

From agro-industrial waste to polyhydroxyalkanoates (PHA): submerged fermentation using mixed and pure bacterial cultures

Dora Bramberger¹, Viktoria Bačić¹, Karlo Grgurević¹, Laura Jaklenec¹, Vesna Ocelić Bulatović¹, Marinko Markić¹, Anja Rakas¹, Lidija Furač¹, Matija Cvetnić¹, Tomislav Bolanča¹, Šime Ukić^{1†}, Stela Jokić², Krunoslav Aladić², Ivica Strelec², Jasmina Ranilović³, Dajana Kučić Grgić¹

¹University of Zagreb, Faculty of Chemical Engineering and Technology, Trg Marka Marulića 19, 10 000 Zagreb, Croatia

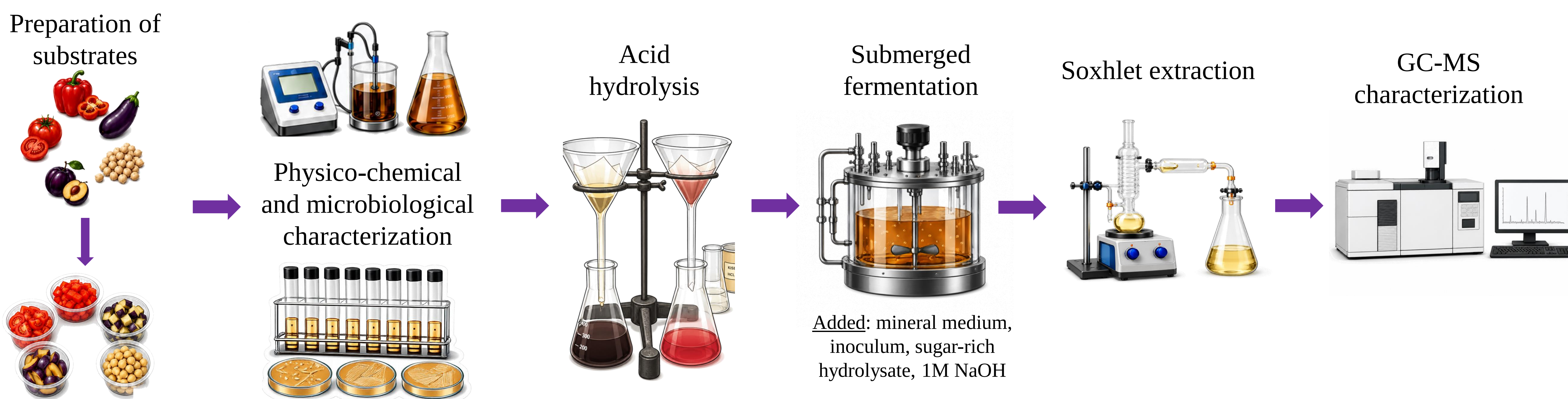
²University of Osijek, Faculty of Food and Technology Osijek, Ulica Franje Kuhača 18, 31 000 Osijek, Croatia

³Podravka d.d., Ulica Ante Starčevića 32, 48 000 Koprivnica, Croatia

INTRODUCTION

Polyhydroxyalkanoates (PHA) are biodegradable polymers synthesized by microorganisms as intracellular carbon and energy storage compounds. Due to their biodegradability and similar properties to conventional plastics, PHA represent a promising sustainable alternative for various applications. This study explored the production of poly(3-hydroxybutyrate)/poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/PHBV) from agro-industrial waste (chickpeas, plums, eggplants, peppers, and tomatoes) using acid hydrolysis and submerged fermentation. The fermentation process was performed in a bioreactor with pure culture *Pseudomonas putida* and mixed bacterial cultures *Cupriavidus necator* and *Pseudomonas putida*. During fermentation, optical density, pH value, dissolved oxygen concentration, and reducing sugar concentration were monitored.

