E- PROCEEDINGS

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EDITORS' PREFACE

The **5th International Conference on Small and Decentralized Water and Wastewater Treatment Plants (SWAT**) was co-organized by the Department of Chemistry (Laboratory of Chemical & Environmental Technology), Aristotle University of Thessaloniki, the Department of Civil Engineering (Division of Hydraulics and Environmental Engineering), Aristotle University of Thessaloniki, Greece and the Food Technology Department, Alexander Technological Educational Institute of Thessaloniki.

It follows the tradition of previously successfully organized relevant SWAT Conferences (2006 in Portara/Pelion/Central Greece; 2008 in Skiathos Island; 2010 also in Skiathos Island; and 2013 in Volos/Central Greece).

The Conference took place in **Thessaloniki, Greece, in August 26-29, 2018**. It aimed to present the best available drinking water and wastewater treatment techniques, as applied to small municipalities, communities, settlements, hotels or industrial plants, based on environmental, financial, technical and social criteria.

The Conference Proceedings includes totally **145 papers**.

These papers have been categorized in **9 thematic areas**:

- **Small and Decentralised wastewater treatment plants and management** (18 papers)
- **Drinking water treatment and management for small settlements – municipalities** (23 papers)
- **Urban and industrial wastewater treatment** (27 papers)
- **Natural systems for wastewater treatment** (13 papers)
- **Sludge and waste management** (21 papers)
- **Water quality, monitring and pollution issues** (13 papers)
- **Recycling, Reuse and Reclamation of effluents** (9 papers)
- **Resource recovery and circular economy** (16 papers)
- **Legislation and policy issues** (5 papers)

Well known Greek and foreign scientists, researchers and administrative employees (from research, technical, construction and consulting companies) participated at the Conference, as well as representatives from local authorities.

Professor **Matthias Barjenbruch** from the Technical University of Berlin was invited to give a on

Professor **Yannis Krestenitis** from the Aristotle University of Thessaloniki and currently serving as EYATh (Water and Sewerage Authority of Thessaloniki) President

The editors would like to thank particularly:

- The sponsors of the conference for their financial support.
- The authors of the papers for the substantive and original character of their contribution.
- The scientists who were engaged in the review process of the submitted papers.
- All participants for their involvement in the exchange of knowledge, serving the conference's purpose and ensuring its success.

A. Zouboulis

A. Kungolos

P. Samaras

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Small and Decentralised wastewater treatment plants

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5th SWAT
INTERNATIONAL CONFERENCE ON SMALL AND DECENTRALIZED WATER AND WASTEWATER TREATMENT PLANTS

ISOLATION OF HYDROLYTIC MICROBIOTA FOR THE VALORIZATION OF ORANGE JUICE PROCESSING WASTEWATERS

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ABSTRACT

The orange juice processing industry is one of the most dynamic sectors of the Greek economy, producing 1.5% of orange juice worldwide. Although the produced wastewaters are characterized by high organic load, a significant part of this organic effluent has a high concentration of slowly hydrolyzed biomolecules, which resist biodegradation. For energy recovery from these agro-industrial wastewaters, the application of novel specialized microbiota capable of effectively hydrolyzing such substrates appears to be more economically feasible than the implementation of chemical methods. In this work, microorganisms capable of degrading slowly hydrolyzed biomolecules were isolated by the use of selective media and their enzymatic activities were determined through the performance of polygalacturonase assays. Among a total of 41 microbial strains, selected isolates exhibited high intracellular activities, indicating that such microbes can be used as starter cultures for the valorization of orange juice processing wastewaters. In conclusion, the biotechnological potential of these indigenous microorganisms was uncovered since these microbial agents can find applicability in this key agro-industrial sector of the Mediterranean region.

Keywords: *orange juice processing wastewater; microbial hydrolysis; polygalacturonase activity.*

1. INTRODUCTION

Agro-industrial wastes, which are produced in large quantities in Greece, are characterized by a high organic load, which makes their management particularly demanding [1]. In addition, the variability in the produced quantities, the dispersal of the production areas and the fragmentation of the cultivated areas make unfavorable the use of a centralized management system, and the operation of smaller processing systems appears to be a feasible approach.

