ANALYSIS OF SCIENTIFIC WORK

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CONTENTS

A. PUBLICATIONS IN REFEREED JOURNALS

1. **Sarris D,** Stoforos N. G., Mallouchos A., Kookos I. K., Koutinas A. A., Aggelis G., Papanikolaou S. (2017). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. Eng Life Sci (In Press; DOI: 10.1002/elsc.201600225).

   Yarrowia lipolytica ACA-YC 5033 was grown on glucose-based media in which high amounts of olive mill wastewaters (OMWs) had been added. Besides shake-flask aseptic cultures, trials were also performed in previously pasteurized media while batch bioreactor experiments were also done. Significant decolorization (58%) and remarkable removal of phenolic compounds (51% w/w) occurred, with the latter being amongst the highest ones reported in the international literature, as far as yeasts were concerned during their growth on phenol-containing media. In nitrogen-limited flask fermentations the microorganism produced maximum citric acid quantity   19.0 g/L [simultaneous yield of citric acid produced glucose consumed (YCit/Glc)   0.74 g/g]. Dry cell weight (DCW) values decreased at high phenol-containing media, but, on the other hand, the addition of OMWs induced reserve lipid accumulation.Maximumcitric acid concentration achieved (52.0 g/L; YCit/Glc   0.64 g/g) occurred in OMW-based high sugar content media (initial glucose added at   80.0 g/L). The bioprocess was successfully simulated by a modified logistic growth equation. A satisfactory fitting on the experimental data occurred while the optimized parameter values were found to be similar to those experimentally measured. Finally, a non-aseptic (previously pasteurized) trial was performed and its comparison with the equivalent aseptic experiment revealed no significant differences. Yarrowia lipolytica hence can be considered as a satisfactory candidate for simultaneous OMWs bioremediation and the production of added-value compounds useful for the food industry.

2. Dourou M., Kancelista A., Juszczyk, P., **Sarris D.**, Bellou S., Triantaphyllidou I-E, Rywinska A., Papanikolaou S., Aggelis G (2016). Bioconversion of olive mill wastewater into high-added value products. J Cleaner Prod, 169, 957-969.

   Olive mill wastewater (OMW) contains a variety of assimilable carbon sources, and therefore can be regarded as fermentation medium for the production of added-value products, rather than as a waste material. In this study, the biotechnological valorization of OMW (enriched with other low cost carbon sources) for lipid, mannitol, citric acid and ethanol production was conducted using selected yeast strains. *L. starkeyi* and *Y. lipolytica* (strains A6 and S11), showed a noteworthy ability to produce lipids cultivated on OMW based media (i.e. 24.5%, 14.9% and 16.5%, respectively). Oleic (Δ9C18:1) acid was the major fatty acid in lipids produced by the above mentioned strains, suggesting a selective uptake of Δ9C18:1 from OMWs lipids. A6 strain produced also mannitol in considerable amounts (i.e. 13.4 g/L) during fermentation on OMW enriched with glycerol. *Y. lipolytica* LGAM S (7) produced high quantities of citric acid (i.e. 30.3 g/L), cultivated in flasks on OMW enriched with glycerol. *C. tropicalis* LFMB 16 produced 21.9 g/L of ethanol cultivated in bioreactor on OMW enriched with glucose, while S. cerevisiae MAK-1 produced 31.3 g/L of ethanol cultivated under non-aseptic conditions in bioreactor on OMW based media. Remarkable phenolic removal was performed by *Y. lipolytica* (strains A6 and S11), *C. tropicalis* and *S. cerevisiae* under non-aseptic conditions, while color removal was performed by *C. tropicalis* and *S. cerevisiae*. It is concluded that *L. starkeyi*, *Y. lipolytica*, *C. tropicalis* and *S. cerevisiae* could be used utilized for the production of high-value metabolites with biotechnological interest using OMW based media, in parallel with color and phenolic removal, providing another option in the OMW management.

3. **Sarris, D.**, Papanikolaou, S. (2015). Biotechnological production of ethanol: Biochemistry, processes and technologies. Eng Life Sci, 16 (4), 307-329.

