



# D-lactic acid production from hydrothermally pretreated, alkali delignified and enzymatically saccharified rockrose with the metabolic engineered *Escherichia coli* strain JU15

Maria C. Fernandes<sup>1,2</sup> · Júnia Alves-Ferreira<sup>1,2,3</sup> · Luís C. Duarte<sup>3</sup> · Helena Pereira<sup>4</sup> · Florbela Carvalheiro<sup>3</sup> · Alfredo Martínez<sup>5</sup>

Received: 11 August 2021 / Revised: 3 December 2021 / Accepted: 6 December 2021  
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

## Abstract

Rockrose lignocellulosic residues (RR) were selectively fractionated for hemicellulose separation using autohydrolysis, followed by an alkaline treatment to solubilize the lignin. The cellulose-enriched solids were used to study the effect of solid loading (SL: 2–10%) and enzyme dosage (ED: 6.34–23.66 FPU/g dry biomass) on saccharification using a Doehlert experimental design, followed by fermentation with the metabolic engineered *Escherichia coli* strain JU15 to produce D-lactic acid (DLA). Pretreatment increased glucan content and enzymatic digestibility up to 84%. A significant positive effect of SL and ED was found for glucose production, but SL negatively impacted glucose yield. DLA concentrations and productivity varied from 8.85 to 32.98 g/L and 1.11 to 2.17 g/(Lh), respectively. Overall process efficiency strongly depended on saccharification yield and varied from 33 to 71%. These results indicate that sequential autohydrolysis, delignification, and fermentation of RR may be a potential relevant strategy for D-lactic production in the biorefinery framework.

**Keywords** Autohydrolysis · D-lactic acid · Doehlert design · Lactogenic *Escherichia coli* · Rockrose (*Cistus ladanifer*) · Sequential saccharification and fermentation

## Highlights

- D-lactic acid was obtained from pretreated rockrose with a high productivity.
- Autohydrolysis and alkaline treatment quadrupled enzymatic hydrolysis (EH) performance.
- Solid loading and enzyme dosage significantly effect glucose production in EH.
- Saccharification yield is the major factor affecting overall process efficiency.

✉ Maria C. Fernandes  
maria.fernandes@cebal.pt

<sup>1</sup> Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL)/Instituto Politécnico de Beja (IPBeja), Apartado 6158, 7801-908 Beja, Portugal

<sup>2</sup> MED-Mediterranean Institute for Agriculture Environment and Development /Centro de Biotecnologia Agrícola e Agro-Alimentar do Alentejo (CEBAL), Apartado 6158, 7801-908 Beja, Portugal

## 1 Introduction

Lactic acid is an enantiomeric GRAS (Generally Recognized as Safe) chemical that is usually chemically synthesized as a racemic mixture of L-lactic acid and D-lactic acid. This organic acid has many applications in the food, cosmetic, medical, and pharmaceutical industries [1]. Most noteworthy, lactic acid is the precursor of polylactic acid (PLA), which is used for the manufacture of biodegradable

<sup>3</sup> LNEG-Laboratório Nacional de Energia e Geologia, Unidade de Bioenergia e Biorrefinaria, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal

<sup>4</sup> Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal

<sup>5</sup> Departamento de Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México., Av. Universidad 2001, Col. Chamilpa, Cuernavaca, Morelos 62210, México