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A one-stage cultivation process for the production of poly-3-(hydroxybutyrate-*co*-hydroxyvalerate) from olive mill wastewater by *Haloferax mediterranei*

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ABSTRACT

Olive mill wastewater (OMW), a highly polluting waste from the olive oil industry, was utilized as sole carbon source for the production of polyhydroxyalkanoate (PHA) by extremely halophilic *Haloferax Mediterranei* (*H. mediterranei*) in a one stage cultivation step. *H. mediterranei* showed remarkable cell growth and tolerated the inhibitory effect of polyphenols present in medium containing 25% of OMW. *H. mediterranei* cultivation conditions were optimized in medium containing 15% OMW by investigating several parameters that affect the production of PHA. The highest polymer yield (0.2 g/L) and PHA content (43% PHA/cell dry mass) were achieved at 37 °C, 170 rpm and 22% salt concentration. Analysis of the produced PHA revealed the production of copolyester poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) containing 6.5 mol% 3-hydroxyvalerate (3HV). The production of PHBHV was observed without the need for fermentation step or adding external carbon source. The PHBHV displayed reduced melting points at 140.1 °C and 154.4 °C when compared to homopolymer polyhydroxybutyrate.

1. Introduction

The overproduction and accumulation of petroleum-based plastics have turned research efforts toward the production of biodegradable plastics. These efforts have been focused on the production of bacterial polymers, polyhydroxyalkanoates (PHAs) and in particular the homopolymer polyhydroxybutyrate (PHB) [1,2]. PHB intracellularly accumulated as carbon and energy sources by several bacteria and archaea under unfavorable conditions [3]. However, the industrial application of PHB is restricted by its poor mechanical and physical properties such as brittleness and stiffness [4]. Studies have suggested that the incorporation of other monomeric units such as 3-hydroxyvalerate (3HV) into PHB chains yields co-polymer with improved properties [5,6]. Therefore, the copolyester of 3HB and 3HV, poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBHV) has been intensively investigated with expectations of practical use. PHBHV showed better mechanical properties when compared to PHB homopolymer and similar properties to polypropylene such as high impact resistance, toughness and flexibility [7–9].

The high production cost of PHBHV compared to that of polypropylene prevents further market penetration [10]. A cost reduction in PHBHV biosynthesis process could be obtained by using inexpensive carbon sources and developing new PHBHV accumulation process with cheap and simple purification steps. Industrial by-products such as glycerol (from the biodiesel industry), [4,11] rice bran [12], molasses [13] and cheese whey [14] have been used as cheap carbon sources for PHBHV production. The effluent of the olive oil industry, olive mill wastewater (OMW) can also be considered as a potential no-cost substrate for PHBHV production. OMW is rich in high amounts of readily consumable carbon source such as carbohydrates, lipids and volatile fatty acids which are the most direct substrates for PHBHV production [15]. Annually, high levels of OMW were recorded, particularly in the Mediterranean counties, which produced about 30 million m^3 [16]. In Jordan, there are approximately 130 olive mills serving olive plantation and generating around 200 thousand m^3 of OMW per year [17]. The OMW creates serious environmental problems such as changes in soil microbial populations, threat to surface and groundwater sources and pollution of the air through phenol and sulfur dioxide emissions [18]. Thus, their employment as feedstock for PHBHV production could represent an alternative solution for their disposal.

The production of PHAs from OMW is currently based on multi-stage processes [15,19–21]. These processes involve OMW pre-treatment, which consists of polyphenols removal, followed by an acidogenic fermentation step to obtain volatile fatty acids (VFAs).

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After that, the VFAs stream is employed for PHAs accumulation, using a pure [21] or mixed culture cell methodology [22,23]. In the pure cells methodology, high cell densities and PHAs contents were obtained. However, the selection of mixed cultures has more economic advantages than pure strains where the production process performed under non-sterile conditions [23]. The previous processes clearly showed that the OMW requires prior steps before it is utilized as sole carbon source for PHA production, due to the incompatibility between OMW medium and biological components. One approach to tackle the incompatibility between OMW and biological components is to employ extremophile organisms, which exhibit tolerance to a range of environmental stressors and provide many biotechnological applications [24–26].

