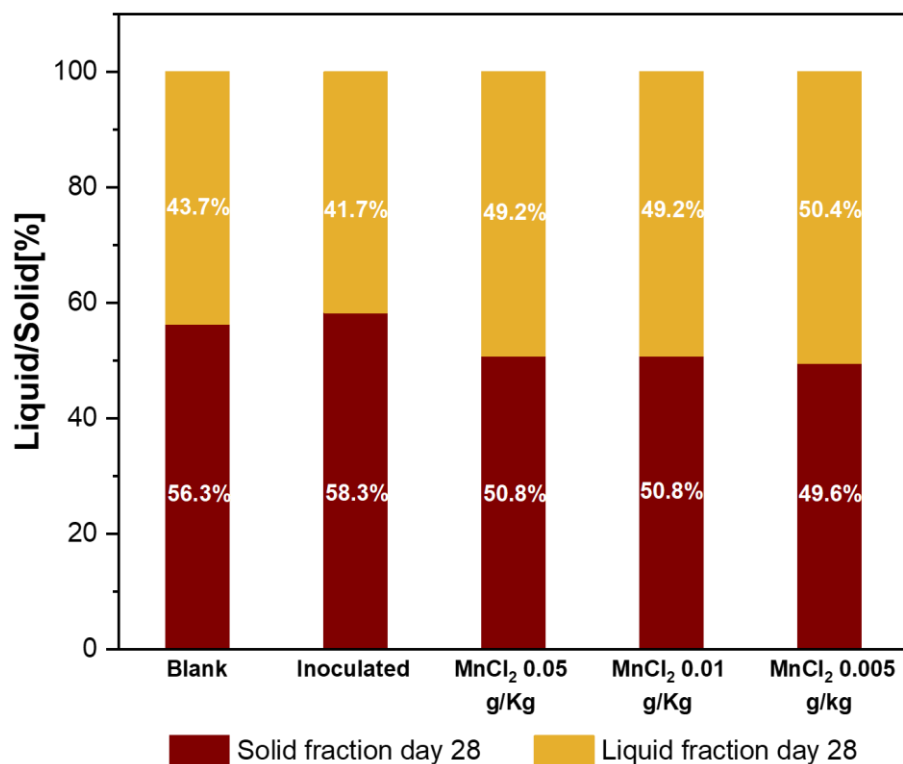


Figure 5. Incidence of the solid and liquid fraction at the end of the ensiling processes.

Table 2. Volatile solids recovery after the ensiling process (28 days) and in the solid fraction, separated by centrifugation, with respect to the VS initially present

Batch	Ensiling mode	VS Recovery after ensiling		VS recovery by centrifugation (solid fraction)	
		Replicates	Average	Replicates	Average
1	Blank	60,5%	60,1%	39,6%	41,4%
2		59,6%		43,1%	
3	Inoculated	68,0%	64,5%	46,9%	46,8%
4		61,0%		46,7%	
5	MnCl ₂ 0.05 g/kg	50,1%	53,3%	37,6%	37,5%
6		56,5%		37,4%	
7	MnCl ₂ 0.01 g/kg	54,9%	55,4%	38,2%	38,2%
8		55,9%		38,2%	
9	MnCl ₂ 0.005 g/kg	59,3%	57,8%	37,5%	37,2%
10		56,2%		37,0%	

Indeed, Table 2 outlines how the VS recovery in the solid fraction separated by centrifugation is larger (+23-26%) in inoculated batches with respect to manganese chloride supplemented reactors.

Furthermore, at the day 10, acids concentrations (especially lactic acid) are largely higher in the inoculated reactor with respect to the others, as witnessed also by the statistical analysis (Table 3). This trend is partially confirmed, in the case of lactic acid, by experiments at day 28. In this case, $MnCl_2$ supplemented reactors performed quite well (Table 4). It is interesting to highlight that the lactic acid concentration detected at both 10 and 28 days is higher on using the manganese chloride supplemented reactors compared to the blank ones. Acetic acid concentrations, measured at the end of experiments, are more uniform. For what ethanol is concerned, concentrations are similar in all the batches both at day 10 and 28, as also outlined by statistical analysis.

