**Biomass composition of *R. marinus***

The macromolecular composition of the biomass in *R. marinus* was obtained from *R. marinus* specific data found in literature (Cordova, 2017, Sardari, 2017, Lutneas, 2004), experimentally obtained in this study and when data on *R. marinus* was not available, experimental data for *E. coli* was used (Feist, 2007). Amino acid (Cordova, 2017), EPS (Sardari, 2017), lipid (Nunes, 1991 and Moreira, 1996), carotenoid (Lutneas, 2004) and polyamine (Hamana, 1992) compositions for *R. marinus* were available in literature. DNA and RNA compositions were calculated from the DSM 4252 genome.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Component** | | **Fraction (% w/w)** | **Molar mass (g mol-1)** | **Coefficient (mmol gDW-1)** | **Source** |
| Protein | PROT\_RMAR\_c | 45,8% | 128,0 | 3,5815 | *R. marinus* (Cordova, 2017) |
| Glycogen | glycogen\_c | 14,0% | 162,1 | 0,8643 | *R. marinus* (Cordova, 2017) |
| Peptidoglycan | peptido\_c | 2,50% | 991,0 | 0,0253 | *E. coli* (Feist, 2007) |
| EPS | EPS\_RMAR\_c | 0,36% | 1121,8 | 0,0032 | *R. marinus* (Sardari, 2017) |
| LPS | lipidAds\_c | 3,40% | 1323,7 | 0,0257 | *E. coli* (Feist, 2007) |
| DNA | DNA\_RMAR | 2,50% | 304,2 | 0,0823 | *R. marinus* (This study) |
| RNA | RNA\_RMAR | 13,0% | 322,7 | 0,4032 | *R. marinus* (Cordova, 2017) |
| Lipids | LIP\_RMAR\_c | 16,2% | 970,6 | 0,1671 | *R. marinus* (Cordova, 2017) |
| Carotenoids | CAROT\_RMAR\_c | 0,04% | 955,4 | 0,0004 | *R. marinus* (Lutneas, 2004) |
| Polyamines | POLAM\_RMAR\_c | 0,43% | 178,6 | 0,0241 | *E. coli* (Feist, 2007) |
| Soluble pool | SOL\_c | 0,67% |  | 1 | *E. coli* (Feist, 2007) |
| Inorganic ions | IONS\_c | 1,00% |  | 1 | *E. coli* (Feist, 2007) |
|  | Total | 100% |  | 3,5815 |  |

**Biomass reaction:**

0.0004 CAROT\_RMAR\_c + 0.0822 DNA\_RMAR\_c + 0.0032 EPS\_RMAR\_c + IONS\_c + 0.167 LIP\_RMAR\_c + 0.0241 POLAM\_RMAR\_c + 3.5814 PROT\_RMAR\_c + 0.4032 RNA\_RMAR\_c + SOL\_c + 20.0 atp\_c + 0.8643 glycogen\_c + 20.0 h2o\_c + 0.0257 lipidAds\_c + 0.0252 peptido\_c --> 20.0 adp\_c + 20.0 h\_c + 20.0 pi\_c

**Protein**

Amino acid composition obtained mainly from *R. marinus* (Cordova, 2017).

|  |  |  |  |
| --- | --- | --- | --- |
| **Amino acid** | **Fraction (mmol/gprotein)** | **Molar ratio** | **Molar mass (g mol-1)** |
| Ala | 0,90 | 9,9% | 89,1 |
| Arg | 0,51\* | 5,7% | 174,2 |
| Asx | 0,71 | 7,8% |  |
| Asp |  | 3,9% | 132,1 |
| Asn |  | 3,9% | 132,1 |
| Cys | 0,16\* | 1,8% | 121,2 |
| Glx | 1,09 | 12,1% |  |
| Glu |  | 6,0% | 146,6 |
| Gln |  | 6,0% | 146,6 |
| Gly | 0,82 | 9,1% | 75,1 |
| His | 0,41\* | 4,5% | 155,2 |
| Ile | 0,16 | 1,8% | 131,1 |
| Leu | 0,80 | 8,8% | 131,1 |
| Lys | 0,45 | 5,0% | 146,2 |
| Met | 0,20 | 2,2% | 149,2 |
| Phe | 0,31 | 3,4% | 165,2 |
| Pro | 0,53 | 5,8% | 115,1 |
| Ser | 0,47 | 5,2% | 105,1 |
| Thr | 0,54 | 6,0% | 119,1 |
| Trp | 0,10\* | 1,1% | 204,2 |
| Tyr | 0,30 | 3,3% | 181,2 |
| Val | 0,62 | 6,8% | 117,2 |
| Total | 9,08 | 100% | 128,0 |

