

# ROBOX

This project is funded by the European Union (grant agreement 635734) under EU's Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1



**Proposal Acronym: ROBOX**

**Project title: Expanding the industrial use of Robust Oxidative Biocatalysts for the conversion and production of alcohols**

**Project № 635734**

**Funding Scheme Horizon 2020 Research and Innovation actions H2020-LEIT**

**Coordinator: RUG**

**Project start date: 01 April 2015**

**Duration 48 months**

**DOCUMENT CONTROL SHEET**

Title of Document:	Deliverable report 1.6: Quorum sensing technology and micro fluidics platform available	
Work Package:	WP1	
Deliverable №:	D1.6	
Last version date:	31-01-2018	
Status:	For EU Deliverable	
Document Version:	1	
File Name	ROBOX_D1.6_Quorum_sensing_technology_&_fluidics	
Number of Pages		
Dissemination Level	Confidential	
Responsible Authors	Marco Fraaije	RUG
Project Coordinator	Marco Fraaije	RUG

**“The ROBOX project has received funding from the European Union (EU) project ROBOX (grant agreement n° 635734) under EU's Horizon 2020 Programme Research and Innovation actions H2020-LEIT BIO-2014-1.”**

# Table of contents

**TABLE OF CONTENTS.....2**

**Deliverable report 1.6: Quorum sensing technology and micro fluidics platform available (FIA, M30).....3**

**Summary .....3**

**Introduction.....3**

**Results.....3**

**Conclusions.....4**

## **Deliverable report 1.6: Quorum sensing technology and micro fluidics platform available (FIA, M30)**

### **Summary**

For developing tailor-made alcohol oxidases, the ROBOX project included research activities that would enable more efficient engineering of oxidases. One approach for this was to exploit the effect of quorum sensing signaling molecules. Such natural regulation system could form the basis for a very effective system to select for interesting oxidases. Along developing the “quorum sensing” approach, micro fluidic platforms would be explored for performing screening for improved enzymes (oxidases).

Yet, the preceding work (in the first 24 months of the ROBOX project) on engineering a glucose oxidase did not yield any suitable alcohol oxidase, while other partners were successful in delivering suitable alcohol dehydrogenases for the glucose oxidase target reaction. This severely limited the value of pursuing the work originally planned for Fraunhofer, and therefore the work on glucose oxidase screening and related methodology was stopped. As a consequence, Fraunhofer decided to step out of the project and this deliverable 1.6 was not fulfilled.

### **Introduction**

Within ROBOX, alcohol oxidases are being explored as novel biocatalysts for industrial processes. One of the target oxidases was the fungal glucose oxidase which would be engineered to act on lactols. As part of the scheme for delivering a tuned oxidase, the project would also focus on developing a new and effective method for identifying new oxidases or screening for superior oxidase mutants. This new method would be based on the natural process of so-called ‘quorum sensing’ and the use of micro fluidic devices. By destroying quorum sensing signalling molecules, microbes would become available for discovery of new oxidases. The micro fluidic methodology would enable swift selection of recombinant organisms that produce an (improved) oxidase.

### **Results**

Fraunhofer would work on the above-mentioned technology development. It would be an extension of their aim to discover & redesign glucose oxidase. However, in the first 24 months the engineering of glucose oxidase did not result in any improved variant. In fact, after 2 years of expressing and screening for glucose oxidase mutants that perform well on the targeted lactol, it had to be concluded that no significant progress had been made (see M24 report). In fact, it was difficult to reproduce data obtained in the beginning of the project which could be partly explained by the erratic behavior of glucose oxidase in the host *Pichia pastoris*. Usage of an alternative host and probing other glucose oxidases did not improve the situation. It also seemed extremely unlikely that an effective biocatalyst could be developed from glucose oxidase within a reasonable amount of time. On the other hand, activities elsewhere in the ROBOX project had already delivered alcohol dehydrogenases that showed decent activities on the target lactol.

Considering the fact that no suitable glucose oxidase had been engineered and that alternative enzymes (alcohol dehydrogenases) could be used for the target reaction, it was decided that work on glucose oxidase should be concluded. The discovery and engineering of alternative oxidases in the project

(glycerol oxidase and eugenol oxidase) had already delivered alternative alcohol oxidases that could be transferred to other WPs for alternative substrate targets and technology development.

The decision to stop developing glucose oxidase had as a consequence that the work originally targeted in this deliverable was no longer relevant. For this reason, the basis for Fraunhofer as research partner in the project diminished to such extent that Fraunhofer decided to leave the project. As a consequence, no work has been done on the development of quorum sensing technology and micro fluidics platform.

## **Conclusions**

Due to the fact that no glucose oxidase with significant activity on the target compound could be engineered, it was decided to stop the work on engineering glucose oxidase. As a result, the work originally planned for this deliverable was cancelled and the involved beneficiary, Fraunhofer, has decided to leave the project. Alternative alcohol oxidases, that already showed industrially relevant activities, will be developed in ROBOX instead.