Table 3. Ethanol, Lactic and Acetic acid concentration in the separated liquid at day 10. The same superscript letter denotes statistical similarity at $p < 0.05$.

Day 10 Ensiling condition	Lactic acid [g/L]		Acetic acid [g/L]		Ethanol [g/L]	
	Replicates	Average	Replicates	Average	Replicates	Average
Blank	2.16 1.64	1.90 ^A	1.23 1.18	1.21 ^A	26.19 26.53	26.36 ^A
Inoculated	13.50 13.67	13.59 ^B	5.65 5.69	5.67 ^B	22.79 24.91	23.85 ^A
$MnCl_2$ 0.05 g/kg	1.90 2.16	2.03 ^A	1.30 1.53	1.42 ^A	-* 25.61	25.61 ^A
$MnCl_2$ 0.01 g/kg	2.13 3.41	2.77 ^A	2.21 2.10	2.16 ^C	25.51 27.30	26.41 ^A
$MnCl_2$ 0.005 g/kg	3.03 2.33	2.68 ^A	2.28 2.19	2.24 ^C	27.26 24.97	26.12 ^A

*outlier value

Table 4. Ethanol, Lactic and Acetic acid concentration in the separated liquid at day 28. The same superscript letter denotes statistical similarity at $p < 0.05$.

Day 28 Ensiling condition	Lactic acid [g/L]		Acetic acid [g/L]		Ethanol [g/L]	
	Replicates	Average	Replicates	Average	Replicates	Average
Blank	8.72 8.20	8.46 ^A	4.58 4.53	4.56 ^A	26.32 26.42	26.37 ^A
Inoculated	17.90 18.15	18.03 ^B	6.87 6.67	6.77 ^B	23.25 25.95	24.60 ^A
MnCl ₂ 0.05 g/kg	12.36 12.11	12.24 ^C	6.25 5.94	6.10 ^C	-* 27.53	27.53 ^A
MnCl ₂ 0.01 g/kg	12.92 13.69	13.31 ^C	6.51 6.39	6.45 ^{B,C}	26.84 25.51	26.18 ^A
MnCl ₂ 0.005 g/kg	14.23 13.17	13.70 ^C	6.40 6.59	6.50 ^{B,C}	26.37 25.67	26.02 ^A

*outlier value

Table 5. Ethanol, Lactic and Acetic acid yields (day 28).

Day 28	Lactic acid [g kg _{TS} ⁻¹]	Acetic acid [g kg _{TS} ⁻¹]	Ethanol [g kg _{TS} ⁻¹]
Blank	28.7±2.6	15.4±1.0	89.2±5.4
Inoculated	54.3±0.1*	20.4±0.4*	74.1±3.7
MnCl ₂ 0.05 g/kg	51.8±0.4*	25.8±0.6*	117.0±0.0
MnCl ₂ 0.01 g/kg	54.3±1.5*	26.3±0.3*	106.8±2.9
MnCl ₂ 0.005 g/kg	55.0±2.0*	26.1±0.4*	104.5±1.1

* Indicate a statistical difference respect to control ($p < 0.05$)

Yields from TS added at the beginning of the ensiling (Table 5) demonstrate the clear superiority of inoculated and MnCl₂ supplemented batches in terms of lactic and acetic acids production. In the case of ethanol, blank and inoculated batches show a similar behaviour while those chemically stimulated give a better result. Dunnett test confirms that the performance of the inoculated and manganese supplemented batches is statistically different from blank.

4. Discussion

Data on TS, VS and biogas production demonstrate that the stabilization of the OPW for blank and inoculated reactors occurs quickly, in agreement with previous reports (Calabrò et al., 2020; Calabrò and Panzera, 2018). The process is characterised by a significant biogas production that stops in about 10-15 days. The difference in biogas production observed between the blank and the inoculated reactors confirms that the microbial consortium already adapted to the OPW rapidly and intensely guides the process. Manganese chloride supplemented reactors behave differently, the VS consumption (witnessed by the steeper decreasing trend in days 10-28) continues for a longer period but the total biogas production is significantly lower. These findings makes possible to hypothesize that the manganese chloride supplementation induces changes in the microbial consortium and/or in its metabolism leading to a reduced CO₂ production. This hypothesis is supported if we take into account that MnCl₂ was reported to modify the bacterial community composition during the anaerobic digestion (Cai et al., 2018) affecting homofermentative and heterofermentative LAB (Raccach, 1985) and influencing, at the same time the CO₂ production.