On the other hand, agro-industrial wastes should not only be considered as a facing issue, but also as an opportunity to recover high added value products, taking into account that the cost of raw materials during the valorization of such waste is limited to the cost of transports. The exploitation of agro-industrial wastes is now feasible through the installation of small technologically specialized treatment systems.

Thus, the various agro-industrial wastes, through the implementation of the appropriate processing and management practices, can be used to recover components of commercial

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value, to identify novel biological factors, or to constitute the initial raw materials for the production of innovative high added value products. Therefore, agro-industrial wastes can be used for the recovery of antioxidants, the identification of novel micro-organisms of biotechnological interest, the production of alternative protein sources for animal feed, the generation of soil bulking agents and fertilizers and for energy recovery [2].

The cultivation of citrus and in particular oranges is considered as an important sector of the Greek economy [3]. The majority of these quantities is directed to the orange processing manufactures for the production of juice [4], while 50% of the dry weight of the oranges remains unprocessed as a residue (both in liquid and solid form) [5]. The main factor influencing the composition of orange juice waste is the quality of the fruits that depends on the cultivation practice, the degree of ripening, the citrus variety and the climate conditions. These residues are either disposed on site without treatment, a fact that causes deterioration of the soil quality and threatens the quality of local water reserves [6], or get used as a livestock feed, which, however, is considered as an economically infeasible management practice due to the low protein content of these wastes (below 6% in protein) [2].

Although the residues from the orange juice processing industry contain high concentrations of sugars, they also consist of various non-easily biodegradable biomolecules, like pectins. Thus, their hydrolysis is feasible only through the hydrolytic activity of specialized microbial strains [7]. In this work, we attempt to isolate and assess novel specialized microorganisms capable of hydrolyzing pectins, which can be used either as starter cultures or for the extraction of novel pectinases that can assist the hydrolysis step during energy recovery from such agro-industrial residues.

2. MATERIAL AND METHODS

Wastewater from orange juice processing industry, which was subjected to fermentation, was used as the raw material for the isolation of pectin-degrading microorganisms. The basic characteristics of the fermented orange juice processing wastewater are illustrated in Table 1.

Table 1. Physicochemical characterization of fermented orange juice processing wastewater.

Characteristics	Value
pH	4.22
SS (g/L)	4.45
VSS (g/L)	4.32
tCOD (mg/L)	96,000
dCOD (mg/L)	89,600
BOD_5 (mg/L)	18,270
TKN (mg/L)	518
Dissolved carbohydrates (mg/L)	2,209

The physicochemical characterization of fermented orange juice processing wastewater was carried out through the performance of the respective protocols for determination of SS, VSS, total COD (tCOD), dissolved COD (dCOD), BOD_5 and TKN described in detail in “Standards Methods for the Examination of Water and Wastewater” [8], whereas the estimation of carbohydrates in the fermented effluent was estimated by the sulfuric acid-

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phenol method of Dubois et al. [9].

To isolate pectinolytic microbial population from fermented orange juice processing wastewater, the spreadplate technique was employed, where, after tenfold dilution series, 0.2 ml from each dilution were spread onto appropriate agar plates. All Petri dishes were placed for 2 weeks at 28 °C and colonies were purified after successive spreading. The agar plates consisted of 10 g pectin, 1.4 g $(NH_4)_2SO_4$, 6 g K_2HPO_4, 2 g KH_2PO_4, 0.1 g $MgSO_4.7H_2O$ and 17 g agar per liter of medium prepared.

To determine polygalacturonase hydrolytic activities, each purified strain was inoculated into liquid medium comprising of 2 g/L polygalacturonic acid and the abovementioned ingredients (apart from agar) applied in the same concentrations. After effective growth of the cultures at 28^{o}C, cultures were centrifuged at high centrifuge speed and their cells were resuspended in acetate buffer. Furthermore, cell suspensions were lysed through sonication [10]. The polygalacturonase hydrolytic activities were estimated after incubation of the sonicated cell suspensions at 50^{o}C and determination of the free monomers by the DNS method, as previously presented in Miller [11].

