BIOTHERMODYNAMIC ANALYSIS OF MYCOPLASMA PATHOGEN-HOST INTERACTIONS

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During pathogenesis of infections, changes occur in different state properties of microorganisms, as well as damage to host cells. Damage to host cells is proportional to the rate of multiplication of microorganisms. The rate of multiplication of microorganisms is directly proportional to the driving force of biosynthesis of microorganism molecules. The driving force of multiplication of microorganisms is Gibbs energy of biosynthesis. Based on the value of Gibbs energy of biosynthesis, it is possible to assess the virulence of a microorganism. A microorganism characterized with a more negative Gibbs energy exhibits faster kinetics of biosynthesis and greater degree of damage to host cells. In this research, the atom counting method, molecular composition method and Patel-Erickson-Battley model were used to determine empirical formula, molar mass and thermodynamic properties of live matter of Mycoplasma cells. The determined properties were used to formulate the biosynthesis reaction and find thermodynamic properties of biosynthesis of Mycoplasma cells. Furthermore, a mechanistic model was developed of pathogen-host interactions of Mycoplasma, based on nonequilibrium thermodynamics.

Key words*: Biosynthesis; Epidemic; Mechanistic model; Microorganism; Nonequilibrium thermodynamics; Virulence; Bacteria; Gibbs energy; Enthalpy; Entropy*

1. Introduction

Microorganisms can be viewed as biological systems, but also as physicochemical systems. As such microorganisms can be characterized with chemical formulas. Chemical formulas of viruses and bacteria have been reported in the literature [1,2]. The chemical formula of Poliovirus is $C_{332652}H_{492388}O_{131196}N_{98245}P_{7501}S_{2340}$ [1]. Organisms perform biological processes. Moreover, these processes in their essence represent chemical processes, which obey the laws of chemistry and are led by a driving force [2,3]. Driving force of metabolism is a physical force that constantly influences the functioning of the organism and is related to the flow of energy and matter. For most metabolic processes the driving force is change in Gibbs energy. This holds for subcellular and cellular organisms. These circumstances allow the application of the methods of physics and chemistry to calculate properties of microorganisms and host cells, as well as changes in properties during processes. This is particularly

important when microorganisms are not available in sufficient amounts or purity to be classified and recognized. Nucleic acid sequences can be used to identify microorganisms even in low abundance like microorganisms in the rare biosphere, but does not provide complete information about their metabolism [67,68].

Physicochemical properties of microorganisms include chemical formulas, molar masses, enthalpy, entropy, Gibbs energy, biosynthesis reactions etc. [4,5]. Knowledge of these properties allows development of mechanistic models of processes performed by microorganisms. Knowing the mechanisms of processes opens the way to influence the dynamics of interactions of microorganisms with host organisms or the environment. For example, if we observe the lifecycles of microorganisms and changes caused in host cells as processes, then knowledge of their mechanisms can allow us to influence their dynamics, with the goal to decrease the negative influence of microorganisms on their host organisms. This means that by influencing the empirical formula of a microorganism we can change their thermodynamic properties, for example Gibbs energy as the driving force of biological/chemical reactions performed by microorganisms. Moreover, microorganisms often live in complex multi-species biofilm communities where individual microorganism cells interact [69]. Interactions between microorganisms in microorganism communities have been analyzes with the methodology of biothermodynamics [38,62]. This is why the biothermodynamic methodology is useful in analysis of microbial communities [38,62].

The biothermodynamic approach was applied in studies of a wide range of microorganisms, which include bacteria [6-8,64,65,66], fungi [9-11], algae [12-14] and viruses [2,15,16,63]. Moreover, biothermodynamics was applied in research on human tissues [17,18].

Organisms represent open thermodynamic systems with the property of growth. They consist of a certain amount of live matter clearly bordered from their surroundings (environment). Live matter represents all mater in an organism except water, which includes DNA, RNA, proteins, lipids etc. [19,20]. This is why organisms as systems are characterized by thermodynamic properties, like amount of substance, mass, volume, temperature, enthalpy, entropy, Gibbs energy etc. [4,21,22]. Moreover, these systems interact with their environment and other organisms. Interactions of organisms represent thermodynamic processes [2,4]. To perform thermodynamic analysis of biological processes, it is necessary to know thermodynamic properties of organisms.

Thermodynamic properties of organisms can be calculated based on their empirical formulas with biothermodynamic models [54,55]. Development of models for calculation of thermodynamic properties of substances began in the 19th century with equations for the energy content of coal (e.g. Dulong equation). In the early 20th century, the models were extended to all organic substances (e.g. Thornton's rule). In the late 20th century bioengineering became an important topic due to its high potential for sustainable develpment. This is why the models were improved to give better results for live matter that comprises organisms and provide more properties: enthalpy, entropy and Gibbs energy. Among the most important models are the Patel-Erickson-Battley [40,41], Sandler-Orbey [5,45] and Roels models [46,47]. The Patel-Erickson-Battley model calculates enthalpy and entropy directly from empirical formulas. Gibbs energy is determined indirectly from enthalpy and entropy with the equation $\Delta G = \Delta H - T\Delta S$. Entropy is Sandler-Orbey and Roels models calculate enthalpy and Gibbs energy directly, based on which entropy is calculated indirectly.

Mycoplasmas are among the smallest and simplest bacteria [23,24]. Mycoplasma cells possess only the most basic structures and metabolic machinery for growth and multiplication, which include a

double-stranded circular DNA genome, ribosomes, glycolytic enzymes, cell membrane etc. [23,25]. However, Mycoplasma cells lack cell walls [26,27].

Mycoplasmas are obligatory parasitic microorganisms [28,29]. Many Mycoplasmas, including *Mycoplasma pneumoniae*, can enter into host cells and multiply as intracellular pathogens [30,31]. *Mycoplasma pneumoniae* can perform infection as an extracellular or an intracellular pathogen [30,32]. The ability to infect host cells is important for Mycoplasmas to achieve immune evasion and obtain additional resources for multiplication [30,31,33].

The aim of this paper is to perform an analysis of multiplication and pathogen-host interactions of Mycoplasma with the methodology of biothermodynamics. Chemical and thermodynamic properties of Mycoplasma were determined, which include empirical formulas, molar masses, thermodynamic properties (enthalpy, entropy and Gibbs energy) of live matter, biosynthesis reactions and thermodynamic properties of biosynthesis. Based on the determined properties, a model was developed of interactions of Mycoplasma cells with host cells, based on nonequilibrium thermodynamics.