\*Taken from *E. coli* (Feist, 2007)

**Protein reaction:**

0.0986 alatrna\_c + 0.0565 argtrna\_c + 0.0389 asntrna\_c + 0.0389 asptrna\_c + 0.306 atp\_c + 0.0175 cystrna\_c + 0.0603 glntrna\_c + 0.0603 glutrna\_c + 0.0908 glytrna\_c + 2.0 gtp\_c + 2.306 h2o\_c + 0.0452 histrna\_c + 0.0181 iletrna\_c + 0.0876 leutrna\_c + 0.0496 lystrna\_c + 0.0217 mettrna\_c + 0.034 phetrna\_c + 0.0579 protrna\_c + 0.0517 sertrna\_c + 0.0599 thrtrna\_c + 0.0109 trptrna\_c + 0.0335 tyrtrna\_c + 0.0682 valtrna\_c --> PROT\_RMAR\_c + 0.306 adp\_c + 2.0 gdp\_c + 2.306 h\_c + 2.306 pi\_c + 0.0986 trnaala\_c + 0.0565 trnaarg\_c + 0.0389 trnaasn\_c + 0.0389 trnaasp\_c + 0.0175 trnacys\_c + 0.1206 trnaglu\_c + 0.0908 trnagly\_c + 0.0452 trnahis\_c + 0.0181 trnaile\_c + 0.0876 trnaleu\_c + 0.0496 trnalys\_c + 0.0217 trnamet\_c + 0.034 trnaphe\_c + 0.0579 trnapro\_c + 0.0517 trnaser\_c + 0.0599 trnathr\_c + 0.0109 trnatrp\_c + 0.0335 trnatyr\_c + 0.0682 trnaval\_c

**DNA**

Calculated from DSM 4252 genome

|  |  |  |
| --- | --- | --- |
| **Nucleotide** | **Molar ratio** | **Molar mass (g mol-1)** |
| dAMP | 17,8% | 331,2 |
| dCMP | 32,2% | 289,2 |
| dGMP | 32,2% | 304,2 |
| dTMP | 17,8% | 304,2 |
| Total | 100% | 304,2 |

**DNA reaction:**

1.37 atp\_c + 0.178 datp\_c + 0.322 dctp\_c + 0.322 dgtp\_c + 0.178 dttp\_c --> DNA\_RMAR\_c + 1.37 adp\_c + 1.37 h\_c + 1.37 pi\_c + ppi\_c

**RNA**

Calculated from DSM 4252 genome, assuming equal transcription

|  |  |  |
| --- | --- | --- |
| **Nucleotide** | **Molar ratio** | **Molar mass (g mol-1)** |
| CMP | 32,2% | 305,2 |
| GMP | 33,0% | 345,2 |
| UMP | 17,6% | 306,2 |
| AMP | 17,2% | 329,2 |
| Total | 100% | 322,7 |

**RNA reaction:**

0.572 atp\_c + 0.322 ctp\_c + 0.33 gtp\_c + 0.176 utp\_c --> RNA\_RMAR\_c + 0.4 adp\_c + 0.4 h\_c + 0.4 pi\_c + ppi\_c

**Lipids**

Fatty acid composition in *R. marinus* obtained from (Moreira, 1996)

|  |  |
| --- | --- |
| **Fatty acid** | **Molar ratio** |
| iC14 | 1,6% |
| nC14 | 0,2% |
| iC15 | 8,4% |
| aC15 | 19,1% |
| nC15 | 0,2% |
| iC16 | 13,0% |
| nC16 | 5,4% |
| iC17 | 26,4% |
| aC17 | 18,4% |
| nC17 | 0,7% |
| iC18 | 4,7% |
| nC18 | 0,9% |
| iC19 | 0,2% |
| aC19 | 0,4% |