METHODOLOGY



RESULTS

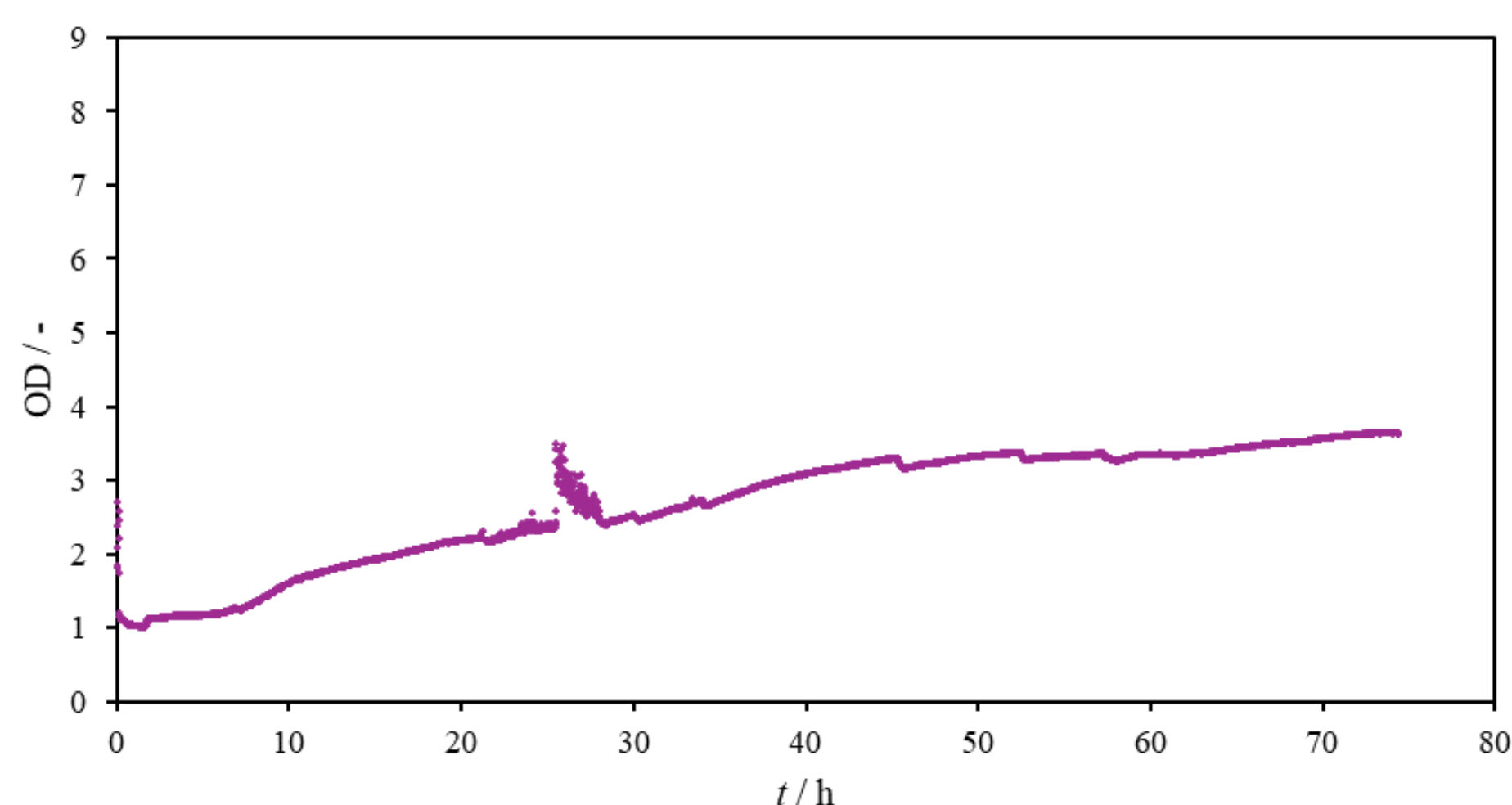


Fig 1. Change in optical density during fermentation with *Pseudomonas putida*.