2. Methods

Based on the macromolecular composition of a Mycoplasma species, the empirical formula of macromolecular constituents and live matter of Mycoplasma were calculated with the molecular composition method. Then, thermodynamic properties of live matter were calculated with the Patel-Erickson-Battley, Sandler-Orbey and Roels models. Moreover, biosynthesis reactions were formulated with the methodology of stoichiometry and thermodynamic properties of biosynthesis were calculated with Hess's law.

2.1. Data sources

The genetic sequence of Mycoplasma was taken from the NCBI database [34]. The genetic sequence of *Mycoplasma gallisepticum* can be found under the NCBI accession number LS991952.1. The macromolecular composition of Mycoplasma cells, nucleotide composition of Mycoplasma RNA, amino acid composition of Mycoplasma proteins and composition of Mycoplasma lipids were taken from [35]. The molecular formulas of the lipid components were taken from the PubChem database [36] under the accession numbers: 11006 for saturated hydrocarbons, 246520 for cholesterol esters, 131755699 for triglycerides, 985 for free fatty acids, 5997 for cholesterol, 5282283 for diglycerides, 5283523 for glycerophosphatidic acid, 71728373 for cephalins and inositides, 5497103 for inositides and lecithin and 9939941 for sphingomyelin.

Standard Gibbs energies of biosynthesis, $\Delta_{bs}G^o$, of other bacteria were taken from the literature: *Bordetella pertussis* [37], *Escherichia coli* [38], *Pseudomonas fluorescens* [38] and *Streptococcus thermophilus* [38]. Standard Gibbs energies of biosynthesis of viruses were taken from the literature: SARS-CoV-2, Coxsackievirus, Ebola virus and West Nile virus [2].

2.2. Chemical composition of Mycoplasma DNA

The molecular formula, empirical formula and molar mass of Mycoplasma DNA was obtained with the atom counting method, as described in [39]. The atom counting method is a computational approach that calculates chemical properties of macromolecules and macromolecular assemblies, based on nucleic acid sequences, protein sequences and particle morphology [39]. The sequence and

morphology data are given to the computer program, which calculates the molecular formula, empirical formula and molar masses of the particles [39]. The program goes along the genetic and proteins sequences and calculates the numbers of atoms of constituent elements that come from nucleotide and amino acid residues [39]. The atom counting method is applicable to macromolecules and macromolecular assemblies. To apply the atom counting method to macromolecules, the sequences of monomer residues must be known (e.g. nucleotide and amino acid sequences for nucleic acids and proteins). To apply the atom counting method to macromolecular assemblies, the numbers of copies of macromolecules in the macromolecular assemblies must be known.

2.3. Chemical composition of Mycoplasma RNA and proteins

The empirical formulas of the RNA and proteins of Mycoplasma were calculated with a modified molecular composition method, which is described in [37,39]. RNA and proteins are biopolymers comprised of monomer residues (ribonucleotide residues for RNA and amino acid residues for proteins) [19]. Every monomer residue has a well-defined chemical formula [19]. The chemical formulas of the monomer residues can be combined with their amounts in the biopolymer to find the chemical formula of the biopolymer. This can be done with the equation

$$n_J(poly) = \sum_X x_X \cdot n_J(X) \quad (1)$$

where $n_J(poly)$ is the number of atoms of element J in the empirical formula of the biopolymer, x_X the mole fraction of monomer X in the biopolymer and $n_J(X)$ number of atoms of element J in the empirical formula of monomer X [37,39]. The sum is over all X monomers that comprise the biopolymer [37,39].

2.4. Chemical composition of lipids and live matter

Empirical formulas of lipids and live matter of Mycoplasma were obtained with the molecular composition method, as described in [37,39]. The molecular composition method gives the empirical formula of a complex substance made of components with known empirical formulas [37,39]. The empirical formula of the complex substance can be found with the equation

$$n_J = \sum_Y x_Y \cdot n_J(Y) \quad (2)$$

where n_J is the number of atoms in the empirical formula of the complex substance, x_Y mole fraction of component Y and $n_J(X)$ number of atoms of element J in the empirical formula of component Y [37,39]. The sum is over all Y components [37,39].

The components of Mycoplasma live matter are DNA, RNA, lipids and proteins [35]. They were reported in mass fractions. To apply the molecular composition method, the composition of mycoplasma lipids and live matter was converted from mass fractions to mole fractions with the equation

$$X_Y = \frac{w_Y / M_r(Y)}{\sum_Z w_Z / M_r(Z)} \quad (3)$$

where w_Y is the mass fraction of component Y, $M_r(Y)$ molar mass of component Y, w_Z is the mass fraction of component Z, $M_r(Z)$ molar mass of component Z [37,39]. The summation is over all Z components [37,39].

2.5. Patel-Erickson-Battley model

Thermodynamic properties of Mycoplasma DNA, RNA, proteins, lipids and live matter were calculated with the Patel-Erickson-Battley model, as described in [2,40,41]. The Patel-Erickson-Battley model gives thermodynamic properties (enthalpy, entropy and Gibbs energy) of live matter, based on chemical composition (empirical formula) [40-42]. The Patel-Erickson-Battley model directly calculates enthalpy and entropy, which are used to find Gibbs energy with the equation $\Delta G = \Delta H - T\Delta S$ [40-42].

From the empirical formula of live matter, the degree of reduction, E, is calculated

$$E = 4n_C + n_H - 2n_O - 0\,n_N + 5n_P + 6n_S \quad (4)$$

where n_C, n_H, n_O, n_N, n_P and n_S are the numbers of atoms of C, H, O, N, P and S in the empirical formula, respectively [40,42]. The degree of reduction represents the number of electrons transferred to oxygen during complete oxidation of live matter [40].

The degree of reduction is used to calculate the enthalpy of combustion of live matter with the Patel-Erickson equation

$$\Delta_C H^0_{PEB}(bio) = -111.14\ \frac{kJ}{C-mol} \cdot E \quad (5)$$

where $\Delta_C H_{PEB}{}^o$ is standard enthalpy of combustion calculated with the Patel-Erickson-Battley model [40,42].

Standard enthalpy of combustion, $\Delta_C H^o$, is used to calculate standard enthalpy of formation, $\Delta_f H^o$, of live matter with Hess's law [40]

$$\Delta_f H^0(bio) = n_C\ \Delta_f H^0(CO_2) + \frac{n_H}{2}\ \Delta_f H^0(H_2O) + \frac{n_P}{4}\ \Delta_f H^0(P_4O_{10}) + n_S\ \Delta_f H^0(SO_3) - \Delta_C H^0 \quad (6)$$

Standard molar entropy, $S_m{}^o$, of live matter can be calculated from its empirical formula with the Battley equation

$$S^0_{m,PEB}(bio) = 0.187\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (7)$$

where $S^o{}_m(J)$ is standard molar entropy of element J, a_J number of atoms of element J in its standard state elemental form, and n_J the number of atoms of element J in the empirical formula of live matter [41,43]. The summation is over all J elements that form the live matter [41,43]. The sum term is multiplied with the constant 0.187, which takes into account the changed environment of the atoms of constituent elements in live matter.