The present work was dedicated to evaluating the possibility of producing PHA from OMW in a one-stage cultivation process (not multi-stage processes), using an extremely halophilic organism *Haloferax Mediterranei* (*H. mediterranei*). *H. mediterranei* was chosen as the biocatalyst due to its capability of accumulating the copolymer PHBHV from cheap carbon sources with chemical structure unrelated to 3-hydroxyvalerate (3HV), such as glycerol [11] and carbohydrates [27–29], and the fact that *H. mediterranei* is extremely resilient to contamination as the high salt concentration (2–5 M NaCl) for its optimal growth prevents almost any other organisms from replicating [30]. Moreover, *H. mediterranei* like some halophiles [31–33] could grow on in media that contains phenolic compounds such as OMW medium. Additionally, the obtained polymer can be easily recovered by hypo-osmatic shock of cells after decreasing the salinity of the external medium [34]. Finally, *H. mediterranei* has simple growth requirements, with relatively rapid doubling time in comparison to other haloarchaea [35,36]. Many studies in the literature have tested *H. mediterranei* to produce PHBHV from different carbon sources [12–14,28,37]. However, to the best of our knowledge, this work represents the first attempt to produce PHAs from OMW by employing *H. mediterranei*. In order to develop a novel culture method for the production of PHBHV from OMW, the effects of different conditions on the growth of *H. mediterranei* were investigated and the produced polymer was fully characterized.

2. Materials and methods

2.1. Chemical reagent and standards

All chemical reagents, unless stated otherwise, were purchased as analytical grade. The standard solutions used in volatile fatty acids (VFAs) analysis were prepared from concentrated formic acid (≥98%, Sigma), acetic acid (glacial) (100%, Merck), butyric acid (≥99%, Merck), pentatonic acid (≥98%, Merck), propionic acid (>99%, Merck) and hexanoic acid (≥99.5%, Merck). Poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) 8 mol% PHV; natural origin, methyl (*R*)-3-hydroxybutyrate 99% and methyl (*R*)-3-hydroxyvalerate ≥98.0% were purchased from Sigma-Aldrich.

2.2. Microorganisms and growth conditions

Haloferax mediterranei DSM 1411 and *Cupriavidus necator* DSM 545 were obtained from German Collection of Microorganisms and Cell cultures (DSMZ). *Haloferax mediterranei* (*H. mediterranei*) was cultivated first in 100 ml of nutrient-rich AS-168 medium [35] containing (per liter) 200 g NaCl, 20 g $MgSO_4{\cdot}7H_2O$, 2 g KCl, 3 g trisodium citrate, 1 g sodium glutamate, 50 mg $FeSO_4{\cdot}4H_2O$, 0.36 mg $MnCl_2{\cdot}4H_2O$, 5 g casamino Acids, 5 g yeast extract, pH 7.2. The culture was incubated in shaking incubator with constant shaking (170 rpm) for 3 days at 37 °C. For cultivation with OMW, three milliliter of the culture was transferred into 100 ml of a nutrient-limited MST medium [35] containing (per liter) 200 g NaCl, 20 g $MgSO_4{\cdot}7H_2O$, 2 g KCl, 1 g sodium glutamate, 37.5 mg KH_2PO_4, 50 mg $FeSO_4{\cdot}7H_2O$, 0.36 mg $MnCl_2{\cdot}4H_2O$, 1 g yeast extract and different concentrations (5, 15, 25, 50 and 75% V/V) of OMW. The culture was incubated for 4 days at 170 rpm, 37 °C. *Cupriavidus necator* (*C. necator*) was cultivated in seed mineral medium, as previously described [38].

2.3. Analytical methods and OMW characterization

Total dissolve solid (TDS), total suspended solid (TSS), total solid (TS), chemical oxygen demand (COD), total Phenols, total nitrogen (T-N), Total Kjeldhal nitrogen (TK-N), ammoniacal nitrogen (NH_4—N) and density (ρ) (at least three repetitions for each sample) were determined according to Standard Methods [39]. Volatile fatty acids (VFAs) namely acetic acid, propionic acid, butyric acid and hexanoic acid concentrations were determined using Agilent 1100 HPLC equipped with a diode array detector and 8 mm Rezex ROA-organic acid H column (Phenomenex). VFAs were eluted using 0.013 N H_2SO_4 at a flow rate of 0.6 ml/min and 35 °C operating temperature. The detection wavelength was set at 210 nm.

Fresh OMW was collected from an olive oil mill located in Madaba in central Jordan. Sample was stored in dark plastic containers at 4° C. The physicochemical characterization for OMW is described in Table 1.