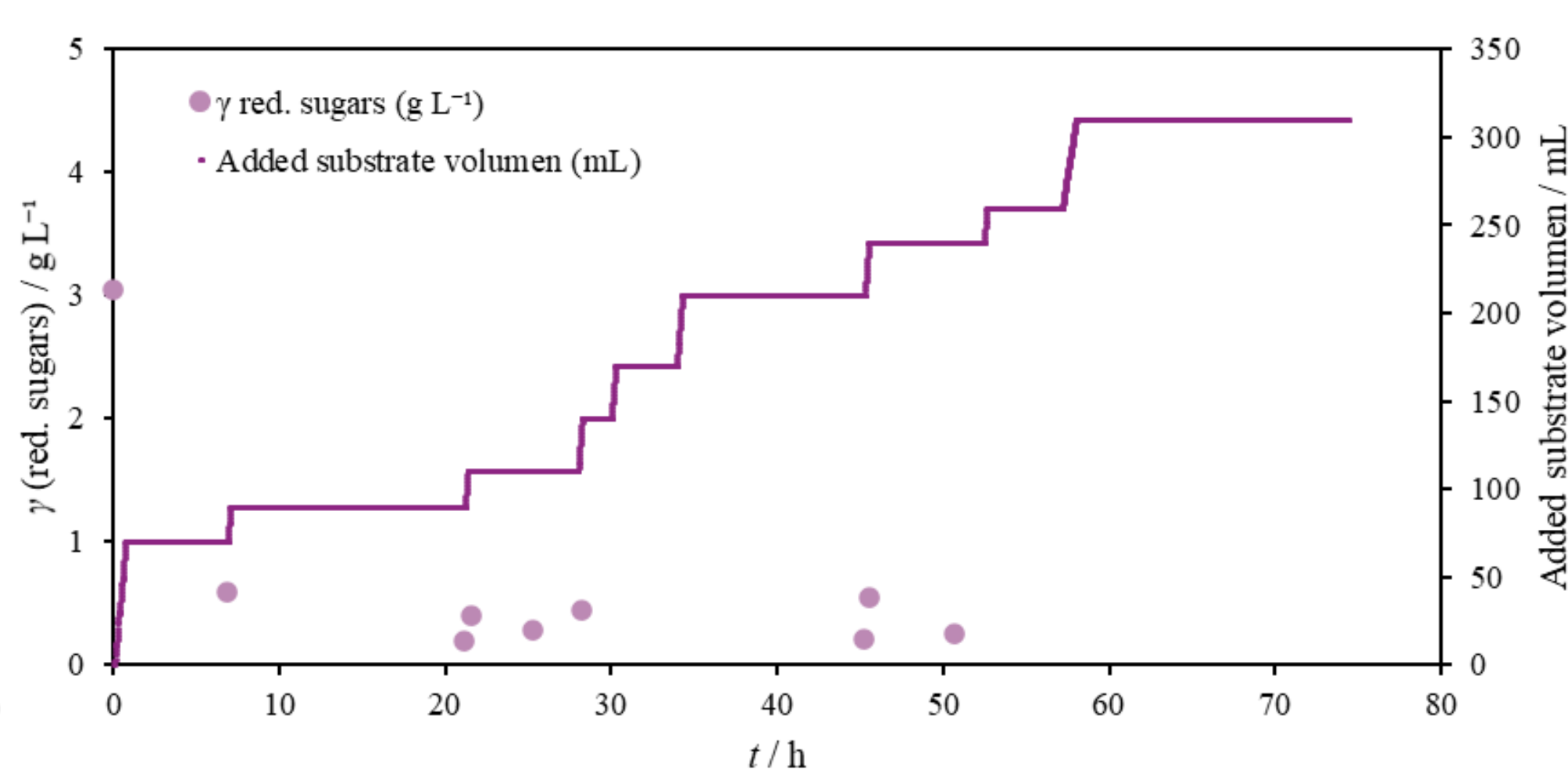


Fig 2. Change in reducing sugars concentration during fermentation with *Pseudomonas putida*.