The Battley equation can be modified to give standard entropy of formation, $\Delta_f S^o$, of live matter if the constant 0.187 is changed to -0.813 [41,43]

$$\Delta_f S^0_{PEB}(bio) = -0.813\ \sum_J \frac{S^0_m(J)}{a_J} n_J \quad (8)$$

Standard Gibbs energy of formation, $\Delta_f G^o$, was calculated from the standard enthalpy of formation, $\Delta_f H^o$, and standard entropy of formation, $\Delta_f S^o$, with the equation

$$\Delta_f G^0(bio) = \Delta_f H^0(bio) - T\Delta_f S^0(bio) \quad (9)$$

where T is temperature [44].

2.6. Sandler-Orbey model

The model proposed by Sandler and Orbey can be used to calculate thermodynamic properties of live matter [5,45]. The Sandler-Orbey model directly calculates enthalpy and Gibbs energy, which are then used to find entropy with the equation $\Delta G = \Delta H - T\Delta S$ [5,45].

The empirical formula of live matter is used to calculate the degree of reduction, *E*, with equation (4). Then *E* is used to calculate standard enthalpy of combustion obtained with the Sandler-Orbey model, $\Delta_C H_{SO}{}^o$, with the equation [5,45]

$$\Delta_C H_{SO}^0(bio) = -109.04\ \frac{kJ}{C-mol} \cdot E \quad (10)$$

The $\Delta_C H^o$ value is then used to calculate standard enthalpy of formation, $\Delta_f H^o$, with equation (6).

The degree of reduction *E* is also used to calculate standard Gibbs energy of combustion obtained with the Sandler-Orbey model, $\Delta_C G_{SO}{}^o$, with the equation [5,45]

$$\Delta_C G_{SO}^0(bio) = -110.23\ \frac{kJ}{C-mol} \cdot E \quad (11)$$

The obtained $\Delta_C G^o$ value is converted into standard Gibbs energy of formation, $\Delta_f G^o$, with the Hess's law [44]

$$\Delta_f G^0(bio) = n_C\ \Delta_f G^0(CO_2) + \frac{n_H}{2}\ \Delta_f G^0(H_2O) + \frac{n_P}{4}\ \Delta_f G^0(P_4O_{10}) + n_S\ \Delta_f G^0(SO_3) - \Delta_C G^0(bio) \quad (12)$$

$\Delta_f H^o$ and $\Delta_f G^o$ are combined to calculate standard entropy of formation, $\Delta_f S^o$, with equation (9). Then $\Delta_f S^o$ is used to calculate standard molar entropy, $S_m{}^o$, with the Hess's law

$$S_m^0(bio) = \Delta_f S_m^0(bio) + \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (13)$$

where $S^o{}_m(J)$ is standard molar entropy of element *J* in its standard state elemental form, a_J number of atoms of element *J* in its standard state elemental form, and n_J the number of atoms of element *J* in the empirical formula of live matter [41,44].

2.7. Roels model

The Roels model calculates thermodynamic properties of live matter based on its elemental composition [46,47]. The Roels model directly calculates enthalpy and Gibbs energy, which are used to find entropy with the equation $\Delta G = \Delta H - T\Delta S$ [46,47].

Standard enthalpy of combustion obtained with the Roels model, $\Delta_C H_R{}^o$, is calculated as

$$\Delta_C H_R^0(bio) = -115\,\frac{kJ}{C-mol} \cdot E \quad (14)$$

where *E* is the degree of reduction calculated with equation (4) [46,47]. Then $\Delta_C H^o$ is used to find standard enthalpy of formation, $\Delta_f H^o$, with equation (6) [46,47].

Standard Gibbs energy of combustion can be calculated with the Roels model with the equation

$$\Delta_C G_R^0(bio) = -86.6\,\frac{kJ}{C-mol} - 94.4\,\frac{kJ}{C-mol} \cdot E \quad (15)$$

where $\Delta_c G^o{}_R$ is standard Gibbs energy of combustion calculated with the Roels model [46,47]. $\Delta_c G^o{}_{SO}$ is then used to find standard Gibbs energy of formation, $\Delta_f G^o$, with equation (12). $\Delta_f H^o$ and $\Delta_f G^o$ are then combined to find standard entropy of formation of live matter, $\Delta_f S^o$, with equation (9). Finally, $\Delta_f S^o$ is used to calculate standard molar entropy of live matter, $S^o{}_m$, with equation (13).

2.8. Biosynthesis reactions

Biosynthesis reactions of Mycoplasma DNA, RNA, proteins, lipids and live matter were formulated based on empirical formulas, as described in [38,40]. Biosynthesis reactions are chemical reactions that show how new live matter and additional biosynthesis products are produced from nutrients by organisms [4,40]. The biosynthesis reactions of Mycoplasma have the general form

$$\text{(Amino acid)} + CH_2O + O_2 + HPO_4^{2-} + HCO_3^- \rightarrow \text{(Bio)} + SO_4^{2-} + H_2O + HCO_3^- + H_2CO_3 \quad (16)$$

where (Amino acid) represents amino acids with the empirical formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$, CH_2O carbohydrates and (Bio) is the empirical formula of live matter [2,37]. Amino acids represent the source of energy, carbon, nitrogen and sulfur [37,48]. Carbohydrates are an additional energy source [37,48]. O_2 is the electron acceptor [37,38,49]. HCO_3^- forms a bicarbonate buffer (together with H_2CO_3) that absorbs the H^+ ions that are produced in the biosynthesis process [2,37]. SO_4^{2-} is an additional metabolic product, which takes excess sulfur atoms that are not incorporated into new live matter [2,37]. H_2CO_3 is a part of the bicarbonate buffer and also takes excess carbon atoms, which are not incorporated into new live matter [2,37].

