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Nishu Goyal

In silico Modeling and Experimental Validation for Improving Methanogenesis from CO₂ via *M. maripaludis*

Doctoral Thesis accepted by
National University of Singapore, Singapore

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Supervisor's Foreword

It is my great pleasure to introduce the Ph.D. thesis of Dr. Nishu Goyal, which has been selected as a Springer Thesis for outstanding original work. Dr. Goyal was conferred Ph.D. on 17 December 2015 by the Department of Chemical and Biomolecular Engineering of the National University of Singapore. She began her Ph.D. in July 2011, after securing an M.Tech. (Biomedical Engineering) from the Indian Institute of Technology Bombay, India, and gaining some valuable work experience in Strand Life Sciences Pvt Ltd., a leading bioinformatics company in Bangalore, India. For her graduate studies at NUS, she was awarded the highly prestigious President Graduate Fellowship. With interest and experience in microbiology, genomics, and informatics, she chose the area of systems biology as her Ph.D. topic under my supervision. Given the global issue of increasing CO₂ emissions and a need for sustainable energy, she decided to focus on the possibility of converting carbon dioxide into a cleaner energy fuel such as methane via biochemical pathways. To this end, she conducted an exhaustive search and identified the potential for using hydrogenotrophic methanogens to convert CO₂ to methane. She selected *M. maripaludis*, a mesophilic, gram-negative anaerobe with the ability to utilize CO₂ as the sole carbon substrate for growth and methanogenesis, as the most attractive microbe.

Her first task in the thesis was to develop the first constraint-based genome-scale metabolic model (*i*MM518) for *M. maripaludis* to quantify the various intra- and extracellular fluxes during methanogenesis from CO₂. She used *i*MM518 to explain a variety of experimental phenotypes known in the literature. Then, she identified several genetic manipulations that can enhance methanogenesis. Since virtually no experimental flux data existed in the literature for more accurate validation, Nishu performed anaerobic batch cultivations and measured key extracellular fluxes along with specific growth rates in the presence of CO₂ as the sole carbon substrate. For analysing her data and calibrating her model, she developed a novel process simulation approach to estimate the key maintenance energy parameters of a cell. Her experiments revealed the beneficial impact of free nitrogen on methanogenesis. Therefore, she studied the effect of diazotrophy (growth on free N₂) on

methanogenesis in *M. maripaludis*. Her experiments have suggested 2.5 times higher conversion yield for CO₂ to methane during diazotrophy. During her work, she also constructed a detailed, integrated, and comprehensive picture of the entire metabolism of *M. maripaludis*, which is also missing in the literature.

In brief, her work demonstrates a synergistic mix of modelling and experiments. Its framework for systematically and quantitatively studying the metabolism in *M. maripaludis* at the genome-scale by combining constraint-based metabolic modelling and experimental flux data under different culture conditions is general, and applicable to any other CO₂-fixing methanogen. She has published three papers in international refereed high-impact journals and presented her work at several prestigious international conferences.

Singapore
August 2016

Prof. I.A. Karimi

Parts of this thesis have been published in the following journal articles:

Publications and Conferences

Journal Publications

1. **N. Goyal**, Z. Zhi, and I. A. Karimi. “Genome-scale metabolic model of *M. maripaludis* S2 for CO₂ capture and conversion to methane”. *Molecular BioSystems* (2014), 10, 1043–1054.
2. **N. Goyal**, Z. Zhi, and I. A. Karimi. “Flux measurements and maintenance energy for CO₂ Utilization by *M. maripaludis*”. *Microbial Cell Factories* (2015), 14, 146.
3. **N. Goyal**, Z. Zhi, and I. A. Karimi. “Metabolic processes in *M. maripaludis* for industrial and environmental applications”. *Microbial Cell Factories* (2016), 15, 107.
4. **N. Goyal**, Z. Zhi, and I. A. Karimi. “Diazotrophy enhances CO₂ to methane conversion in *M. maripaludis*” (Under review by *Biomass and Bioenergy*).
5. M. Padhiary, K. Walczak, **N. Goyal**, and Z. Zhou. “Bioelectrocatalyzed reduction of CO₂ to higher alcohols and acids using acetogens and acetate-utilizing *Clostridium* strains” (Under review by *Energy and Environmental Science*).

Conference Proceedings

1. **N. Goyal**, H. Widiastuti, I. A. Karimi and Z. Zhi. “Genome-scale metabolic network reconstruction and *in silico* analysis of *M. maripaludis* S2” *Computer-Aided Chemical Engineering* (2013), Vol 32, 181–186. Lappeenranta, Finland.
2. **N. Goyal**, I. A. Karimi and Z. Zhi. “Genome based metabolic flux analysis (MFA) of *M. maripaludis* for improved methane productivity” (264e), *AIChE Annual Meeting* (2014), Atlanta, Georgia, USA.
3. **N. Goyal**, I. A. Karimi and Z. Zhi. “Experimental validation of *in silico* flux predictions from a genome-scale model (*i*MM518) for CO₂ utilization by *M. maripaludis*” *Computer-Aided Chemical Engineering* (2015), Vol 37, 2153–2159. Copenhagen, Denmark.
4. **N. Goyal**, I. A. Karimi and Z. Zhi. “Influence of nitrogen limitation on methanogenesis and growth in *M. maripaludis*” (250). *International Conference on Carbon Dioxide Utilization* (2015), Singapore.

