

COST Action

Final Achievement Report

CM1303: Systems Biocatalysis (20/11/2013 to 19/11/2017)

The Action was approved by the Committee of Senior Officials (CSO) on 16-5-2013 and has the MoU reference COST 008/13.

This report was submitted on 10-01-2018 by the Action Chair on behalf of the Management Committee in fulfilment of the requirements of the rules for COST Action Management, Monitoring and Final Assessment.

Action leadership and participants

Leadership positions

Position	Name	Contact details	Country of work affiliation
Chair	Prof Stefano Servi	stefano.servi@polimi.it +390223993047	Italy

Position	Name	Contact details	Country of Nomination
Vice Chair	Prof Isabella Arends	i.w.c.e.arends@tudelft.nl +31152784423	Netherlands

Working groups

#	WG Title	# of participants	WG Leader	Country of nomination
1	New modes of creation and interconversion of chemical functionality in metabolites	23	Prof John Ward j.ward@ucl.ac.uk	United Kingdom
2	Routes to New Key Metabolites	25	Dr Roland WOHLGEMUTH rwohlgemuth@catv.rol.ch	Switzerland
3	Constructing pathways towards complex lipid conjugates	7	Prof Wolf-Dieter Fessner fessner@tu-darmstadt.de	Germany
4	Enzyme engineering for novel and optimized functions	29	Prof Uwe Bornscheuer uwe.bornscheuer@uni-greifswald.de	Germany
5	Process intensification	24	Prof László Poppe poppe@mail.bme.hu	Hungary

Other key leadership positions

Position	Name	Contact details	Country of work affiliation
STSM Coordinator	Dr Sergio Riva	sergio.riva@icrm.cnr.it	Italy

Participants

COST members having accepted the MoU

AT	01/08/2013	BE	14/10/2013	HR	14/06/2013	CZ	18/06/2013	DK	17/06/2013
FI	23/09/2013	FR	20/06/2013	DE	19/06/2013	EL	01/10/2013	HU	20/06/2013
IE	27/06/2013	IL	16/07/2013	IT	09/07/2013	LT	15/09/2014	NL	16/07/2013
NO	21/10/2013	PL	05/07/2013	PT	16/09/2013	RO	12/09/2013	RS	16/03/2014
SK	23/06/2013	SI	25/08/2013	ES	12/06/2013	SE	02/09/2013	CH	30/10/2013
TR	19/09/2013	UK	30/05/2013						

Other participants

Institution Name	Country
------------------	---------

Summary

Main aim/ objective

The main objective of the Action is to build up a scientific platform to design and construct optimized modular biocatalytic systems for the sustainable synthesis of valuable products.

The Action addressed this as described below

The MC since the beginning of the Action has taken decisions which have been orienting the whole activity. Thus WG with competences in complementary domains are intended to work together. No funds have been allocated for WG meetings disconnected from a scientific meeting of general interest. This decision has favoured continuous integration between groups and among the participants. No Action meeting isolated from an international high level congress have been organised. COST participants have gathered according to the COST rules and have partially supported conferences on topics central for the Action: Novel enzymes (Gent and Groeningen), Multi-step enzyme catalysed reactions (Madrid) Oxidative biocatalysis (Wageningen) continuous flow reactors (Opatija, Bled) Biotrans (Vienna and Budapest). Support for participation to these events has been offered to selected active participants with a particular attention to all the inclusiveness principles and ITC. As a consequence of this policy a strong integration with the scientific community has been developed with obvious dissemination both of COST and of SysBiocat. Moreover the ESI participants have had the opportunity to interact with an open qualified audience larger and reacher than the Action family. Each WG leader has offered access to knowledge, techniques, apparatus, software. What kind of collaboration has been set up and how ESIs have been moving appears from the joint papers gathered in this report and in the STSMs stories published on the website (<http://cost-sysbiocat.fkit.hr/stsm.html>)