2.9. Thermodynamic properties of biosynthesis

Thermodynamic properties of biosynthesis of Mycoplasma Mycoplasma DNA, RNA, proteins, lipids and live matter were determined with the methodology of thermochemistry, based on the biosynthesis reactions, as described in [38,40]. Thermodynamic properties of biosynthesis are changes in thermodynamic properties during biosynthesis reactions [4,40]. They can be determined by application of the Hess's law to biosynthesis reactions

$$\Delta_{bs}H^0 = \sum_{products} \nu\, \Delta_f H^0 - \sum_{reactants} \nu\, \Delta_f H^0 \quad (17)$$

$$\Delta_{bs}S^0 = \sum_{products} \nu\, S_m^o - \sum_{reactants} \nu\ S_m^o \quad (18)$$

$$\Delta_{bs}G^0 = \sum_{products} \nu\, \Delta_f G^0 - \sum_{reactants} \nu\, \Delta_f G^0 \quad (19)$$

where $\Delta_{bs}H^o$ is standard enthalpy of biosynthesis, $\Delta_{bs}S^o$ is standard entropy of biosynthesis, $\Delta_{bs}G^o$ is standard Gibbs energy of biosynthesis and ν stoichiometric coefficient [2,40,44].

2.10. Uncertainties

Thermodynamic properties of live matter and biosynthesis of Mycoplasma cells and their macromolecular constituents were calculated based on the Patel-Erickson-Battley model [40-43]. The uncertainty in standard enthalpy of combustion, $\Delta_C H^o$, calculated with the Patel-Erickson-Battley model is 5.36% [2,38,52]. The uncertainty in standard molar entropy, $S_m{}^o$, calculated with the Patel-Erickson-Battley model is 19.7 % or less [41,43]. The uncertainties in the $\Delta_C H^o$ and $S_m{}^o$ values were used to calculate uncertainties in thermodynamic properties of live matter ($\Delta_f H^o$ and $\Delta_f G^o$) and biosynthesis ($\Delta_{bs}H^o$, $\Delta_{bs}S^o$ and $\Delta_{bs}G^o$), through classical error propagation, as described in [53].

3. Results

The molecular formula of genomic DNA of *Mycoplasma gallisepticum* was calculated with the atom counting method, based on the genetic sequence. Molecular formulas show total numbers of atoms present in a macromolecule or macromolecular assembly. The molecular formula of genomic DNA of *Mycoplasma gallisepticum* is $C_{1.93\times10^7}H_{2.42\times10^7}O_{1.18\times10^7}N_{7.18\times10^6}P_{1.96\times10^6}$. The molar mass of the entire genomic DNA *Mycoplasma gallisepticum* is 606 MDa (1.01×10^{-15}g).

Table 1 shows the empirical formulas of macromolecular constituents and live matter of *Mycoplasma gallisepticum*. Empirical formulas are also known as unit carbon formulas or C-mole formulas. Empirical formulas show the numbers of atoms of constituent elements per carbon atom. They have the general form $CH_{nH}O_{nO}N_{nN}P_{nP}S_{nS}$, where n_H, n_O, n_N, n_P and n_S are the numbers of H, O, N, P and S atoms in the empirical formula, respectively, which are given in this table. The table gives molar

masses of empirical formulas, *Mr*. The empirical formulas were determined with the atom counting and molecular composition methods, as described in [39].

Table 2 gives thermodynamic properties of macromolecular constituents and live matter of *Mycoplasma gallisepticum*. Thermodynamic properties of live matter are inherent properties of live matter and do not depend on the way in which the live matter is produced by organisms (biosynthesis reactions) [2,40]. $\Delta_f H^o$ is standard enthalpy of formation, $S_m{}^o$ standard molar entropy and $\Delta_f G^o$ standard Gibbs energy of formation. Thermodynamic properties are given for the macromolecular constituents (genomic DNA, RNA, proteins and lipids) and live matter of the entire cell. Thermodynamic properties of live matter were determined with the Patel-Erickson-Battley model, as described in [2,40,41].

Table 3 presents biosynthesis reactions of macromolecular constituents and live matter of *Mycoplasma gallisepticum*. Biosynthesis reactions are chemical reactions that show how new live matter and additional biosynthesis products are produced from nutrients by organisms [4,40]. The biosynthesis reactions have the general form: (Amino acid) + CH_2O + O_2 + HPO_4^{2-} + HCO_3^- → (Bio) + SO_4^{2-} + H_2O + HCO_3^- + H_2CO_3, where (Amino acid) represents amino acids with the empirical formula $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$, CH_2O carbohydrates and (Bio) is the empirical formula of live matter. Table 3 presents the stoichiometric coefficients of the biosynthesis reactions. The biosynthesis reactions were determined based on the empirical formulas, with the rules of stoichiometry, as described in [38,40].

Table 4 gives thermodynamic properties of biosynthesis of macromolecular constituents and live matter of *Mycoplasma gallisepticum*. Thermodynamic properties of biosynthesis are changes in thermodynamic properties that occur during production of new live matter by organisms in biosynthesis reactions [4,40]. $\Delta_{bs}H^o$ is standard enthalpy of biosynthesis, $\Delta_{bs}S^o$ standard entropy of biosynthesis and $\Delta_{bs}G^o$ standard Gibbs energy of biosynthesis. They were determined based on the biosynthesis reactions and thermodynamic properties of live matter, with the methodology of thermochemistry, as described in [38,40].

Table 1: Empirical formulas of the macromolecular constituents and live matter of *Mycoplasma gallisepticum*.

Name	n_H	n_O	n_N	n_P	n_S	Mr (g C-mol^{-1})
Genomic DNA	1.2540	0.6096	0.3716	0.1016	0.000000	31.38
RNA	1.2306	0.7360	0.3989	0.1049	0.000000	33.86
Proteins	1.5857	0.3794	0.2718	0.0000	0.003801	23.61
Lipids	1.8842	0.1564	0.0122	0.0147	0.000000	17.04
Live matter	1.6008	0.3725	0.2434	0.010733	0.002929	23.42

Table 2: Thermodynamic properties of macromolecular constituents and live matter of *Mycoplasma gallisepticum*.

Name	$\Delta_f H^o$ (kJ C-mol^{-1})				$S_m{}^o$ (J C-mol^{-1} K^{-1})				$\Delta_f G^o$ (kJ C-mol^{-1})		
Genomic DNA	-144.25	±	27.06		35.48	±	6.99		-98.25	±	27.14
RNA	-172.25	±	25.51		38.14	±	7.51		-122.82	±	25.61
Proteins	-82.63	±	28.89		32.57	±	6.42		-40.40	±	28.95
Lipids	-46.47	±	33.63		27.38	±	5.39		-10.98	±	33.66
Live matter	-83.92	±	29.35		32.20	±	6.34		-42.19	±	29.41

4. Discussion

Based on the available data on macromolecular composition of a Mycoplasma species, this paper reports for the first time the empirical formulas (unit carbon formulas) of macromolecular constituents and live matter of entire cells of Mycoplasma (Table 1). The empirical formula of Mycoplasma cells is $CH_{1.6008}O_{0.3725}N_{0.2434}P_{0.010733}S_{0.002929}$ (Table 1). It was calculated using the molecular composition method, as described in [37,39].

