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Research Article

Recycling of Waste Streams of the Biotechnological Poly(hydroxyalkanoate) Production by *Haloferax mediterranei* on Whey

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For manufacturing "bioplastics" such as poly(hydroxyalkanoates) (PHA), the combination of utilization of inexpensive carbon sources with the application of robust microbial production strains is considered a decisive step to make this process more cost-efficient and sustainable. PHA production based on surplus whey from dairy industry was accomplished by the extremely halophile archaeon *Haloferax mediterranei*. After fermentative production of PHA-rich biomass and the subsequent cell harvest and downstream processing for PHA recovery, environmentally hazardous, highly saline residues, namely spent fermentation broth and cell debris, remain as residues. These waste streams were used for recycling experiments to assess their recyclability in subsequent production processes. It was demonstrated that spent fermentation broth can be used to replace a considerable part of fresh saline fermentation medium in subsequent production processes. In addition, 29% of the expensive yeast extract, needed as nitrogen and phosphate source for efficient cultivation of the microorganism, can be replaced by cell debris from prior cultivations. The presented study provides strategies to combine the reduction of costs for biomediated PHA production with minimizing ecological risks by recycling precarious waste streams. Overall, the presented work shall contribute to the quick economic success of these promising biomaterials.

1. Introduction

Replacing contemporarily produced and exhaustively consumed plastics of petrochemical origin by sustainable "bioplastics" constitutes a challenging task both in terms of polymer quality and economics of the process [1, 2]. In the case of poly(hydroxyalkanoates) (PHA), a group of microbial biopolyesters with excellent perspectives to penetrate divers sectors of the global plastic market in the near future, product quality can be tailored according to the costumers demands by fine-tuning the PHA composition on the molecular level [3], or by designing novel composite materials and blends of PHA and compatible inorganic or bio-based materials [4]. Here, innovative methods to create PHA co- and terpolyesters [5], or blocky structured polyesters with enhanced physical properties [6], most preferably at low cost, are described. These efforts resulted in the production of a variety of advanced materials with various conceivable applications in packaging, nutrition, medicine, or other fields [7]. Also regarding the optimization of PHA productivities, for decades a cumbersome item for biotechnologists, solutions are already available based on the improvement of the process design [8], application of growth factors enhancing microbial growth rates [9], and genetic engineering [10]. Figure 1 provides the general structure of PHA.

Two remaining crucial tasks in PHA production are identified in the long-term stability of the production strains, and in the allocation of inexpensive raw materials to be used as carbon source for microbial growth and PHA biosynthesis. Regarding strain stability, one can focus on genetic engineering to equip the cells with resistances against antibiotics active against microbial competitors; alternatively, the natural antibiotic resistance of PHA producers was investigated [11], and antibiotic feeding strategies were developed that use

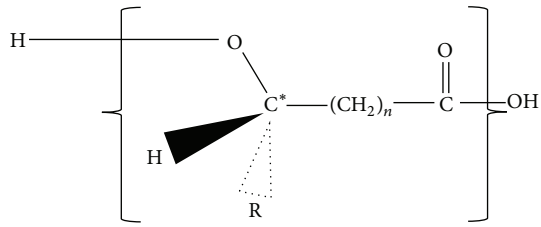

Figure 1: General structure of PHA. R indicates the side chain of monomers and *n* the number of methylene groups in the monomer's backbone. The asterisk spots indicate the chiral center of most PHA building blocks.

such antibiotics merely against frequently occurring and well-known contaminants like, for example, *Bacillus* sp. [12]. A more promising route is expected by the implementation of robust production strains that either are cultivated exclusively in ecological niches, characterized by extreme salinity, temperature, substrate concentration, or pH-value [13], or at least tolerate such extreme environments without severe losses of growth and product formation rates. Processes based on such strains are carried out without the need for genetic engineering, without supply of antibiotics, and often even at minimum sterility precautions. On the substrate side, there is an increasing number of frequently occurring carbon-rich industrial waste streams that perform well as feed stocks for PHA; here, the value-added upgrading of whey lactose, crude glycerol phase from the biodiesel production, CO_2 from effluent gases, hydrolysates of lignocellulosics, nonedible starch, or waste lipids to raw materials for biopolyester production is exhaustively reported in the literature [14–16]. The work at hands concentrates on the conversion of surplus whey from dairy industry, a lactose-rich liquid accruing at enormous quantities, especially in Europe and North America. PHA production from surplus whey is, beside the generation of other bioproducts such as ethanol, sophorolipds, and others, one of the most promising strategies to convert this ecopollutant in a value-generating way [16].

