**Data sheet 2. Biomass composition of *P. pastoris***

**Table 1. Macromolecular composition:**
0.410 PROTEIN + 0.066 RNA + 0.130 DNA + 0.22 PHOSPHOLIPID + 0.314 CARBOHYDRATE +
0.01 COF + ATP -> BIOMASS + ADP + PI

**Table 2. Proteins composition:**

| Amino acid | % protein (w/w) | MW[a], g/mol | mmol/g protein |
|---|---|---|---|
| Alanine | 6.62 | 71.09 | 0.931 |
| Arginine | 9.03 | 156.20 | 0.578 |
| Asparagine | 5.03 | 114.12 | 0.441 |
| Aspartate | 5.03 | 115.10 | 0.437 |
| Cysteine | 0.13 | 103.16 | 0.013 |
| Glutamate | 7.43 | 128.15 | 0.580 |
| Glutamine | 7.43 | 129.13 | 0.575 |
| Glycine | 4.07 | 57.07 | 0.713 |
| Histidine | 2.65 | 137.16 | 0.193 |
| Isoleucine | 3.87 | 113.18 | 0.342 |
| Leucine | 7.51 | 113.18 | 0.664 |
| Lysine | 7.95 | 128.19 | 0.620 |
| Methionine | 1.59 | 131.21 | 0.121 |
| Phenylalanine | 4.07 | 147.19 | 0.277 |
| Proline | 4.89 | 97.13 | 0.503 |
| Serine | 5.92 | 87.09 | 0.680 |
| Threonine | 6.59 | 101.12 | 0.652 |
| Tryptophan | 2.2 | 186.23 | 0.118 |
| Tyrosine | 3.27 | 163.19 | 0.200 |
| Valine | 4.59 | 99.15 | 0.463 |
| Energy requirement for polymerisation (ATP): | | | 40 |

[a] water is substracted from MW to account for water excretion during peptide bond formation

*Protein biosynthesis equation is therefore (in mmol for synthesis of 1 g protein):*
0.931 ALA + 0.578 ARG + 0.441 ASN + 0.437 ASP + 0.013 CYS + 0.580 GLN + 0.575 GLU + 0.713
GLY + 0.193 HIS + 0.342 ILE + 0.664 LEU + 0.620 LYS + 0.121 MET + 0.277 PHE + 0.503 PRO +
0.68 SER + 0.652 THR + 0.118 TRP + 0.2 TYR + 0.463 VAL + 40.0 ATP -> 40.0 ADP + 40.0 PI +

**Table 3. DNA composition:**
The composition of DNA was calculated from the genomic sequence of *P. pastoris*. Energy requirement for polymerisation of triphosphates was from (Ingraham et al., 198

| Nucleotide | mol/mol DNA | MW[a], g/mol | mmol/g DNA |
|---|---|---|---|
| dAMP | 0.293 | 313.2 | 0.950 |
| dCMP | 0.207 | 289.2 | 0.669 |
| dTMP | 0.293 | 304.2 | 0.949 |
| dGMP | 0.207 | 329.2 | 0.669 |
| 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

*DNA biosynthesis equation is therefore (in mmol for synthesis of 1 g DNA):*
0.946 DATP + 0.666 DCTP + 0.945 DTTP + 0.666 DGTP + 4.4 ATP -> 4.4 ADP + 4.4 PI + DNA

**Table 4. RNA composition:**
It was assumed that RNA consisted of 5% mRNA, 75% rRNA and 20% tRNA (molar). The nucleotide composition of mRNA was taken as for genomic DNA. The nucleotide composition of rRNA was calculated from the sequences the ribosomal RNA units. tRNA composition was found from sequences of leucine and glycine transporting RNAs. Energy requirement for polymerisation of triphosphates was from (Ingraham et al., 1983)

| Nucleotide | mol/mol RNA mRNA 5% | rRNA 75% | tRNA 20% | MW[a], g/mol | mol/mol RNA | mmol/g RNA |
|---|---|---|---|---|---|---|
| AMP | 0.293 | 0.303 | 0.198 | 329.2 | 0.281 | 0.873 |
| GMP | 0.207 | 0.227 | 0.342 | 345.2 | 0.249 | 0.773 |
| CMP | 0.293 | 0.210 | 0.289 | 305.2 | 0.230 | 0.714 |
| UMP | 0.207 | 0.260 | 0.171 | 306.2 | 0.240 | 0.744 |
| 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

*RNA biosynthesis equation is therefore (in mmol for synthesis of 1 g RNA):*
0.600 ATP + 0.826 GTP + 1.031 CTP + 0.662 UTP + 1.25 ATP -> 1.25 ADP + 1.25 PI + RNA