The majority of the environmental problems arise from the use of conventional energy sources. The liability of such problems along with the reduction of fossil energy resources has led to the global need for alternative renewable energy sources. Using renewable biofuels as energy sources is of remarkable and continuously growing importance. Producing bioethanol through conversion of waste and residual biomass can be a viable and important perspective. In the first part of this review, general concepts, approaches and considerations concerning the utilization of the most important liquid biofuels, namely biodiesel and bioethanol, are presented. Unlike biodiesel (specifically first generation biodiesel), the production of bioethanol is exclusively based on the utilization of microbial technology and fermentation engineering. In the second part of this review, the biochemistry of ethanol production, with regards to the use of hexoses, pentoses or glycerol as carbon sources, is presented and critically discussed. Differences in the glycolytic pathways between the major ethanol-producing strains (*Saccharomyces cerevisiae* and *Zymomonas mobilis*) are presented. Regulation between respiration and fermentation in ethanol-producing yeasts, viz. effects "Pasteur", "Crabtree", "Kluyver" and "Custers", is discussed. Xylose and glycerol catabolism related with bioethanol production is also depicted and commented. The technology of the fermentation is presented along with a detailed illustration of the substrates used in the process and in pretreatment of lignocellulosic biomass, and the various fermentation configurations employed (separate hydrolysis and fermentation, simultaneous saccharification and fermentation, simultaneous saccharification and co-fermentation, and consolidated bioprocessing). Finally, the production of bioethanol under non-aseptic conditions is presented and discussed.

4. **Sarris, D.**, Matsakas, L., Aggelis, G., Koutinas, A. A., Papanikolaou, S. (2014). Aerated vs non-aerated conversions of molasses and olive mill wastewaters blends into bioethanol by *Saccharomyces cerevisiae* under non-aseptic conditions. Ind Crops Prod, 56, 83-93.
(First report in international literature for the biotechnological treatment and valorization of blends of molasses and olive mill waste-waters)

The ability of *Saccharomyces cerevisiae* MAK-1 to convert blends of molasses and olive mill wastewaters (OMWs) into compounds of higher added-value under aerated and non-aerated conditions was studied in the current investigation. Noticeable decolorization (up to 60%) and moderate removal of phenolic compounds (up to 28%, w/w) was observed. Under aerated conditions in non-sterile shake-flask cultures, cultures in molasses-based media in which supplementation with OMWs had been performed did not significantly decrease ethanol and biomass production in comparison with control experiments (cultures in which no OMWs had been added). Ethanol of 34.3 g L^{-1} (with simultaneous yield of ethanol produced per sugar consumed of ~0.40 g g^{-1}) and biomass of 7.3 g L^{-1} (with yield of ~0.08 g g^{-1}) was observed. Under similar aerated bioreactor cultures, biomass production (up to 5.7 g L^{-1} with yield of biomass produced per sugar consumed of ~0.07 g g^{-1}) decreased while, on the other hand, ethanol biosynthesis was notably enhanced (up to 41.8 g L^{-1} with yield of ethanol produced of ~0.49 g g^{-1} – value very close to the maximum theoretical one). Comparing non-sterile aerated with non-aerated bioreactor experiments, biomass production showed some slight increase and ethanol production slightly increased in the latter case. It is concluded that *S. cerevisiae* MAK-1 is a microorganism of importance amenable for simultaneous OMWs remediation and production of added-value compounds.

5. Bellou, S., Makri, A., **Sarris, D.**, Michos, K., Rentoumi, P., Celik, A., Papanikolaou, S., Aggelis, G. (2014). The olive mill wastewater as substrate for single cell oil production by Zygomycetes. J Biotechnol, 170, 50-59.

   The conversion of olive mill wastewater (OMW) into high added value lipids containing polyunsaturated fatty acids (PUFA), in parallel with a significant phenolic removal by selected strains of Zygomycetes, is reported here for the first time. The growth of *Mortierella isabellina*, *Mortierella ramanniana*, *Cunninghamella echinulata*, *Mucor* sp., *Thamnidium elegans* and *Zygorhynchus moelleri* on solidified media was not significantly affected by the presence of OMW used in the growth medium up to 50% (v/v). Kinetic parameter values and conversion yields, estimated using a mathematical model which was fitted on the experimental data originated from submerged cultures, shows the ability of some Zygomycetes (i.e. *T. elegans* and *Z. moelleri*) to grow on OMW and accumulate storage material, i.e. lipids rich in PUFA, and these findings open new perspectives in OMW management and valorization. In liquid media containing OMW as sole carbon source, *T. elegans* and *Z. moelleri* produced 4.4 and 3.5 g/L cell mass in surface (SC) and submerged (SMC) cultures, respectively, containing around 60% (w/w) of lipids. Oleic and palmitic acids were the predominant fatty acids. Gamma-linolenic acid was found in high percentages (up to 17.7%, w/w) in the lipid of *Z. moelleri*, in SMC with OMW as sole carbon source, while PUFA biosynthesis was not favored in SC.

6. **Sarris, D.**, Giannakis, M., Philippoussis, A., Komaitis, M., Koutinas, A. A., Papanikolaou, S. (2013). Conversions of olive mill wastewater-based media by *Saccharomyces cerevisiae* through sterile and non-sterile bioprocesses. J Chem Technol Biotechnol, 88, 958-969.