Starting from the empirical formulas, thermodynamic properties of macromolecular constituents and live matter of entire cells of Mycoplasma were calculated (Table 2). Live matter of Mycoplasma cells has standard enthalpy of formation of -83.92 kJ/C-mol, standard molar entropy of 32.20 J/C-mol K and standard Gibbs energy of formation of -42.19 kJ/C-mol (Table 2). They were calculated with the Patel-Erickson-Battley model, as described in [37,40-42].

Table 3: Biosynthesis reactions of the macromolecular constituents and live matter of *Mycoplasma gallisepticum*.

Name	Reactants					→	Products				
	Amino acid	CH_2O	O_2	HPO_4^{2-}	HCO_3^-		Bio	SO_4^{2-}	H_2O	HCO_3^-	H_2CO_3
Genomic DNA	1.6537	0.0000	0.9175	0.1016	0.0000	→	1	0.0372	0.3210	0.1289	0.5249
RNA	1.7753	0.0000	1.1334	0.1049	0.0000	→	1	0.0399	0.3226	0.1300	0.6453
Proteins	1.2096	0.0000	0.2894	0.0000	0.0468	→	1	0.0234	0.0615	0.0000	0.2564
Lipids	0.0541	1.3440	0.0000	0.0147	0.0000	→	1	0.0012	0.0732	0.0269	0.3712
Live matter	1.0832	0.0000	0.1131	0.0107	0.0214	→	1	0.0214	0.0847	0.0000	0.1046

Table 4: Thermodynamic properties of biosynthesis of macromolecular constituents and live matter of *Mycoplasma gallisepticum*.

Name	$\Delta_{bs}H^o$ (kJ C-mol^{-1})				$\Delta_{bs}S^o$ (J C-mol^{-1} K^{-1})				$\Delta_{bs}G^o$ (kJ C-mol^{-1})		
Genomic DNA	-417.35	±	40.09		-80.53	±	10.02		-394.54	±	40.21
RNA	-516.03	±	39.07		-103.77	±	10.39		-486.33	±	39.19
Proteins	-138.58	±	41.35		-22.30	±	9.63		-131.91	±	41.45
Lipids	-38.03	±	44.79		54.96	±	8.98		-53.24	±	44.87
Live matter	-55.83	±	41.67		-6.55	±	9.58		-54.00	±	41.77

Based on the empirical formulas, biosynthesis reactions were formulated, as a part of the mechanistic model of the microorganism life cycle and interactions with host organisms presented in this paper. The biosynthesis reaction of Mycoplasma cells is

$$1.0832\ CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472} + 0.1131\ O_2 + 0.0107\ HPO_4^{2-} + 0.0214\ HCO_3^- \rightarrow CH_{1.6008}O_{0.3725}N_{0.2434}P_{0.010733}S_{0.002929} + 0.0214\ SO_4^{2-} + 0.0847\ H_2O + 0.1046\ H_2CO_3 \quad (21)$$

where $CH_{1.798}O_{0.4831}N_{0.2247}S_{0.022472}$ is the empirical formula of amino acids and $CH_{1.6008}O_{0.3725}N_{0.2434}P_{0.010733}S_{0.002929}$ is the empirical formula of Mycoplasma cells (Table 3). Gibbs energy of biosynthesis of Mycoplasma cells is -54 kJ/C-mol (Table 4).

Biosynthesis is a process that is necessary for the replication of microorganisms and growth of colonies or populations of microorganisms within a host organism. A microbial population represents a thermodynamic system within the environment that is formed by host cells. The size of a thermodynamic system defined in this way (growing population of microorganisms) depends on the driving force of the biosynthesis reaction (Gibbs energy of biosynthesis) and is given by well-established phenomenological equations [2,4,50]. Phenomenological equations belong to nonequilibrium thermodynamics and give

rates of processes based on their driving force [4,21,50]. The biosynthesis phenomenological equation calculates the biosynthesis rate, r_{bs}, based on Gibbs energy of biosynthesis, $\Delta_{bs}G$,

$$r_{bs} = -\frac{L_{bs}}{T}\Delta_{bs}G \quad (22)$$

where L_{bs} is the biosynthesis phenomenological coefficient [2,37,38].

4.1. Thermodynamic properties of live matter

Thermodynamic properties of live matter of Mycoplasma are given in Table 2 and Figure 1. The figure shows (a) standard enthalpies of formation $\Delta_f H^o$, (b) standard molar entropies $S_m{}^o$ and (c) standard Gibbs energies of formation $\Delta_f G^o$. Thermodynamic properties are given for the macromolecular constituents (RNA, genomic DNA, proteins and lipids) and live matter of the entire cell. Standard Gibbs energies of formation of the macromolecular components of Mycoplasma are: -122.82 kJ C-mol^{-1} for RNA, -98.25 kJ C-mol^{-1} for DNA, -40.40 kJ C-mol^{-1} for proteins and -10.98 kJ C-mol^{-1} for lipids (Figure 1c). Lipids are characterized by the least negative (greatest) Gibbs energy of formation. This means that lipids have the highest usable energy content. This is why degradation of lipids releases large amounts of energy. For this reason most organisms use lipids as energy storage molecules. Proteins have a more negative (lower) Gibbs energy than lipids and therefore a lower usable energy content. This is why proteins are used more for structural and enzymatic roles by organisms and only in emergency (lack of lipids) as energy storage. The most negative (lowest) Gibbs energies are those of nucleic acids (DNA and RNA), which means that they have the lowest usable energy content. The lowest usable energy content limits the role of nucleic acids as energy storage molecules, but decreases the energetic burden for their production.

