SIMULTANEOUS EXTRACTION AND ACTIVATED-CARBON PURIFICATION OF POLY(3-HYDROXYNONANOATE) FOR MEDICAL APPLICATIONS

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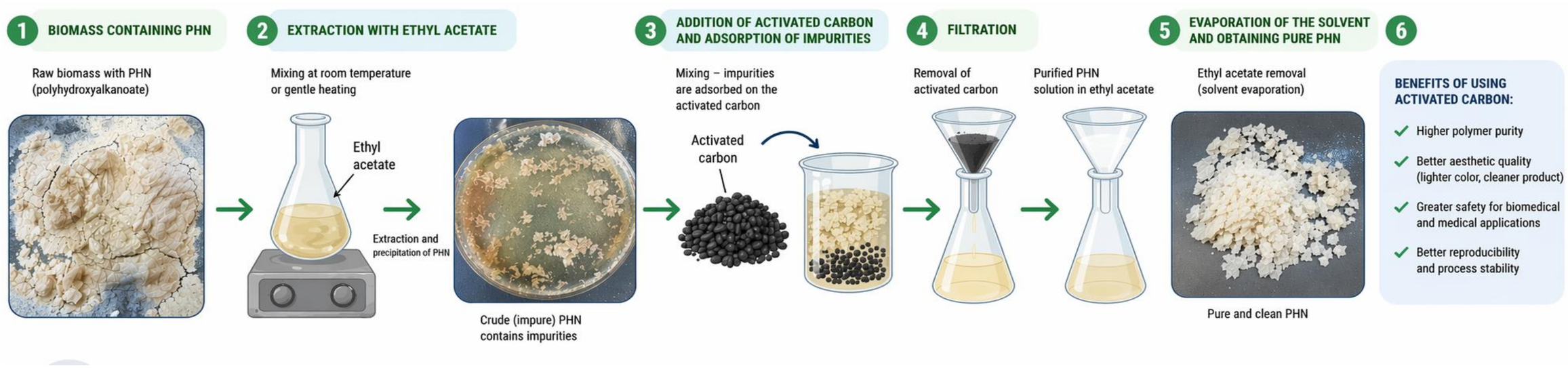
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INTRODUCTION

Poly(3-hydroxynonanoate) PHN) is a medium-chain-length polyhydroxyalkanoate (mcl-PHA) valued in biomedical applications (such as resorbable implants and drug delivery systems) due to its high flexibility, biocompatibility, and biodegradability(1). A critical challenge is the contamination of PHA, produced by Gram-negative bacteria (such as *Pseudomonas putida* KT2440), with lipopolysaccharides (LPS)—endotoxins that provoke inflammatory responses(2). Achieving medical-grade purity therefore requires rigorous purification. This study compares the effectiveness of different types of activated carbon in removing these impurities during a one-pot simultaneous extraction process.

MATERIALS AND METHODS

PHN was synthesized by *Pseudomonas putida* KT2440 cultivated in mineral salts medium with nonanoic acid under nitrogen-limited conditions. Ten grams of freeze-dried biomass were suspended in 100 mL ethyl acetate with activated carbon and stirred for 24 h at 150 rpm. Three carbons were tested: Black Pearls® 700, Black Pearls® 2000 and Norit® granules. After filtration, the polymer mass and UV–Vis absorbance of its solution were measured.

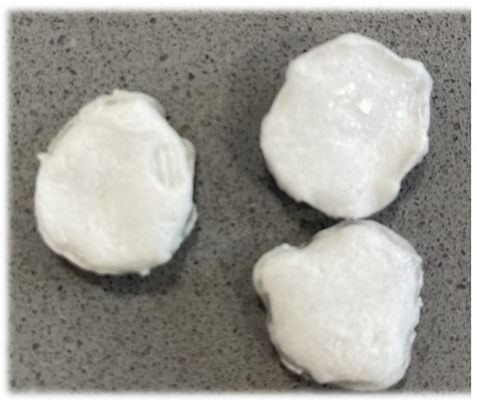


RESULTS

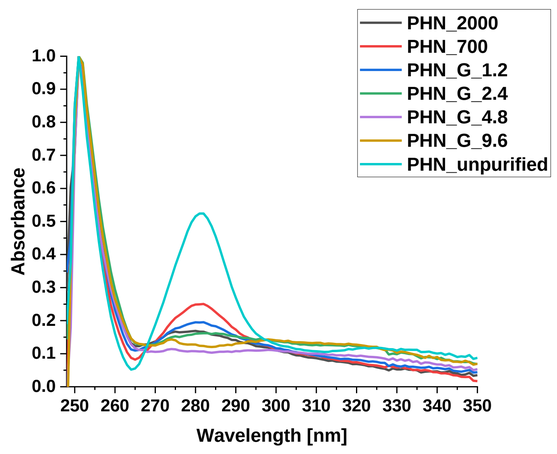
Comparison of purification with different activated carbons (1.2 g of carbon for 10 g of biomass)

