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Biovalorization of Olive Mill Waste Water for the Production of Gellan Gum from *Sphingomonas paucimobilis*

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Authors' contributions

This work was carried out in close collaboration between the authors which designed and executed this study. Author KP designed this research at an initial stage and was responsible for the treatments of olive mill wastewater prior to fermentation, and author IG performed the literature search, the bioprocessing and fermentation experiments, managed the analyses of the study, and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of this work was to investigate and optimize the potential of olive mill waste water (OMWW) to be utilized as a substrate for the production of gellan gum by *Sphingomonas paucimobilis*.
Study Design: The study was divided in two phases, a preparatory phase for the removal of olive polyphenols and/or condensation of OMWW, and a bioprocessing-fermentation phase.
Place and Duration of Study: The study was conducted at the TEI of Thessaly from January 2013 to August 2015.
Methodology: OMWW was used as a fermentation substrate for production of gellan after dephenolization by microfiltration, thermal condensation, and addition of minerals/nitrogen sources

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and glycerol. *S. paucimobilis* was grown in this substrate under controlled process conditions in shake flasks and a 15 lt bioreactor. Biomass, gellan, sugars, phenol concentration and apparent viscosity of OMWW were evaluated.

Results: The results show that although *S. paucimobilis* can degrade olive polyphenols in OMWW, the removal of polyphenols is beneficial to gellan synthesis. The condensation (2/1, i.e. to the half of its initial volume) of the dephenolized OMWW also improved gellan production as it offered more sugars for polysaccharide production. After both dephenolization and 2/1 condensation gellan production increased by 50%. Yeast extract (1 g/l) was the preferable nitrogen source supplementation as it stimulated both cell growth and gellan synthesis. Glycerol (5 g/l) increased gellan formation and viscosity of the fermentation broth, which may indicate a key role of glycerol in gellan biosynthesis. An agitation of 500 rpm and aeration of 1 vvm resulted in the highest gellan production of 9.5 g/l in 63 h in the optimized and fortified OMWW after dephenolization and condensation. At 500 rpm an intense aeration of 2 vvm increased cell growth at the expense of gellan formation and resulted in a reduced viscosity.

Conclusion: After dephenolization, condensation, addition of some nitrogen source and glycerol, OMWW can be utilized as a substrate for efficient gellan production.

Keywords: Gellan gum; Sphingomonas paucimobilis; olive mill waste water utilization; olive polyphenol; biovalorization; dephenolization and phenol degradation; bioprocess optimization; microbial polysaccharide.

1. INTRODUCTION

Olive mill waste water (OMWW) is the dark liquid by-product of olive oil production after mechanical pressing of the olive fruit and centrifugation of the olive pulp, which contains the aqueous part of the olive fruit including sugars such as glucose, fructose, saccharose, as well as polyphenols, polyalcohols, minerals and other water-soluble components [1-4]. Its composition varies depending on the type and maturity of the olive fruit and the type of the processing system used in olive oil production (two-phase or three-phase system, the latter producing a larger volume of a more dilute waste water). This by-product is abundant in Mediterranean countries where most of the olive oil is produced and it is a major environmental pollutant due to illegal disposal to lakes, rivers, and land where it exhibits toxicity to plants and aquatic organisms, while its disposal without prior processing is accompanied with highly unpleasant odour [1-4]. The biodegradation of this by-product in conventional biological wastewater treatment unit is problematic, firstly because this waste has a high organic load (high COD and BOD values), secondly because it contains polyphenols at high concentrations which restrict microbial growth, and thirdly because most olive oil producing plants are small and scattered factories with seasonal operation which cannot afford the investment and processing costs of a biological treatment unit. These conditions show that the handling of OMWW is an environmental and economical issue that needs to be addressed. Most of the solutions proposed so far have focused on the improvement of the degradation of OMWW by chemical, enzymatic or biological means, in order to facilitate a safer disposal to the environment [4-8]. However, as mentioned above, this substrate contains fermentable sugars and can be used as a costless substrate for microbial fermentations, in order to produce valuable microbial metabolites, in other words to utilize or valorize this waste [9-13]. Microorganisms resistant to polyphenols or polyphenol-degrading can be used for this purpose or alternatively polyphenols can be removed or reduced by chemical methods or via microfiltration. Also, the relatively low sugar composition of the OMWW can be increased after appropriate condensation [1,4].

This strategy was at the core of our study, in other words the attempt to make the OMWW a more suitable substrate for fermentative production of gellan gum, by removing a large proportion of its polyphenol content and by increasing its sugar concentration. More specifically, we investigated the production of gellan gum by the polyphenol-degrading bacterium *Sphingomonas paucimobilis* in OMWW after several treatments or amplifications of the initial waste. Gellan gum is an industrial microbial exopolysaccharide of relatively high market value which is used as thickener, gelling agent, stabilizer, coating/encapsulating agent or in the formulation of edible films and has numerous applications in food, cosmetics and

pharmaceuticals [14-16]. It is composed of a repeating unit of two residues of D-glucose, and one of each residues of L-rhamnose and D-glucuronic acid, along with side chains of acyl groups (glycerate and acetate) and has a high molecular weight (~500.000 Dalton on average), which results in very high viscosity of the process medium or the solutions where it is added [14-17]. Gellan solutions, as well the fermentation broth during gellan production are pseudoplastic exhibiting strong shear-thinning behavior. The viscosity of gellan broth is a crucial quality parameter for gellan production (higher viscosity corresponds to higher thickening or gelling properties) which can be used for process monitoring and it is affected mainly by gellan concentration and molecular weight [14-18]. In order to achieve a high production of gellan a high carbon concentration and a low nitrogen concentration is usually desired [15-18], and from this point of view OMWW may be suitable as it is poor in nitrogen, while its sugar content can be increased after condensation. The aim of this work was to study the feasibility and to optimize the conditions of utilizing OMWW for gellan production.

2. MATERIALS AND METHODS

2.1 Olive Mill Waste Water (OMWW) as a Fermentation Substrate

The OMWW derived from different olive oil producing companies from the region of Thessaly, Greece, all of which used a three-phase production system. The OMWW from these sources was initially collected and kept refrigerated by the company Polyhealth S.A. (olive polyphenol producer, Larisa, Greece) which then supplied the necessary OMWW for our studies. The OMWW was refrigerated before following further potential treatments, which included dephenolization (partial but not complete removal of phenols) without use of organic solvent, via microfiltration through macroporous resins (XAD-4 and AMBERLITE FPX66), and also thermal condensation at 90℃ to produce half of the initial volume of the waste (condensation 2/1) and to increase its sugar content. Since this condensation also increases polyphenol concentration which may adversely affect the bioprocess, a diluted OMWW with an equal volume of distilled water (1/2 dilution) was also used. Several additional nutrients were added in some cases to the OMWW substrate, such as nitrogen sources which are scarce in this substrate, as well as glycerol, which may be able to enhance gellan synthesis. In all processes, the substrate was also supplemented with 2 g/l of potassium phosphate (1 g/l KH_2PO_4 + 1 g/l K_2HPO_4), which has been shown to stabilize the pH of the process medium and also enhance gellan synthesis [19], and the initial pH was adjusted to pH 7.0, in order to support cell growth. All chemicals used were of analytical grade.