The search for new powerful and robust microbial production strains that come along without genetic engineering is a task of contemporarily high significance [14]. Among these wild-type strains, the archaeon *Haloferax mediterranei*, an extreme halophile, is of outstanding importance. Its excellent robustness, genetic stability, broad substrate spectrum, superior quality of the produced PHA, and marketable side products such as bacterioruberins, extracellular polysaccharides, or halocins attract considerable attention by the scientific community [13]. Its extremophile character, manifested by the high salt requirement for growth, enables the exclusive cultivation of this strain even under nonsterile conditions without endangering the fermentation batch by microbial contamination [5, 17]. The adaptation of *H. mediterranei* cells to high salinity offers the possibility to run such processes at low operation costs (low energy for sterilization, no solvents needed for product recovery from the cells grace to their high inner osmotic pressure); cost estimations report a production price below 3 € per kg PHA based on the raw material whey lactose [18].

As the other side of the medal, the required quantities of salt (approximately 200 g NaCl per kg of fermentation broth) need to be allocated and, especially, need to be disposed of after cell harvest and product recovery. Hence, the recycling of the major waste streams from PHA production batches with *H. mediterranei* is of major interest not only for economic reasons but also for diminishing ecological risks stemming from the enormous salt lots. When PHA-rich cells of *H. mediterranei* are separated from the liquid phase of the cultivation medium (supernatant), they subsequently undergo a process for PHA recovery; here, PHA is isolated from biomass by disruption in hypotonic medium. As a consequence, the following waste streams from the entire production process can be pointed out as follows:

(i) salty supernatant of the fermentation broth (spent fermentation broth, "SSF");

(ii) cell debris plus the intracellular salts ("SF," obtained by drying together cell debris plus released salts after disruption of cells and separation of PHA);

(iii) salt-free cell debris (obtained when saline debris SF is washed after disruption and separation of PHA, "SD").

Aim of the Work. The paper reports for the first time the experimental assessment of these waste streams to be reutilized in subsequent fermentation batches. In detail, how SSF can act to replace fresh saline fermentation medium and how SF and SD can be used to partly substitute the costly cosubstrate yeast extract that is needed to obtain optimum growth of *H. mediterranei* were investigated. Hence, as a novelty, the work combines the utilization of whey, a waste material from dairy industry, as main carbon source for biopolymer synthesis with the recycling of waste streams stemming from the polymer production process itself.

Figure 2 illustrates the proposed "ideal" fermentation strategy for PHA production using *H. mediterranei* and whey as carbon source; an integral process is proposed, characterized by keeping all waste streams in closed cycles within the production lines.

2. Materials and Methods

2.1. Enzymatic Treatment of Whey Permeate. Whey permeate was kindly provided by the dairy company Latterie Vincentine, Italy, with a lactose concentration of about 200 g/L. The permeate was subjected towards enzymatic degradation towards glucose and galactose using a *Maxilact 2000* formulation (DSM Food Specialities, UK) according to [18].

2.2. Microbial Strain, Preculture, and Inoculum Preparation. *H. mediterranei* DSM1411, obtained as lyophilized sample from DSMZ culture collection, Germany, was maintained on solid agar-agar slants containing the following medium ingredients (g/L): NaCl, 150; $MgCl_2 * 6\ H_2O$, 13; $CaCl_2 * 2\ H_2O$, 0.69; KCl, 4; NH_4Fe(III)citrate, 0.063; SL6, 1.25; $MgSO_4 * 7\ H_2O$, 20; $NaHCO_3$, 0.25; NaBr, 0.5; yeast extract, 6.25; enzymatically hydrolyzed whey permeate, 50; and agar-agar, 15.