Material	Yield [g]	Absorbance (282nm)	Activated carbon used	Specific surface area of activated carbon [m^2 g^{-1}]
PHN_unpurified	3.64	0.524	-	-
PHN_700	3.37	0.250	Black Pearls® 700	~200
PHN_2000	2.73	0.167	Black Pearls® 2000	~1500
PHN_G	**3.43**	**0.195**	**Norit®**	**~1400**

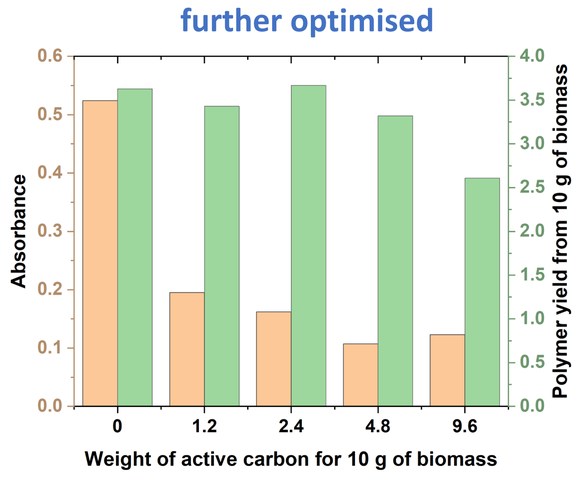
Purified polymer



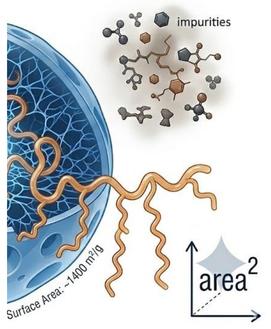
Absorbances were measured using 6.67 mg ml^{-1} polymer solutions)

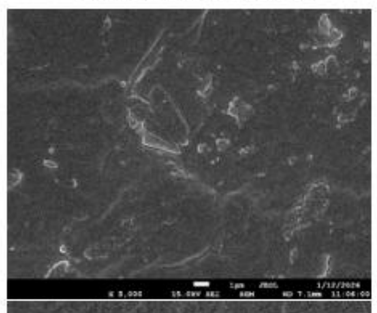
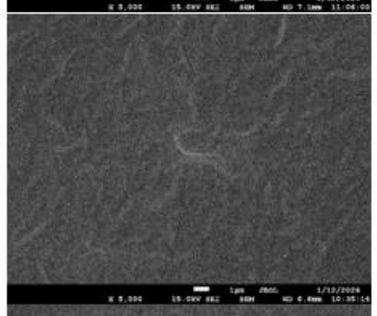
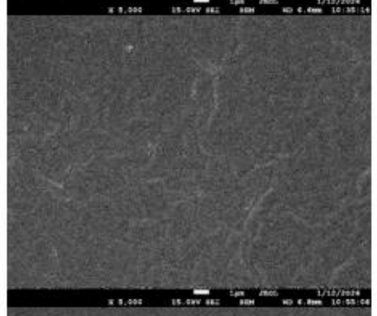
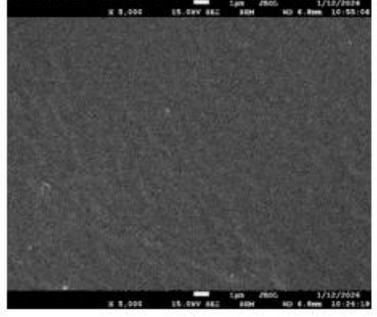


Mass of chosen activated carbon was further optimised



Purification was evaluated also by microstructure observations



Material	Microstructure
PHN_unpurified	
PHN_700	
PHN_2000	
PHN_G	

KEY TAKEAWAY: Activated carbon **acts as a "magnet"** for impurities, enabling the isolation of clean, high-quality PHN biopolymer suitable for advanced applications.

CONCLUSIONS

The simultaneous extraction and carbon treatment process effectively removes protein-origin impurities from PHN. Comparing different activated carbons it was established that Norit® was the easiest to filter and offered the best compromise between impurity removal, polymer recovery, and operational simplicity. In second optimization step, the minimal amount of selected activated carbon was determined as 0.48 gram of activated carbon for each gram of biomass. Future work will involve performing quantitative endotoxin analysis using the LAL (Limulus Amebocyte Lysate) test to confirm medical suitability.

REFERENCES:

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[2] A. C. K. Wu *et al.*, Biomaterials, 2006, 27, 4715-4725.

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