Phospholipid composition in *R. marinus* obtained from (Nunes, 1992)

|  |  |
| --- | --- |
| **Lipid** | **Molar ratio** |
| PE | 50,0% |
| DPG | 40,0% |
| PG | 10,0% |

Taken together:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Lipid** | **Molar ratio** | **Molar mass** |  | **Lipid** | **Molar ratio** | **Molar mass** |
| pgi14 | 0,16% | 666,9 |  | pei17 | 13,25% | 720,0 |
| pg140 | 0,02% | 666,9 |  | pea17 | 9,24% | 720,0 |
| pgi15 | 0,84% | 694,9 |  | pe170 | 0,35% | 720,0 |
| pga15 | 1,92% | 694,9 |  | pei18 | 2,36% | 748,1 |
| pg150 | 0,02% | 694,9 |  | pe180 | 0,45% | 748,1 |
| pgi16 | 1,31% | 723,0 |  | pei19 | 0,10% | 776,1 |
| pg160 | 0,54% | 723,0 |  | pea19 | 0,20% | 776,1 |
| pgi17 | 2,65% | 751,0 |  | dpgi14 | 0,64% | 1241,7 |
| pga17 | 1,85% | 751,0 |  | dpg140 | 0,08% | 1241,7 |
| pg170 | 0,07% | 751,0 |  | dpgi15 | 3,37% | 1297,8 |
| pgi18 | 0,47% | 779,1 |  | dpga15 | 7,67% | 1297,8 |
| pg180 | 0,09% | 779,1 |  | dpg150 | 0,08% | 1297,8 |
| pgi19 | 0,02% | 807,1 |  | dpgi16 | 5,22% | 1353,9 |
| pga19 | 0,04% | 807,1 |  | dpg160 | 2,17% | 1353,9 |
| pei14 | 0,80% | 635,9 |  | dpgi17 | 10,60% | 1410,0 |
| pe140 | 0,10% | 635,9 |  | dpga17 | 7,39% | 1410,0 |
| pei15 | 4,22% | 663,9 |  | dpg170 | 0,28% | 1410,0 |
| pea15 | 9,59% | 663,9 |  | dpgi18 | 1,89% | 1466,1 |
| pe150 | 0,10% | 663,9 |  | dpg180 | 0,36% | 1466,1 |
| pei16 | 6,53% | 692,0 |  | dpgi19 | 0,08% | 1522,2 |
| pe160 | 2,71% | 692,0 |  | dpga19 | 0,16% | 1522,2 |
|  |  |  |  | Total | 100% | 970,6 |

**Lipid reaction:**

0.0008 dpg140\_c + 0.0008 dpg150\_c + 0.0217 dpg160\_c + 0.0028 dpg170\_c + 0.0036 dpg180\_c + 0.0767 dpga15\_c + 0.0739 dpga17\_c + 0.0016 dpga19\_c + 0.0064 dpgi14\_c + 0.0337 dpgi15\_c + 0.0522 dpgi16\_c + 0.106 dpgi17\_c + 0.0189 dpgi18\_c + 0.0008 dpgi19\_c + 0.001 pe140\_c + 0.001 pe150\_c + 0.0271 pe160\_c + 0.0035 pe170\_c + 0.0045 pe180\_c + 0.0959 pea15\_c + 0.0924 pea17\_c + 0.002 pea19\_c + 0.008 pei14\_c + 0.0422 pei15\_c + 0.0653 pei16\_c + 0.1325 pei17\_c + 0.0236 pei18\_c + 0.001 pei19\_c + 0.0002 pg140\_c + 0.0002 pg150\_c + 0.0054 pg160\_c + 0.0007 pg170\_c + 0.0009 pg180\_c + 0.0192 pga15\_c + 0.0185 pga17\_c + 0.0004 pga19\_c + 0.0016 pgi14\_c + 0.0084 pgi15\_c + 0.0131 pgi16\_c + 0.0265 pgi17\_c + 0.0047 pgi18\_c + 0.0002 pgi19\_c --> LIP\_RMAR\_c