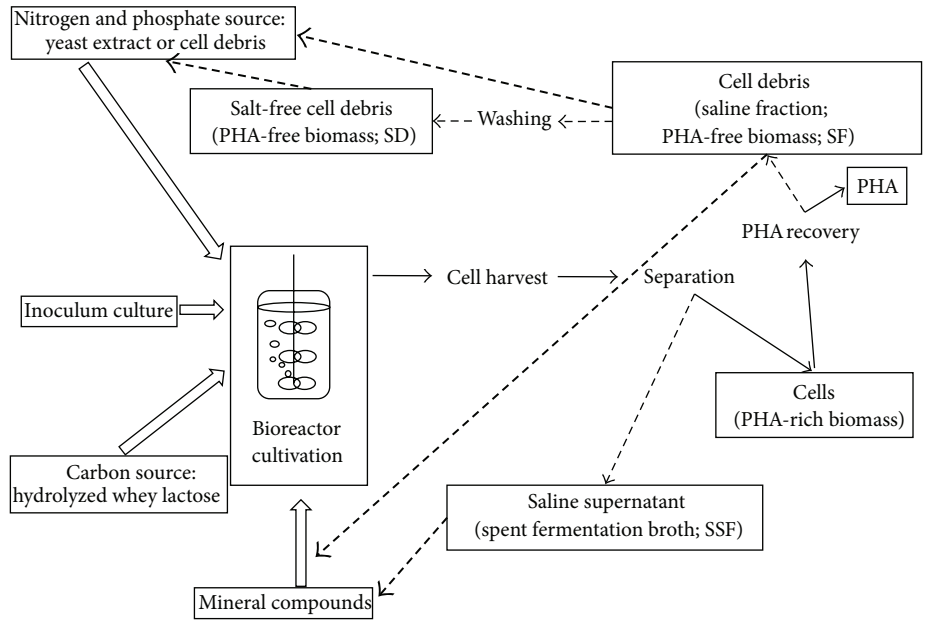

Figure 2: Scheme of a closed recycling system for reutilization of waste streams from fermentative PHA production process by *H. mediterranei* based on the feedstock whey. The saline supernatant SSF should be used to partly replace mineral compounds in subsequent fermentation processes, whereas salty (SF) or salt-free (SD) cell debris should be used as nitrogen and phosphate source in order to reduce the required amount of yeast extract.

From the agar slants, single colonies where transferred into 100 mL of the same liquid medium (without agar-agar) adjusted to a pH-value of 7.0 and agitated on a reciprocal laboratory shaker at 130 rpm in baffled shaking flasks (*precultures*) at 37°C. When the cells had reached the late exponential phase (monitored by the achievement of an $OD_{420\,nm}$ of app. 20), 5 mL of a selected preculture was transferred to 250 mL fresh culture medium in baffled shaking flasks (*inoculum cultures*).

2.3. Bioreactor Cultivation. The *inoculum cultures* where cultivated at 37°C on a reciprocal laboratory shaker at 130 rpm. After 48 h of growth, the cells had reached the late exponential phase and were added as inoculum to 7 liters of nonsterilized growth medium (same composition as for inocula preparation) in a 300 L bioreactor (L 1523, *Bioengineering*, Wald, CH), operated with a working volume of 220 L. The bioreactor was equipped with a two-axial propeller stirrer. Temperature was controlled with a bioengineering temperature controller SPC, the pH-value with a Hamilton electrode, and the oxygen partial pressure (pO_2) using an Ingold electrode. pO_2 was controlled and maintained at about 20% of air saturation by varying both agitation speed (~300–650 rpm) and the aeration rate. Temperature was kept constant at 41°C and the pH-value in a narrow range between 6.8 and 7.4. 10% (w/v) *Structol* was used as antifoam agent, 10% (w/v) H_2SO_4 as acid and 10% (w/v) NaOH as hydroxide for correction of the pH-value. The fermentation was designed as a fed-batch process; refeeding of carbon source was accomplished by pulse feeding of enzymatically hydrolyzed whey permeate (sugar content 20% w/w) according to the conversion of the substrate by the cells (monitored by consumption of dissolved oxygen and by regular HPLC analysis). Samples were taken at regular time periods to analyze optical density, cellular protein, concentration of glucose and galactose, and concentration and composition of intracellular PHA. The fermentation was stopped after 64.25 h after depletion of the majority of remaining sugars.

2.4. Determination of Optical Density ($OD_{420\,nm}$). Optical density ($OD_{420\,nm}$) was measured at a wavelength of λ = 420 nm against cell-free supernatant as zero reference using a Hitachi U-1100 spectrophotometer.

2.5. Determination of Cellular Protein. Protein determination was done, after ultrasonic disruption (20 min at 35 kHz; Sonorex Digitec, Bandelin) of the cells in pellets obtained after centrifugation of 5 mL fermentation broth in plastic tubes and subsequent washing with water, according to Lowry's method [19].

2.6. PHA Analysis. The PHA detection method was based on the simultaneous extraction and transesterification of PHA. The frozen and lyophilized biomass pellets in glass tubes were used for measuring the PHA concentration and composition in microbial biomass by gas chromatography with flame ionization detection (GC-FID) after acidic methanolysis as described previously [5, 9, 18]. Pure poly(3-*R*-hydroxybutyrate-*co*-3-*R*-hydroxyvalerate) (PHBV) copolyester with a 3-*R*-hydroxyvalerate (3HV) content of 19.1% from Biopol (UK) was used for calibration; thus, the contents of 3-*R*-hydroxybutyrate (3HB) and 3HV were determined; PHA was defined as the sum of 3HB and 3HV.

The PHA content in cells (% w/w) was defined as the ratio of PHA concentration to the sum of PHA and cellular protein.