2.4. Evaluation of the inhibitory effect of OMW

The inhibitory effect of OMW was evaluated by monitoring the cell growth of *H. mediterranei* in MST medium and different concentrations (percent, v/v) of 5%, 15%, 25% 50% and 75% of OMW. The culture was incubated for 4 days at 170 rpm, 37 °C. The cell growth was analyzed spectrophotometrically by measuring the optical density at 520 nm (OD_{520} nm) [40], using a Biochrom Libra S50 UV–visible spectrophotometer. Blank samples (media without culture) were performed to eliminate the matrix interference. *C. necator* the most studied PHA producer was used as reference cell to estimate the inhibitory effect of OMW. *C. necator* was cultivated in seed mineral medium with 5% to 75% of OMW. The culture was incubated for 4 days at 170 rpm, 30 °C. The cell growth was monitored by measuring the OD_{600} nm.

2.5. Optimization of PHA production

H. mediterranei was cultivated in 100 ml of nutrient-rich AS-168 medium in a 250 ml Erlenmeyer flask for 3 days with shaking at 170 rpm, 37 °C. To develop a concentrated inoculum, the

Table 1
The physicochemical characteristics of OMW.

Parameters	
pH-value	5.2 ± 0.1
EC (μS/cm)	3790 ± 57
TDS (mg/L)	17450 ± 698
TSS (mg/L)	12500 ± 813
TS (mg/L)	39920 ± 800
COD (mg/L)	58850 ± 5002
Total Phenols (mg/L)	2417 ± 12
T-N (mg/L)	544 ± 35
TK-N (mg/L)	543 ± 33
NH_4—N (mg/L)	43.7 ± 3
Acetic acid (mg/L)	6367 ± 540
Propionic acid (mg/L)	9852 ± 810
Butyric acid (mg/L)	2055 ± 180
Hexanoic acid (mg/L)	3070 ± 270
VFAs (mg/L)	21344 ± 810
ρ (g/cm^3)	0.99 ± 0.05

culture was centrifuged at 1957 × g for 10 min using Sigma laboratory centrifuge. Inoculum (0.5 ± 0.05 g wet cell mass) was transferred into 100 ml of 15% OMW and the other components of MST medium. The production was carried out in 250 ml Erlenmeyer flasks for 4 days at different cultivation conditions (temperature: 25 °C, 37 °C and 45 °C; agitation speed: 100 rpm, 170 rpm and 220 rpm; and salt concentration: 15%, 22% and 30%).

2.6. Determination of cell dry mass

At the end of PHA production, broth containing *H. mediterranei* cells (five milliliters) were pelleted by centrifugation (10 min; 1957 × g). The cell dry mass (CDM) was determined by drying the cell at 100 °C until constant mass. After that, the sample was heated for 4 h at 400 °C and cooled at room temperature using glass desiccator. The mass of resulted ash was recorded and the CDM was calculated by subtracting the mass of the ash from the mass of dry cell.

2.7. Isolation and purification of PHA

Following PHA production, the broth containing *H. mediterranei* cells were harvested by centrifugation (10 min; 1957 × g). The cell pellets were suspended in distilled water with 0.1% sodium dodecyl sulfate (SDS) [41]. The cells were disrupted by Vortex until the lysate appeared transparent and the suspension was incubated for 3 h at 25 °C. The lyzed suspension was centrifuged at 6340 × g for 15 min following centrifugation, black substance was obtained. The substance was dissolved in 3% sodium hypochlorite and the solution was incubated for 30 min at 25 °C. The mixture was centrifuged at 6340 × g for 15 min. A white colored substance was obtained and washed by water and ethanol. The whitish substance resulting from this treatment was dissolved in 10 ml chloroform. Most of the material was dissolved and the undissolved remains were removed by simple filtration. Finally, the chloroform was evaporated and recycled. A thin film of PHA was obtained and characterized using further spectroscopic analysis.