Action website

<http://cost-sysbiocat.fkit.hr/>

Achievement of MoU objectives, deliverables and additional outputs/ achievements

MoU objectives

The Action had the following specific objectives:

MoU objective	Level of achievement	Further information (hyperlink or other)
develop methods for creating enzyme modules with novel functions to establish a plug-and-play toolbox concept	76 - 100%	See papers 1, 4, 6, 7, 14, 25, 38, 53 in Action publications in the report see also: ImPap1, ImPap6, ImPap8, ImPap9, ImPap10 http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf , - and ImpTalks, ImpTalks5, ImpTalks16, ImpTalks19, ImpTalks20 http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf
develop the assembly of biocatalytic modules from the toolbox to create novel artificial metabolic pathways in vitro	76 - 100%	Please see papers 23, 24, 25 in Action Publications in this report and ImPap2, ImPap4, ImPap9, ImPap22 in http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf also ImpTalks4, ImpTalks5, ImpTalks15, ImpTalks16, ImpTalks19 in http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf
develop enzyme modules with new/modified/improved substrate tolerance from the systems perspective	51 - 75%	Please see papers 60,70,71 in Action Publications in this report ImPap18, ImPap23 http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf ImpTalks22, ImpTalks30, ImpTalks33, ImpTalks38 http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf
develop a better understanding of regulation of enzyme function, to avoid inhibition in optimized	51 - 75%	Please see papers 10, 26, 32 in Action Publications in this report

modules		<p>ImPap8, ImPap11, ImPap30, ImPap11</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf</p> <p>ImpTalks29, ImpTalks33</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf</p>
develop systems approaches to cofactor-dependent biocatalysis by new/improved/cofactor-independent biocatalysts	76 - 100%	<p>Please see papers n° 15, 23, 69 in Action Publications in this report</p> <p>ImPap 42 in http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf</p> <p><u>ImpTalks 35</u> http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf</p>
develop standardized interfaces between important standard enzyme modules	26 - 50%	<p>see paper n° 23 in Action Publication2</p> <p>-</p> <p>ImPap1, ImPap2</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf</p> <p>ImpTalks19</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf</p>
develop improved expression systems for biocatalytic modules	76 - 100%	<p>The progress towards this objective is impressive. See:</p> <p>See papers n° 9, 10, 15, 23, 31, 34, 55 in Actions Publications in this report</p>
develop improved mutagenesis strategies for debottlenecking enzyme modules from the systems perspective	26 - 50%	<p>This topic was partially covered only in poster communications in Biotrans 2015 in Vienna</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf</p>
develop smart assay concepts for high-throughput screening of enzyme function	76 - 100%	<p>Several contribution to reach the objective are found with a narrow applicative range. However there are a few examples quite significant:is quite significant:</p> <p>Please see paper n° 11 in Action Publications section in this report and ImPap37, ImPap49 in</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf,</p>
develop improved reaction technology, devices for flow synthesis and in situ reaction monitoring	76 - 100%	<p>A very interdisciplinary topic concerning enzyme engineering, protein stabilisation, process engineering</p>