2.7. PHA Recovery. After stop of the bioreactor fermentation, the fermentation was *in situ* pasteurized (20 min, 70°C) and harvested. The volume of this broth was reduced by 90% using a Westphalia separator; finally, solid cell pellets and the saline spent supernatant (SSF) were obtained by using a *Sorvall RC-5B Refrigerated Superspeed* centrifuge at 4,000 rpm for 20 min. The wet cell pellets were frozen and subsequently dried by lyophilization (lyophilizator *Christ Alpha 1-4 B*).

By using water for disrupting the cells, their high intracellular osmotic pressure of *H. mediterranei* leads to a suspension of unscratched PHA granules and cell debris in a salty solution. PHA granules and cell debris were separated by profiting from the different densities by centrifugation (*Sorvall RC-5B Refrigerated Superspeed* centrifuge, 4,000 rpm, 20 min), enabling the convenient separation of PHA granules by simple skimming. Water was removed from the salty cell debris suspension by heat treatment to produce a solid material (saline fraction SF).

2.8. Recycling of the Salty Supernatant (SSF-Experiment). For this experiment, a bioreactor (10 L scale, *Bioengineering*, Wald, CH) was used according to [18]. In order to quantify the inorganic compounds, samples of the concentrated salty recycle stream (supernatant, called SSF) were incinerated, and the ash content was quantified. Based on the data thereof, a fermentation medium was created containing a total amount of salts equivalent to the entire salt content of the *H. mediterranei* medium (see Microbial Strain, Preculture, and Inoculum Preparation). Nitrogen and phosphates were added by supplementing yeast extract, not by supplementing cell debris (SF or SD) in order to change only one parameter (recycled salts instead of new salts) compared with the "original" fermentation on 300 L scale. Carbon source was provided to the cells by supplementation with hydrolyzed whey permeate. Temperature (37°C) and pH-value (7.0) were adjusted corresponding to the "original experiment" on 300 L scale.

2.9. Recycling of Salty Cell Debris (SF-Experiment). The salty material (saline fraction (SF)) was resuspended and used for shaking flask experiments, where, starting from the *H. mediterranei* medium described above, parts of NaCl (25% or 50%, resp.) or all of it (100%) were replaced by the salty waste material SD that stemmed from a previous cultivation. Control flasks with completely fresh medium (0%) were also prepared. All flasks were inoculated with 5% (v/v) of dense cultures from the late exponential phase ($OD_{420\,nm}$ approximately 20) prepared on fresh medium at a temperature of 37°C and a pH-value of 7.0.

2.10. Recycling of Salt-Free Cell Debris (SD-Experiment). Shaking flasks containing 5 g/L of complex nitrogen and phosphate source (yeast extract or SD or mixtures thereof) and 10 g/L of sugars from hydrolyzed whey permeate were incubated with 1% (v/v) of a dense preculture of *H. mediterranei* DSM 1411 and cultivated at $T = 37$°C at a pH-value of 7.0. Different percentages of yeast extract were substituted by SD as obtained by repeated washing of the saline fraction SF (calculated by the dry matter): 0, 25, 50, 75, and 100%.

3. Results and Discussion

3.1. Recycling of the Salty Supernatant (SSF-Experiment). This bioreactor experiment aimed to investigate the possible recycling of the huge amounts of salts remaining in the supernatant SSF, constituting the major salt fraction accruing at *H. mediterranei* cultivations after cell harvest. The supernatant was obtained from cultivation on 300 L scale using *H. mediterranei* on hydrolyzed whey permeate as carbon source (see Section 2). The fermentation broth was *in situ* pasteurized in the 300 L bioreactor before cell harvest.

The type of polymer that was produced during the fermentation on SSF on 10 L scale was a copolyester consisting of 3HB and 3HV monomers; approximately 10% of 3HV was incorporated into the polyester during the entire cultivation time. This is a typical feature for PHA produced by *H. mediterranei* from simple carbon sources like sugars [5, 18] and in huge contrast to the polymer accumulated by common PHA producers like *Cupriavidus necator* that produces poly(3-*R*-hydroxybutyrate) (PHB) homopolyester from similar [9] or the same [18] substrates.

The low production of protein in the recycling experiment resulted in a sudden increase of the polymer content in the entire cell mass already at the beginning of the cultivation. This is in contrast to prior fermentations with this organism on fresh media, where production rates for protein and PHA are similar during growth phase and a sharp increase of the PHA contents does only occur after protein formation has ceased [5, 18]. The final PHA content in cells amounted to approximately 70% (w/w) that is nearly identical to the "original" fermentation on 300 L scale (see Table 1).