2.2 Microorganisms

Sphingomonas paucimobilis ATCC 31461 was used for these experiments. The pure stock cultures were cultivated and maintained in test tubes with Yeast Malt Broth (YMB) containing 10 g/l of each of Yeast Extract and Malt Extract and in petri dishes with Yeast Malt Gellan (YMG) containing an additional 1.2% of Gelzan, an industrial form of gellan used as an agar substitute. The use of Gelzan in the YMG instead of agar was useful for the identification of potential colonies that may produce gellan lyases after prolonged storage. The potential gellan lyase-producing colonies will gradually liquefy the solid substrate in the petri dishes, and then be rejected as undesirable for gellan production, because they hydrolyse gellan and reduce its molecular weight and thus its gelling capacity [14,20]. The stock cultures were incubated at 30℃ for 3 days aerobically.

2.3 Production of Inoculum

In order to facilitate the adaptation and swift growth of *S. paucimobilis* to OMWW, the inoculum contained cells that were previously grown (pre-adapted) in OMWW-containing substrate. Specifically, solid substrates of YMG containing 25% OMWW were used for plating single colonies, one of which was used to inoculate a test tube with 10 ml of YMB containing 25% OMWW. After growth at 30℃ for 3 days this was used to inoculate 300 ml OMWW-based substrate in flasks in the shake flask experiments. In the experiments carried out in a 15 lt bioreactor with a 10 lt working volume, inoculated flasks containing YMB with 25% OMWW were used as inoculums of the bioreactor.

2.4 Fermentation Process and Production of SCP

The production of gellan was carried out mostly in 500 ml shake flasks containing 300 ml substrate using an Innova 40R shaker (New

Brunswick) at a constant temperature and agitation rate. After optimization of culture condition and substrate composition, the optimal conditions were applied in a scaled-up batch fermentation using a 15 lt stirred tank bioreactor (Bioflo 415, New Brunswick) with 10 lt working volume. Unless stated otherwise, the standard fermentation conditions were 30℃ temperature, 350 rpm agitation rate, pH 7, 10% inoculum (with cells grown on YMB + 25% OMWW). The pH was adapted to 7 by addition of 2M NaOH. All substrates were sterilized in an autoclave at 121℃ for 15 min before use.

2.5 Analyses

For the measurement of biomass concentration 30 ml of the fermentation broth were heated at 60℃ for 15 min in order to dissociate gellan molecules from the cell wall at which they may be attached and then centrifuged at 5000 rpm for 45 min. The sediment of the first centrifugation was washed with water and re-centrifuged in order to remove any gellan molecules that may be still attached to the cells. The sediment of the second centrifugation was then dried at 105℃ overnight until constant weight and the biomass concentration was counted after the weighing of the dried cell debris. Gellan gum was contained (mostly) in the supernatant of the first centrifugation, but its concentration was determined as follows: 10 ml of fermentation broth were mixed with a double volume (20 ml) of absolute ethanol which precipitated not only the polysaccharide, but both gellan and cells. After centrifugation at 5000 rpm for 45 min this sediment contained the sum of both gellan and cells and was dried at 105℃ overnight until constant weight in order to measure the sum of gellan+cells. Gellan concentration was measured as the difference between this sum of gellan+cells and the amount of biomass determined earlier. The supernatant of this centrifugation which contained ethanol/water was used for the determination of sugars and phenols. Total sugars and polyphenols were measured spectrophotometrically. Reducing sugars were measured spectrophotometrically according to the DNS (dinitrosalicylic acid) method at 540 nm wavelength [21], while total polyphenols were measured according to the Folin-Ciocalteu method [22] and expressed as gallic acid equivalent after measurement of the absorbance at 750 nm, using a Hach Lange UV-VIS DR 6000 spectrophotometer. The apparent viscosity of the process fluid (fermentation broth) was measured at 25℃ with a RAYPA rotational viscometer (RP1) set at 200 rpm, using an L2 cylindrical probe of 5 mm height, which was immersed into 30 ml of fermentation broth inside a 50 ml falcon plastic tube. All chemicals used were of analytical grade, and all analyses described above were carried out in triplicate and the mean values are reported here.

3. RESULTS AND DISCUSSION

3.1 Effect of the Dilution and Condensation of OMWW

In order to examine the ability of *S. paucimobilis* to grow in OMWW and to degrade polyphenols, as well to see whether the sugar concentration is adequate for sufficient gellan production, three OMWW-based substrates were prepared: a standard untreated substrate (with only pH adjustment to pH 7), a diluted substrate (OMWW 1/2) where an equal volume of distilled water was added, and a condensed substrate (OMWW 2/1) where the initial substrate was reduced to the half of the initial volume via thermal condensation.

As illustrated in Fig. 1 (a and b) both biomass and gellan concentration are optimal in the 2/1 condensed OMWW, reaching a maximum value of 3.5 g/l (at 48h) and 4.6 g/l (at 48 h), respectively. The 1/2 diluted OMWW limited gellan synthesis, as well as cell growth, showing that it is poor in carbon source (fermentable sugars) for this kind of fermentation. Also, the fact that *S. paucimobilis* grew better in the condensed medium (OMWW 2/1) indicates that the presence of polyphenols in OMWW is probably not a severe limiting factor for growth and that the sugar concentration is the most decisive factor regulating cell growth and gellan synthesis. As observed in other studies, gellan synthesis is parallel to cell growth for most of the fermentation process [15-19]. Viscosity reached a maximum of 18 mPa.sec at 48 h (relatively low for this type of fermentation broth) in process where condensed 2/1 OMWW was used (Fig. 2a). After 48 h the viscosity dropped abruptly in all bioprocesses, possibly due to gellan degradation, since gellan concentration did not recede significantly after 48 h. The residual sugar concentration curves (Fig. 2b) show that sugars were not fully utilized in any of the three bioprocesses. The fastest and most efficient sugar assimilation was observed in the condensed 2/1 OMWW substrate which had an initial sugar concentration of 34.8 g/l, but even in that case there were at least 18.3 g/l sugars that

were not utilized during this fermentation. Phenol concentration (Fig. 2c) was reduced during every fermentation, illustrating the ability of *S. paucimobilis* to gradually degrade olive polyphenols in OMWW. The maximum phenol consumption of approximately 1.8 g/l phenols was attained in the 2/1 condensed medium, corresponding to a 40 % reduction of the initial phenol concentration. This is in agreement with previous reports describing the capacity of *Sphingomonas paucimobilis* or other *Sphingomonas* species to degrade polyphenols, and the first report to our knowledge which relates *Sphingomonas paucimobilis* with the biodegradation of olive mill waste polyphenols [23-25].

