Supplemenal information 2 Biomass composition

Table I Macromolecule composition:

Component	Composition[a] (g/g DCW)	Comments
Protein	0.500	Determined in this study[b]
DNA	0.028	Determined in this study
RNA	0.200	Taken from Neidhardt et al. (1996)
Phospholipid	0.049	Taken from Michael et al. (2005)
Cofactors and vitar	0.030	Assumption
Lipopolysaccharide	0.025	Taken from Conrad et al. (1996)
Peptidoglycan	0.227	Taken from Neidhardt et al. (1996)

[a]Calculated for an average macromolecular composition of *M. succiniciproducens* in complex medium with glucose. Biomass composition was experimentally measured at the exponential growth phase of anaerobic batch cultivation (specific growth rate: 0.81 h^{-1}), which is the average of three samples. MW of 1 water was subtracted from MW of each molecule to account for esterification or peptide bonding.

[b] We determined the protein content in the cells as described by Han et al (2005) and Bradford. (1976). The analyzed protein is 40.48% of the total biomass. Membrane proteins are difficult to be solubilized due to their hydrophobicity, and so it is not likely that they are accounted for in the above Bradford assay. Therefore, we included in the total protein composition by estimating their relative content as approximately 20% of total protein content, which is an average value of the gram negative cell (Brock Biology of Microorganisms 11th ed. by Madigan and Martinko). As a result, the total protein composition became 50.6% of the total biomass

Table II Proteins composition:

The amino acids composition determined for *P. putida KT2440* by a Waters HPLC systems (Waters Corporation, Milford, MA). Absorbance was measured at 254nm.

Amino acid	% protein (w/w)	MW[a], g/mol	mmol/g protein
Alanine	9.6	71.09	0.02543
Arginine	5.5	156.20	0.01166
Asparagine	4.5	114.12	0.02236
Aspartate	4.5	115.10	0.02236
Cysteine	1.7	103.16	0.00080
Glutamate	4.9	128.15	0.02839
Glutamine	4.9	129.13	0.02839
Glycine	11.5	57.07	0.02489
Histidine	1.8	137.16	0.00491
Isoleucine	5.4	113.18	0.01179
Leucine	8.4	113.18	0.02085
Lysine	6.4	128.19	0.01367
Methionine	2.9	131.21	0.00461
Phenylalanine	3.5	147.19	0.00776
Proline	4.1	97.13	0.01183
Serine	4	87.09	0.01174
Threonine	4.7	101.12	0.01260
Tryptophan	1.1	186.23	0.00002
Tyrosine	2.6	163.19	0.00267
Valine	7.9	99.15	0.01851
Energy requirement for polymerisation (ATP):			40

Table III DNA composition:

The composition of DNA was calculated from the genomic sequence of *P. putida* . Energy requirement for polymerisation of triphosphates was from (Ingraham et al., 1983).

Nucleotide	mol/mol DNA	MW[a], g/mol	mmol/g DNA
dAMP	0.192	313.2	0.6213431
dCMP	0.308	289.2	0.9967379
dTMP	0.192	304.2	0.6213431
dGMP	0.308	329.2	0.9967379
Energy requirement for polymerisation (ATP):			4.40

[a] the molecular weight is the weight of the nucleotide monophosphate substracted 1 water, which is lost during esterification

Table IV RNA composition:

It was assumed that RNA consisted of 5% mRNA, 80% rRNA and 15% tRNA (molar). The nucleotide composition of mRNA was taken as for genomic DNA. The nucleotide composition of rRNA was calculated from the sequences of 16S, 23S and 5S ribosomal RNA units. tRNA composition was also taken from sequences of tRNA. All the sequences were obtained from TIGR (http://cmr.tigr.org/tigr-scripts/CMR/GenomePage.cgi?org=gpp). Energy requirement for polymerisation of triphosphates was from (Ingraham et al., 1983).

Nucleotide	mol/mol RNA			MW[a], g/mol	mol/mol RNA	mmol/g RNA
	mRNA 5%	rRNA 80%	tRNA 15%			
AMP	0.192	0.313	0.307	329.2	0.306	0.380
GMP	0.308	0.281	0.219	345.2	0.273	0.339
CMP	0.192	0.192	0.261	305.2	0.202	0.250
UMP	0.308	0.213	0.212	306.2	0.218	0.270
Energy requirement for polymerisation (ATP):						1.25

[a] the molecular weight is the weight of the nucleotide monophosphate substracted 1 water, which is lost during esterification

Table V Phospholipids composition:

The composition of phospholipids was taken from Pinkhart and White, 1997

Component	g/g phospholipids	mmol/g phospholipids
Phosphatidylethanolamin	0.65	0.688
Phosphatidylglycerol	0.22	0.225
Cardiolipin	0.10	0.054

Table VI Molecular weights of phospholipids components:

Constituent	MW, g/mol		
	backbone	# of fatty acids residues	total
Phosphatidylethanolamin	181.128	2	944.66
Phosphatidylglycerol	212.139	2	975.67
Cardiolipin	332.183	4	1859.25

Table VII Composition of fatty acids in phospholipids:

The composition of fatty acids tails in phospholipids was measured using gas chromatographic analysis (6890 GC system, Agilent Technologies, Palo Alto, CA)

Fatty acid	g/g total fatty acids	MW[a], g/mol	mmol/g total fatty acids	mol/mol total fatty acids
C10	0.030	172.265	0.18	0.067
C12	0.095	200.318	0.48	0.182
C14	0.011	228.371	0.05	0.018
C16	0.394	256.424	1.54	0.587
C18:0	0.034	284.477	0.12	0.046
C18:1	0.074	282.461	0.26	0.100
Average molecular weig		382	SUM:	1.00

Table VIII Peptidoglycan composition:

Peptidoglycan is the main component of the bacterial cell wall. Its composition was taken from the peptidoglycan composition of Pseudomonas aerugnosa (Heilmann, 1972).

Component	molar ratio in peptidoglycan of: *P. aeruginosa (Heilmann 1972)*	Average	MWa, g/mol	mmol/g peptidoglycan
N-acetylmuramic acid	0.4	0.4	275	1.010
N-acetylglucosamine	0.4	0.4	203	1.012
Alanine	1.0	1.0	71	2.185
Diaminopimelinic acid	0.5	0.5	154	1.168
D-glutamate	0.5	0.5	129	1.157
Lysine	0.1	0.1	146	0.129
Energy requirement for polymerisation (ATP):				6.660

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