Figure 3 shows the growth curve (expressed as the $OD_{420\,nm}$) of the "original" fermentation on 300 L scale (fresh medium) together with the growth curve from the 10 L fermentation (recycling of the supernatant SSF). It is clearly visible that there are inhibitory effects when recycling streams are applied. The final $OD_{420\,nm}$ after 64.25 hours amounted to 62.7 (see Figure 3) in the recycling fermentation in comparison with an $OD_{420\,nm}$ of approximately 120 that was obtained after the same cultivation time in the "original" fermentation on 300 L scale (see Figure 3 and Table 1).

The most significant process-relevant outcomes of the recycling experiment in comparison with the "original" fermentation are collected in Table 1. The final concentration of PHA in the recycling experiment amounted to 2.28 g/L (see Table 1). The "original" fermentation in 300 liter scale reached 7.2 g/L after the same time (64.25 hours) and 11.86 after the stop of the experiment at $t = 80$ hours. The total biomass concentration was calculated with 3.22 g/L for the recycling fermentation ("original": 13.63 g/L; see Table 1). It was not possible to reach a higher protein concentration than 0.94 g/L under the investigated conditions (at the "original" experiment: ca. 6 g/L; see Table 1). The calculated values for

Table 1: Comparison of important results from *H. mediterranei* DSM 1411 fermentation on recycled salty supernatant (10 L scale) and the original cultivation on 300 L scale.

	Original fermentation	Recycling experiment	Factor recycling experiment/original experiment
μ_{max} [1/h]	0.18	**0.08**	0.44
Duration of lag-phase [h]	17	**21**	1.24
Final protein concentration [g/L]	6.0	**0.94**	0.16
Final PHA concentration [g/L]	7.20	**2.28**	0.32
Final PHA in biomass [%]	66	**70**	1.06
Final optical density [$OD_{420\,nm}$]	120	**62.7**	0.52
Mass fraction 3HV/PHA [g/g]	0.1	**0.1**	1.00

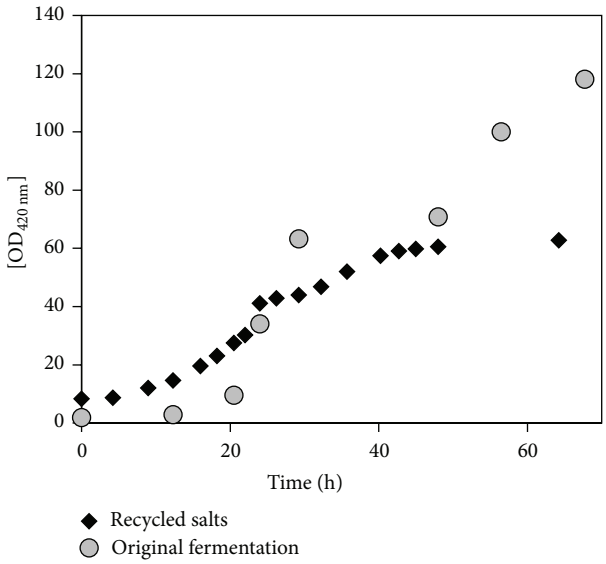

Figure 3: Comparison of time curves of optical density [$OD_{420\,nm}$] of the "original" fermentation and the recycling experiment on salty spent supernatant (SSF); cultivations of *H. mediterranei* DSM 1411 on whey as main carbon source.

the maximum specific growth rate μ_{max} amounted to 0.18 h^{-1} for the "original" fermentation, but to only 0.08 h^{-1} for the recycling experiment. It is well visible that the adaptation phase (lag-phase) was extended in the recycling experiment (approximately 21 hours versus approximately 17 hours, resp.; see Table 1). The final concentrations of 3HV amounted to approximately 0.24 g/L; in the "original" experiment, about 0.43 g/L could be obtained within the same time (see Table 1). In both fermentation setups, the produced copolyesters displayed mass fractions of about 0.9 g/g for 3HB and 0.1 g/g for 3HV as typical for the applied production strain.

As a conclusion, it was demonstrated that SSF principally can act as saline medium for subsequent cultivations of *H. mediterranei*, but lower productivities and growth rates were observed if compared to the original setup on fresh medium. Therefore, a processing step for the salty supernatant "SSF" is suggested, because the inhibitory components are expected to derive thereof. The inhibitory effects clearly affect the protein formation, not the polyester production (high rates of PHA production from the beginning of the cultivation, extremely low protein formation, and specific growth rate). An additional drying and incineration step in order to destroy all remaining organic material might be the solution of the problem. The same should be tried with the salty residues released from the biomass after disruption (saline fraction (SF)). Of course, these additional steps demand further energy, but a possible recycling of the very high salt amounts should nevertheless result in an economic progress and, in any case, in an ecological improvement. Further experiments could also be done by supplementing only parts of the needed salts by supernatant.