**Carotenoids**

Carotenoid composition in *R. marinus* obtained from (Lutneas, 2004)

|  |  |  |  |
| --- | --- | --- | --- |
| **Carotenoid** | | **Molar ratio** | **Molar mass (g mol-1)** |
| hglpyrcarote | Non-acetylated | 3,30% | 745,0 |
| ddcahglpyrcarote | Iso-C12:0 | 5,08% | 927,3 |
| myrshglpyrcarote | n-C12:0 | 0,56% | 955,4 |
| palmhglpyrcarote | Iso-C13:0 | 7,34% | 983,4 |
| odechglpyrcarote | Anteiso-C13:0 | 9,03% | 1011,5 |
| pdechglpyrcarote | Iso-C14:0 | 4,51% | 969,4 |
| 10mudechglpyrcarote | n-C14:0 | 2,26% | 927,3 |
| 12mtdechglpyrcarote | Iso-C15:0 | 5,64% | 955,4 |
| 14mpdechglpyrcarote | Anteiso-C15:0 | 10,72% | 983,4 |
| 11mddechglpyrcarote | n-C15:0 | 0,56% | 941,3 |
| 13mmyrshglpyrcarote | Iso-C16:0 | 3,95% | 969,4 |
| 15mpalmhglpyrcarote | n-C16:0 | 3,39% | 997,5 |
| 10mddechglpyrcarote | Iso-C17:0 | 0,56% | 941,3 |
| 12mmyrshglpyrcarote | Anteiso-C17:0 | 1,69% | 969,4 |
| glpyrcarote | Non-acetylated | 2,50% | 729,0 |
| ddcaglpyrcarote | Iso-C12:0 | 3,50% | 911,3 |
| myrsglpyrcarote | n-C12:0 | 0,39% | 939,4 |
| palmglpyrcarote | Iso-C13:0 | 6,22% | 967,4 |
| odecglpyrcarote | Anteiso-C13:0 | 7,00% | 995,5 |
| pdecglpyrcarote | Iso-C14:0 | 2,33% | 953,4 |
| 10mudecglpyrcarote | n-C14:0 | 1,56% | 911,3 |
| 12mtdecglpyrcarote | Iso-C15:0 | 3,89% | 939,4 |
| 14mpdecglpyrcarote | Anteiso-C15:0 | 7,39% | 967,4 |
| 11mddecglpyrcarote | n-C15:0 | 0,39% | 925,3 |
| 13mmyrsglpyrcarote | Iso-C16:0 | 1,95% | 953,4 |
| 15mpalmglpyrcarote | n-C16:0 | 2,33% | 981,5 |
| 10mddecglpyrcarote | Iso-C17:0 | 0,39% | 925,3 |
| 12mmyrsglpyrcarote | Anteiso-C17:0 | 1,17% | 953,4 |
| 14mpalmglpyrcarote | n-C18:0 | 0,39% | 981,5 |
| Total | | 100% | 955,4 |

**Carotenoid reaction:**

0.004 10mddecglpyrcarote\_c + 0.006 10mddechglpyrcarote\_c + 0.016 10mudecglpyrcarote\_c + 0.023 10mudechglpyrcarote\_c + 0.004 11mddecglpyrcarote\_c + 0.006 11mddechglpyrcarote\_c + 0.012 12mmyrsglpyrcarote\_c + 0.017 12mmyrshglpyrcarote\_c + 0.039 12mtdecglpyrcarote\_c + 0.056 12mtdechglpyrcarote\_c + 0.019 13mmyrsglpyrcarote\_c + 0.04 13mmyrshglpyrcarote\_c + 0.004 14mpalmglpyrcarote\_c + 0.074 14mpdecglpyrcarote\_c + 0.107 14mpdechglpyrcarote\_c + 0.023 15mpalmglpyrcarote\_c + 0.034 15mpalmhglpyrcarote\_c + 0.035 ddcaglpyrcarote\_c + 0.051 ddcahglpyrcarote\_c + 0.025 glpyrcarote\_c + 0.033 hglpyrcarote\_c + 0.004 myrsglpyrcarote\_c + 0.006 myrshglpyrcarote\_c + 0.07 odecglpyrcarote\_c + 0.09 odechglpyrcarote\_c + 0.062 palmglpyrcarote\_c + 0.073 palmhglpyrcarote\_c + 0.023 pdecglpyrcarote\_c + 0.045 pdechglpyrcarote\_c --> CAROT\_RMAR\_c