		Please see papers n° 13, 14, 28, 33 in the section Action papers in this report and proposal n° 4 in the section Proposal & Projects in this report.
identify useful reaction sequences	76 - 100%	This objective is in some way the core of SysBiocat and it has been developed extensively with some significant success please see papers n° 6, 7, 36, 48, 49, 66 in the section Action Publications in this report
optimize enzyme performance by directed evolution	76 - 100%	Directed evolution techniques are tools to improve enzyme performance. Most of the work around protein application deals with this problem. Excellent results have been obtained here and outside in the community. Please see papers n° 9, 11, 15, 23, 34, 37, 41, 43, 54, 55, 68, 70 in the section Action Publications in this report and ImPap1, ImPap11, ImPap15, ImPap16, ImPap17, ImPap23, ImPap30 in http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf , and ImpTalks7, ImpTalks8, ImpTalks10, ImpTalks20, ImpTalks22, ImpTalks23, ImpTalks24, ImpTalks36 in http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf
engineer non-natural enzyme reactivity	76 - 100%	Enzymes can show promiscuous activity and this propensity to catalyse similar or completely different reactions is extensively studied. From the understanding of observed enzyme promiscuity comes the opportunity of engineer a non-natural activity: please see papers n° 8, 15, 70 in the section Action Publications in his report ImPap49, ImPap37 http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf ImpTalks18 http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf
design and optimize multistep artificial biosynthetic pathways in vitro	76 - 100%	Please see papers n° 4, 5, 15, 16 in the section Action Publication in this report. ImPap1, ImPap2, ImPap6, ImPap8, ImPap18, ImPap19, ImPap43 in

		<p>http://cost-sysbiocat.fkit.hr/final%20report/ImpPap.pdf,</p> <p>and ImpTalks26, ImpTalks28, ImpTalks32, ImpTalks33 in</p> <p>http://cost-sysbiocat.fkit.hr/final%20report/ImpTalks.pdf</p>
assemble optimized modules in a plug-and-play fashion for specific synthetic targets	76 - 100%	<p>Similar to objective n 14 however the optimization to an industrial level is at the moment out of reach</p> <p>see papers n° 23, 25,26, 32, 33 in the section Action Publications in this report</p> <p>ImpPap54in</p> <p>http://cost-sysbiocat.fkit.hr/PR2/important_papers.html</p> <p>ImpTalks15 in</p> <p>http://cost-sysbiocat.fkit.hr/PR2/meeting_programs.html</p>
apply process engineering and intensification tools for competitive applications	76 - 100%	<p>see papers n° 13, 24, 28, 29, 43 in the section Action publications in this report</p> <p>and ImpTalks16, ImpTalks19 in http://cost-sysbiocat.fkit.hr/PR2/meeting_programs.html</p>

Deliverables

The Action reported the following deliverables:

Deliverable	Timing of deliverable	Further information (hyperlink or other)
New Metabolisms: Newly designed reaction sequences going beyond the single-enzyme catalyzed step for the synthesis of valuable products.	Not delivered, but foreseen within 2 years	see papers n° 1, 6, 7, 14, 19, 21, 25, 28, 32, 46 In the section Action Publications in this report
Best Biocatalysts: Proteins with required and complementary catalytic functions obtained from directed evolution techniques optimized towards substrates loading, solvent tolerance, broadened pH and T range with as high as possible specific activity.	Not delivered, but foreseen within 2 years	see papers n° 3, 6, 8, 14, 22, 46, 60 In the section Action Publications in this report
Engineered Bioprocesses: Process conditions allowing successive catalytic steps to occur in the same reactor or in successive regions of a continuous reactor without product isolation, solvent removal, and purification.	Delivered	please see papers n° 13, 14, 24, 33, 43 in the Section Action Publications in this report and project n° 4 in the section Proposal/Projects
Novel enzyme modules, new reaction systems, new molecules and new tools in multistep enzyme catalyzed reactions	Not delivered, but foreseen within 2 years	please see papers n° 1, 14, 23, 25, 43, 48, 49, 66 in the Section Action Publications in this report, ImPap2 in http://cost-sysbiocat.fkit.hr/final%20report/ImPap.pdf and project n° 1-4 in the section Proposal/Projects

Additional outputs/ achievements

N/A

Projects

N/A

Other outputs / achievements

N/A

Impacts

The Action reported the following impact(s):