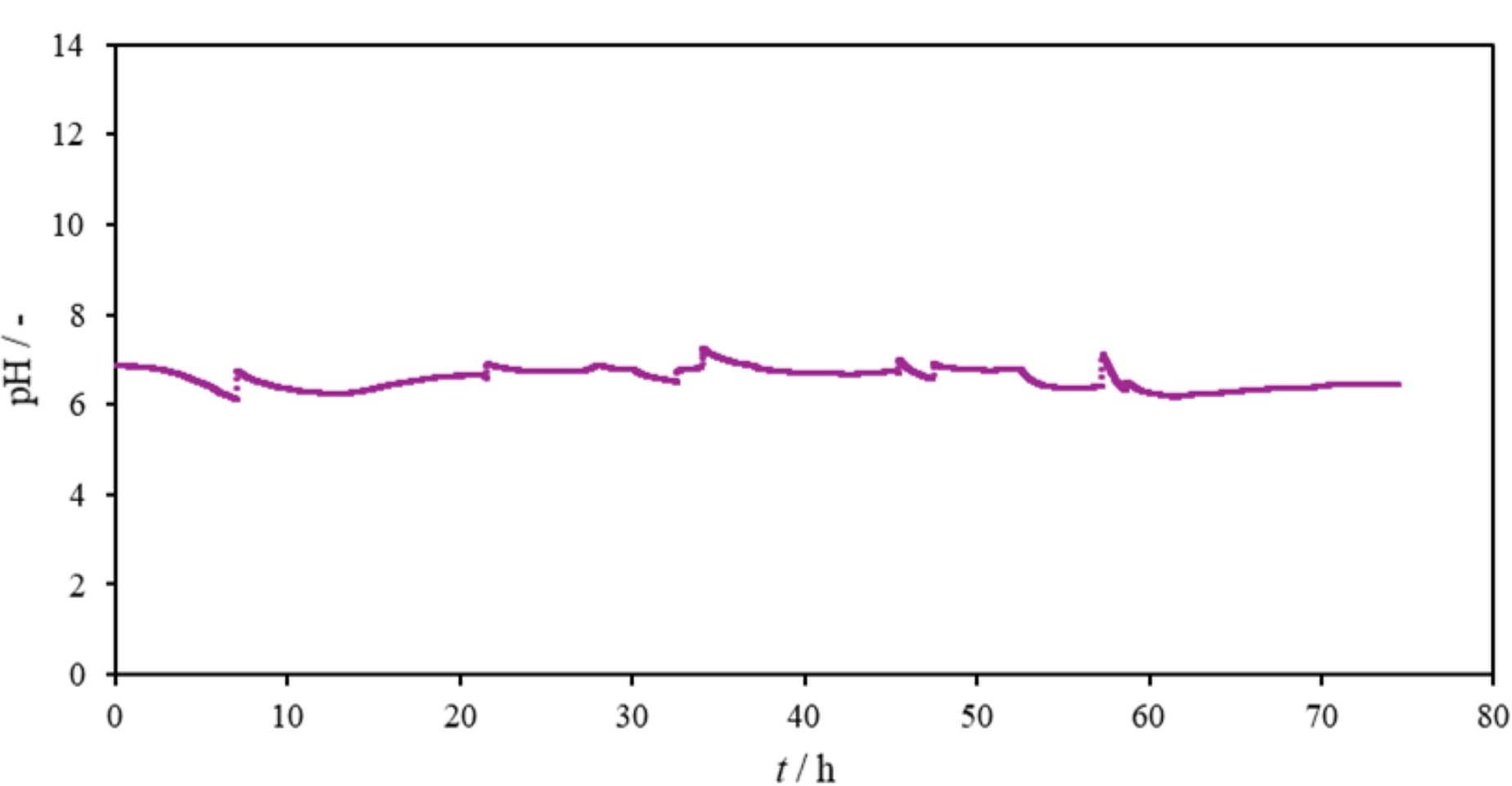


Fig 3. Change in pH value during fermentation with *Pseudomonas putida*.