   Olive mill wastewaters (OMWs) are an important residue and several methods have been proposed for their treatment. Remarkable decolorization (~63%) and phenol removal (~34% w/w) from OMW was achieved. In glucose-based flask sterile cultures, enrichment with OMWs increased ethanol and biomass production compared with cultures without OMWs added. Flask sterile and un-sterilized cultures demonstrated similar kinetic results. Batch-bioreactor trials performed showed higher ethanol and lower biomass quantities compared with the respective shake-flask experiments, while cultures used under un-sterilized conditions revealed equivalent results to the sterile ones. In non-sterile bioreactor cultures, OMWs addition enhanced biomass production in comparison with culture with no OMWs added, whereas ethanol biosynthesis was not affected. The maximum ethanol quantity achieved was 52 g L^{-1} (conversion yield per sugar consumed of 0.46 g g^{-1}) in a batch bioreactor non-sterilized trial with OMW–glucose enriched medium used as substrate, that presented initial reducing sugars concentration at ~115 g L^{-1}. Fatty acid analysis of cellular lipids demonstrated that in OMW-based media, cellular lipids containing increased concentrations of oleic and linoleic acid were produced in comparison with cultures with no OMWs added. *S. cerevisiae* simultaneously produced bio-ethanol and biomass and detoxified OMWs, under non-sterile conditions.

7. **Sarris, D.**, Galiotou-Panayotou, M., Koutinas, A. A., Komaitis, M., Papanikolaou, S. (2011). Citric acid, biomass and cellular lipid production by *Yarrowia lipolytica strains* cultivated on olive mill wastewater-based media. J Chem Technol Biotechnol, 86, 1439-1448.

   Olivemill wastewaters (OMWs) are an important residue and several physico-chemical and/or biotechnological methods have been proposed for their treatment. The ability of three *Yarrowia lipolytica* strains to grow on and convert glucose-enriched OMWs into added-value compounds in carbon- and nitrogen-limited shake-flask cultures was assessed. Remarkable decolorization (up to 63%) and non-negligible removal of phenolic compounds (up to 34%, w/w) occurred. In nitrogen-limited cultures, the accumulation of cellular lipids was favored by OMW addition into the medium. In contrast, although remarkable quantities of citric acid (Cit) were produced in control experiments (cultures without OMW addition), in which Cit up to 18.9 g L^{-1} was produced with yield of Cit synthesized per sugar consumed ~0.73 g g^{-1}), adaptation of cultures to media supplemented with

OMWs reduced the final Cit quantity and conversion yield values achieved. In OMW-based media, the highest concentration of citric acid produced was 18.1 g L^{-1}, with conversion yield ~0.51 g g^{-1}. In carbon-limited cultures, despite the presence of inhibitory compounds in the medium (e.g. phenols), biomass production was enhanced with the addition of OMWs. The highest biomass concentration achieved was 12.7 g L^{-1}, with biomass conversion yield per sugar consumed ~0.45 g g^{-1}. Fatty acid analysis of cellular lipid produced demonstrated that adaptation of all strains in OMW-based media favored the synthesis of cellular lipids that contained increased concentrations of cellular oleic acid. The *Y. lipolytica* strains tested can be regarded as possible candidates for simultaneous OMWs remediation and production of added-value compounds.

8. André, A., Diamantopoulou, P., Philippoussis, A., **Sarris, D.**, Komaitis, M., Papanikolaou, S. (2010). Biotechnological conversions of bio-diesel derived waste glycerol into added-value compounds by higher fungi: production of biomass, single cell oil and oxalic acid. Ind Crops Prod, 31(2), 407-416.

   Waste bio-diesel derived glycerol was used as the sole carbon source by higher fungi; two *Lentinula edodes* strains were flask cultured in carbon-limited conditions and displayed satisfactory growth in media presenting weak agitation, pH 4.0 and temperature 25◦C. Maximum biomass of 5.2 g/l was produced. Mycelia were synthesized, containing around 0.1 g of fat per g of biomass, with linoleic acid ($^{\Delta 9,12}$C18:2) being the principal cellular fatty acid produced. Two *Aspergillus niger* strains were grown in nitrogen-limited flask cultures with constant nitrogen and two different initial glycerol concentrations into the medium. In 250-ml flask cultures, large-sized pellets were developed, in contrast with the trials performed in 2-l flasks. Nitrogen limitation led to oxalic acid secretion and intra-cellular lipid accumulation; in any case, sequential production of lipid and oxalic acid was observed. Initially, nitrogen limitation led to lipid accumulation. Thereafter, accumulated lipid was re-consumed and oxalic acid, in significant quantities, was secreted into the medium. In large-sized pellets, higher quantities of intra-cellular total lipid and lower quantities of oxalic acid were produced and vice versa. Maximum quantities of oxalic acid up to 20.5–21.5 g/l and lipid up to 3.1–3.5 g/l (corresponding to 0.41–0.57 g of fat per g of biomass) were produced. Lipid was mainly composed of oleic ($^{\Delta 9}$C18:1) and linoleic ($^{\Delta 9,12}$C18:2) acids.