Description of the impact, i.e. what will change, and for whom, as a result of what the Action achieved	Type of impact	Timing of impact
<p>Access to purified proteins with well defined purity and properties. Systems Biocatalysis aims at the formation of reaction networks in which series of events are triggered from a series of specific enzymes. The recent advances in protein engineering have made possible to give for granted the availability of (almost) any enzymatic activity. This require the leading orientation of specialized laboratories and skills not usually present in synthetic laboratories where the synthetic sequence and the synthetic strategies are mostly addressed. The Action has allowed to connect the enzyme producing centers with the more synthetically oriented ones. This simplified approach from one side will become established in ESI being educated to consider biocatalytic tools as real competitors of organic solvent chemistry. At the same time will help to modify industrial processes forcing to evaluate the economics and ecological aspects of enzyme catalysis.</p>	<ul style="list-style-type: none"> • Scientific / Technological • Economic • Societal 	<p>Achieved</p>
<p>Identify possible enzyme catalysed synthetic reaction sequences with an adaptation of the concept of the traditional retro-synthetic approach. The retro-synthetic approach to design the way reactants ==> products considers for each step the available reagent tool from the whole of the known instruments. In replacing chemical steps with enzyme catalysed ones, the reaction sequence might be sensibly or completely different. The Action results on this field are exemplified by the enormous improvement in amine and lactones formation employing advanced preparation of new modified aminotransferases, imine reductases, Bayer-Villigerases among others. Moreover enzymes previously considered as strictly dependent from cofactors and well defined reaction conditions and medium are in the process of being modified by genetic engineering to more generalistic and easy tu use biocataysts. The new way of designing reaction sequences will at large influence the economic and societal aspects of oganic synthesis.</p>	<ul style="list-style-type: none"> • Scientific / Technological • Economic • Societal 	<p>Achieved</p>
<p>Process intensification: continuous flow reactor can be applied in multi-step enzyme catalysed reaction sequences going beyond enzyme immobilisation and compartmentalisation. The activity occurs at different level acting on enzyme immobilization: novel immobilization methods especially suitable for continuous flow systems (nanoparticles, electrospinning, monolith systems, magnetic biocatalysts). Flow systems: Novel continuous flow mini and microreactors (e.g. microreactor chips, membrane reactors, monolith reactors, disc reactors) for consecutive enzymic and/or chemical steps; Miniaturization and integration with downstream and process monitoring elements in a single continuous flow system. The progress in miniaturization in continuous systems is parallel to what i s actually happening in non-enzyme catalysed processes already reaching the industrial scale. It is expected that also in the case on enzymatic catalysis the same level of efficiency will be reached.</p>	<ul style="list-style-type: none"> • Scientific / Technological • Economic 	<p>Foreseen within two years</p>

Dissemination and exploitation of Action results

Dissemination and exploitation approach of the Action

The Action's dissemination and exploitation approach as well as all activities undertaken to ensure dissemination and exploitation of Action results and the outcomes of these activities are described below.

Dissemination instruments are expressly designed meetings to present initiative to a specific audience, compilation of a devoted publication usually expensive and not suitable for advanced basically oriented research, sometimes not near to commercialisation. We have been active in the most important european conferences where also industrial representative are present with hundreds of contribution, a form of presence that we have considered more efficient. The set-up of the web-tool described under "other outputs" in this report, i.e. the SysBiocat project in the portal Research Gate (<https://www.researchgate.net/project/SystemsBiocatalysis>) is going more in the direction of a traditional dissemination but oriented to stimulate collaboration and exchange of scientific experience. Considering exploitation of knowledge and scientific reputation and accountability: the success of 5 projects awarded with more than 24M€ and where Industry are present is certainly to be considered as an (in)direct result of the SysBiocat community.