With respect to the declared objectives of this paper, it seems that the biological stimulation, induced by the use of the already evolved microbial inoculum (contained in the liquid coming from a previous ensiling process) is beneficial for a faster stabilization of OPW with a concurrent better preservation of the VS (Table 2). Infact, as expected, the blank and the inoculated reactors exhibit a similar trend. This was predictable since the liquid used to inoculate the reactors derives from the ensiling of the same OPW matrix used for the blank reactors. Clearly, the lower values observed for the blank reactors indicates how the autochthonous microbial population needs time to stabilize and to evolve while it is already established in the inoculated reactors. These results confirm the advantage of bioaugmentation in the ensiling similarly to the anaerobic digestion process (Calabrò et al., 2018; Yu et al., 2016).

264 The more prolonged and intense use of VS in manganese chloride supplemented reactors is
265 coherent with a lower recovery of VS at the end of the ensiling and after centrifugation (Table
266 2).

267 The addition of the leachate, coming from a previous ensiling cycle does not significantly
268 influence the concentrations of lactic and acetic acids at day 10, since the volume of liquid
269 present in the batch is about four times higher than that added at day 0. By means of a mass
270 balance, it is possible to calculate that the leachate added at day 0 (whose composition was
271 assumed equal to that of the leachate collected at the end of the ensiling in the blank reactor)
272 contains 3% of the lactic acid, 5% of the acetic acid and 26% of the ethanol found at day 10.

273 This confirms that the production of acids in the first 10 days in the inoculated reactor is fast
274 and larger than in the other reactors. In the case of lactic acid (Table 4), at day 28, its
275 concentration in the inoculated reactor is 113% higher than that of the blank, while in
276 manganese chloride supplemented reactors the concentration is increased to a lower extend
277 (+45/+62%) with respect to the blank. It is worth to underline that a minor amount of MnCl_2
278 has a positive effect on the lactic acid production. The better performance of inoculated reactors
279 with respect to the blank is related to the rapid degradation activity of the naturally evolved
280 microbial consortium originated from previous OPW ensiling (Sivagurunathan et al., 2016; Yan
281 et al., 2012). The improved lactic acid production in manganese chloride supplemented reactors
282 compared to the blank ones confirms the positive effect of the inorganic salt on the growth and
283 metabolic activities of LAB (Raccach, 1985) even if an excessive concentration has toxic
284 effects (Nsair et al., 2020) and this justifies the better results obtained with more limited
285 supplementation of MnCl_2 . However, it must be pointed out that if the lactic acid yield is
286 considered instead of its concentration, , the production is similar in inoculated and chemically
287 supplemented reactors (the higher amount of liquid fraction available in manganese
288 supplemented reactors needs to be taken into account). The uniformity of the amount of ethanol

found in reactors containing different treatments as well as in the two sampling times (day 10 and day 28) supports the idea that its is suppressed very early, before day 10. It is plausible that the autochthonous yeasts population (mainly ethanol producers) behave similarly to that analysed during ensiling experiments carried out by some of the authors on analogous samples. It was shown that this population reaches the maximum load after seven days of the ensiling and then gradually decreases (Calabrò et al., 2020). Moreover, it seems that the yeasts inhabiting fresh OPW are, to some extent, naturally resistant to the D-limonene with differentiation of species throughout the ensilage time. In this context, it has been already reported (Calabrò et al., 2020; Wilkins et al., 2007) the ethanol production from the citrus peel waste using *Saccharomyces cerevisiae* and enzymes such as β -glucosidase that hydrolyse polysaccharides into sugars ready to be used by yeasts. Engineered *S. cerevisiae* for β -glucosidase, able to use cellobiose, have also been reported (Adam and Polaina, 1991; Tokuhito et al., 2008). Considering that yeasts belonging to different species naturally occurring in vegetal material can possess, at different rates, the β -glucosidase activity (Sidari et al., 2019), it cannot be excluded that the autochthonous species of yeast, inhabiting the OPW, contribute to the process by saccharification and/or fermentation.