9. André, A., Chatzifragkou, A., Diamantopoulou, P., **Sarris, D.**, Philippoussis, A., Galiotou-Panayotou, M., Komaitis, M., Papanikolaou, S. (2009). Biotechnological conversions of bio-diesel-derived crude glycerol by *Yarrowia lipolytica* strains. Eng Life Sci, 9(6), 468-478.

   In the present report, crude glycerol, waste discharged from bio-diesel production, was used as carbon substrate for three natural *Yarrowia lipolytica* strains (LFMB 19, LFMB 20 and ACA-YC 5033) during growth in nitrogen-limited submerged shake-flask experiments. In media with initial glycerol concentration of 30 g/L, all strains presented satisfactory microbial growth and complete glycerol uptake. Although culture conditions favored the secretion of citric acid (and potentially the accumulation of storage lipid), for the strains LFMB 19 and LFMB 20, polyol mannitol was the principal metabolic product synthesized (maximum quantity 6.0 g/L, yield 0.20–0.26 g per g of glycerol consumed). The above strains produced small quantities of lipids and citric acid. In contrast, *Y. lipolytica* ACA-YC 5033 produced simultaneously higher quantities of lipid and citric acid and was further grown on crude glycerol in nitrogen-limited experiments, with constant nitrogen and increasing glycerol concentrations (70–120 g/L). Citric acid and lipid concentrations increased with increment of glycerol; maximum total citric acid 50.1 g/L was produced (yield 0.44 g per g of glycerol) while simultaneously 2.0 g/L of fat were accumulated inside the cells (0.31 g of lipid per g of dry weight). Cellular lipids were mainly composed of neutral fraction, the concentration of which substantially increased with time. Moreover, in any case, the phospholipid fraction was more unsaturated compared with total and neutral lipids, while at the early growth step, microbial lipid was more rich in saturated fatty acids (e.g. C16:0 and C18:0) compared with the stationary phase.

10. **Sarris, D.**, Kotseridis, Y., Linga, M., Galiotou-Panayotou, M., Papanikolaou, S. (2009). Enhanced ethanol production, volatile compound biosynthesis and fungicide removal during growth of a newly isolated *Saccharomyces cerevisiae* strain on enriched pasteurized grape musts. Eng Life Sci, 9(1), 29-37.

    The kinetic behavior of a newly isolated *Saccharomyces cerevisiae* strain, grown on pasteurized grape musts enriched with industrial sugars, was studied after the addition of various concentrations [0.0 (reference), 0.4 and 2.4 mg/L] of the fungicide quinoxyfen to the medium. Batch-flask cultures were carried out. Significant quantities of biomass (10.0±0.8 g/L) were produced regardless of quinoxyfen addition to the medium; therefore, the addition of the fungicide did not seriously inhibit biomass production. Ethanol was synthesized in very high quantities in all trials (highest concentrations 106.4–119.2 g/L). A slight decrease of ethanol production in terms of both absolute value and conversion yield of ethanol produced per sugar consumed was, however, observed when the quinoxyfen concentration was increased. The addition of quinoxyfen led to significantly lower ethylic ester levels, which also pertains to the acetates analyzed in this study. Fusel alcohol synthesis seemed to be activated when 0.4 mg/L quinoxyfen was added, but at 2.4 mg/L of added fungicide, no statistically significant differences were observed compared with the control trial. Volatile acid levels did not present a uniform trend in relation with the added fungicide. Finally, the fermentation was accompanied by a significant reduction of the fungicide concentration (79–82 wt% fungicide removal).