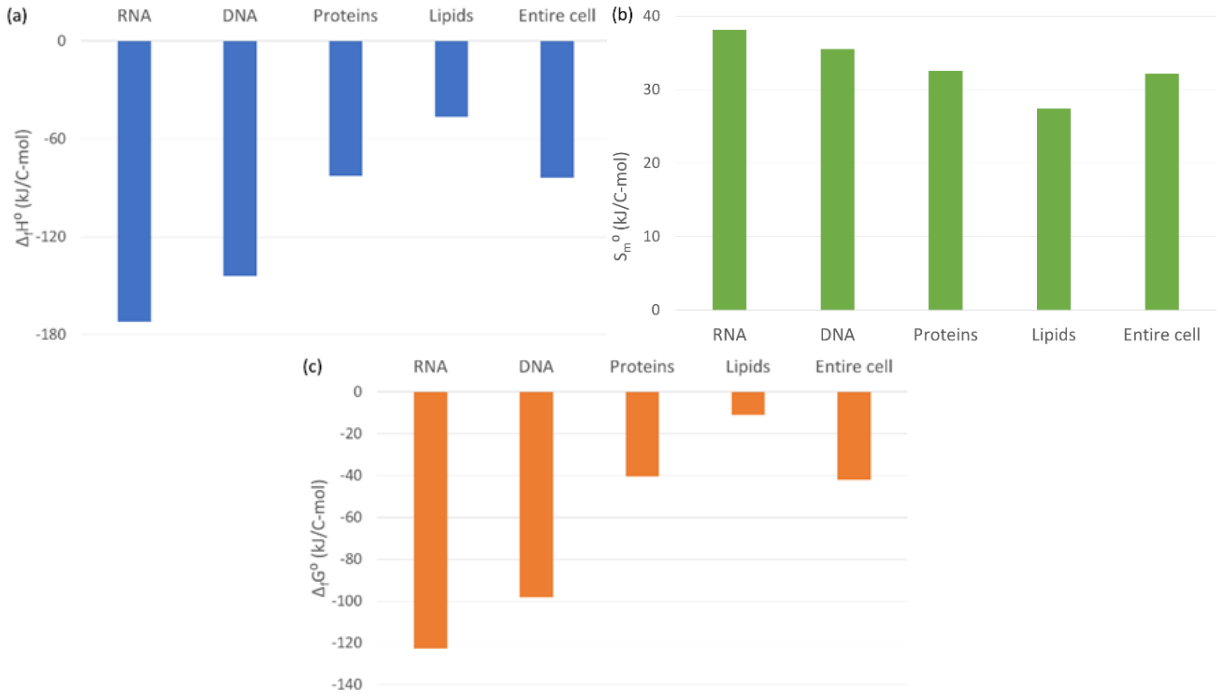

Figure 1: Thermodynamic properties of live matter of macromolecular components and cells of Mycoplasma.

Gibbs energies have an enthalpic and entropic component. Enthalpies of Mycoplasma cells and macromolecular constituents are presented in Figure 1a. The standard enthalpies of formation of the macromolecular components are: -172.25 kJ C-mol^{-1} for RNA, -144.25 kJ C-mol^{-1} for DNA, -82.63 kJ

C-mol^{-1} for proteins and -46.47 kJ C-mol^{-1} for lipids (Table 2). The enthalpy of lipids is the least negative (greatest), which means that they have the greatest total energy content. The enthalpy of nucleic acids (DNA and RNA) is the lowest, which means the they have the lowest total energy content. The differences in enthalpy values of the macromolecular components originate from their degrees of reduction, according to the Patel-Erickson equation. The different degrees of reduction come from the differences in chemical composition (empirical formulas) of the macromolecular components.

Entropies of Mycoplasma cells and macromolecular constituents are presented in Figure 1b. Standard molar entropies of the macromolecular components are: 38.14 J C-mol^{-1} K^{-1} for RNA, 35.48 J C-mol^{-1} K^{-1} for DNA, 32.57 J C-mol^{-1} K^{-1} for proteins and 27.38 J C-mol^{-1} K^{-1} for lipids (Table 2). The lowest entropy value of lipids means that they have the lowest unusable energy content. The highest entropy values of nucleic acids (DNA and RNA) means that they have the greatest unusable energy content. The differences in entropy values originate from different chemical composition (empirical formulas), according to the Battley equation.

Standard Gibbs energy of formation of Mycoplasma cells is -42.19 kJ C-mol^{-1} (Table 2). It is more negative than that of lipids and proteins, but less negative than that of RNA and DNA. Gibbs energy of formation Mycoplasma cells is the closest to that of proteins (-40.40 kJ C-mol^{-1}). The reason for this is that proteins are the most abundant macromolecular component of Mycoplasma cells [35]. Macromolecular composition of microorganisms determines their empirical formulas [37,39]. Empirical formulas determine thermodynamic properties, which include Gibbs energy [40-42]. Gibbs energy is the driving force of multiplication and determines biosynthesis rate [38,47]. This is why microorganisms can adapt their chemical composition to achieve the optimal multiplication rate in given environmental conditions.

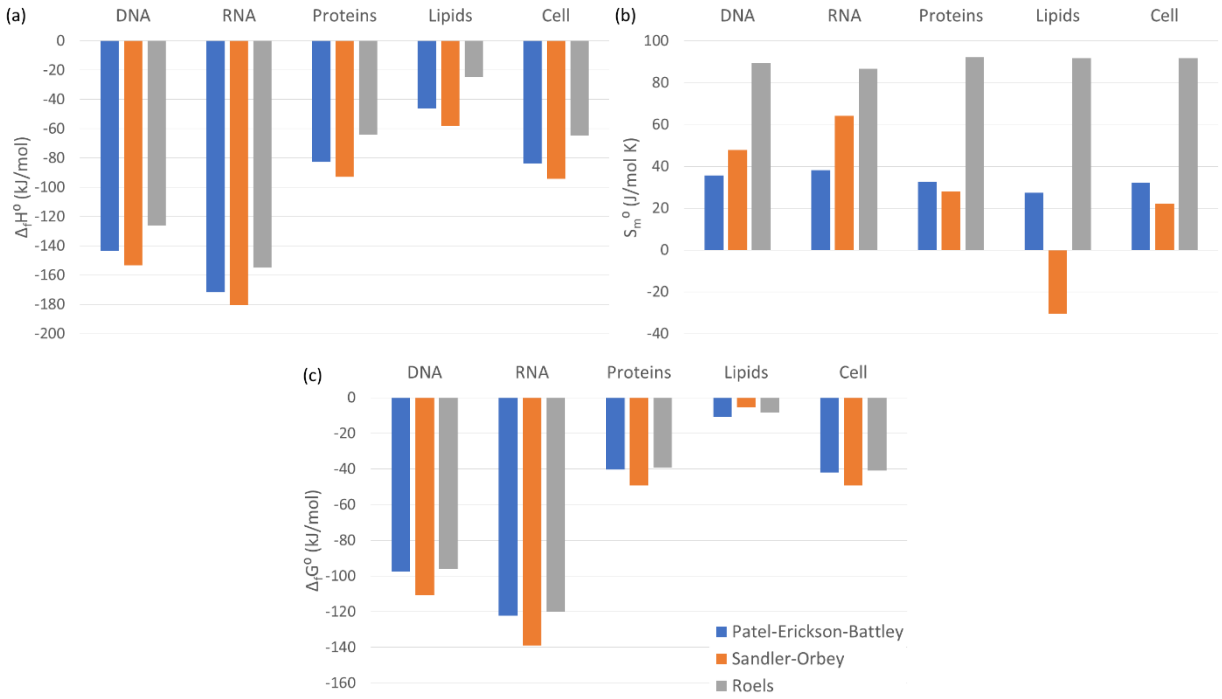

Figure 2: Biothermodynamic properties of Mycoplasma live matter calculated with the Patel-Erickson-Battley, Sandler-Orbey and Roels models.