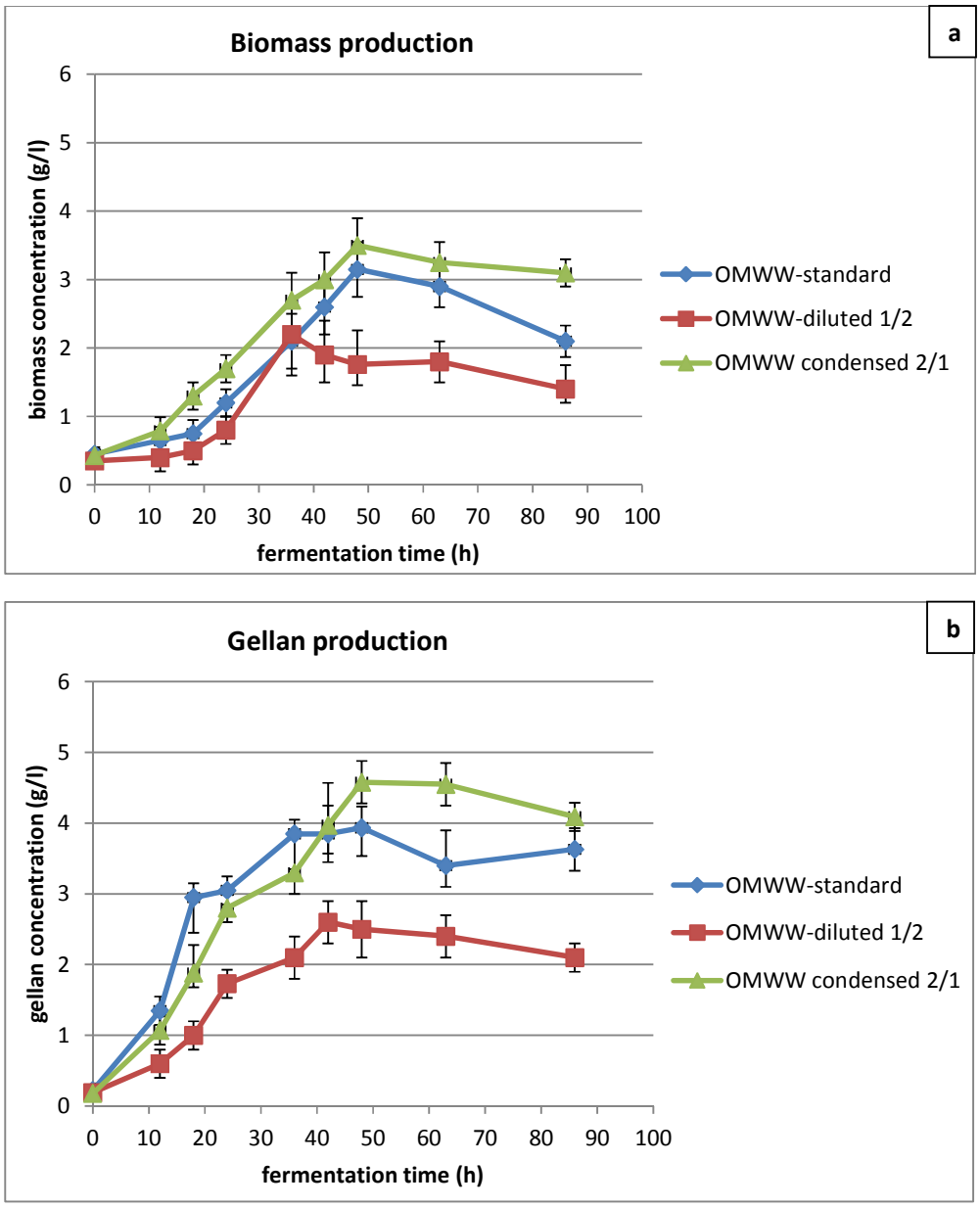

Fig. 1. Biomass (a) and Gellan (b) concentration in OMWW fermentation substrate with and without dilution or condensation, during growth of *S. paucimobilis*