2.8. Characterization of PHA

The thermal property of the PHA was examined by differential scanning calorimetry (DSC), using a DSC 200 F3 Maia. PHA sample was exposed to a temperature profile of over −30 °C to 220 °C, at a heating rate of 10 °C/min. Nitrogen gas was purged into the sample with a flow rate of 20 ml/min. Fourier transform infrared (FT-IR) spectra for PHA was obtained in the range of 400–4000 cm^{-1}, using IR-Prestige-21 Shimadzu FT-IR spectrophotometer. PHA content and purity were quantified by gas chromatography (GC). The method developed by Braunegg et al. [42] was used to simultaneously extract and derivatize PHA to the 3-hydroxyalkanoate methyl esters of the monomers. PHA sample (5 mg) was digested by adding 2 ml of digest solution and incubating at 100 °C for 4 h. The digest solution contained 1 ml chloroform, 0.85 ml methanol and 0.15 ml sulfuric acid (98 v/v%). After cooling, 2 ml of water was added to the sample and the contents were shaken vigorously for 1 min. The bottom layer (chloroform and methyl esters) was collected and placed in a GC vial. The methyl ester in the organic phase was analyzed using GC Shimadzu 2010 equipped with ionization detector (FID) and capillary column (Agilent DB 23, 60 m × 0.25 mm, 0.15 μm). The injection volume was 1 μl and the temperature program started at 50 °C for 8 min, then increased by 3 °C per min to a final temperature of 146 °C for 8 min. The injector and detector temperatures were 230 °C and 240 °C, respectively. Standard calibration was established using standard poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) 8 mol% PHV from Sigma-Aldrich with benzoic acid as internal standard.

3. Results and discussion

3.1. Inhibitory effect of OMW on H. mediterranei

The growth profile of *H. mediterranei* in Fig. 1 shows that up to 25% OMW concentration had no inhibitory effect on the growth of *H. mediterranei*. The optimal cell growth was recorded at 5% of OMW. Increasing the concentrations of OMW in the range of 50% to 75% inhibits the growth of *H. mediterranei* (Fig. 1). In contrast to *H. mediterranei*, the most studied PHA producer, *C. necator* was completely inhibited in the presence of 5% OMW (Data not shown). The results indicate that *H. mediterranei* can tolerate the inhibitory effect of 'toxic' compounds present in OMW, such as phenolic compounds.

3.2. Effect of substrate concentration on PHA production

The effects of various OMW at 5, 15, and 25% (V/V) on the production of PHA were investigated (Fig. 2). The results indicated that the concentration of OMW significantly affected the polymer production. The highest PHA production (0.2 g/L) was recorded in the medium containing 15% of OMW. Therefore, this concentration was selected for further experiments to optimize PHA accumulation by *H. mediterranei*.

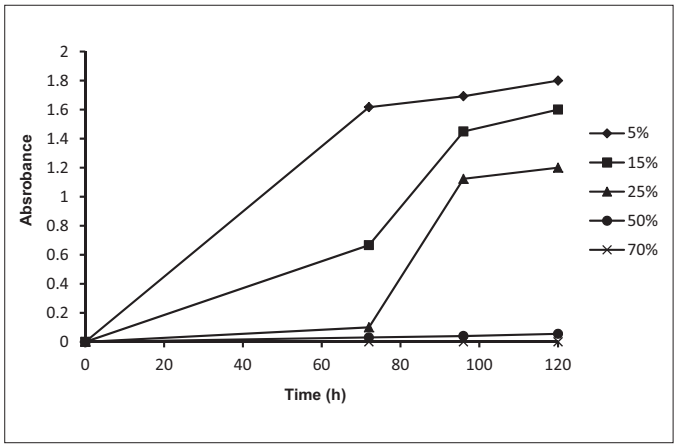

Fig. 1. Growth time profile of *H. mediterranei* at different concentrations of OMW.

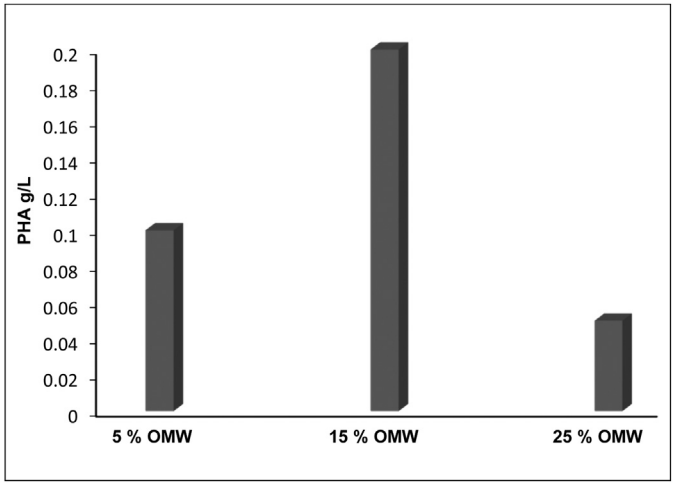

Fig. 2. PHA production by *H. mediterranei* in the presence of different concentrations of OMW.