At the best of the authors' knowledge, there is only one paper in the scientific literature that allows a partial comparison with the results of this research. For what the lactic acid yield is concerned (Ricci et al., 2019), used rewetted OPW for a solid state fermentation at a laboratory scale (5 g of dry substrate used in each replicate) by selected microbial strains, in pH and temperature controlled conditions, and obtained a maximum of 209.65 g kg⁻¹. Although higher, this production is fully comparable with those of the experiments presented in this paper (Table 5) obtained without the need of a specialized inoculum, pH and temperature control with the obvious benefits in terms of process economy and simplicity.

5. Conclusions

This research outlines how it is possible to produce high added-value chemicals such as lactic acid without any significant modification of the currently applied procedure for the ensiling process.

In particular, the inoculation with the leachate produced during the ensiling improved both VS recovery (+7%) and concentration of lactic acid (+113%) with respect to the uninoculated one (blank).

The yields of the process are noticeable, up to about $55 \text{ g} \cdot \text{kg}_{\text{TS}}^{-1}$ of lactic acid, $26 \text{ g} \cdot \text{kg}_{\text{TS}}^{-1}$ of acetic acid and $120 \text{ g} \cdot \text{kg}_{\text{TS}}^{-1}$ of ethanol have been produced.

The chemical stimulation carried out supplementing manganese chloride, enhances the production of value-added chemicals during the ensiling process but also the VS loss. Experiments on the long-term effects of the manganese chloride supplementation are necessary for a more comprehensive evaluation and for the optimization of the process as a function of the maximization of the most desired output (i.e. feedstock production or VS preservation).

Additional research experiments are currently ongoing on the valorisation of the solid OPW, obtained by centrifugation, and on the analysis the effect of a prolonged ensiling on the yields of the process as well as on the possibility of combining chemical and biological stimulation.

The practical implications of this research are very promising since its full-scale implementation would allow, at the same time, to produce value-added chemicals during the ensiling and to store the OPW for further valorisations processes (e.g. animal feed, pectin or biomethane production).

References

- Adam, A.C., Polaina, J., 1991. Construction of a *Saccharomyces cerevisiae* strain able to ferment cellobiose. *Curr. Genet.* 20, 5–8. <https://doi.org/10.1007/BF00312758>
- Ahmadi, F., Zamiri, M.J., Khorvash, M., Banihashemi, Z., Bayat, A.R., 2015. Chemical composition and protein enrichment of orange peels and sugar beet pulp after fermentation by two *Trichoderma* species. *Iran. J. Vet. Res.*
- Ángel Siles López, J., Li, Q., Thompson, I.P., 2010. Biorefinery of waste orange peel, *Critical Reviews in Biotechnology*. <https://doi.org/10.3109/07388550903425201>
- APHA, AWWA, WEF, 2012. *Standard Methods for the Examination of Water and Wastewater*, 22nd Edition. American Public Health Association, American Water Works Association, Water Environment Federation.
- Archibald, F., 1986. Manganese: Its acquisition by and function in the lactic acid bacteria. *Crit. Rev. Microbiol.* 13, 63–109. <https://doi.org/10.3109/10408418609108735>
- Ashbell, G., Lisker, N., 1987. Chemical and microbiological changes occurring in orange peels and in the seepage during ensiling. *Biol. Wastes* 21, 213–220. [https://doi.org/10.1016/0269-7483\(87\)90127-3](https://doi.org/10.1016/0269-7483(87)90127-3)
- Benito, D., Benito, D., Ruiz, B., de Benito, A., Rivera, J.D., Flotats, X., 2016. Assessment of different pre-treatment methods for the removal of limonene in citrus waste and their effect on methane potential and methane production rate. *Waste Manag. Res.* 34, 1249–1257. <https://doi.org/10.1177/0734242X16661053>
- Büyükkılıç Beyzi, S., Ülger, İ., Kaliber, M., Konca, Y., 2018. Determination of Chemical, Nutritional and Fermentation Properties of Citrus Pulp Silages. *Turkish J. Agric. - Food Sci. Technol.* <https://doi.org/10.24925/turjaf.v6i12.1833-1837.2229>
- Cai, Y., Zheng, Z., Zhao, Y., Zhang, Y., Guo, S., Cui, Z., Wang, X., 2018. Effects of molybdenum, selenium and manganese supplementation on the performance of