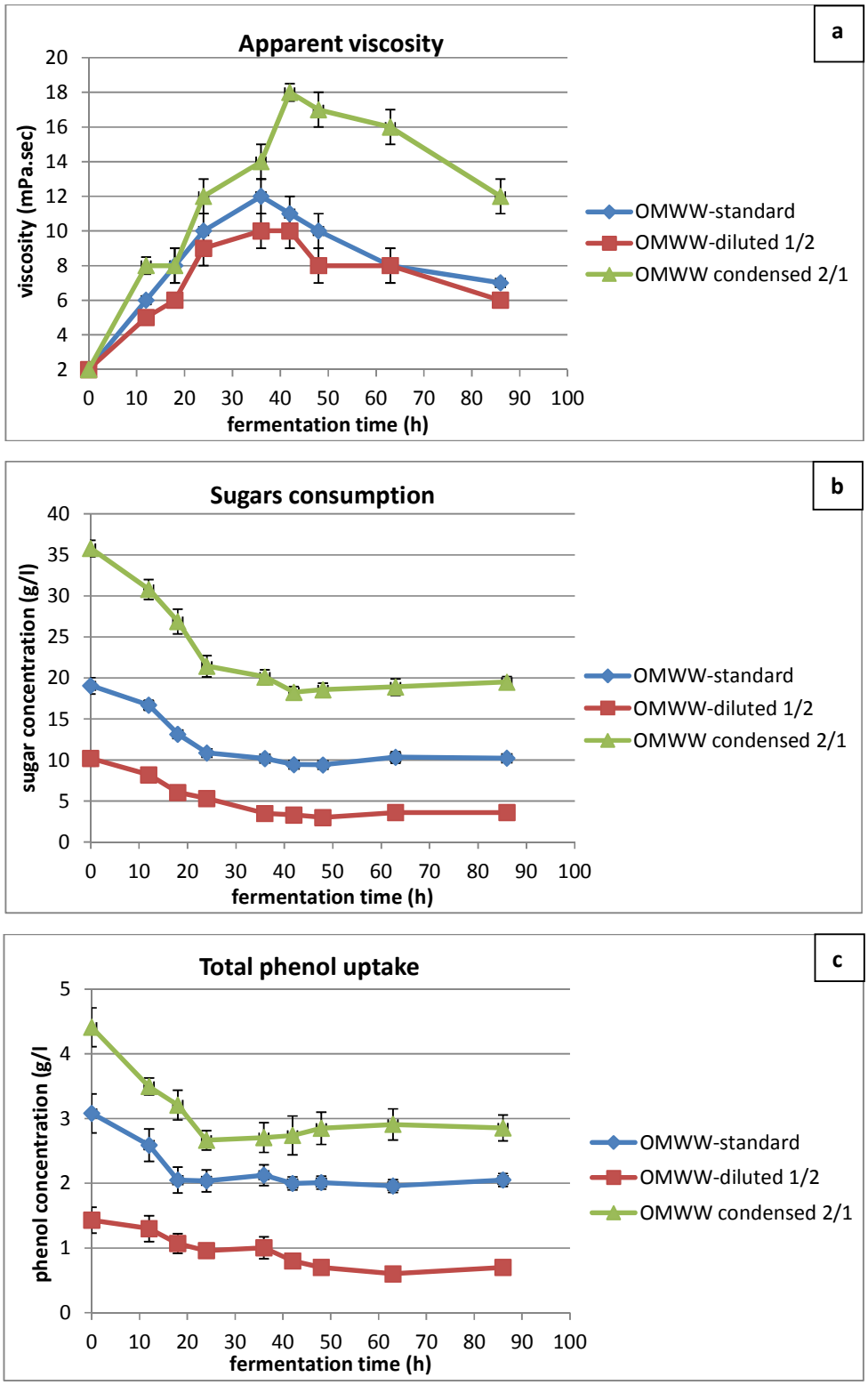

Fig. 2. Apparent viscosity, sugar consumption and phenol concentration in OMWW fermentation substrate, with or without dilution or condensation, during growth of *S. paucimobilis*

3.2 Effect of the Dephenolization of OMWW

Despite the fact that the culture may degrade a significant amount of the polyphenols contained in process fluid, we examined whether this is done at the expense of gellan synthesis and whether a higher gellan production can be attained after (partial) removal of the phenolic compounds. For this purpose we used a standard OMWW substrate, a dephenolized (via microfiltration) OMWW substrate and a dephenolized and condensed 2/1 (to the half of the initial volume) OMWW substrate. The profile of biomass production (Fig. 3a) was similar among the three substrates in the first 48 h and was maximal at 63 h in the dephenolized and 2/1 condensed medium where it reached 3.9 g/l. Gellan concentration reached a peak of 6.2 g/l at 48 h in the same substrate, followed by a peak at 5.6 g/l at the same time in the dephenolized (but non-condensed) substrate. Viscosity (Fig. 4a) followed the profile of gellan and biomass concentration for the three substrates tested here and an optimal value of 31 mPa.sec was obtained at 63 h in the dephenolized and 2/1 condensed medium. Sugar consumption (Fig. 4b) was more efficient in the same substrate where 17 g/l of sugars were consumed, but even then, there were 12 g/l of residual sugars at the end of the fermentation which were not utilized, probably to some bottleneck that exists. Phenol uptake by the culture was dependent on the initial phenol concentration and thus this uptake was highest in the standard OMWW substrate (Fig. 4c). Dephenolization alone increased the utilization of sugars (Fig. 4b) in the OMWW substrate from 11.2 g/l in the standard OMWW substrate to 12.7 g/l in the dephenolized OMWW, even though the dephenolized medium had a lower initial sugar concentration, due to some losses of sugars during the microfiltration process for phenol removal (this loss of sugars during the microfiltration process was due to the type of macroporous resins used, which were chosen based on their efficiency in phenol removal, but they also allowed the capture of some sugar molecules in their pores). These results suggest that dephenolization allows the more efficient assimilation of sugars for gellan synthesis, possibly because the presence and the degradation of the antimicrobial polyphenols requires the expenditure of extra energy, which can be diverted to gellan biosynthesis after dephenolization. This strategy of phenol removal prior to fermentation of olive mill waste has also been successful in increasing product yield in other bioprocesses such as the production of hydrogen by *Rhodopseudomonas palustris* [26]. Based on the above experimental findings, a dephenolized and 2/1 condensed medium was used in the following experiments.

3.3 Effect of the Supplementation of Nutrients to the OMWW

Nitrogen is necessary for cell growth and OMWW is poor in nitrogen sources. On the other hand, in the gellan bioprocess, as well as other exopolysaccharide production processes, a high carbon/nitrogen ratio is desired, so that after a sufficient growth cells are primarily utilizing an existing surplus of sugars for exopolysaccharide synthesis and not for cell growth (in other words, after the cells achieve sufficient growth at the end of the growth phase, there must be a nitrogen depletion and a simultaneous surplus of sugars, in order to boost gellan biosynthesis) [14,17-19,27-28]. In this experiment the nitrogen concentration of the OMWW was fortified by addition of 1 g/l yeast extract (which also offers vitamins and minerals), or 1 g/l ammonium nitrate and the effects of the two nitrogen sources on the fermentative production of gellan were examined. Also, the effect of glycerol (a cheap by-product of biodiesel production) on gellan synthesis was studied. Although there are no reports for the uptake of glycerol as a sole carbon source in bioprocesses by *Sphingomonas paucimobilis*, other *Sphingomonas* members can readily assimilated glycerol and it is possible that *S. paucimobilis* may utilize glycerol when it is not the sole carbon source [29]. Glycerol may be useful to gellan synthesis as a promoter of lipid synthesis, since the biosynthetic route for gellan synthesis involves lipid carrier molecules which transport the sugar moieties of the molecule to the cell membrane where polymerization then takes place [14], and this hypothesis was tested in this study.