B. PRESENTATIONS IN CONFERENCES

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2. **Sarris, D.**, Georgousis, M., Psarianos, D., Gardeli, Ch., Koutinas, A.A., Aggelis, G., Papanikolaou, S. Selection of yeast strains capable to assimilate xylose for the production of microbial lipids. 6th Greek lipid forum, 2015, p. 60.
3. **Sarris, D.**, Koutinas, A.A., Mallouchos, A., Aggelis, G., Papanikolaou, S. Production of biomass and cellular lipids during growth of yeasts on substrates based on blends of xylose and raw glycerol. 6th Greek lipid forum, 2015, p. 61.
4. **Sarris, D.**, Matsakas, L., Koutinas, A.A., Komaitis, M., Papanikolaou, S. Bio-ethanol production during growth of *Saccharomyces cerevisiae* MAK 1 on mixtures of molasses and olive mill wastewaters under non-sterile conditions. 5th Greek lipid forum, 2009, page 51.
5. **Sarris, D.**, Giannakis, M., Galiotou-Panayotou, M., Komaitis, M., Papanikolaou, S. Bioethanol and biomass production during growth of *Saccharomyces cerevisiae* MAK 1 on Olive oil Mill Wastewater-based media. Greek lipid forum, 2009.
6. **Σαρρής, Δ.**, Γιαννάκης, Μ., Γαλιώτου-Παναγιώτου, Μ., Κωμαΐτης, Μ., Παπανικολάου Σ. Βιοτεχνολογική παραγωγή αιθανόλης κατά την αύξηση του στελέχους *Saccharomyces cerevisiae* ΜΑΚ-1 σε υποστρώματα με βάση τα υγρά απόβλητα ελαιουργίας. 1ο Συνέδριο Γεωπονικής Βιοτεχνολογίας, 2009, σ. 47.
7. **Σαρρής, Δ.**, Γαλιώτου-Παναγιώτου, Μ., Κωμαΐτης, Μ., Παπανικολάου Σ. Βιοτεχνολογική παραγωγή κιτρικού οξέος και μικροβιακού λίπους κατά την επεξεργασία υποστρωμάτων με βάση τα υγρά απόβλητα ελαιουργίας από το στέλεχος *Yarrowia lipolytica* ACA-YC 5033. 1ο Συνέδριο Γεωπονικής Βιοτεχνολογίας, 2009, σ. 38.
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9. André, A., Diamantopoulou, P., **Sarris, D.**, Galiotou-Panayotou, M., Philippoussis, A., Papanikolaou, S. Bioconversion of crude glucerol, waste discharged after bio-diesel production process, into biomass, oxalic acid and microbial lipid. 6th Euro Fed Lipid, 2008, page 121.

10. **Sarris, D.**, Kotseridis, Y., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M. Production of bio-ethanol and removal of fungicide during growth of a newly isolated *Saccharomyces cerevisiae* strain on enriched grape musts. 4ο Διεθνές Συνέδριο Βιοτεχνολογίας (IGBF 4), 2007. **With distinction as the best conference publication**.
11. **Sarris, D.**, Kotseridis, Y., Rodis, P., Galiotou-Panayotou, M., Papanikolaou, S. Studies on the alcoholic fermentation of enriched grape musts by a newly isolated *Saccharomyces cerevisiae* strain: High production of bio-ethanol and fungicide removal. 2ο Πανελλήνιο Συνέδριο Βιοτεχνολογίας, 2007, pages 301-304.

C. IMPACT FACTOR OF PUBLICATIONS (Google Scholar)

Journal	Year	Impact Factor	Number of Publications	Total Impact Factor	Citations	Self-citations
Engineering in Life Sciences	2009, 2016, 2017	2.119	4	8.476	109	8
Industrial Crops and Products	2010, 2014	3.449	2	6.898	104	2
Journal of Chemical Technology and Biotechnology	2011, 2013	2.738	2	5.476	53	8
Journal of Cleaner Production	2016	4.959	1	4.959	2	0
Journal of Biotechnology	2014	2.667	1	2.667	23	2
	Grand Total		**10**	**28.476**	**293**	**28**
	h-index = 8 **i10-index = 8**					

D. CITATIONS (Google Scholar - Total: 259; Self-citations: 20)

Sarris D, Stoforos N. G., Mallouchos A., Kookos I. K., Koutinas A. A., Aggelis G., Papanikolaou S. (2017). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. Eng Life Sci (In Press; DOI: 10.1002/elsc.201600225).

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Dourou M., Kancelista A., Juszczyk, P., **Sarris D.**, Bellou S., Triantaphyllidou I-E, Rywinska A., Papanikolaou S., Aggelis G (2016). Bioconversion of olive mill wastewater into high-added value products. J Cleaner Prod, 139, 957-969.

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2. Sarris, D., Stoforos, N. G., Mallouchos, A., Kookos, I. K., Koutinas, A. A., Aggelis, G., & Papanikolaou, S. (2017). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. Eng Life Sci.