362 anaerobic digestion and the characteristics of bacterial community in acidogenic stage.
 363 Bioresour. Technol. 266, 166–175. <https://doi.org/10.1016/j.biortech.2018.06.061>
 364 Calabrò, P.S., Fazzino, F., Folino, A., Scibetta, S., Sidari, R., 2019. Improvement of semi-
 365 continuous anaerobic digestion of pre-treated orange peel waste by the combined use of
 366 zero valent iron and granular activated carbon. Biomass and Bioenergy 129, 105337.
 367 <https://doi.org/10.1016/J.BIOMBIOE.2019.105337>
 368 Calabrò, P.S., Fazzino, F., Sidari, R., Zema, D.A., 2020. Optimization of orange peel waste
 369 ensiling for sustainable anaerobic digestion. Renew. Energy 154, 849–862.
 370 <https://doi.org/10.1016/j.renene.2020.03.047>
 371 Calabrò, Paolo S., Fòlino, A., Tamburino, V., Zappia, G., Zema, D.A., 2018. Increasing the
 372 tolerance to polyphenols of the anaerobic digestion of olive wastewater through
 373 microbial adaptation. Biosyst. Eng. 172, 19–28.
 374 <https://doi.org/10.1016/j.biosystemseng.2018.05.010>
 375 Calabrò, P.S., Panzera, M.F., 2018. Anaerobic digestion of ensiled orange peel waste:
 376 Preliminary batch results. Therm. Sci. Eng. Prog.
 377 <https://doi.org/10.1016/j.tsep.2017.12.011>
 378 Calabrò, P.S., Panzera, M.F., 2017. Biomethane production tests on ensiled orange peel
 379 waste. Int. J. Heat Technol. 35, S130–S136. <https://doi.org/10.18280/ijht.35Sp0118>
 380 Calabrò, P. S., Paone, E., Komilis, D., 2018. Strategies for the sustainable management of
 381 orange peel waste through anaerobic digestion. J. Environ. Manage. 212, 462–468.
 382 <https://doi.org/10.1016/j.jenvman.2018.02.039>
 383 de la Torre, I., Martin-Dominguez, V., Acedos, M.G., Esteban, J., Santos, V.E., Ladero, M.,
 384 2019. Utilisation/upgrading of orange peel waste from a biological biorefinery
 385 perspective. Appl. Microbiol. Biotechnol. <https://doi.org/10.1007/s00253-019-09929-2>
 386 Gou, C., Yang, Z., Huang, J., Wang, H., Xu, H., Wang, L., 2014. Effects of temperature and

organic loading rate on the performance and microbial community of anaerobic co-digestion of waste activated sludge and food waste. *Chemosphere*.
<https://doi.org/10.1016/j.chemosphere.2014.01.018>

Gumina, B., Espro, C., Galvagno, S., Pietropaolo, R., Mauriello, F., 2019. Bioethanol Production from Unpretreated Cellulose under Neutral Selfsustainable Hydrolysis/Hydrogenolysis Conditions Promoted by the Heterogeneous Pd/Fe₃O₄ Catalyst. *ACS Omega* 4, 352–357. <https://doi.org/10.1021/acsomega.8b03088>

Liang, S., Gliniewicz, K., Gerritsen, A.T., McDonald, A.G., 2016. Analysis of microbial community variation during the mixed culture fermentation of agricultural peel wastes to produce lactic acid. *Bioresour. Technol.* 208, 7–12.
<https://doi.org/10.1016/j.biortech.2016.02.054>