The results of biomass formation and gellan production (Figs. 5a and 5b) show that both yeast extract and ammonium nitrate stimulated cell growth considerably, and biomass reached a maximum level of 4.4 g/l at 48 h after yeast extract addition. This also had a positive impact on gellan synthesis which was enhanced in the substrates enriched with either yeast extract or ammonium nitrate. Interestingly, when both yeast extract and glycerol were added, cell growth did not improve compared to the standard, non-fortified OMWW substrate (OMWW-dephenolized) and the additional glycerol certainly did not enhance cell growth (it appears

it was not utilized for biomass formation). However, without promoting cell growth, the addition of glycerol seems to improve gellan synthesis from 6.8 g/l at 63 h (OMWW with yeast extract only) to 7.9 g/l at 63 h (OMWW with yeast extract and glycerol). Similarly, the broth viscosity (Fig. 6a) is optimal in the OMWW with yeast extract and glycerol (58 mPa.sec at 63 h), probably as a result of improved gellan synthesis in this bioprocess. Sugar consumption (Fig. 6b) was most efficient after yeast extract addition, practically regardless of the presence of additional glycerol up to 63 h, but after that point in the substrate with additional glycerol sugar concentration was almost zeroed (only 1.8 g/l of residual sugars at 83 h, from an initial 19.2 g/l concentration) in contrast to the other bioprocesses, possibly due to the higher biomass growth and gellan synthesis from 63 to 83 h in this bioprocess. Overall, the above findings show that the addition of a modest concentration (1 g/l) of nitrogen source such as yeast extract or ammonium nitrate enhances both biomass and gellan formation and the addition of glycerol at 5 g/l can improve gellan synthesis and increase significantly the viscosity of the process fluid. Similar results on the beneficial effect of adding only low (0,5g/l) concentrations of nitrogen sources, such as yeast extract and ammonium nitrate in the process fluid for gellan production have been previously reported [28]. Also, the enhanced polysaccharide formation after glycerol supplementation as observed in the gellan bioprocess was also reported for exopolysaccharide biosynthesis from *Enterobacter* species [30].

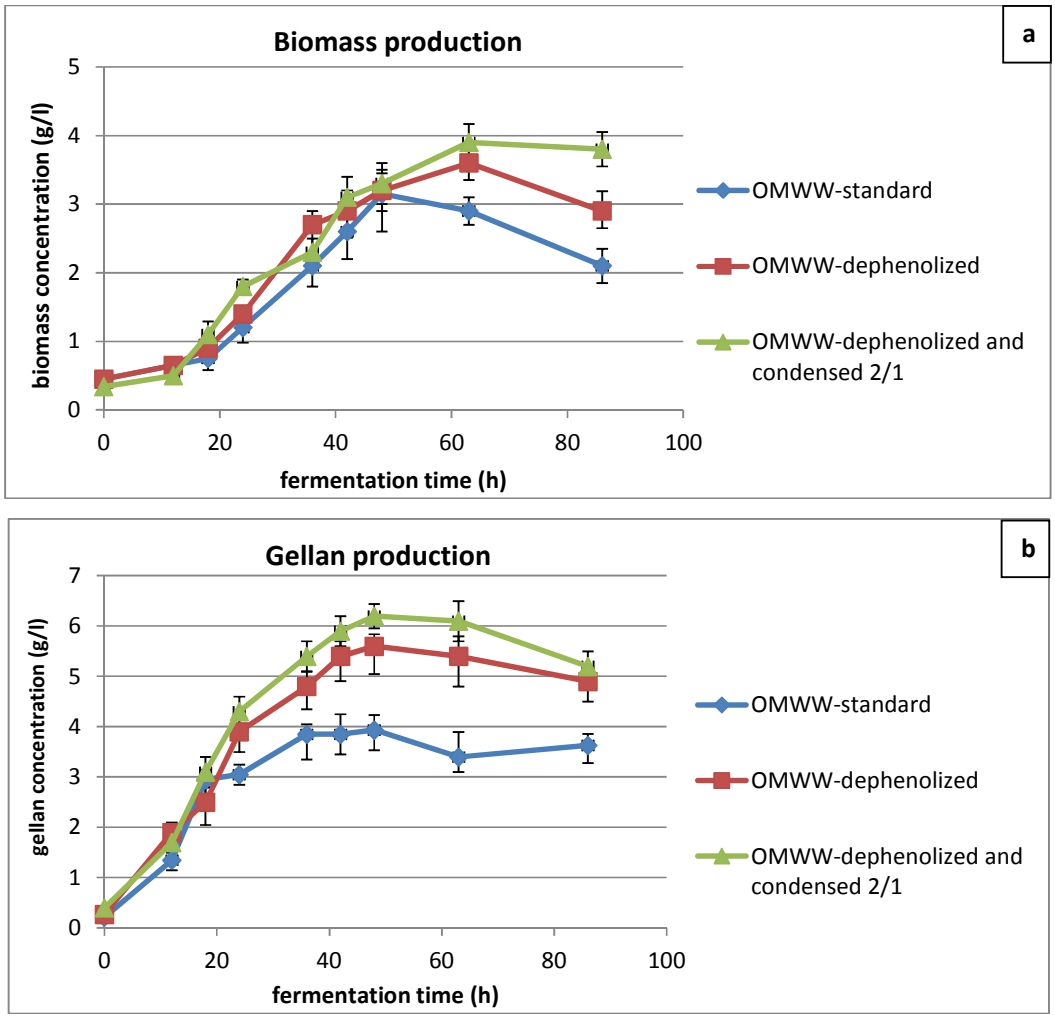

Fig. 3. Biomass (a) and gellan (b) concentration in OMWW fermentation substrate, before and after dephenolization (OMWW-standard and OMWW-dephenolized, respectively), and after condensation 2/1 of the dephenolized substrate, during growth of *S. paucimobilis*