Dissemination meetings funded by the Action

The Action did not fund any Dissemination Meetings

Other dissemination activities

The Action also undertook the following dissemination activities:

Activity	W.D. Fessner (DE) Systems Biocatalysis: back to the future. Symposium: Systems Biocatalysis for Asymmetric synthesis Concepts, challenges and Rewards, Darmstadt October 23rd 2015 Seminar centered on the Scientific significance of SysBiocat
Target	Scientific audience composed of senior scientist from German chemical companies, graduate students, academics from Darmstadt University
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://carbzymes.com/news-and-events/news?start=15

Activity	Symposium: Systems Biocatalysis for Asymmetric synthesis Concepts, challenges and Rewards, Darmstadt October 23rd 2015 Seminar centered on the Scientific significance of SysBiocat L. Hecquet FR Carbologation reaction by trankeolase: from past to present challenges
Target	Scientific audience composed of senior scientist from German chemical companies, graduate students, academics from Darmstadt University
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://carbzymes.com/news-and-events/news?start=15

Activity	Symposium: Systems Biocatalysis for Asymmetric synthesis Concepts, challenges and Rewards, Darmstadt October 23rd 2015 Seminar centered on the Scientific significance of SysBiocat N. Turner UK Biocatalytic retrosynthesis: working back from the answer
Target	Scientific audience composed of senior scientist from German chemical companies, graduate students, academics from Darmstadt University
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://carbzymes.com/news-and-events/news?start=15

Activity	Symposium: Systems Biocatalysis for Asymmetric synthesis Concepts, challenges and Rewards, Darmstadt October 23rd 2015. Seminar centered on the Scientific significance of SysBiocat. S. Servi IT Metabolic like reaction sequences in Systems Biocatalysis
Target	Scientific audience composed of senior scientist from German chemical companies, graduate students, academics from Darmstadt University
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://carbazymes.com/news-and-events/news?start=15

Activity	Symposium: Systems Biocatalysis for Asymmetric synthesis Concepts, challenges and Rewards, Darmstadt October 23rd 2015. Seminar centered on the Scientific significance of SysBiocat P. Clapès (ES) Substrate promiscuity of aldolases by minimalist active site redesign
Target	Scientific audience composed of senior scientist from German chemical companies, graduate students, academics from Darmstadt University
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://carbazymes.com/news-and-events/news?start=15

Activity	Conference by W.Kroutil (AT) at Emory University, Atlanta, USA, September 2, 2015
Target	Graduate students, academics of the Emory university
Outcome	Raise the attention on the Systems Biocatalysis approach and at the European funding institution (COST)
Link	none

Activity	W.Kroutil AT : Biotrans award lecture at Biotrans '15 in Vienna
Target	The largest community ever gathered in biocatalysis, over 600 scientists from all over the world
Outcome	A compendium of a (young) life achievement with particular focus on Systems Biocatalysis
Link	http://biotrans2015.book-of-abstracts.com/programme/confirmed-oral-presentations/

Activity	Conference by W.Kroutil AT et al. Asymmetric Pictet-Spengler Reaction via Strictosidine Synthases to Access Tetrahydro- β -carbolines - Protein Expression and Substrate Scope Austrian Chemistry Days, Innsbruck, Austria, September 21, 2015.
Target	Meeting of the Austrian Chemical Society
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://www.chemietage.at

Activity	Conference by W.Kroutil AT et al. Biocatalytic oxidation and C-C bond formation Seminar at Merck & Co., Inc., Rahway, USA, August 31, 2015.
Target	Scientific audience composed of senior scientist from Merck corporation
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	not available

Activity	Conference by W.Kroutil (AT) Biocatalysts and organic synthesis Seminar at the California Institute of Technology (CalTech), Pasadena, USA, August 18, 2015
Target	Scientific audience composed of graduate students, academics from CALTECH and senior scientists of local chemical companies
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions

Link	not available
-------------	---------------

Activity	Presentation by M. Tudorache (RO) "Tuning the bio-polymer properties based on lignin residues and biocatalytic process" 12th International Conference on Renewable Resources & Biorefineries, Gent, Belgium, May-Jyne, 2016
Target	The conference audience
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	not available

