IMMOBILISATION OF *LACTOBACILLUS RHAMNOSUS* ON INDUSTRIAL CARRIERS IN COMBINATION WITH CALCIUM ALGINATE FOR L-(+)-LACTIC ACID FERMENTATION

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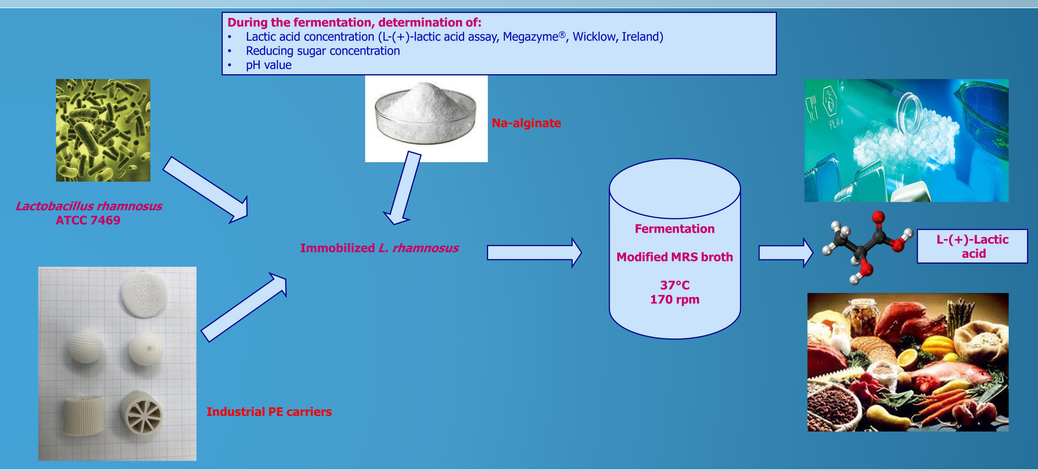
1.Introduction

Lactic acid (LA) is the most important hydrocarboxylic acid widely distributed in nature, that has numerous applications in variety of industries such as food, pharmaceutical, textile, leather, and chemical industries. LA bacteria represent one of the most promising microorganisms for application in extensive bioconversion of various substrates into value added products. Although LA production is majorly accomplished by batch fermentation using free cells, application of immobilized cells (biocatalyst) offers various advantages, such as biocatalyst (BC) reusability, higher fermentation rate, easier separation of microorganism from the fermented media, protective effect against substrate and product inhibition, and cheaper inoculum preparation. Sodium alginate (Na-alginate) is a natural biopolymer, composed of polysaccharide backbone with two repeating monosaccharide units (guluronic acid, and manuronic acid), with hydroxyl and carboxyl groups in its structure that enable further structure modifications.

2. Materials and Methods

Three types of industrial carriers (IC) were investigated, and possibility of biofilm formation was observed. Originally ICs were made for waste water treatment (at Faculty of Technology and Metallurgy) from polyethylene (PE) in the shape of ribbed sphere, round leaf (with cavities) and hollow gear. To further enhance cell attachment the ICs were coated with Ca-alginate.



3. Results and Discussion

The formation of biofilm on all ICs was observed and the highest viability of *L. rhamnosus* cells was 9.8 log CFU/g of the carrier. It must be noted that the *L. rhamnosus* is exopolysaccharide producing strains and thus has the ability to form stable biofilms. *L. rhamnosus* cells immobilised in ICs coated with Ca-alginate had even higher viability reaching values of 11.7 log CFU/g of the carrier. In LA fermentation, the highest LA yield and volumetric productivity of 97.4 % and 2.1 g/l h^{-1}, respectively, was achieved with IC (round leaf) coated with Ca-alginate (Table 1). With application of IC biocatalysts, especially with Ca-alginate coating, there was significant increase in reducing sugar conversion (up to 87.5%), LA concentration (101.6%), yield (7.6%) and volumetric productivity (90.9%) in comparison to free cell fermetnation.

Table 1. Reducing sugar conversion, L-(+)-LA concentration, yield, volumetric productivity, and cell viability (log CFU/ml and logCFU/g of carrier) in batch fermentations of modified MRS broth (55 g/L of reducing sugars), by immobilized *L. rhamnosus* cells on IC without and with Ca-alginate coating.*

		Without Ca-alginate coating			With Ca-alginate coating		
	Free cells	Ribbed sphere	Round leaf (with cavities)	Hollow gear	Ribbed sphere	Round leaf (with cavities)	Hollow gear
Reducing sugar conversion (%)	51.4 ± 1.0^{a}	87.3 ± 1.1^{b}	88.6 ± 1.1^{b}	87.9 ± 0.08^{b}	94.3 ± 1.4^{c}	96.4 ± 0.08^{c}	95.1 ± 1.2^{c}
L-(+)-concentration (g/L)	25.6 ± 0.7^{a}	44.1 ± 0.8^{b}	44.9 ± 0.08^{b}	44.6 ± 0.7^{b}	50.1 ± 1.1^{c}	51.6 ± 1.3^{c}	50.6 ± 1.3^{c}
L-(+)-LA yield (%)	90.5 ± 1.3^{a}	91.9 ± 0.8^{a}	92.1 ± 1.1^{a}	91.8 ± 1.0^{a}	96.5 ± 1.2^{b}	97.4 ± 1.1^{b}	96.8 ± 1.3^{b}
Volumetric productivity (g/L h^{-1})	1.1 ± 0.01^{a}	1.8 ± 0.01^{b}	1.9 ± 0.02^{b}	1.8 ± 0.01^{b}	2.1 ± 0.02^{c}	2.1 ± 0.02^{c}	2.1 ± 0.02^{c}
Cell viability (log CFU/g)	-	9.8 ± 0.9^{a}	9.7 ± 0.8^{a}	9.7 ± 0.9^{a}	11.5 ± 0.7^{b}	11.7 ± 0.8^{b}	11.6 ± 0.8^{b}
Cell viability (log CFU/ml)	9.1 ± 0.6^{a}	8.8 ± 0.9^{a}	8.7 ± 0.8^{a}	8.7 ± 0.9^{a}	10.5 ± 0.7^{b}	10.7 ± 0.8^{b}	10.6 ± 0.8^{b}

*Values represent means ± standard deviation calculated from three parallel tests. Means written with different small letter in a row are significantly different (p<0.05); Small letters (a-c) represent statistically significant different values;

5. Conclusion

Obtained results suggest the possibility of highly efficient LA fermentation by application of ICs without (preferred biofilm forming microorganisms) and with Ca-alginate coating.

6. Acknowledgements

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