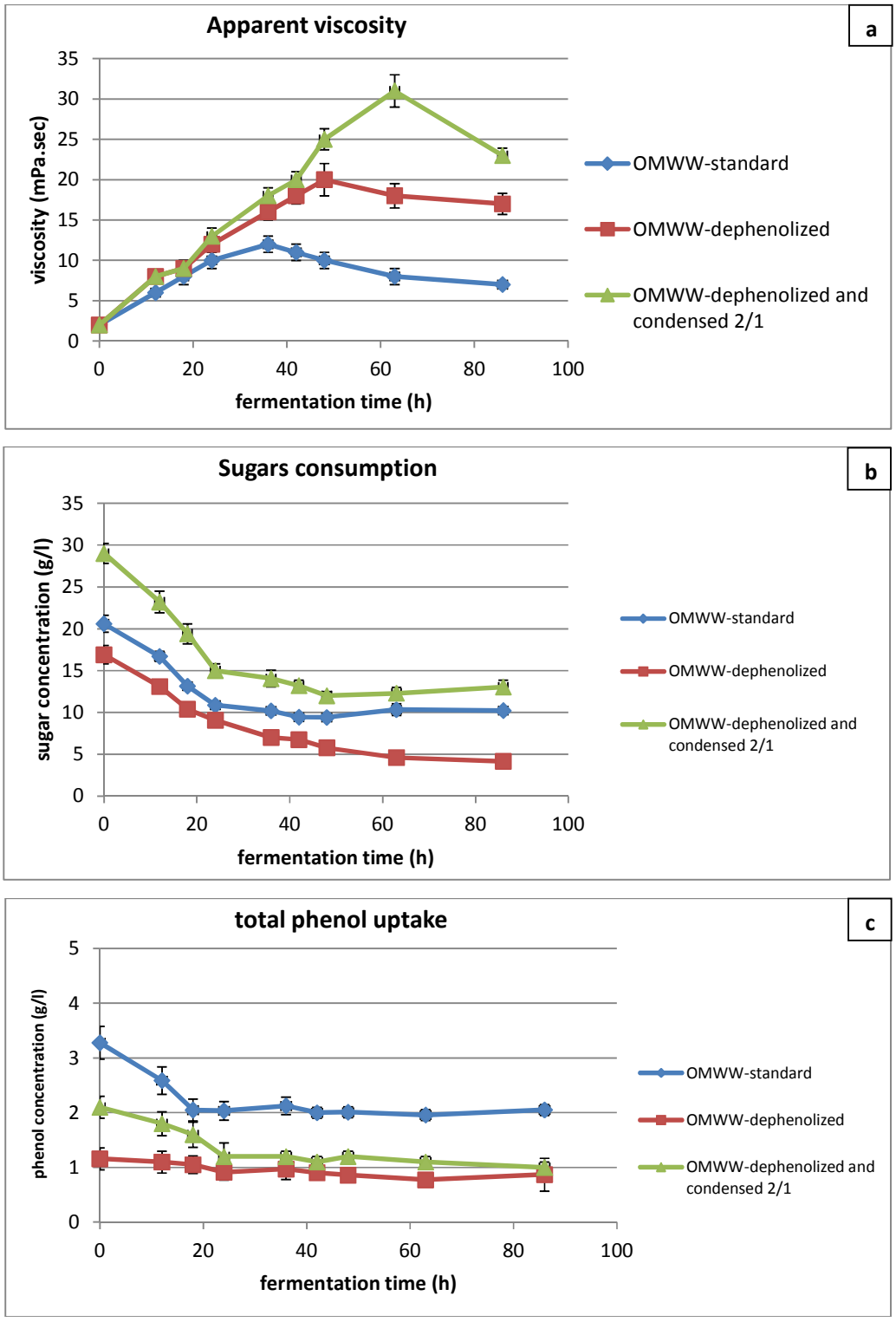

Fig. 4. Apparent viscosity (a), sugars concentration (b) and phenol concentration (c) in OMWW fermentation substrate, before and after dephenolization (OMWW-standard and OMWW-dephenolized, respectively), and after condensation 2/1 of the dephenolized substrate, during growth of *S. paucimobilis*

3.4 Effect of the Agitation and the Aeration Rate during an Optimized and Scaled-Up Process in a Bioreactor

The most favorable conditions and OMWW substrate formulations observed in the above shake flasks experiments were combined in a scaled-up bioprocess in a 15 lt bioreactor with a 10 lt working volume. Therefore, based on the previous findings as described in Figs. 1-6 the optimal fermentation medium (OMWW-opt.) in order to achieve an optimum gellan synthesis was the OMWW-dephenolized, and 2/1 condensed medium, supplemented with 1 g/l yeast extract and 1 g/l glycerol. In addition, in the scaled-up process the effect of agitation rate (350 rpm and 500 rpm) and aeration rate, namely 1 vvm (volume of air/volume of bioreactor/minute) and 2 vvm were comparatively studied.

As can been seen in Fig. 7 (a and b) biomass is generally enhanced by the increase of both aeration and agitation and is maximal (4.3 g/l) at 63 h in the process with 2 vvm and 500 rpm, where the average growth rate (up to 48 h) reaches a peak of 0.083 g/l/h. The lower biomass