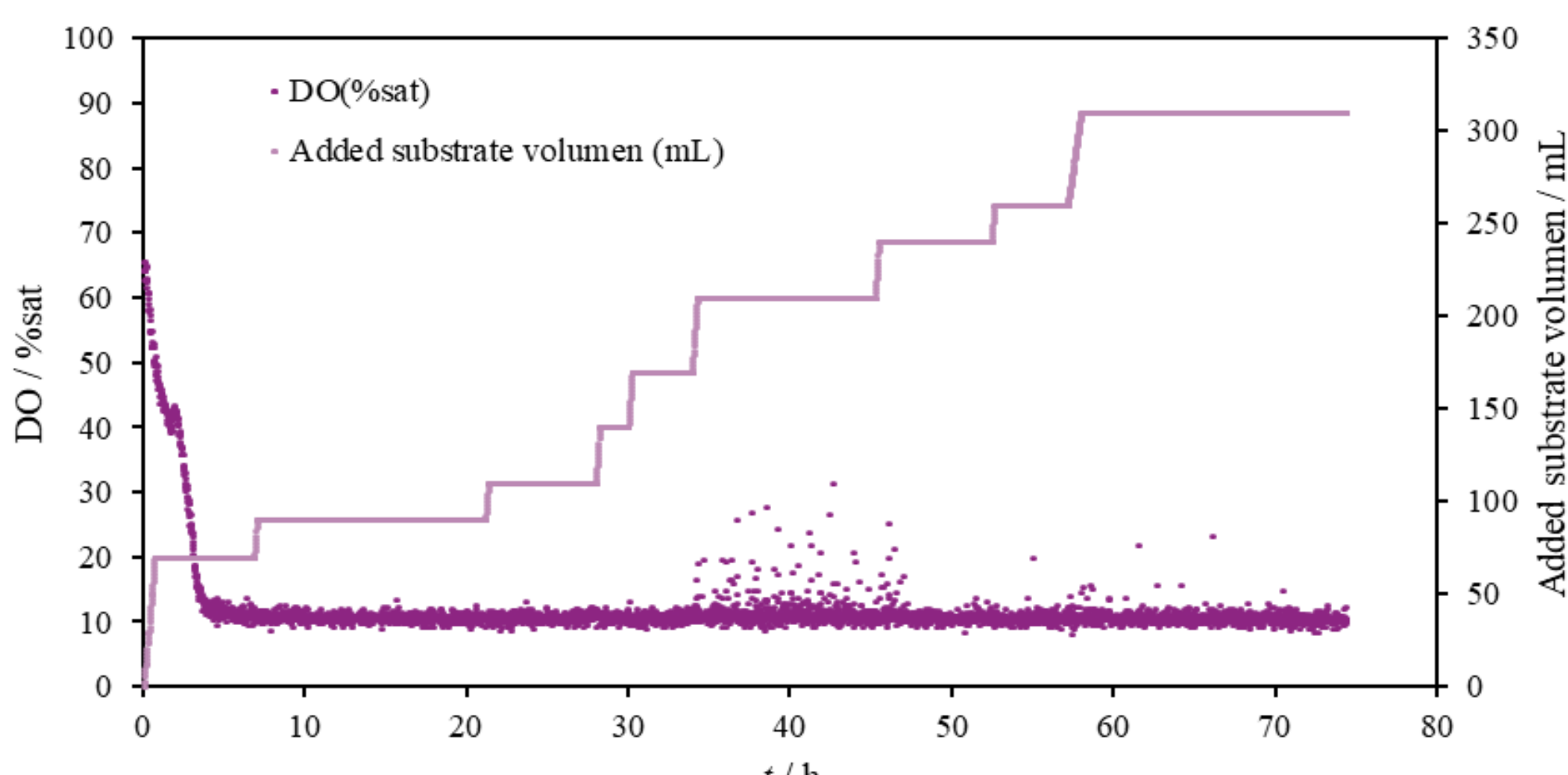


Fig 4. Change in the amount of dissolved oxygen and addition of hydrolyzate during fermentation with *Pseudomonas putida*.