Figure 2 shows thermodynamic properties of Mycoplasma live matter obtained with the Patel-Erickson-Battley, Sandler-Orbey and Roels models. The figure shows: (a) standard enthalpy of

formation $\Delta_f H^o$, (b) standard molar entropy $S_m{}^o$, and (c) standard Gibbs energy of formation $\Delta_f G^o$. The blue, orange and gray columns represent the results obtained with the Patel-Erickson-Battley, Sandler-Orbey and Roels models. Figure 2a presents standard enthalpies of formation of live matter. From Figure 2a it can be seen that Patel-Erickson-Battley, Sandler-Orbey and Roels models give very similar results for standard enthalpy of formation. Figure 2b shows standard molar entropies. The Roels model gives higher standard molar entropies than the Patel-Erickson-Battley and Sandler-Orbey models. Also, the result for entropy of lipids given by the Sandler-Orbey model is negative, which is meaningless according to the third law of thermodynamics. The reason why the Sandler-Orbey model gives a meaningless result for entropy of lipids is that it was developed to directly calculate enthalpy and Gibbs energy, from which entropy is calculated indirectly. For this reason the results for entropy given by the Sandler-Orbey model are less accurate than for enthalpy and Gibbs energy. This is why the results for enthalpy and Gibbs energy of lipids given by the Sandler-Orbey model are correct, but the result for entropy is meaningless. However, the entropies of other substances calculated with the Sandler-Orbey model are correct. The result from the Patel-Erickson-Battley model should be the most accurate, since it was developed to calculate entropy of live matter directly with the Battley equation. The results from the Sandler-Orbey and Roels models should be less accurate, since they calculate entropy as difference between enthalpy and Gibbs energy. Figure 2c shows standard Gibbs energies of formation. From Figure 2c it can be seen that Patel-Erickson-Battley, Sandler-Orbey and Roels models give very similar results for standard Gibbs energy of formation. Due to the greatest accuracy in calculation of entropy, the results obtained with the Patel-Erickson-Battley model will be applied in the rest of the discussion. Thermodynamic properties in Tables 2 and 4 were obtained with the Patel-Erickson-Battley model.

4.2. Pathogen-host interactions

The microorganism-host interaction proceeds using metabolic machinery. The biosynthesis reactions of microorganisms and their host organisms are competitive [2,3]. According to the phenomenological equations, the reaction characterized with a greater driving force (more negative Gibbs energy) exhibits a faster dynamics and uses more nutrients [2,3]. Gibbs energy of biosynthesis of Mycoplasma is -54.00 kJ C-mol^{-1} (Table 4).

Figure 3 shows Gibbs energies of biosynthesis of bacteria and viruses. Gibbs energy of biosynthesis of Mycoplasma is shown in orange. Gibbs energies of biosynthesis of other bacteria are in green: *Bordetella pertussis*, *Escherichia coli*, *Pseudomonas fluorescens* and *Streptococcus thermophilus*. Gibbs energies of biosynthesis of viruses are shown in blue: SARS-CoV-2, Coxsackievirus, Ebola virus and West Nile virus. Gibbs energy of biosynthesis of Mycoplasma is within the range of values of other bacteria. Mycoplasma has a less negative Gibbs energy of biosynthesis than *Escherichia coli* and *Streptococcus thermophilus*, but more negative Gibbs energy of biosynthesis than *Bordetella pertussis* and *Pseudomonas fluorescens*.

Mycoplasma and other pathogenic microorganisms perform infection of host organisms. They multiply inside host organisms, from which they take nutrients. During infection, inside host organisms processes of biosynthesis proceed of host cell building blocks and of new microorganisms cells, all of which consume nutrients as reactants. Since nutrients are limited, biosynthesis of host cell building blocks and new microorganism cells are competitive chemical reactions. The reaction with a higher rate will consume more nutrients and dominate in the competition. According to phenomenological equations, the rate of a reaction is proportional to its driving force – Gibbs energy. Gibbs energy of biosynthesis of the human lung tissue is -49.76 kJ C-mol^{-1} [2]. This is very similar to Gibbs energy of

biosynthesis of Mycoplasma -54.00 kJ C-mol^{-1} and is within the range of values of bacteria. However, bacteria have a metabolic machinery that includes catabolism. Catabolic processes can release additional energy for microorganism multiplication. This provides additional driving force for multiplication of microorganisms inside host organism during infection. The additional driving force from catabolism allows microorganisms to dominate in the competition with the host organism.