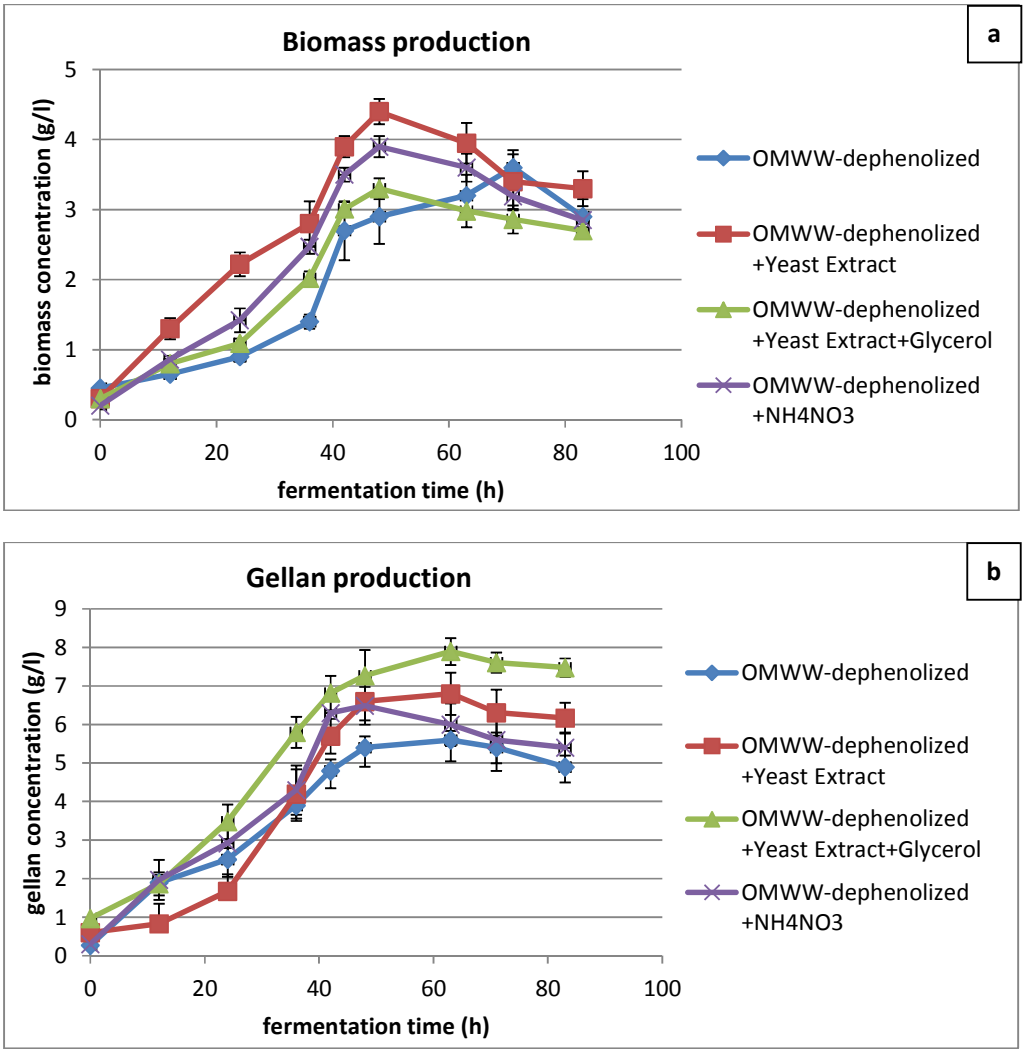

Fig. 5. Biomass (a) and gellan (b) concentration in OMWW without or with supplementation of 1 g/l yeast extract, 1 g/l yeast extract and 5 g/l glycerol, or 1 g/l ammoniunm nitrate, during growth of *S. paucimobilis*

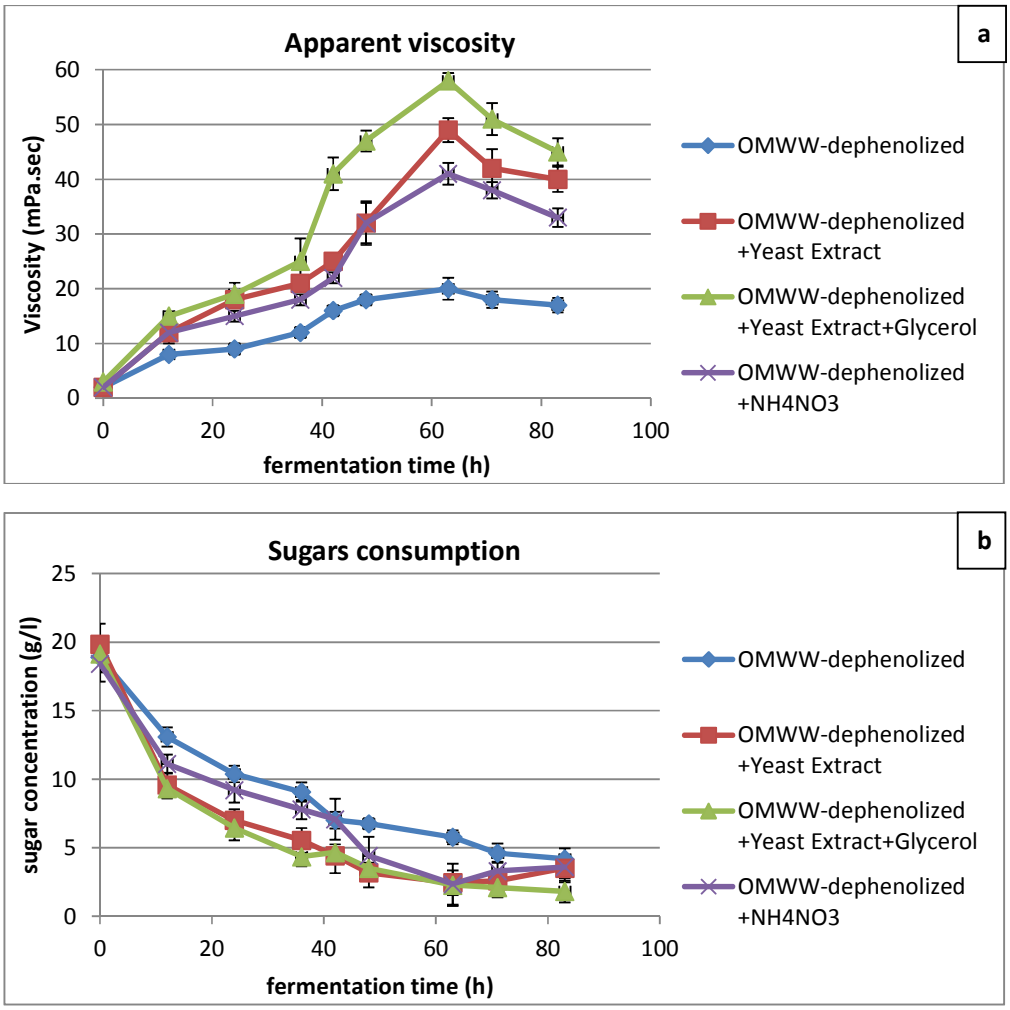

Fig. 6. Apparent viscosity (a) and sugars concentration (b) in OMWW fermentation substrate, without or with supplementation of 1 g/l yeast extract, 1 g/l yeast extract and 5 g/l glycerol, or 1 g/l ammoniunm nitrate, during growth of *S. paucimobilis*

concentration is attained at 1 vvm and 350 rpm, suggesting that both a good mixing and mass transfer and sufficient oxygenation of the substrate are essential to cell growth. Nevertheless, gellan formation does not follow exactly the same trend. It is indeed enhanced by the increase of aeration from 1 vvm to 2 vvm when agitation is modest (350 rpm), since the maximum gellan concentration increases from 8 g/l to 8.9 g/l and the gellan productivity (gellan concentration per hour) rises from 0.100 g/l/h to 0,128 g/l/h, but gellan synthesis is negatively influenced when the aeration increases from 1 vvm to 2 vvm in the vigorously stirred process with 500 rpm. Thus, the optimal gellan concentration of 9.5 g/l and gellan productivity of 0.141 g/l/h was achieved at 500 rpm and 1 vvm. These values are somewhat lower but comparable to the ones attained in glucose-based synthetic media (~12-20 g/l) which are well optimized but more expensive [14-16,18-19, 27,31]. The gellan yield (gellan produced/sugars consumed) also reached a peak of 0.34 in this bioprocess. The above results illustrate the inevitable interconnection that exists between aeration and agitation as both affect each other, and indicates that although at modest agitation rates (350 rpm) a high aeration (2 vvm) may benefit gellan synthesis, the intense oxygenation (2vvm) combined with high agitation (500 rpm) may reduce gellan biosynthesis and/or gellan viscosity, even if cell growth is enhanced. This was also observed previously in synthetic media were *S. paucimobilis* was grown [18].