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Singapore
July 2015

Nishu Goyal

Declaration

I hereby declare that this thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

July 2015

Nishu Goyal

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Nomenclature

Symbols

I	Number of metabolites
J	Number of reactions
t	Time (h)
v	Vector of reaction fluxes (mmol/gDCW-h)
b	$I \times 1$ vector of net metabolite fluxes (mmol/gDCW-h)
S	Matrix of stoichiometric coefficient of the reactants and products (dimensionless)
Z	Objective function
μ	Specific growth rate (/h)
c_j	Weight associated with the reaction fluxes (dimensionless)
v_j	Reaction flux (mmol/gDCW-h)
X	Biomass (gram dry cell weight)
C_i	Specific concentration of metabolite i (mmol/gDCW)
α_j	Lower bound for reaction flux (mmol/gDCW-h)
β_j	Upper bound for reaction flux (mmol/gDCW-h)

Abbreviations

3-HP	3-Hydroxypropionate
ACS	Acetyl-CoA synthetase
ADP	Adenosine diphosphate
ADTH	2-amino-3,7-dideoxy-D-threo-hept-6-ulosonate
AMP	Adenosine monophosphate
AroAAs	Aromatic amino acids
ATP	Adenosine triphosphate
BCAAs	Branched-chain amino acids
CBB	Calvin benson-bassham
CoA	Coenzyme A
COBRA	COntstraint-Based Reconstruction and Analysis

CODH	Carbon monoxide dehydrogenase
CoM	Coenzyme M
COM-S-S-COB	Coenzyme M 7-mercaptoheptanoylthreonine-phosphate heterodisulfide
CTP	Cytidine triphosphate
CUR	Carbon dioxide uptake rate
CysRS	CysteinyI-tRNA synthetase
Cys-tRNA	CysteinyI-tRNA
DCP	Dry Cell Protein
DCW	Dry Cell Weight
DHQ	3-dehydroquinate
DKFP	6-deoxy-5-ketofructose 1-phosphate
E4P	Erythrose-4-phosphate
Ech	Energy-converting hydrogenase
EMP	Embden–Meyerhof–Parnas
EPC	Electronic pneumatic control
F420(ox)	Oxidized coenzyme F420
F420(rd)	Reduced coenzyme F420
F6P	Fructose-6-phosphate
FBA	Flux balance analysis
FBP	Fructose-bisphosphatase
Fd(rd)	Reduced ferredoxin
Fd(ox)	Oxidized ferredoxin
Fdh	Formate dehydrogenase
Fru/Frc	F420 reducing hydrogenase
Ftr	Formyltransferase
FVA	Flux variability analysis
Fwd/Fmd	Tungsten/Molybdenum containing formylIMFR dehydrogenase
G3P	Glyceraldehyde-3-phosphate
GAM	Growth-associated maintenance
GAPDH	G3P dehydrogenase
GAPN	NADP ⁺ -dependent G3P dehydrogenase
GAPOR	Fd-dependent G3P dehydrogenase
GHG	Greenhouse gas
GK	Glucokinase
GMP	Guanosine monophosphate
GPR	Gene–protein reaction
Hdr	Heterodisulfide reductase
HHV	High Heating Value
Hmd	5,10-methenylTHMPT hydrogenase
HS-COM	Coenzyme M (2-mercaptoethanesulfonate)
HUR	Hydrogen Uptake Rate
IPMS	2-isopropylmalate synthase
LNG	Liquefied natural gas
LP	Linear programming

McC	Complex medium
Mch	Methylene-THMPT cyclohydrolase
McN	Minimal medium
McNA	Minimal medium plus sodium acetate
Mcr	Methyl-COM reductase
MECs	Microbial electrolysis cells
MER	Methane evolution rate
Mer	Methylene-THMPT reductase
Methyl-S-COM	2-(Methylthio)-coenzymeM
MFA	Metabolic flux analysis
MFCs	Microbial fuel cells
MFR	Methanofuran
Mtd	Methylene-THMPT dehydrogenase
Mtr	Methyltransferase
NAD	Nicotinamide adenine dinucleotide
NGAM	Non-growth-associated maintenance
NOPPP	Non-oxidative PPP
OD	Optical density
ORFs	Open-reading frames
PABA	p-aminobenzoic acid
PEG	Polyethylene glycol
PFK	Phosphofructokinase
PFORs	Pyruvate: ferredoxin oxidoreductases
PMF	Proton motive force
PORs	Pyruvate oxidoreductases
PPC	Phosphoenolpyruvate carboxylase
PPP	Pentose phosphate pathway
PPS	Phosphoenolpyruvate synthase
ProRS	Prolyl-tRNA synthetase
PRPP	5-Phosphoribosyldiphosphate
PYC	Pyruvate carboxylase
rDNA	Recombinant DNA technology
SBML	Systems Biology Markup Language
SepCysS	Sep-tRNA:Cys-tRNA synthase
Sep-tRNA	O-phosphoseryl-tRNA
SH-CoB	Thio-coenzyme B
SSE	Sum of Squares of Errors
TCA	Tricarboxylic acid
THMPT	Tetrahydromethanopterin
TKL	Transketolase
TSSE	Total sum of squares of errors
UMP	Uridine monophosphate
Vhu/Vhc	F420 non-reducing hydrogenase

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