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2. Chen, H. Z., & Liu, Z. H. (2016). Enzymatic hydrolysis of lignocellulosic biomass from low to high solids loading. Eng Life Sci.
3. Dourou, M., Kancelista, A., Juszczyk, P., Sarris, D., Bellou, S., Triantaphyllidou, I.-E., Rywinska A., Papanikolaou S., Aggelis, G. (2016). Bioconversion of olive mill wastewater into high-added value products. *J Cleaner Prod*, 139, 957-969.
4. Francesconi, J. A., Oliva, D. G., & Aguirre, P. A. (2016). Flexible heat exchanger network design of an ethanol processor for hydrogen production. A model-based multi-objective optimization approach. Int J Hydrogen Energy.
5. Gohel, V., Ranganathan, K., & Duan, G. (2016). Single Temperature Liquefaction Process at Different Operating Phs to Improve Ethanol Production From Indian Rice and Corn Feedstock. Preparative Biochem Biotechnol. doi: x.doi.org/10.1080/10826068.2016.1244687
6. Jiang, L., Liu, H., Mu, Y., Sun, Y., & Xiu, Z. (2016). High tolerance to glycerol and high production of 1, 3-propanediol in batch fermentations by microbial consortium from marine sludge. Eng Life Sci.
7. Lee, J., Oh, J.-I., Ok, Y. S., & Kwon, E. E. (2017). Study on susceptibility of CO 2-assisted pyrolysis of various biomass to CO 2. Energy.
8. Lohri, C. R., Diener, S., Zabaleta, I., Mertenat, A., & Zurbrügg, C. (2017). Treatment technologies for urban solid biowaste to create value products: a review with focus on low-and middle-income settings. Rev Environ Sci Biotechnol, 1-50.
9. Papanikolaou, S., Rontou, M., Belka, A., Athenaki, M., Gardeli, C., Mallouchos, A., Zeng, A. P. (2016). Conversion of biodiesel-derived glycerol into biotechnological products of industrial significance by yeast and fungal strains. Eng Life Sci.
10. Sarris, D., Stoforos, N. G., Mallouchos, A., Kookos, I. K., Koutinas, A. A., Aggelis, G., & Papanikolaou, S. (2017). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. Eng Life Sci.
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E. ABSTRACT OF PhD THESIS

Biotechnological treatment of olive mill wastewaters-based media: production of added-value compounds with the use of strains of yeasts *Yarrowia lipolytica* and *Saccharomyces cerevisiae*.

The ability of two yeast species, *Yarrowia lipolytica* [strains ACA-YC 5028, ACA-YC 5033 and W29 (ATCC 20460)] and *Saccharomyces cerevisiae* (strain MAK-1), to simultaneously bioremediate (reduce phenolic content and color) olive mill wastewater (OMW)-based media and produce (high-) added value products (yeast biomass, citric acid, ethanol, cellular lipids) was assessed at the present study, which is divided in four main parts: In the first part of this work, the ability of three *Yarrowia lipolytica* strains to grow on and convert glucose-enriched OMWs into microbial mass, cellular lipids and citric acid in aseptic shake-flask cultures was assessed. Decolorization (~63%) and removal of phenolic compounds (~34% *w/w*) occurred. In nitrogen-limited cultures citric acid in non-negligible quantities was produced [maximum citric acid (Cit_{max}) ~18.1 g L^{-1}; total citric acid yield on glucose consumed ($Y_{Cit/Glc}$) ~0.51 g g^{-1}] but adaptation of cultures to media supplemented with OMWs reduced the final citric acid quantity and conversion yield values achieved. In contrast, the accumulation of cellular lipids was favored by OMWs addition compared to the control experiment (no OMWs addition). On the other hand, in carbon-limited cultures, insignificant amounts of citric acid were produced (as excpected) whereas, despite the presence of inhibitory compounds into the medium, biomass production [maximum biomass (X_{max}) ~13.0 g L^{-1}; dry cell weight yield on glucose consumed ($Y_{X/Glc}$) ~0.45 g g^{-1}] was enhanced with the addition of OMWs into the synthetic medium, as compared with the control experiment (no OMW addition). Fatty acid analysis of total cellular lipids produced demonstrated that for all strains, cultures in media supplemented with OMWs favored the biosynthesis of cellular lipids that contained increased concentrations of cellular oleic acid.