**Polyamine**

Polyamine composition in *R. marinus* obtained from (Hamana, 1992)

|  |  |  |  |
| --- | --- | --- | --- |
| **Polyamines** | **Fraction (mmol gDW-1)** | **Molar ratio** | **Molar mass (g mol-1)** |
| Putrescine | 0,000245 | 1,02% | 90,2 |
| Cadaverine | 0,000245 | 1,02% | 104,2 |
| Spermidine | 0,012840 | 53,30% | 148,3 |
| Spermine | 0,007337 | 30,46% | 206,4 |
| Thermopentamine | 0,000978 | 4,06% | 261,5 |
| N4-Aminopropylspermidine | 0,000611 | 2,54% | 206,4 |
| N4-bis(aminopropyl)spermidine | 0,001834 | 7,61% | 247,5 |
| Total | 0,024091 | 100% | 178,6 |

**Exopolysaccharides**

Exopolysaccharide composition in *R. marinus* obtained from (Sardari, 2017)

|  |  |  |
| --- | --- | --- |
| **EPS unit** | **Molar ratio** | **Molar mass (minus udp/dtdp) (g mol-1)** |
| UDP-glucose | 0,5 | 160,125 |
| dTDP-glucose | 0,5 | 163,149 |
| UDP-arabinose | 2,45 | 130,099 |
| UDP-xylose | 4,93 | 130,099 |
| Total |  | 1121,768 |

**EPS reaction:**

0.5 dtdpglu\_c + h2o\_c + 2.45 udparab\_c + 0.5 udpg\_c + 4.93 udpxyl\_c --> EPS\_RMAR\_c + 0.5 dtdp\_c + 7.88 udp\_c

**Vitamins, cofactors and inorganic ions**

Soluble pool and inorganic ions adapted from *E. coli* (Feist, 2007)

**Soluble pool reaction:**

0.00022 10fthf\_c + 0.00022 5mthf\_c + 0.00028 accoa\_c + 0.00022 adocbl\_c + 0.00022 btn\_c + 0.00022 chor\_c + 0.00017 coa\_c + 0.00022 fad\_c + 0.00022 gthrd\_c + 0.00022 hemeA\_c + 0.00022 hemeO\_c + 3e-05 malcoa\_c + 0.00022 mlthf\_c + 0.00179 nad\_c + 4e-05 nadh\_c + 0.00011 nadp\_c + 0.00034 nadph\_c + 0.00022 pheme\_c + 0.00022 pydx5p\_c + 0.00022 ribflv\_c + 0.00022 sheme\_c + 0.0001 succoa\_c + 0.00022 thf\_c + 6e-05 udcpdp\_c --> SOL\_c

**Inorganic ions reaction:**

0.0045 ca2\_c + 0.003 cobalt2\_c + 0.003 cu2\_c + 0.0068 fe2\_c + 0.0068 fe3\_c + 0.1704 k\_c + 0.0076 mg2\_c + 0.003 mn2\_c + 0.003 mobd\_c + 0.0038 na1\_c + 0.0114 nh4\_c + 0.0038 pi\_c + 0.0038 so4\_c + 0.003 zn2\_c --> IONS\_c

**GAM and NGAM**

No data for energy requirements in *R. marinus* is available. NGAM (non-growth associated maintenance) was set to 1 mmol/gDW/h but GAM (growth associated maintenance) was estimated by constraining the model with experimental uptake- and secretion rates of glucose, pyruvate, lactate and acetate, and growth rate of *R. marinus*. The GAM was estimated to be 20 mmol/gDW/h. This is relatively low compared to many other models, which can be explained by the energy accounted for in biosynthetic reactions for the macromolecules. The dataset used for this estimation (supplementary file 2) was not used again for validation.

**Sensitivity analysis**

The sensitivity of predicted growth rate to variation in biomass and energy components was investigated. The coefficient of each component was varied by 50% at different glucose uptake rates while the growth rate was predicted. Predicted growth rate was most sensitive to changes in the protein component, followed by the lipid component.

|  |  |  |
| --- | --- | --- |
| Growth rate (h-1) |  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  | Glucose uptake rate (mmol gDW-1 h-1) | |