Activity	Presentation by M. Tudorache RO Guideline Of Alpha-Pinene To Value-Added Products Using Biocatalytic Tools", Third International Conference Catalysis For Renewable Sources: Fuel, Energy, Chemicals, Catania, Sicily, 2015
Target	The conference audience
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	not available

Activity	F. Rudroff / TU Wien, Vienna, Austria CATALYTIC CASCADES – 'EN ROUTE' TO APPLIED BIOCHEMICAL CELL-FACTORIES Monday, 2017-09-25, Austrian Chemistry Days, Salzburg, (AT)
Target	Austrian Chemical Society
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://www.chemietage.at/index.php?id=1708

Activity	M.D. Mihovilovic / TU Wien, Vienna, Austria FLAVOENZYME BIOCATALYSIS – CHALLENGES AND OPPORTUNITIES Austrian Chemistry days, Monday, 2017-09-25, Salzburg, (AT)
Target	Austrian Chemical Society
Outcome	Highlight on the methods of Systems Biocatalysis and on the tools provided by COST Actions
Link	http://www.chemietage.at/index.php?id=1708

Activity	Horizon 2020 Summer School 2017 Biocatalysis as a Key Enabling Technology
Target	ESI from COST Countries
Outcome	10 ESI from CM1303 have participated to the workshop held in Siena 3-7 October 2017 funded by the ITC Conference Grant
Link	http://cost-sysbiocat.fkit.hr/report/CORDIS_project_Carbazymes.pdf

Exploitation activities

The Action undertook the following activities to ensure exploitation (use, in particular in a commercial context) of the Action's achievements:

No input provided by the Action

Action Success(es)

The Action's two most significant successes were the following:

- Numbers: 4 projects started and financed to CM1303 SysBiocat members in the Action period gathering almost 25.000.000 euro. Coordinators belong to WG 1, WG3 and WG5 71 publications authored by at least 2 Cost members from different Countries with an average IF of 5.5 (see this report). 57 additional publications acknowledging SysBiocat and relevant for the Action success <http://cost-sysbiocat.fkit.hr/publications.html> 38 lectures at Biotrans 2015 Vienna + 2017 Budapest http://cost-sysbiocat.fkit.hr/PR2/meeting_programs.html 200 posters presented in workshops, meeting, 66 STSMs + conf grants (<http://cost-sysbiocat.fkit.hr/stsm.html>) budget 130.000 (25 % of the budget) Trainee composition: Gender M 37 (10 from ITC) F 29 (20 from ITC) Schools budget 90.000 (15 % of budget). on total expenditures M / F = 51 / 49 ESI participation to Activities has been globally of 40%, mainly in STSMs and TS on total expenditures ITC = 38% Total 350 supported in different forms. I believe that these numbers accompanied by the quality of the research should speak of a very successful Action
- Providing access to european excellence centres in biotechnology. Within the promoters of the Action are present laboratories which can be considered as expressing excellence on several topics, and not only at the european level. The exchange of ESI has allowed young researchers from ITC but not only (40:60 %) to have the possibility to enter the sanctuary and eventually obtain a temporary position for a longer period. This has had a profound effect on the confidence and the relationships of ESI with senior scientists and among themselves. A great mix. These is the social educational aspects. As a fundamental complement the deep research carried out on protein engineering and the innovation in metabolic like pathways design adds quality to the scientific aspect.

Action Expenditure

The table below shows the budget allocated to the Action for each Grant Period:

#	Grant Period	Start Date	End Date	Budget allocated to Action (EUR)
1	CM1303-20131204	1-1-2014	30-11-2014	162,000.00 (EUR)
2	CGA-CM1303-2	2-12-2014	30-9-2015	137,323.73 (EUR)
3	CGA-CM1303-2B	1-10-2015	30-4-2016	84,794.56 (EUR)
4	AGA-CM1303-4	1-5-2016	30-4-2017	135,720.00 (EUR)
5	AGA-CM1303-5	1-5-2017	19-11-2017	83,150.47 (EUR)