**Table 5. Phospholipids composition:**

| Component | g/g phospholipids | mmol/g |
|---|---|---|
| Phosphatidylethanolamin | 0.052 | 0.274 |
| Phosphatidylcholine | 0.100 | 0.431 |
| Phosphatidylinositol | 0.002 | 0.006 |
| Phosphatidylserine | 0.002 | 0.009 |
| Phosphatidic acid | 0.001 | 0.004 |
| Cardiolipin | 0.003 | 0.009 |
| Ergosterol | 0.268 | 0.676 |

*Phospholipids biosynthesis equation is therefore (in mmol for synthesis of 1 g phospholipids):*
0.1 PE + 0.324 PC + 0.229 PI + 0.038 PS + 0.324 PA + -> PHOSPHOLIPID

**Table 5.1 Molecular weights of phospholipids components:**

| Constituent | MW, g/mol | | |
|---|---|---|---|
| | backbone | # of fatty acids residues | total |
| Phosphatidylethanolamin | 181.128 | 2 | 190.01 |
| Phosphatidylcholine | 223.2066 | 2 | 232.09 |
| Phosphatidylinositol | 300.1996 | 2 | 309.09 |
| Phosphatidylserine | 223.1205 | 2 | 232.01 |
| Phosphatidic acid | 224.0622 | 2 | 232.95 |
| Cardiolipin | 332.183 | 4 | 349.95 |
| Ergosterol | 396.6484 | 0 | 396.6484 |

**Table 5.2 Composition of fatty acids in phospholipids:**
The composition of fatty acids tails in phospholipids was assumed to be the same as in *S. antibioticus* (Zuneda et al., 1984).

| Fatty acid | g/g total fatty acids | MW[a], g/mol | mmol/g total fatty acids | mol/mol total fatty acids |
|---|---|---|---|---|
| c160 | 17.975 | 255 | 70.49 | 0.313 |
| c161 | 5.100 | 253 | 20.16 | 0.090 |
| c180 | 2.450 | 281 | 8.72 | 0.039 |
| c181 | 35.075 | 279 | 125.72 | 0.559 |
| c182 | 28.525 | 277 | 102.98 | 0.458 |
| c183 | 10.875 | 275 | 39.55 | 0.176 |
| Average molecular weight | | 4 | SUM: | 1.63 |

[a] molecular weight without a proton

*Example: phosphatidylethanolamine biosynthesis equations are therefore (in mol):*
GL3P + 0.094 C140ACP + 0.294 C150ACP + 0.262 C160ACP + 0.293 C170ACP + 0.057 C181ACP -> AGL3P + ACP
AGL3P + 0.094 C140ACP + 0.294 C150ACP + 0.262 C160ACP + 0.293 C170ACP + 0.057 C181ACP -> PA + ACP
PA + CTP <-> CDPDG + PPI
CDPDG + SER <-> CMP + PS
PS -> PE + CO2

**Table 6. Small molecules pool composition:**
For simplification is was assumed that the selected small molecules are equally represented (w/w) in the pool

| Molecule | MW, g/mol | g/g pool of small molecules | mmol/g pool of small molecules |
|---|---|---|---|
| NAD | 664.438 | 0.125 | 0.188 |
| NADP | 744.418 | 0.125 | 0.168 |
| COA | 767.534 | 0.125 | 0.163 |
| THF | 445.434 | 0.125 | 0.281 |
| FMN | 456.348 | 0.125 | 0.274 |
| FAD | 785.557 | 0.125 | 0.159 |

[a] molecular mass of recombinant *E. coli* acyl carrier protein (Sigma-Aldrich)

*Small molecules pool biosynthesis equation is therefore (in mmol for synthesis of 1 g SMALL MOLECULES):*
0.188 NAD + 0.168 NADP + 0.163 COA + 0.012 ACP + 0.146 Q6 + 0.281 THF + 0.274 FMN + 0.159 FAD -> COF

**Table 9. Carbohydrates composition:**
*Carbohydrates biosynthesis equation is therefore (in mmol for synthesis of 1 g carbohydrates):*
2.902 UDPNAG + 3.794 UDPGAL -> 5.691 UDP + CARBOHYDRATE

**References**


Carnicer, M., Baumann, K., Toplitz, I., Sanchez-Ferrando, F., *et al.*, Macromolecular and elemental composition analysis and extracellular metabolite balances of *Pichia pastoris* growing at different oxygen levels. *Microb Cell Fact* 2009, *8*, 65.

Wriessnegger, T., Leitner, E., Belegratis, M. R., Ingolic, E., Daum, G., Lipid analysis of mitochondrial membranes from the yeast *Pichia pastoris*. *Biochim Biophys Acta* 2009, *1791*, 166-172.