3.2. Recycling of Salty Cell Debris (SF-Experiment). A feasible, solvent-free method for isolating PHA from *H. mediterranei* cells was developed at the Institute for Process and Particle Engineering, Graz University of Technology. By using water for disrupting the cells, their high intracellular osmotic pressure leads to a suspension of cell debris in a salty solution. These salts derive from the inside of the disrupted cells or were connected to the cells surface. Water was removed from the salty suspension by heat treatment in order to produce a solid material (see materials and Methods section). It was of interest to examine the possibility to use this waste material as a substitute of new salt for subsequent cultivations.

Figure 4(a) shows growth curves (monitored via $OD_{420\,nm}$) for cultures grown in media with 25, 50, and 100% of NaCl replaced by the waste material (SF), compared with the original medium (bold curve).

It is well visible from Figure 4(a) that the replacement of 25 and 50% of NaCl by SF causes slightly longer adaptation phases. This influence on adaptation phase is more clearly visualized in Figure 4(b) where a Dixon plot for evaluation of the inhibiting amount of the saline fraction is drawn. This graphical evaluation of the growth curves results in an "inhibition constant" K_i of 0.12 g/g, indicating that starting from a substitution of 12% (w/w) of NaCl by SF, a negative impact on growth is manifested. The setups with a total substitution of NaCl by the waste material showed very slow growth of the organism. This might have two possible reasons: first, analytical data obtained by ICP–determination of cations in the waste stream show a shift of the sodium/potassium ratio towards higher amounts of potassium (high intracellular potassium pool) (data not shown). A second reason might be the presence of inhibiting organic material that is left from the original cultivation (product inhibition).

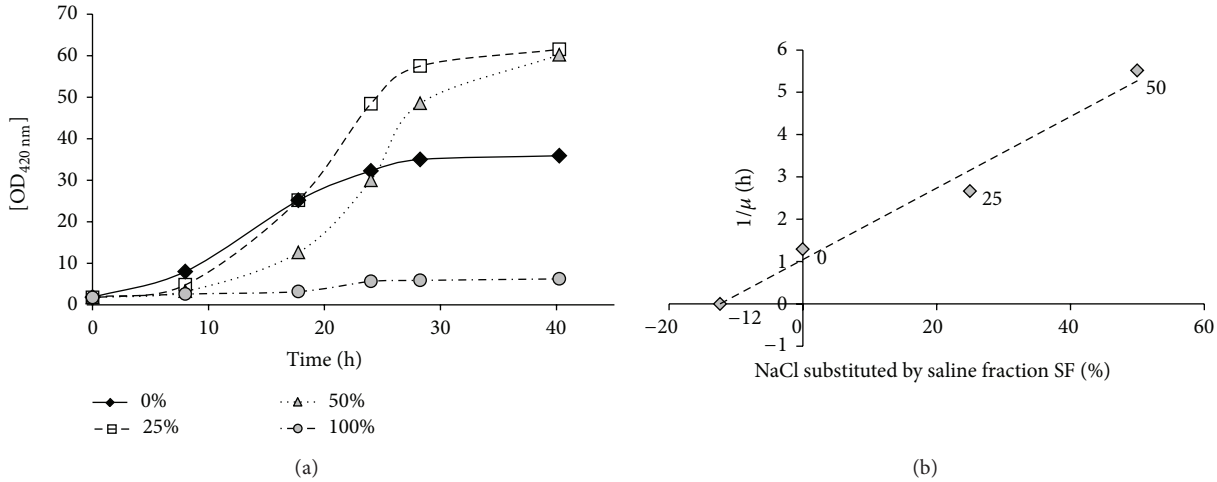

Figure 4: (a) Time curves of optical density: different percentages of NaCl in *H. mediterranei* DSM 1411 medium replaced by saline fraction "SD" from product isolation. (b) Dixon plot for evaluation of the inhibiting amount of saline fraction.

