Bioprocess development for succinic acid production using organic fractions of municipal solid waste

Vasiliki Korka[a], Christos Margioulas[a], Katiana Filippi[a], Eleni Stylianou[a], Dimitrios Ladakis[b], Apostolis Koutinas[a]

[a]Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece

[b]Department of Agricultural Development, Agri-Food and Natural Resources Management, National and Kapodistrian University of Athens, Evripos Campus, 344 00 Psachna, Evia, Greece

Keywords: Succinic Acid, *Actinobacillus succinogenes*, Organic Fraction of municipal solid waste (OFMSW), Neutralizing agents, Nitrogen source

Presenting author email: vkorka727@gmail.com

Introduction

The valorization of renewable resources is crucial for the production of various bio-based products to achieve a smooth transition to the circular economy era (Karmee, 2018). The organic fraction of municipal solid waste (OFMSW) constitutes nearly half of the global waste output. According to estimations, by 2050, the volume of OFMSW will increase from the current 2 billion t to 3.40 billion t (Bandini et al., 2022). Generally, organic wastes are managed by landfilling or incineration, waste management techniques with a huge associated potential loss and environmental and economic cost. It is estimated that 93% of the waste generated is dumped or burned, most of which takes place in underdeveloped nations (Kumar et al., 2023). These environmental issues require proper management of OFMSW. The development of novel approaches to waste management is essential to develop sustainable bioprocesses. A promising one is the utilisation of renewable resources for the production of bio-based products such as succinic acid. Succinic acid is a C-4 dicarboxylic acid with major applications as a precursor for a variety of products such as medicines, cosmetics, food additives, green solvents, and biodegradable polyesters. In the last two decades, bio-based succinic acid production processes have been developed with the aim of a sustainable recovery of different platform chemicals (Li et al, 2021).

The aim of this study is the evaluation of *Actinobacillus succinogenes* for succinic acid production using the OFMSW, focusing mainly on the fermentation's bioprocess optimization. The OFMSW was enzymatically hydrolysed, and the produced hydrolysate was implemented as a fermentation feedstock for succinic acid production via batch fermentations. Different neutralising agents and nitrogen sources were evaluated in order to enhance succinic acid concentration, yield and productivity.

Materials & methods

OFMSW used in this study was obtained from households and supermarkets. Initially, OFMSW main components were identified, including oil (Soxhlet extraction using hexane), starch, protein (Total Kjeldahl Nitrogen), pectin, free sugars, ash, moisture and lignocellulosic content. Cellulose, hemicellulose and lignin were determined according to the method reported by the National Renewable Energy Laboratory (NREL). The estimation of the hydrolysis yield was based on methods reported by Filippi et al. (2021).

Enzymatic hydrolysis of OFMSW was performed by utilizing commercial enzyme preparations containing cellulases, xylanases and arabinases. All hydrolysis experiments were conducted at 50°C for 48 h with 100 g/L solid concentration under continuous mechanical agitation. In all cases, the pH of the

hydrolysis was adjusted to 5.0. The hydrolysate produced after the enzymatic hydrolysis was used as the sole carbon source for succinic acid production with the bacterial strain *Actinobacillus succinogenes* 130Z (DSM-22257). The preculture was prepared using Tryptone Soya Broth (TSB) and was incubated at 37°C for 12-16 h under agitation (180 rpm). Fermentations were carried out in a 1 L bench-top bioreactor with a 500 mL working volume. Inoculum size was 10% (v/v) while the initial sugar concentration in the fermentation was 50 g/L. Fermentation pH was controlled at 6.7 while for pH regulation different neutralizing agents (NaOH, KOH, $Mg(OH)_2$ and NH_4OH) were evaluated. The effect of the nitrogen source was also examined by using either yeast extract or corn-steep liquor. The fermentation broth was also supplemented with 5 g/L $MgCO_3$, 1.16 g/L $NaH_2PO_4 \cdot H_2O$, 0.31 g/L Na_2HPO_4, 1 g/L NaCl, 0.2 g/L $MgCl_2 \cdot 6H_2O$ and 0.2 g/L $CaCl_2 \cdot 2H_2O$. All fermentations were carried out under continuous CO_2 sparging of 0.1 vvm at 37°C and 180 rpm agitation.

Free amino nitrogen (FAN) was determined spectrophotometrically according to the protocol proposed by Lie (1973) based on the ninhydrin method for the determination of free alpha-amino nitrogen.

Results & discussion

OFMSW used in this study is mainly composed of starch (15.3%), glucan (15.5%), hemicellulose (3.6%), lignin (5%), free sugars (12.2%), proteins (15.1%), lipids (12%), pectins (1.3%), ash (10.9%) and organic acids (5.8%). The high polysaccharide content of OFMSW alongside its low lignin content, makes it a perfect candidate for producing a sugar-rich hydrolysate. As a result, the enzymatic hydrolysis obtained resulted in a glucan and hemicellulose conversion yield of more than 65% and 70%, respectively. The produced hydrolysate, which was rich in glucose, was used as carbon source for succinic acid production in different batch cultures in which different neutralizing agents and nitrogen sources were evaluated. According to the results, a maximum succinic acid concentration of more than 30 g/L with yield and productivity higher than 0.6 g/g and 0.87 g/L/h were achieved.

Conclusions

The OFMSW-derived sugar-rich hydrolysate is a promising feedstock for the biotechnological production of succinic acid. Moreover, the influence of nitrogen sources and neutralizing agents used proved significant for the optimal growth of the microorganism.

References

Karmee, S.K., 2018. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. *Waste management*, *72*, pp.240-254.

Bandini, F., Taskin, E., Bellotti, G., Vaccari, F., Misci, C., Guerrieri, M.C., Cocconcelli, P.S. and Puglisi, E., 2022. The treatment of the organic fraction of municipal solid waste (OFMSW) as a possible source of micro-and nano-plastics and bioplastics in agroecosystems: a review. *Chemical and Biological Technologies in Agriculture*, *9*(1), pp.1-17.

Kumar, A., Singh, E., Mishra, R., Lo, S.L., Kumar, S., (2023). Global trends in municipal solid waste treatment technologies through the lens of sustainable energy development opportunity. Energy 275, 127471.

Li, C., Ong, K.L., Cui, Z., Sang, Z., Li, X., Patria, R.D., Qi, Q., Fickers, P., Yan, J. and Lin, C.S.K., 2021. Promising advancement in fermentative succinic acid production by yeast hosts. *Journal of hazardous materials*, *401*, p.123414.

Filippi, K., Georgaka, N., Alexandri, M., Papapostolou, H. and Koutinas, A., 2021. Valorisation of grape stalks and pomace for the production of bio-based succinic acid by *Actinobacillus succinogenes*. *Industrial Crops and Products*, *168*, p.113578.

Acknowledgements

The project is supported by the Circular Bio-based Europe Joint Undertaking and its members. Funded by the European Union (LUCRA project). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or CBE JU. Neither the European Union nor the CBE JU can be held responsible for them. This work was also co-funded by UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee grant number 10082169.