3. WASTEWATER TREATMENT ISSUES

Pectinases are divided into acid and alkaline forms of these enzymes. Acid pectinases, which are mainly produced by *Asperigillus* spp., are applied in the vegetable oil extraction and clarification of pectin from juices as well as in maceration of plant tissues to produce pastes and purées, decreasing the viscosity in juice or vegetable biomass. On the contrary, alkaline pectinases can be used in the pretreatment of vegetable processing wastewater through removal of pectins, in bioscouring process in paper mill industry, in fiber fermentation and in the extraction of vegetable oils [12].

A total of 41 isolates were obtained and analyzed in order to determine their polygalacturonase activities in liquid cultures where pectin served as the sole carbon and energy source. The polygalacturonase activities of the microbial strains isolated from pectin-based media are presented in Figure 1.

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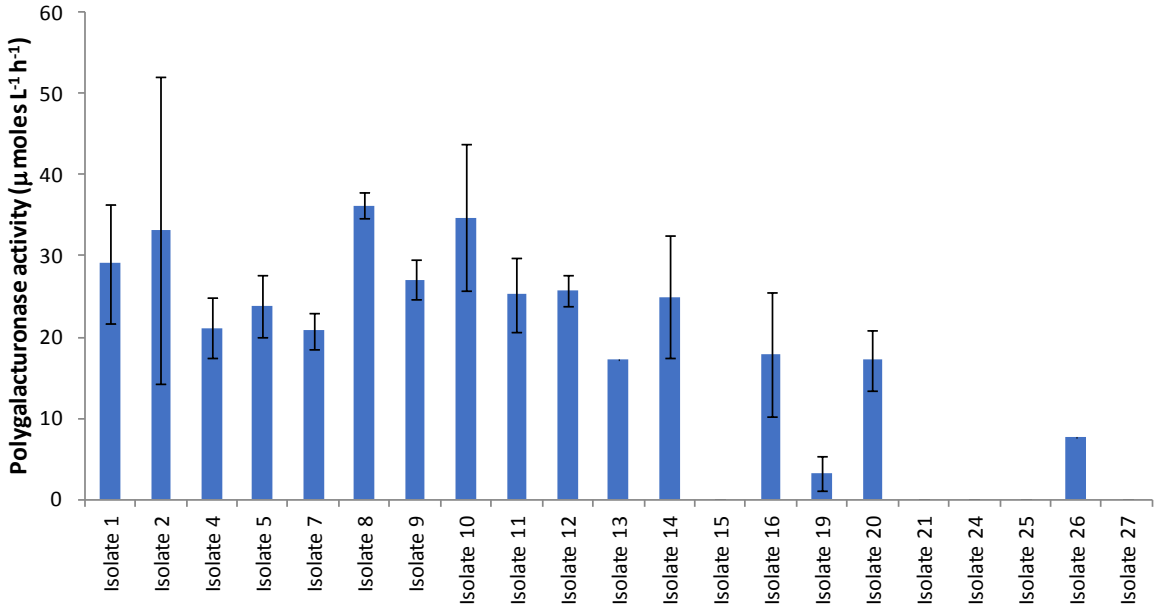
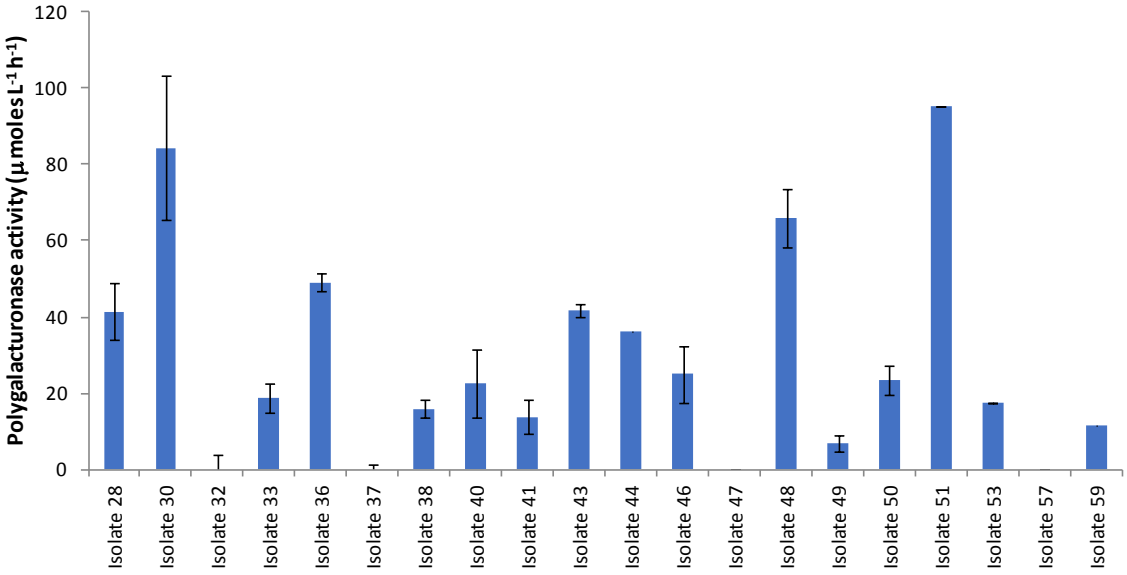