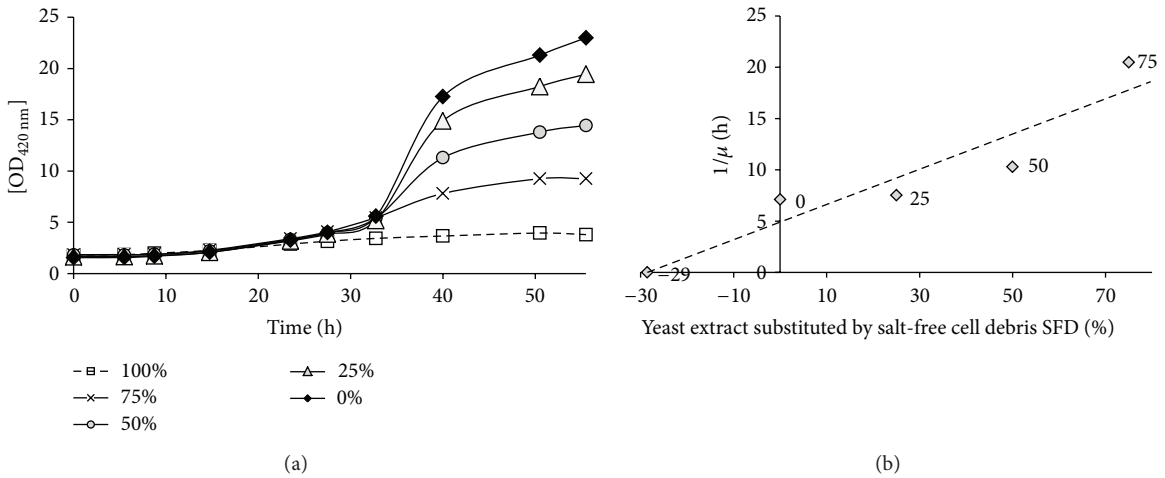

Figure 5: Cultivation of *H. mediterranei* DSM 1411. Time courses of $OD_{420\,nm}$ (a) and maximum specific growth rate μ_{max} (b). The "classical" nitrogen and phosphate source yeast extract was not, partly, and totally substituted by desalinated cell debris SD.

The second half of the cultivation period shows a positive impact of the waste material: in the original medium, the growth curve slows down because of sugar depletion (data not shown); the cultures containing 25 or 50% of waste material SF displayed extended exponential phase of growth. Therefore it is likely that additional substrates from the prior fermentation were available (convertible organic residues). Because of that, it was reasonable to investigate the possible convertibility of salt-free cell debris (SD) in the subsequent experiment.

As a conclusion, the results indicate that the reutilization of the salty waste material SF might not only be of interest for salt recovery but also be an important source of complex substrate (nitrogen, phosphate, and carbon). The obtained data clearly show that the maximum amounts of NaCl that can be replaced by the saline fraction without a major impact on microbial growth are strictly limited to approximately 50%, with negative impacts on growth already at supplementation of about 12%. In any case, the results give first hints that recycling of waste streams from *H. mediterranei* cultivations should be possible to a certain extent and should be investigated in more details in future.

3.3. Recycling of Salt-Free Cell Debris (SD-Experiment). The influence of the salt-free cell debris SD from the cultivation of *H. mediterranei* on 300 L scale on hydrolyzed whey permeate as a substitute or partial substitute for the expensive substrate yeast extract was investigated.

Figure 5(a) shows the times curves of $OD_{420\,nm}$ for the cultures grown in media containing different composition of nitrogen and phosphate source, consisting of yeast extract, or SD, or mixtures of these substrates. Thereof it can be seen that after substituting 25% of yeast extract by SD, 83% of the increase in $OD_{420\,nm}$ from the cultivations on pure yeast extract is obtained. When 75% of the yeast extract is substituted by SD, growth is strongly inhibited. This is more clearly

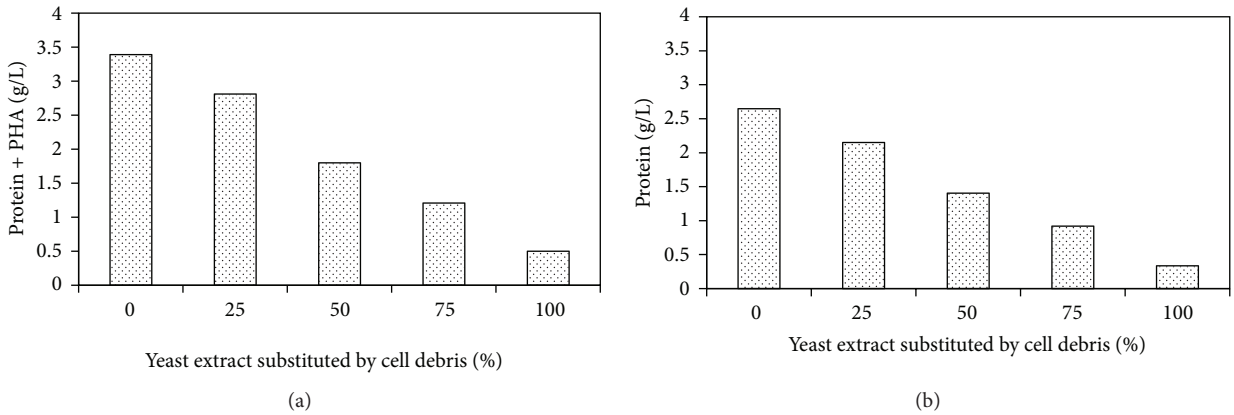

Figure 6: Produced concentrations of protein plus PHA (a) and protein (b): the classic nitrogen and phosphate source yeast extract not (0%), partly (25, 50, and 75%), and totally (100%) substituted by salt-free cell debris (SD). Cultivation of *H. mediterranei* DSM 1411.