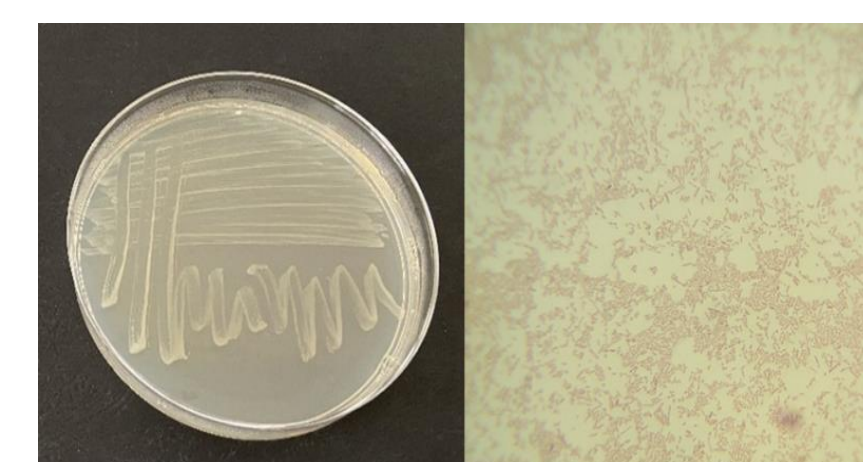


Fig 5. Colonies of the bacterial culture *C. necator* on nutrient agar and micrographs of Gram-stained bacterial cells.

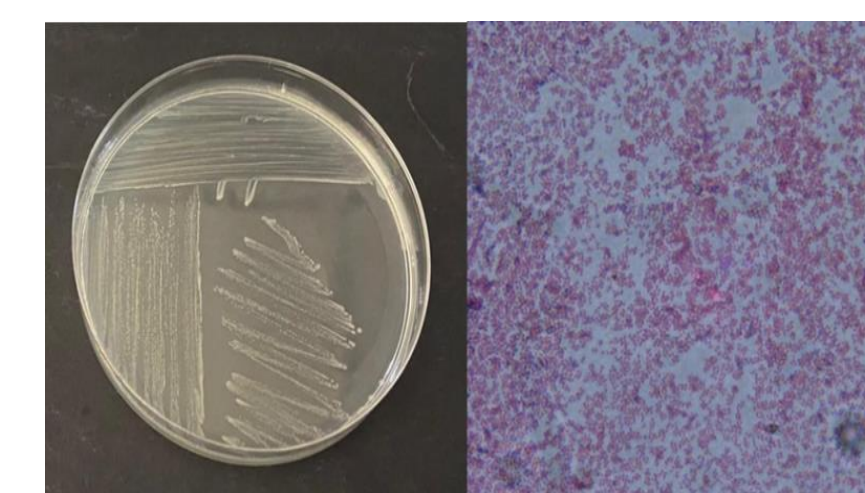


Fig 6. Colonies of the bacterial culture *P. putida* on nutrient agar and micrographs of Gram-stained bacterial cells.

Table 1. Results of PHA extraction from mixed and pure bacterial cultures.

	PHA extraction yield / %
Pure culture	39.44
Mixed culture	40.53

CONCLUSION

- ✓ Submerged fermentation was successfully carried out using both pure and mixed bacterial cultures for the production of PHA biopolymers from agro-industrial waste.
- ✓ The mixed culture showed a slightly higher PHA yield (40.53 %) compared to the pure culture (39.44 %).
- ✓ GC-MS characterization confirmed that the produced biopolymer corresponds to the PHBV copolymer.