Luque, R., Clark, J.H., 2013. Valorisation of food residues: waste to wealth using green chemical technologies. *Sustain. Chem. Process.* 1, 10. <https://doi.org/10.1186/2043-7129-1-10>

Mahato, N., Sharma, K., Sinha, M., Baral, E.R., Koteswararao, R., Dhyani, A., Hwan Cho, M., Cho, S., 2020. Bio-sorbents, industrially important chemicals and novel materials from citrus processing waste as a sustainable and renewable bioresource: A review. *J. Adv. Res.* <https://doi.org/10.1016/j.jare.2020.01.007>

Malara, A., Frontera, P., Bonaccorsi, L., Antonucci, P.L., 2018. Hybrid Zeolite SAPO-34 Fibres Made by Electrospinning. *Mater. (Basel, Switzerland)* 11.
<https://doi.org/10.3390/ma11122555>

Mamlouk, D., Gullo, M., 2013. Acetic Acid Bacteria: Physiology and Carbon Sources Oxidation. *Indian J. Microbiol.* <https://doi.org/10.1007/s12088-013-0414-z>

Moraci, N., Calabrò, P.S., Suraci, P., 2011. Long-term efficiency of Zero-Valent iron - Pumice Granular mixtures for the removal of Copper or Nickel from groundwater. *Soils*

and Rocks 34, 129–138.

Nsair, A., Cinar, S.O., Alassali, A., Qdais, H.A., Kuchta, K., 2020. Operational Parameters of Biogas Plants: A Review and Evaluation Study. *Energies*.

<https://doi.org/10.3390/en13153761>

Ohkouchi, Y., Inoue, Y., 2006. Direct production of l(+)-lactic acid from starch and food wastes using *Lactobacillus manihotivorans* LMG18011. *Bioresour. Technol.* 97, 1554–1562. <https://doi.org/10.1016/j.biortech.2005.06.004>

Paone, E., Beneduci, A., Corrente, G.A., Malara, A., Mauriello, F., 2020. Hydrogenolysis of aromatic ethers under lignin-first conditions. *Mol. Catal.* 497, 111228.

<https://doi.org/10.1016/j.mcat.2020.111228>

Pognani, M., Barrena, R., Font, X., Sánchez, A., 2012. Effect of freezing on the conservation of the biological activity of organic solid wastes, *Bioresource Technology*. Elsevier.

Raccach, M., 1985. Manganese and Lactic Acid Bacteria. *J. Food Prot.* 48, 895–898.

<https://doi.org/10.4315/0362-028x-48.10.895>

Racchach, M., Marshall, P.S., 1985. Effect of Manganese Ions on the Fermentative Activity of Frozen-Thawed *Lactobacilli*. *J. Food Sci.* 50, 665–668. <https://doi.org/10.1111/j.1365-2621.1985.tb13768.x>

Ricci, A., Diaz, A.B., Caro, I., Bernini, V., Galaverna, G., Lazzi, C., Blandino, A., 2019.

Orange peels: from by-product to resource through lactic acid fermentation. *J. Sci. Food Agric.* 99, 6761–6767. <https://doi.org/10.1002/jsfa.9958>

Satari, B., Karimi, K., 2018. Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. *Resour. Conserv. Recycl.* 129, 153–167. <https://doi.org/10.1016/j.resconrec.2017.10.032>

Sharma, K., Mahato, N., Cho, M.H., 2017. Converting citrus wastes into value-added products : Economic and environmently friendly approaches. *Nutrition* 34, 29–46.