illustrated by the produced amounts of protein measured at the end of the cultivation (t = 55.5 hours; see Figure 6(b)). When these values are corrected by the initial protein concentration of the inoculum cells, 2.50 g/L protein is formed using the "classical" medium with pure yeast extract as nitrogen and phosphate source. This can be compared with 1.94 g/L, when 25% of yeast extract is substituted by cell debris. A 50/50 mixture of SD and yeast extract yields in 1.22 g/L protein; when 75% of the complex source amounts to SD, only 0.81 g/L protein is formed. A total replacement of yeast extract by SD results in the generation of only 0.20 g/L protein. The sum of concentrations of PHA and protein for the different setups is illustrated in Figure 6(a), reflecting a similar trend as for protein concentrations (Figure 6(b)).

Figure 5(b) compares the maximum specific rates for total cell mass generation (calculated via $OD_{420\,nm}$) by a Dixon plot for evaluation of the inhibiting percentage of yeast extract if partly replaced by SD. This graphical evaluation evolves an "inhibition constant" K_i of 0.29 g/g; this indicates that, starting from a substitution of 29% (w/w) of yeast extract by SD, a negative impact on growth has to be expected.

According to the results, only small amounts of cell debris can be directly converted into new biomass. Due to the fact that cell debris definitely constitutes a real surplus product and yeast extract contributes enormously to the process costs (market value approximately 50 €/kg) it still might be reasonable to replace certain amounts (according to the results up to 29%) of yeast extract by SD. One could also think about an additional hydrolysis step to make cell debris more accessible (easier convertible) for the cells; here, it is doubtful if the additional costs for enzymatic hydrolysis are justified by an eventual increase of cultivation performance, hence, better microbial growth. Acidic hydrolysis might result in degradation of essential amino acids, as previously demonstrated for supplementation of *H. mediterranei* with acidic hydrolyzed meat-and-bone meal as complex nitrogen source [20].

For the future it would be of interest to investigate the possibility to upgrade cell debris by supplementation, for example, with vitamins that are present in yeast extract, but not in the disrupted cell debris.

4. Conclusion

Based on the presented results, it can be expected that it is possible to at least partly reutilize the main waste streams (salty supernatant, cell debris, and salt from disrupted cells) from PHA production on surplus whey using *H. mediterranei* as extremophile production strain. Some major drawbacks and future challenges in the recycling of the investigated waste streams can be pointed out as follows.

"The salty supernatant SSF very likely includes inhibitory components that are negatively effecting the protein formation."

(i) In future we should try to harvest biomass without prior pasteurization; this could minimize the formation of inhibitory components.

(ii) A drying and incineration step after cell disruption could be useful in order to destroy all remaining organic material. This additional step is energy demanding, but a possible recycling of the enormous amounts of salt should nevertheless make the process significantly cheaper and, in any case, result in an ecological improvement if compared to the alternative direct disposal of the enormous salt loads in aquatic environments.

(iii) Further experiments should also be accomplished to evaluate the maximum amounts of "fresh" salts that could be replaced by supernatant.

(iv) Fine-tuning the ratio of K^+ and Na^+ ions in SSF is needed in order to compensate the K^+-consumption during prior fermentations.

"Only small amounts of cell debris SF and SD can be directly converted into new biomass."

(i) A detailed analysis of amino acids and other cofactors that are not available in cell debris in sufficient amounts when compared with yeast extract should be undertaken. If in fact limitations occur, deficiencies of certain compounds may be compensated by adequate supplementations.

(ii) Due to the high price that is featured by yeast extract, it still appears reasonable to substitute at least parts of the expensive source by cell debris.

(iii) The alternative nitrogen source whey retentate that is directly available at the dairy in its hydrolyzed form constitutes a substrate of major interest due to the higher positive impact on growth of the organism than cell debris [16].

Overall, work combines the utilization of surplus whey as main substrate for biomediated polymer production and the recycling of waste streams stemming from the polymer production process itself; it suggests a new approach towards sustainable, robust, and efficient PHA manufacturing.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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