Table 2
Effect of cultivation conditions on PHA production by *H. mediterranei*, grown in medium supplemented with 15% (v/v) OMW.

Cultivation parameter	Biomass (g/L)[a]	PHA (g/L)	PHA/CDM(%)
Temperature			
25 °C	9.90 ± 0.5	0.14 ± 0.03	25 ± 3
37 °C	10.0 ± 0.5	0.20 ± 0.03	43 ± 4
45 °C	10.8 ± 0.4	0.19 ± 0.04	22 ± 3
Agitation			
100 rpm	9.5 ± 0.3	0.08 ± 0.02	10 ± 2
170 rpm	10.0 ± 0.5	0.2 ± 0.03	43 ± 4
220 rpm	9.5 ± 0.3	0.08 ± 0.02	10 ± 2
SW[b]			
15%	3.5 ± 0.3	ND[c]	ND
22%	10.0 ± 0.5	0.2 ± 0.03	43 ± 4
30%	7.5 ± 0.3	0.03 ± 0.01	6 ± 2

[a] Results were calculated as mean values ± standard deviation (SD) for two independent experiments.
[b] Salt water concentration.
[c] Not detected.

3.3. Optimization of PHA accumulation

In order to optimize PHA production, the influence of cultivation conditions (temperature, agitation speed and salt concentration) on *H. mediterranei* were studied (Table 2).

The data in Table 2 showed that the optimal growth temperature of *H. mediterranei* with the highest biomass (10.8 g/L) was observed at 45 °C. However, *H. mediterranei* cells grown at 37 °C contained the highest amounts of stored polyesters 43% PHA of cell dry mass. In fact, the high temperature at 45 °C promoted *H. mediterranei* cell growth but decreased PHA content in the cell. This result indicates that the optimal temperature of PHA accumulation did not concur with the optimal temperature for *H. mediterranei* growth. Effect of agitation rate on cell growth and PHA production is also shown in Table 2. Both the cell growth and PHA production were increased with the agitation rate of up to 170 rpm. At a high agitation rate (220 rpm), cell autolysis occurred and caused a reduction in PHA productivity. Table 2 showed that the salt water concentration (SW) in the culture medium used for *H. mediterranei* should be maintained around 22% for optimum cell growth and PHA production. When salt concentration was reduced to 15%, the PHA was not detectable due to irreversible cellular damage and cell lysis. Like other halophiles, the mechanism by which *H. mediterranei* contend with high salt concentrations is by intracellular accumulation of KCl in order to balance the osmotic pressure [25]. Cultivation of *H. mediterranei* in media with 30% salt led to a decrease in PHA content. This could be explained by inhibition of PHA biosynthetic pathway and activation of other metabolic processes related to osmotic balance response [24]. At such a high salt concentration, the growth of other organisms is prevented, hence allowing a cultivation process without strict sterile conditions and reducing PHA production cost.

The results in Table 2 revealed that the highest polymer yield (0.2 g/L) and PHA content (43% PHA/CDM) were achieved at 37 °C, 170 rpm and 22% salt concentration. The maximum polymer yield produced in this study at a one-stage process was lower than values previously published when OMW was anaerobically fermented for PHA production (0.35 PHA g/L) [15]. However, the maximum PHA content was higher than the PHA content obtained when a mixed culture rich in PHA-forming bacteria, such as *Pseudomonas sp* [43] and *P. putida* KT2442 [44], was employed for PHA production from OMW. It has been reported that the highest PHA accumulation (64.4% PHA/CDM) was achieved using mixed enriched culture and synthetic wastewater medium as carbon source [43]. However, when the synthetic wastewater was replaced by OMW, the PHAs content significantly dropped to 8.8% PHA/CDM. The authors put this decrease down to the complexity of OMW or to the inhibitory effect of polyphenols compounds present in OMW [43]. In this study, the obtained PHA content is very promising and indicates that OMW can be used without being negative for *H. mediterranei* growth and PHA accumulation.