(A)



(B)

Figure 1. Polygalacturonase activities of bacteria isolated from pectin-based medium.

Despite the fact that a range of 9 microbial isolates grew on pectin-based media, these strains lacked polygacturonase activity, probably either due to their ability to metabolize agar without the assimilation of pectin or to an extremely slow hydrolytic ability, which did not permit the detection of such low hydrolysis rates. By contrast, the isolates 30, 48 and 51 exceed polygalacturonase activities of 60 µmoles L^{-1} h^{-1}, while several other microbial

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isolates (>12) exhibited polygalacturonase activities higher than 30 μmoles L^{-1} h^{-1}.

It is noticed that no reports on the diversity of microbial strains in orange processing wastewater expressing polygalacturonase activities exist in the literature and only well known fungal strains isolated from various (dissimilar) sources have been applied to specific research applications. For instance, Anand et al. [13] purified and characterized an exo-polygalacturonase from *Aspergillus flavus* MTCC 7589 in order to clarify fruit juice through pectin hydlolysis. Similarly, Rangarajan et al. [14] used both orange peel solids and orange peel extract as substrates in order to examine the pectinase production from the fungus *Aspergillus niger.*

The present report is the first that focuses on the functional role of pectinolytic strains isolated from fermented orange juice processing wastewater of low pH and remarkably high C/N ratio, which appears to be of a certain importance due to the polygalacturonase activity exhibited by certain microbial isolates. In addition, this study firstly attempts to quantitatively determine the hydrolytic ability of cultured microbiota that seem to be well acclimatized to the conditions of this wastewater, revealing the strong biotechnological potential of these agro-industrial wastes.

From biotechnological point of view, the isolates 30, 48 and 51 exerted high pectin hydrolysis activities that can be used in the valorization of orange processing wastewater. Such approach can increase the release of monomers in pectin and enhance energy production during anaerobic digestion of such effluents. Miran et al. [15] determined high polygalacturonase activity during the use of orange peel waste as energy substrate in microbial fuel cells. Apart from their use as a starter culture to enhance energy recovery from agro-industrial residues, such microbial isolates can find application in solid state fermentation of vegetative substrates in order to produce and purify valuable enzymes for the food processing industry.

4. CONCLUSIONS

In conclusion, the wastewater from the orange juice processing industry possesses a wide range of microbial strains that exhibited high polygalacturonase activity. In particular, the most effective pectin-degrading isolates obtained in this study can be used in the valorization of such agricultural residues in order to enhance the energy recovery during anaerobic digestion of the orange juice processing industry. Further experiments, which include the molecular characterization of these microbial isolates, will elucidate their phylogenetic position, whereas purification and structural analyses of these polygalacturonases will permit their commercialization.

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