In the second part of this thesis, the ability of a selected *Y. lipolytica* strain that in the previous part had produced significant quantities of citric acid irrespective of the addition of OMWs into the medium, namely ACA-YC 5033, to grow on glucose-enriched OMWs was further studied. Higher quantities of OMWs as compared with the first part of the work were added, trials were also performed in pasteurized media (besides aseptic cultures), while equally batch bioreactor experiments were performed. Decolorization (~58%) and remarkable removal of phenolic compounds [up to 51% *w/w*, at the trial with initial phenolic compounds concentration (Ph_0) ~5.50 g L^{-1}] occurred. Such high value of phenolic compounds removal from the fermentation medium that occurred in the above-mentioned fermentation, was amongst the highest ones reported so far in the international literature concerning growth of yeasts on phenol-containing residues. In nitrogen-limited flask fermentations (in which Cit_{max}~19.0 g L^{-1}; $Y_{Cit/Glc}$~0.74 g g^{-1}), dry cell weight concentration was reduced proportionally to the phenolic content but the addition of OMWs, very interestingly, stimulated proportionally reserve lipid accumulation process [maximum total lipid (L_{max}) ~1.0 g L^{-1}; total lipid yield in biomass ($Y_{L/X}$) ~0.27 g g^{-1}] comparing to control experiments, suggesting that OMWs seemed to be a "lipogenic" medium. The overall maximum total citric acid concentration achieved (Cit_{max}~47.0 g L^{-1}; $Y_{Cit/Glc}$~0.67 g g^{-1}) occurred in the trial with the highest commercial sugar supplementation of OMW-based media (initial glucose concentration, Glc_0, ~80.0 g L^{-1}). On the other hand, cultures performed at high phenol content media (Ph_0~4.50 and 5.50 g L^{-1}) clearly inhibited the growth of the microorganism, but surprisingly enough lipid accumulation seemed to be stimulated by the addition of OMWs at these ratios. In carbon-limited fermentations, biomass production was enhanced by OMW addition. In the aspect of a potential scale-up of the technology and in order to reduce the cost of the proposed bioprocess, shake-flask and batch bioreactor experiments were performed in a previously pasteurized medium; comparing aseptic and pasteurized shake-flasks cultures, no significant differences were observed in kinetics (for both biomass and lipid production) while the assimilation rate of glucose (in g L^{-1} h^{-1}) seemed to be linear for both experiments, with glucose consumption rate being higher in the aseptic than the in pasteurized cultures. On the contrary reduction of citric acid production was observed in the pasteurized trial by both means of Citmax and $Y_{Cit/Glc}$ values. Comparing aseptic shake-flask and the respective aseptic bioreactor fermentations of OMW-based media (that presented almost equal Glc_0 and Ph_0 concentrations), biomass and lipid production were insignificantly enhanced in bioreactor trials whereas the strain reached its kinetics plateau earlier in shake-flasks than in bioreactor cultures. Glucose consumption rate was higher in the

shake-flask cultures. Concerning citric acid production, it seemed to decrease in the bioreactor cultures (by both means of Cit_{max} and $Y_{Cit/Glc}$ values).

At the third part of the manuscript, the ability of *Saccharomyces cerevisiae* strain MAK-1 to grow on and convert glucose-enriched OMWs into biomass, cellular lipids and ethanol in aseptic and non-aseptic shake-flask and batch bioreactor cultures was assessed. In general, aseptic and non-aseptic processes demonstrated similar kinetic results. Decolorization (∼63%) and phenol removal (∼34% *w/w*) from OMWs was achieved. In aseptic shake-flask cultures, enrichment with OMWs increased ethanol and biomass production. Batch-bioreactor trials performed showed higher ethanol [maximum ethanol concentration (EtOHmax) ~52.0 g L^{-1}; ethanol yield on glucose ($Y_{EtOH/Glc}$) ~0.46 g g^{-1}] and lower biomass quantities compared with the respective shake-flask experiments. Moreover, OMWs addition in batch-bioreactor trials significantly enhanced biomass production while it did not remarkably affect ethanol biosynthesis. Fatty acid analysis of cellular lipids demonstrated that in OMW-based media, cellular lipids contained increased concentrations of oleic and linoleic acid in accordance with the repective trials of the first part of this study when *Y. lipolytica* strains were used.

At the fourth part of this thesis, the ability of *Saccharomyces cerevisiae* strain MAK-1 to grow on and convert blends of OMWs and molasses into biomass and ethanol under non-aseptic shake-flask and batch bioreactor cultures was assessed. OMWs were used as simultaneous substrate and process water of the fermentations employed and molasses were used as low-cost substrate to supplement already existing OMWs sugar content for the enhancement of added value compounds production. The rationale of the utilization of OMW and molasses blends was to study the effect of these mixtures of residues upon the physiological and kinetic behavior of the strain, since in a potential scale-up of the process, OMWs could be used as tap water substitute for molasses dilution. This is the first time in the international literature in which such types of blends are used in a fermentation process. Decolorization (~60%) and removal of phenolic compounds (~28% *w/w*) occurred. Under aerobic conditions in shake-flask cultures, adaptation of cultures to molasses media supplemented with OMWs did not significantly decrease ethanol and biomass production. Under similar aerobic bioreactor cultures biomass production (Xmax~5.7 g L^{-1}; yield of dry cell weight per total sugars consumed ($Y_{X/TS}$) ~0.07 g g^{-1}) was reduced whereas ethanol production ($EtOH_{max}$~42.0 g L^{-1}; $Y_{EtOH/TS}$~0.49 g g^{-1}) significantly increased as compared with the flask cultures. Comparing aerobic with anaerobic bioreactor experiments, biomass production showed some slight decrease whereas ethanol production slightly increased in the latter case.

The yeast strains tested in this study could be regarded as possible candidates for simultaneous OMWs remediation and production of (added-) value compounds, in some cases under completely non-aseptic conditions.

