ABSTRACT

The doctoral dissertation consists of four publications, one of which is a review article and three of which are original research articles. Biopolymers have attracted much attention in the last few decades as environmentally friendly alternatives to petrochemical polymers. Due to the unique material properties polyhydroxyalkanoates (PHAs) are particularly attractive. They are produced in biotechnological processes by various microorganisms as carbon and energy reserves under unbalanced growth conditions. PHAs have great potential in industry, nevertheless their production on a large scale is still limited due to the high production costs. Despite the fact that the fermentation technology and polymer extraction process have been improved over the last years, the microbial PHA production is still not economically feasible. Therefore, there is a growing need to develop novel microbial processes and to search for an efficient PHA producer capable of synthesizing these biopolymers using renewable carbon sources. In the literature there are many scientific reports on the microbial production of PHAs, however none of them describes the microbial production of unique PHAs from SMCFAs generated in the anaerobic mixed culture fermentation of acid whey. Also, there was a lack of studies that evaluated in details properties of the biopolyesters extracted in the cultivations with SMCFAs to assess their application potential. Therefore, the aim of the doctoral dissertation was to investigate the use of synthetic and acid whey derived short and medium chain fatty acids (SMCFAs) as feedstocks for PHA production by bacteria belonging to three different genera: *Paracoccus* sp., *Aeromonas* sp. and *Pseudomonas* sp..

The results revealed that all tested bacterial strains were able to grow and synthesize PHAs using individual synthetic SMCFAs, those derived from acidogenic anaerobic mixed culture fermentation or their synthetic mixtures as carbon sources in the growth media. Furthermore, the results indicated that the PHA content in bacterial cells depended on the type and concentration of the substrate, and applied culture conditions. The replacement of the individual SMCFAs by the SMCFAs-rich residual stream was found to be less beneficial for PHA productivity compared to the single, synthetic SMCFAs that suggested more inhibitory effect of waste-derived fatty acids. In the cultivation of *Paracoccus homiensis* quite low PHA productivity could be explained by the presence of butyric acid as the dominant component in the SMCFA-rich stream which as a individual fatty acid in the culture medium resulted in the lowest PHA production efficiency compared with others. Interestingly, a mixture of acetic acid and butyric acid supported better PHA synthesis and accumulation than individual acids in the

cultivation of *Aeromonas* sp. AC_01. Moreover, unbalanced growth conditions positively affected the PHA productivity. In the fermentation process of *Pseudomonas* sp. GL01 PHA productivity reached up to 4.5 mg/L·h when the ammonium was exhausted in the culture medium. Importantly, the monomeric composition of the extracted PHAs depended on the culture conditions used. The data showed that SMCFAs-rich stream used in the cultivation of *Paracoccus homiensis* could support the synthesis and accumulation of the P(3HB-co-3HV) copolymer. However, the 3HV content was low, therefore the induction of the acidogenic fermentation to produce more short and medium chain fatty acids with an odd carbon number has been suggested. Furthermore, *Aeromonas* sp. AC_01 cultured on the same carbon source was able to produce novel short- and medium-chain length (scl-mcl) copolymers of 3-hydroxybutyrate (3HB), 3-hydroxyvalerate (3HV), 3-hydroxytridecanoate (3HtriD) and/or 3-hydroxytetradecaonate (3HTD) with high 3HB and 3HV fractions. Also, *Pseudomonas* sp. GL01 showed the tendency to produce copolymers but with high amounts of 3-hydroxyhexanoate (3HHx) and 3-hydroxydodecanoate (3HDD) as the main monomers and lower content of 3-hydroxybutyrate (3HB), 3-hydroxyoctanoate (3HO), and 3-hydroxydecaonate (3HD). Furthermore, a differential scanning calorimetric, thermogravimetric and water contact angle analysis proved that the extracted biopolymers have useful properties. The melting temperature of the scl-mcl copolymer extracted from the *Pseudomonas* sp. GL01 was the lowest and unusual indicating its amorphous behavior and flexibility at ambient temperature. Also, the presence of the 3HB fraction could have caused its lower thermal resistance. The biopolyesters isolated from *Paracoccus homiensis* and *Aeromonas* sp. AC_01 cells possessed high thermal stability which is beneficial taking into account their processing technology. The obtained data are promising in the perspective of developing novel process of utilizing high SMCFAs content waste-derived streams without necessity for substrate pretreatment for production of value-added PHA copolymers.