The content and purity of the obtained polymer were investigated using GC with standard PHBHV from Sigma-Aldrich. Fig. 3 showed that the polymer sample was highly pure like the Sigma-Aldrich standard. Furthermore, the GC analysis revealed that the polymer was composed of 6.5 mol% of 3-hydroxyvalarate (3HV). In fact, the presence of the 3HV unit in the polymer chain improves its thermal and mechanical properties [5,6]. Previous studies clearly showed that the 3HV content in produced polymer was dependent on the ratio of odd to even VFAs in the fermented OMW medium [15,23,43]. For example, Shimizu et al. [45] suggested that the higher the ratio of odd to even VFAs in the medium the higher the ratio of 3HV units in the final polymer. Therefore, in some studies the 3HV monomeric units were not obtained in the polymer chain, due to the relatively low concentration of VFAs with odd carbon numbers [23,45].

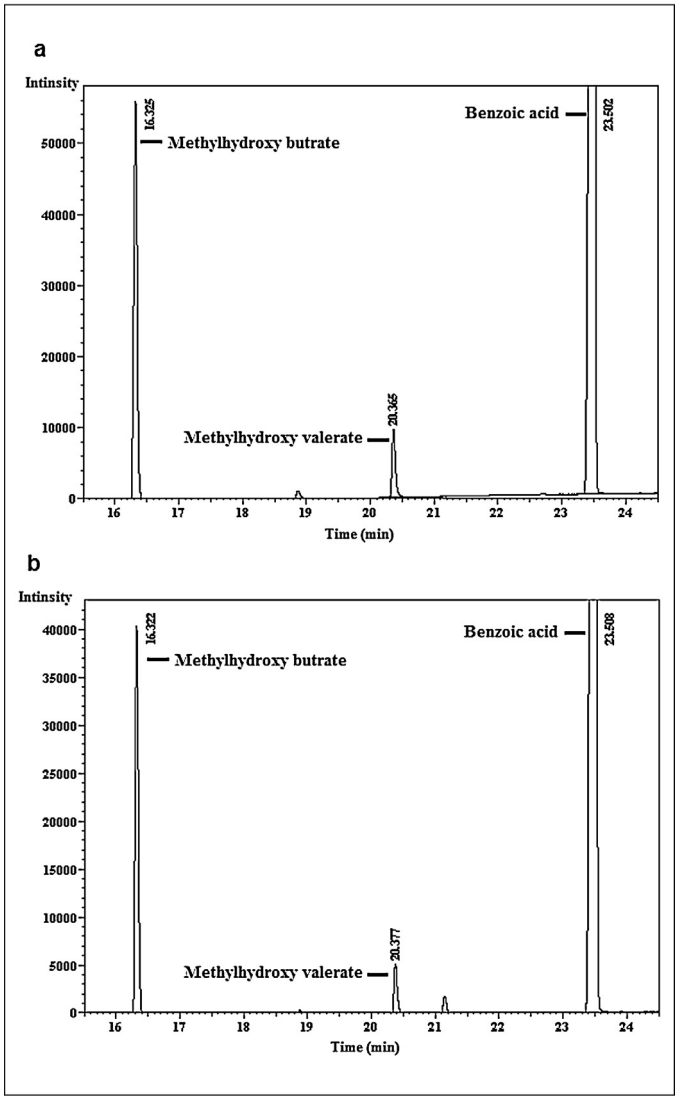

Fig. 3. Gas chromatogram for (a) Standard PHBHV containing 8 mol% of hydroxyvalarate (b) PHA from *H. mediterranei* cultivated in 15% OMW.

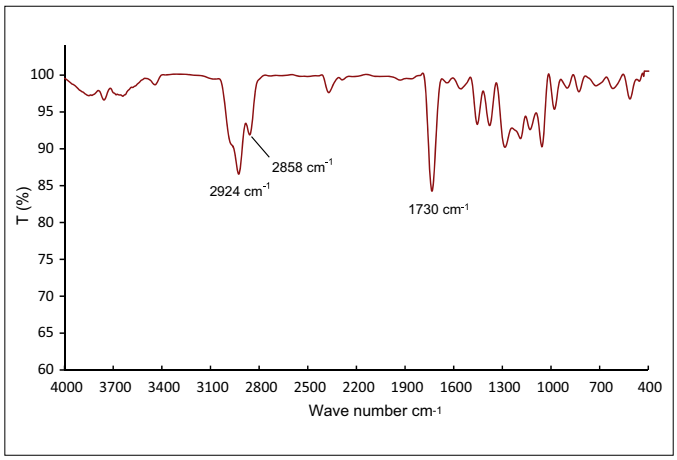

Fig. 4. FT-IR spectra of PHA isolated from *H. mediterranei* grown on 15% OMW.