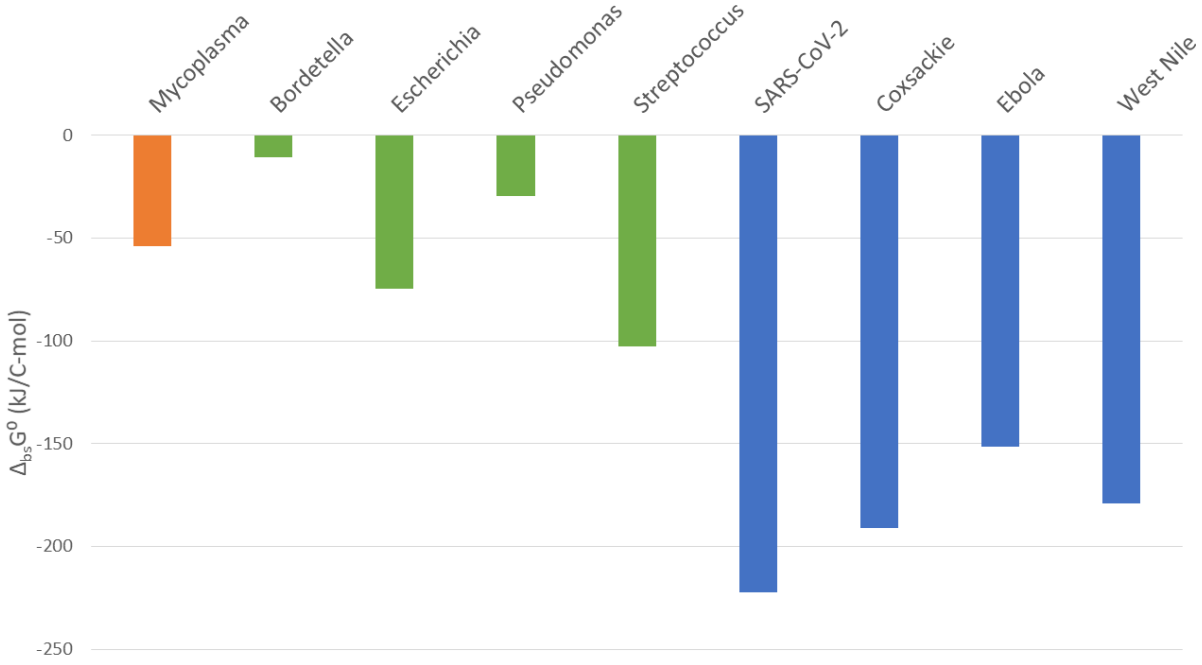

Figure 3: Gibbs energies of biosynthesis of Mycoplasma, other bacteria and viruses.

Viruses have more negative Gibbs energies of biosynthesis than Mycoplasma and bacteria (Figure 3). The reason for this is that viruses must hijack the host cell metabolic machinery in order to multiply. The resources and energy produced by the host cell metabolic machinery can be used to produce host cell building blocks or new virus particles. In order to hijack the host cell metabolism, biosynthesis of new virus particles must proceed at a greater rate than biosynthesis of host cell building blocks. In order to achieve this, viruses must have a greater driving force of biosynthesis than bacteria, according to the biosynthesis phenomenological equation. The driving force of biosynthesis is Gibbs energy of biosynthesis. This is why Gibbs energy of biosynthesis of viruses must be highly negative. On the other hand, bacteria possess their own metabolic machinery and do not need to hijack the metabolism of host cells. This is why Gibbs energies of biosynthesis of bacteria are less negative than those of viruses.

4.3. Gibbs energy and virulence

Virulence is the ability of microorganisms to cause damage to their host [51]. In other words, it represents a measure of severity and harmfulness of a disease. Damage of host organisms due to virulence is a consequence of the advantage in the competition for resources that is achieved by microorganisms compared to host cells, or immune evasion. The rate of multiplication of microorganisms and thereby the result of the competition depends on Gibbs energy of biosynthesis of microorganism components. Inside the host organism, the microorganism produces its own structural elements using the basic building blocks (e.g. amino acids, nucleotides etc.) that are normally used by the host cell for production of its structural elements for reparation of damage caused by the functioning

and the aging process of the host cell. Lower values of Gibbs energy, according to the phenomenological equations enable greater success in hijacking of nutrients contained by the host organism. Due to the hijacking of material, there is insufficiency in reparation of the host organism, namely damage of host tissues. This means that virulence is proportional to Gibbs energy of biosynthesis. Moreover, Gibbs energy of biosynthesis represents the driving force for multiplication of viruses. In case of faster virus multiplication, the damage of host tissues by microorganisms is greater. This is why the relationship between virulence and Gibbs energy seems to be obvious.

Except for growth rate, virulence also depends on other properties of microorganisms, like metabolic efficiency [57-59]. Metabolic efficiency of microorganisms is determined by Gibbs energy [47,56]. This is why Gibbs energy can be used to find the relationship between metabolic efficiency of microorganisms and virulence.

Microorganisms that live in microorganism communities were found to possess properties that originate from interactions between individual microorganism cells and are similar to those of multicellular organisms, like programmed cell death, which are important for survival of microorganism communities [60,61]. Microorganism communities and interactions between microorganisms have been analyzed with the methodology of biothermodynamics [38,62]. This is why biothermodynamic methodology can be useful in discussion of properties of microorganisms that live in microorganism communities [38].

Virulence is related to risk of microbial disease. As was shown above, virulence depends on Gibbs energy and can change during time. It appears as a consequence of changes in chemical composition (empirical formula) that cause changes in Gibbs energy. Changes in Gibbs energy influence the kinetics of multiplication of the microorganism, as well as the kinetics of damage to the host tissue. All of this results in constant changes (or at least possibility of appearance of changes) in microbial risk analysis. This is why it seems that a mechanistic perspective on virulence can be a useful tool for epidemiologists and microbiologists in assessment of possible risks posed by infections caused by different microorganisms (viruses, bacteria, fungi etc.).

4.4. Future research directions

Biothermodynamic analysis of microorganisms and their interactions with host organisms during infection is a field with many applications. However, application of the methodology of chemical thermodynamics to biological processes requires knowledge of chemical and thermodynamic properties of microorganisms. This is why it would be good to have more experimental data on chemical and thermodynamic properties of microorganisms, which include empirical formulas, macromolecular composition, enthalpies, entropies, Gibbs energies etc. These data can be used for improvement of existing and development of new models.

5. Conclusions

Chemical and thermodynamic properties of Mycoplasma cells were determined. They include empirical formula, thermodynamic properties (enthalpy, entropy, Gibbs energy) of live matter, biosynthesis reactions and thermodynamic properties of biosynthesis. The empirical formula of live matter of Mycoplasma is $CH_{1.6008}O_{0.3725}N_{0.2434}P_{0.010733}S_{0.002929}$.

Gibbs energy of biosynthesis of Mycoplasma was calculated and compared with those of other bacteria and viruses. Gibbs energy of biosynthesis of Mycoplasma is -54 kJ C-mol^{-1}, which is within the

range of Gibbs energies of biosynthesis of bacteria. Gibbs energies of biosynthesis of viruses are more negative than those of Mycoplasma and other bacteria. The reason for this is that viruses are subcellular organisms and lack metabolic machinery for multiplication. This is why viruses must hijack the metabolic machinery of host cells. To achieve this, viruses must have highly negative Gibbs energy of biosynthesis, which represents the driving force for multiplication.

Virulence of pathogens was analyzed with the methodology of chemical and nonequilbrium thermodynamics. Virulence depends on Gibbs energy expenditure and can change during time.

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Author statement

Marko E. Popović: Conceptualization; Methodology; Software; Validation; Formal analysis; Investigation; Data Curation; Writing - Original Draft; Writing - Review & Editing; Visualization
Vojin Tadić: Validation; Resources; Writing - Review & Editing; Funding acquisition
Marijana Pantović Pavlović: Validation; Resources; Writing - Review & Editing; Funding acquisition

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