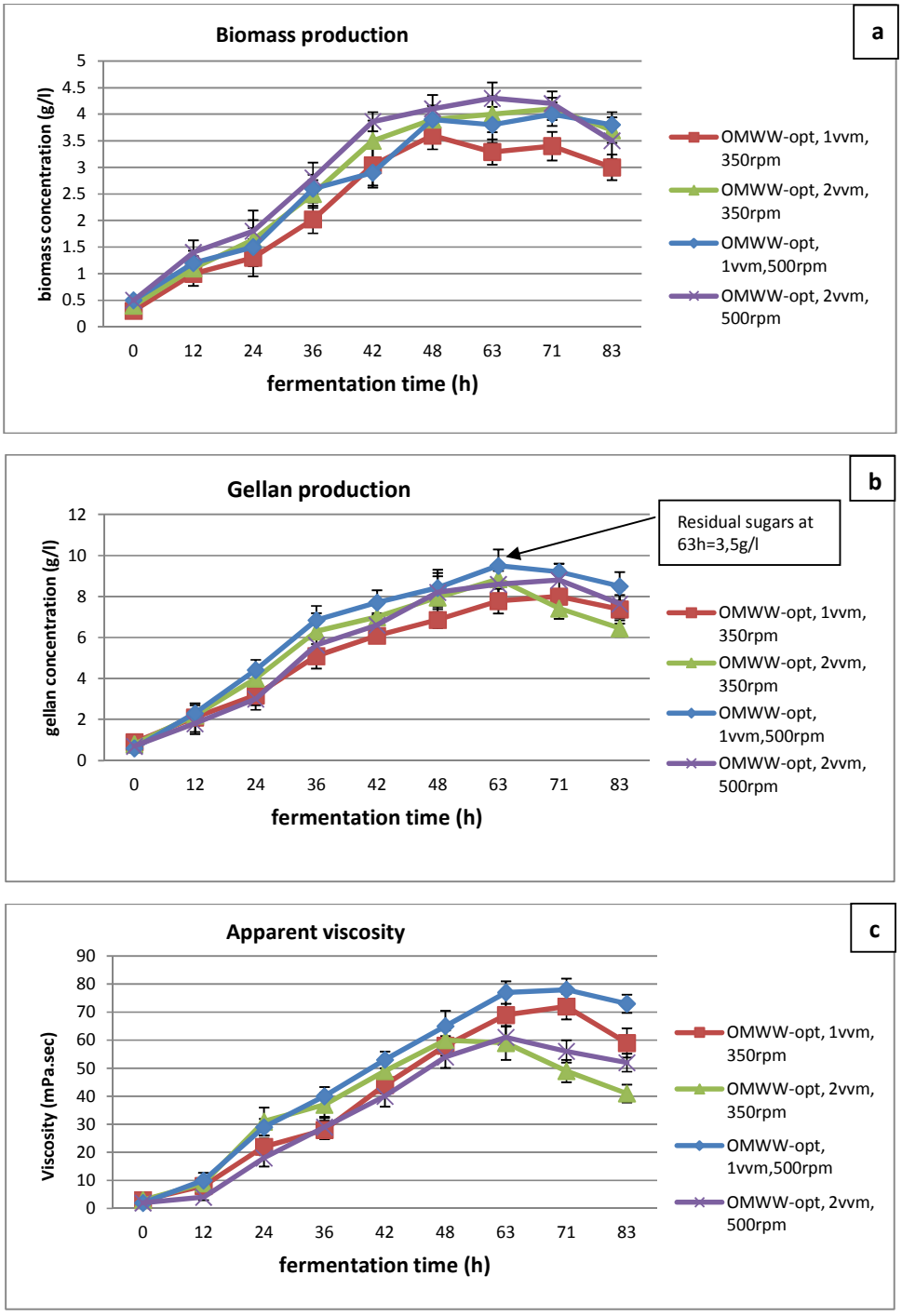

Fig. 7. Biomass (a) and gellan (b) concentration, and viscosity of the process fluid (c) in optimized OMWW-based substrate at 350 rpm and 500 rpm agitation rate, and 1 vvm and 2 vvm aeration rate, during growth of *S. paucimobilis*

The viscosity (Fig. 7c) of the fermentation fluid was also maximal in the process with 500 rpm and 1 vvm aeration and reached a peak of 78 mPa. sec at 71 h. The increase in aeration from 1 vvm to 2 vvm caused a decrease in the viscosity after 48 h, not only in the process with

500 rpm agitation rate (where gellan production was also adversely affected anyway), but also in the process with 350 rpm, although the gellan synthesis there was improved by the increased aeration. This may indicate a possible reduction of the molecular weight of the gellan produced under increased aeration at high agitation rates, which was also observed in a synthetic medium for gellan production [18]. This suggestion is in agreement with Pena et al. [32] who observed a decrease in the molecular weight of alginate produced by *Azotobacter vinelandii*, when dissolved oxygen increased in a process with high agitation rate (700 rpm), which was attributed to increased alginase (polysaccharide-lyase) activity under these conditions. In contrast, xanthan gum production and fermentation broth viscosity, appeared to be enhanced by both high agitation and a simultaneous high aeration rate in bioreactor experiments [33].

4. CONCLUSIONS

Based on the findings of this research, it can be concluded that OMWW can be utilized and valorized as a substrate for gellan production by *S. paucimobilis*, after some modifications. Although *S. paucimobilis* can degrade olive polyphenols, the presence of polyphenols seems to hinder gellan production. Also, the relatively low sugar concentration of OMWW needs to be increased in order to achieve sufficient gellan synthesis. Therefore, dephenolization and condensation (at least 2/1) of the OMWW were crucial parameters in this process optimization. From an industrial point of view, both these two processes and the necessary equipment are readily available and applicable and have minimal operational costs, especially if condensation is carried out without heat treatment, via reverse osmosis. Additionally, the dephenolization process which is based on (energy-efficient) microfiltration can be made more economically viable if the phenolic effluent of this procedure is also utilized for the production of olive polyphenols [34]. Also, the supplementation of the substrate with a modest amount (1 g/l) of nitrogen sources such as yeast extract or ammonium sulphate is beneficial to this fermentation. Interestingly, the addition of 5 g/l glycerol in the above OMWW substrate enhances gellan synthesis even if it is not directly used for energy production, but it may play a key role in gellan biosynthesis.

With regard to the optimization of agitation and aeration, although *S. paucimobilis* could grow better both in a well agitated (500 rpm) and in a highly aerated (2 vvm) process, the combination of high aeration and intense agitation may stimulate cell growth at the expense of gellan formation and may lower the production and the viscosity of gellan. Taking into account the maximum gellan concentration and kinetic parameters such as gellan yield and gellan productivity the optimal process conditions include a high agitation of 500 rpm and a modest aeration of 1 vvm.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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