Interestingly, *H. mediterranei* was able to accumulate 3HV from OMW as carbon source without the need for a fermentation step or additional feeding with costly 3HV-related precursors. Further, the 3HV content accumulated by *H. mediterranei* was higher than the values reported for final polymer produced by mixed cultures grown in fermented OMW (4 mol% of 3HV) [14].

PHA sample was analyzed using FT-IR spectroscopy (Fig. 4). The spectrum showed strong band at 1730 cm^{-1} due to the stretching vibration of the carbonyl group (C=O) of the ester. Relevant peaks were observed at 2924 cm^{-1} and 2858 cm^{-1}. These bands were assigned to CH_3 and CH_2 stretching respectively. The bands for CH_3 bending, CH_2 wagging, C—O, C—C and C—O—C stretching were observed in the range of 1450–1000 cm^{-1}. The presence of these bands confirms the PHBHV nature of the sample.

Thermal properties of polymers were evaluated by DSC (Fig. 5). The DSC thermogram shows a glass transition temperature T_g of 2.6 °C, a cold crystallization point T_c at 65.4 °C and two overlapping melting peaks at 140.1 °C and 154.4 °C. The presence of two overlapping melting peaks could be related to the occurrence of melting-recrystallisation-remelting process during subsequent heating of PHBHV. This trend of double melting behavior of PHBHV has also been reported by Gunaratne et al. [46]. Also, it is noticed that the measured melting points are both lower than the melting point of pure PHB homo-polymer, ca. 175 °C [47]. This indicates that the incorporation of 3HV unit into polymer chain decreases the melting point, which potentially resulted in improvement in the impact strength and flexibility of the polymer.

The results of PHBHV production obtained from this study were compared with PHBHV production from different waste streams using *H. mediterranei* (Table 3). The maximum PHA content (43%) achieved from *H. mediterranei* cultivated in OMW was slightly lower than the range of PHA content for *H. mediterranei* (50.8–76%) cultivation on cheese whey, crude glycerol, whey sugars, enzymatic extruded starch, rice bran-starch, enzymatic extruded starch and rice-based ethanol stillage as substrates [14,11,29,12,28,37]. Although the biomass production (10.0 g/L) was slightly higher