Publications arising from PhD Thesis research:

1. Sarris D, Stoforos N. G., Mallouchos A., Kookos I. K., Koutinas A. A., Aggelis G., Papanikolaou S. (2016). Production of added-value metabolites by *Yarrowia lipolytica* growing in olive mill wastewater-based media under aseptic and non-aseptic conditions. *Eng Life Sci* (In Press).
2. Sarris, D., Papanikolaou, S. (2016). Biotechnological production of ethanol: Biochemistry, processes and technologies. Eng Life Sci, 16 (4), 307-329.
3. Sarris, D., Matsakas, L., Aggelis, G., Koutinas, A. A., & Papanikolaou, S. (2014). Aerated vs non-aerated conversions of molasses and olive mill wastewaters blends into bioethanol by *Saccharomyces cerevisiae* under non-aseptic conditions. *Ind Crops Prod, 56*, 83-93.
4. Sarris, D., Giannakis, M., Philippoussis, A., Komaitis, M., Koutinas, A. A., & Papanikolaou, S. (2013). Conversions of olive mill wastewater-based media by *Saccharomyces cerevisiae* through sterile and non-sterile bioprocesses. *J Chem Technol Biotechnol, 88,* 958-969.
5. Sarris, D., Galiotou-Panayotou, M., Koutinas, A. A., Komaitis, M., & Papanikolaou, S. (2011). Citric acid, biomass and cellular lipid production by *Yarrowia lipolytica* strains cultivated on olive mill wastewater-based media. *J Chem Technol Biotechnol, 86*, 1439-1448.

F. ABSTRACT OF MSc THESIS

Studies on the alcoholic fermentation of enriched grape musts by the newly isolated *Saccharomyces cerevisiae* strain MAK 1: High production of ethanol and fungicide removal.

With the continuous increase of the world's population and the predominance of industrialization, the generation of energy deriving from various renewable or non-renewable resources is of significant importance. Utilization of various renewable bio-fuels, such as bioethanol, as energy sources has become of remarkable and with continuous growing significance.

Ethanol as fuel is considered as one of the most important renewable energy due to its economic and environmental benefits. Therefore, the discovery of new naturally occurring or the "construction" of new "over-producing" strains, as well as the optimisation of ethanol production in various fermentation configurations in order to achieve high yields, final product concentrations and high volumetric productivities is of high importance in our days.

Aim of the current investigation can be divided in two major points: to study the biochemical behaviour of a newly isolated and still not studied Saccharomyces cerevisiae strain MAK 1, as well as to assess the potentiality of the strain to remove the fungicide quinoxyfen, from the culture medium during fermentation.

A non-sterilized, enriched red grape must with initial sugar concentration 240 ± 10 g/L was used as substrate. The concentrations of quinoxyfen in the mixtures were 0, 0.4 and 2.4 mg/L respectively. All fermentations were carried out in batch mode and aerated condition (agitated flasks).

Significant uptake of sugars and quantities of biomass (xmax = 9.5 ± 1.0 g/L) were produced in all cases regardless of the addition of quinoxyfen. In addition, ethanol was synthesized in very high quantities (maximum concentrations ranging between 106.4 and 119.2 g/L, decreasing though when increasing the quinoxyfen concentration).

No significant differences were observed in the production of glycerol, regardless the fungicide addition whilst no citric or no acetic acid were detected.

It was observed a quinoxyfen residue removal (due to agitation) in both of the fungicide control experiments (0.4 mg/L and 2.4 mg/L) at about 20 % (w/w) and 36% (w/w) respectively. Moreover, removals of 79% (w/w), referring to the fermentation done.

Publication arising from MSc Thesis research:

1. Sarris, D., Kotseridis, Y., Linga, M., Galiotou-Panayotou, M., & Papanikolaou, S. (2009). Enhanced ethanol production, volatile compound biosynthesis and fungicide removal during growth of a newly isolated *Saccharomyces cerevisiae* strain on enriched pasteurized grape musts. *Eng Life Sci, 9*(1), 29-37.

G. ABSTRACT OF BSc THESIS

Comparison of fast and classic aging of red wine Aghiorghitiko

Aim of this thesis was the study of fast aging of a red wine with the use of oak wood chips and the comparison with classic aging in an oak cask. Regarding fast aging, oak wood chips of certain dimensions were used and toasted in three levels (light, medium, heavy) in three stainless steel tanks of known volume. The wood chips remained in contact with the wine for thirty two days during which, sampling took place. The samples (conditions of fast aging with wood chips, aging in casks and blank experiment) were analyzed in a GC apparatus via a solid phase micro-extraction (SPME) analysis protocol. The volatiles were taken from the sample head space. Research was focused on the study of furfural, guaiacol, oak lactone and eugenol, which are extracted from wood towards wine.