437 <https://doi.org/10.1016/j.nut.2016.09.006>

438 Sidari, R., Martorana, A., De Bruno, A., 2019. Effect of brine composition on yeast biota
 439 associated with naturally fermented *Nocellara messinese* table olives. *LWT*.
 440 <https://doi.org/10.1016/j.lwt.2019.04.010>

441 Sivagurunathan, P., Kumar, G., Park, Jeong Hoon, Park, Jong Hun, Park, H.D., Yoon, J.J.,
 442 Kim, S.H., 2016. Feasibility of enriched mixed cultures obtained by repeated batch
 443 transfer in continuous hydrogen fermentation. *Int. J. Hydrogen Energy* 41, 4393–4403.
 444 <https://doi.org/10.1016/j.ijhydene.2015.06.133>

445 Terpstra, M., Ahrens, M., Smith, S., 2001. Effect of manganese on *Lactobacillus casei*
 446 fermentation to produce lactic acid from whey permeate. *Process Biochem.* 36, 671–675.
 447 [https://doi.org/10.1016/S0032-9592\(00\)00265-X](https://doi.org/10.1016/S0032-9592(00)00265-X)

448 Tian, Z., Cabrol, L., Ruiz-Filippi, G., Pullammanappallil, P., 2014. Microbial ecology in
 449 anaerobic digestion at agitated and non-agitated conditions. *PLoS One*.
 450 <https://doi.org/10.1371/journal.pone.0109769>

451 Tokuhiro, K., Ishida, N., Kondo, A., Takahashi, H., 2008. Lactic fermentation of cellobiose
 452 by a yeast strain displaying β -glucosidase on the cell surface. *Appl. Microbiol.*
 453 *Biotechnol.* 79, 481–488. <https://doi.org/10.1007/s00253-008-1454-x>

454 United States Department of Agriculture - Foreign Agricultural Service, 2020. Citrus: World
 455 Markets and Trade.

456 Wilkins, M.R., Suryawati, L., Maness, N.O., Chrz, D., 2007. Ethanol production by
 457 *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* in the presence of orange-peel
 458 oil. *World J. Microbiol. Biotechnol.* 23, 1161–1168. [https://doi.org/10.1007/s11274-007-](https://doi.org/10.1007/s11274-007-9346-2)
 459 9346-2

460 Xu, C., Paone, E., Rodríguez-Padrón, D., Luque, R., Mauriello, F., 2020. Reductive catalytic
 461 routes towards sustainable production of hydrogen, fuels and chemicals from biomass

derived polyols. *Renew. Sustain. Energy Rev.* 127, 109852.
<https://doi.org/10.1016/j.rser.2020.109852>

Yan, L., Gao, Y., Wang, Y., Liu, Q., Sun, Z., Fu, B., Wen, X., Cui, Z., Wang, W., 2012. Diversity of a mesophilic lignocellulolytic microbial consortium which is useful for enhancement of biogas production. *Bioresour. Technol.* 111, 49–54.
<https://doi.org/10.1016/j.biortech.2012.01.173>

Yu, J., Zhao, Ye, Liu, B., Zhao, Yubin, Wu, J., Yuan, X., Zhu, W., Cui, Z., 2016. Accelerated acidification by inoculation with a microbial consortia in a complex open environment. *Bioresour. Technol.* 216, 294–301. <https://doi.org/10.1016/j.biortech.2016.05.093>

Zema, D.A., Calabrò, P.S., Folino, A., Tamburino, V., Zappia, G., Zimbone, S.M., 2018. Valorisation of citrus processing waste: A review. *Waste Manag.* 80, 252–273.
<https://doi.org/10.1016/J.WASMAN.2018.09.024>

Zema, D.A.A., 2017. Planning the optimal site, size, and feed of biogas plants in agricultural districts. *Biofuels, Bioprod. Biorefining* 11, 454–471. <https://doi.org/10.1002/bbb.1757>

Zema, D.A.D.A., Andiloro, S., Bombino, G., Tamburino, V., Sidari, R., Caridi, A., 2012. Depuration in aerated ponds of citrus processing wastewater with a high concentration of essential oils. *Environ. Technol.* 33, 1255–1260.
<https://doi.org/10.1080/09593330.2011.618938>

Zerva, I., Remmas, N., Ntougias, S., 2019. Diversity and Biotechnological Potential of Xylan-Degrading Microorganisms from Orange Juice Processing Waste. *Water* 11, 274.
<https://doi.org/10.3390/w11020274>