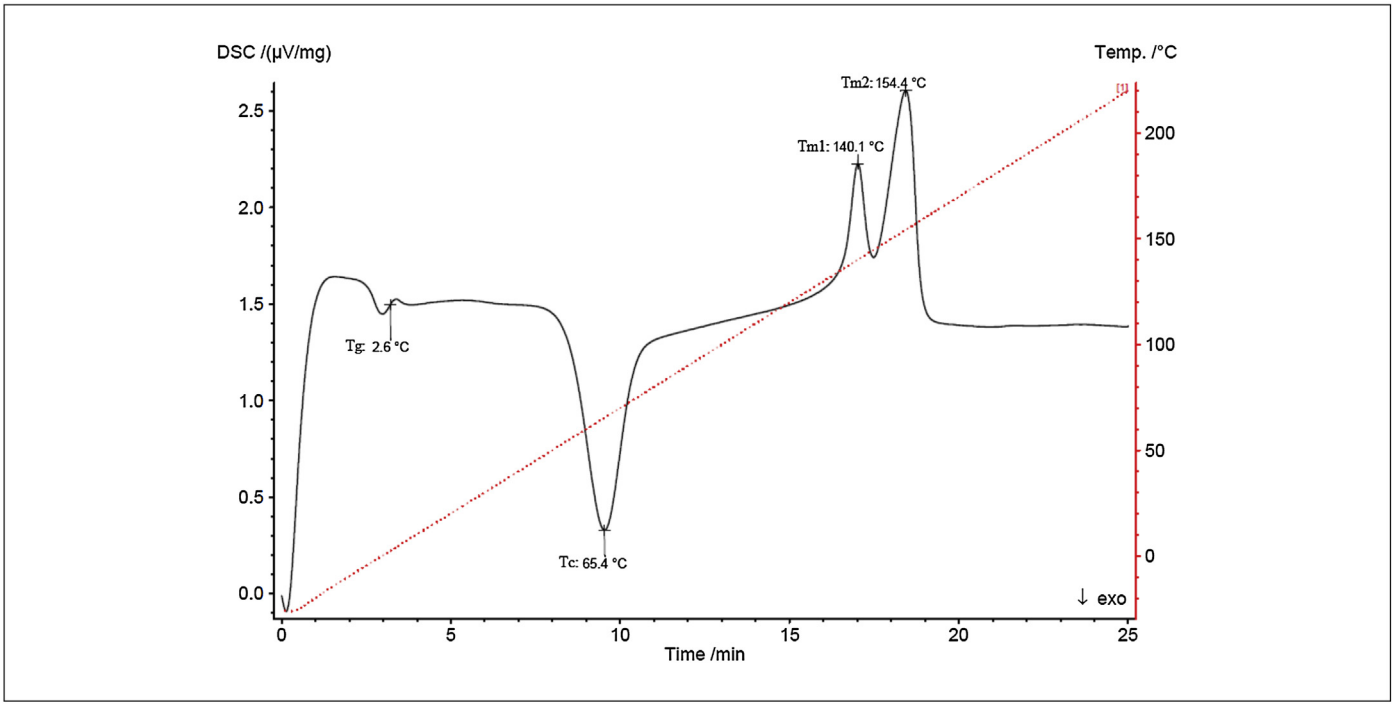

Fig. 5. DSC curve of PHBHV obtained from *H. mediterranei* cultivated in 15% OMW.

Table 3
Cultivation mode and conditions, maximum polymer yields and polymer characterization for cultivations of *H. mediterranei* in different waste streams.

	This study	PHBHV from chemically hydrolyzed cheese whey [14]	PHBHV from crude glycerol (biodiesel industry) [11]	PHBHV from whey sugars [29]	PHBHV from extruded rice bran and starch [12]	PHBHV from enzymatic extruded starch [28]	PHBHV from rice-based ethanol stillage [37]
Cultivation mode	Batch shake flasks	Batch reactor	Fed-batch reactor	Fed-batch reactor	Fed-batch reactor	Fed-batch reactor	Batch shake flasks
Cultivation conditions							
Temperature (°C)	37	37	37	37	37	37	37
pH-value	7.2	7.2	7.0	7.0	6.9–7.1	7	7.2
Agitation (rpm)	170	200	n.r.	n.r.	800	800	180
Polymer yield							
Biomass (g/L)	10.0	7.54	5.5	n.r.	140.0	39.4	n.r.
PHA concentration (g/L)	0.2	7.92	16.2	12.2	77.8	20	16.42
PHA/CDM (%)	43.0	53	76.0	72.8	55.6	50.8	71
Polymer characterization							
3HV content in PHBHV (mol%)	6.5	1.5	10	6	n.r.	10.4	15.4
1st Melting temperature (*Tm*1, °C)	140.1	151.1[a]	128.7	150.8	n.r.	129.1	n.r.
2nd Melting temperature (*Tm*2, °C)	154.4		138.8	158.9	n.r.	144.0	n.r.
Glass transition temperatures (Tg, °C)	2.6		7.0	6	n.r.	−1.2	n.r.

n.r: Not reported.
[a] Single melting temperature was detected.

than those obtained for cheese whey and crude glycerol (7.54 g/L and 5.5 g/L) in batch and fed-batch bioreactor experiments [14,11], the PHA concentration obtained from OMW was lower (Table 3). This might be due to the complexity of OMW streams. It is interesting to note that the 3HV content for the copolyester produced from OMW was close to 3HV content for polymer produced from whey sugars [29]. This very close composition can be confirmed by the similar thermal behavior of the two polyesters (Table 3).

4. Conclusion

We present an effective one-stage cultivation process for the production of PHBHV from OMW through the employment of extremely halophilic organism, *H. mediterranei*. In this process, the zero-cost carbon source of OMW was utilized for PHBHV production directly (no pre-treatment), thereby saving an additional costly dephenolization and fermentation steps. *H. mediterranei* cultivation conditions were optimized for the highest polymer yield and PHA content. The obtained polymer was extracted from microbial biomass by cheap and easy purification steps with minimal quantities of toxic solvents. Furthermore, the production of the copolymer PHBHV with a significant relative amount of HV(6%) was observed without adding any extra carbon source. The proposed process will enhance the valorization of OMW as well as reduce the production cost of valuable biodegradable polymers. Further work is still needed to bring the process steps closer to industrial scale production such as cultivation *H. mediterranei* under controlled conditions and scale-up the process using continuous feeding of the OMW.

Acknowledgments

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