CHEMICAL AND THERMODYNAMIC PROPERTIES OF *BOMBYX MORI* (DOMESTIC SILK MOTH): EMPIRICAL FORMULA, DRIVING FORCE, AND BIOSYNTHESIS, CATABOLISM AND METABOLISM REACTIONS

Marko E. POPOVIĆ[1]*, Marijana PANTOVIĆ PAVLOVIĆ[1,2], Gavrilo ŠEKULARAC[1]

[1]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia

[2] University of Belgrade, Centre of Excellence in Chemistry and Environmental Engineering - ICTM, Belgrade, Serbia

* Corresponding author; E-mail: marko.popovic@ihtm.bg.ac.rs

Biothermodynamics is a discipline which has developed intensely during the last 50 years. Thermodynamic properties have been reported for humans, animals, plants and microorganisms. However, this paper reports for the first time the empirical formula and thermodynamic properties for insects. Thermodynamic properties can be applied in research on thermodynamic interactions between organisms and their environment, as well as between organisms themselves. This paper reports for the first time the empirical formula and reactions of catabolism, biosynthesis and entire metabolism are formulated for Bombyx mori (domestic silk moth), as well as the thermodynamic properties of B. mori. It is shown that growth of B. mori is tightly related to catabolism of carbohydrates and lipids, which represents the driving force for the entire metabolism.

Keywords: Insect; Gibbs energy; Growth; Bioenergetics; Enthalpy; Entropy

1. Introduction

The root of biothermodynamics goes back to the late 18th century in the works of the father and son Carnot [1-3], as well as Lavoisier and Laplace [4,5]. Since then, thermodynamics has crystalized as one of the best based scientific disciplines. Some of the founders of thermodynamics are also the founders of the sub-discipline of biothermodynamics. Lavoisier and Laplace developed the first calorimeter and one of their first measurements was the metabolic heat of a Guinea pig [4,5]. The mathematical and philosophical basis of thermodynamics was laid in the mid-19th century by Clausius [6-8]. The work of Clausius represents the basis of engineering thermodynamics [9,10], which has developed very rapidly in the late 19th and the first half of 20th century. The development of biothermodynamics has been much slower, until the second half of the 20th century. The interest in biothermodynamics was raised by Schrödinger in his famous lecture series and book entitled "What is Life?" [11]. Prigogine has gone a step further with the development of nonequilibrium thermodynamics, as an excellent tool for analysis of processes performed by living organisms [10,12-19]. An important step for development of biothermodynamics was given by Morowitz [20-26]. During the late 20th century, Hansen has analyzed the change in entropy during life processes, as well as applications of calorimetry and thermodynamics in analysis of metabolic processes [27-38]. Von

Stockar has named Gibbs energy of growth as the driving force for growth of microorganisms, and made great contributions to applications of thermodynamics and calorimetry in analysis of metabolism and in bioengineering [39-48]. Battley has developed tools for thermodynamic characterization of microorganisms [49-59]. An important research on thermodynamic characterization of bacteriophages and other microorganisms was made by Maskow [60-70]. Thermodynamic background of microorganism evolution was reported by Hansen [27-29] and Skene [71]. The application of thermodynamics in soil science was intensely developed by Barros [72-76]. Lucia has applied thermodynamics to analyze topics in life sciences, which include virus-host interactions and virus epidemiology [77-84]. The development of biothermodynamics of organisms at different hierarchical levels of organization was made by Özilgen [85-91]. Thermodynamic characterization of organisms was reported by Popovic [92-101].

In the literature, chemical and thermodynamic properties were reported for multicellular organisms, human tissues [101], animals, plants [101], bacteria [95,99] and viruses [77-79,84,91-94,96-98,102-107]. However, until now insects have not yet been a subject of research of biothermodynamics. Thus, chemical and thermodynamic characterization of insects has not yet been performed.

The molecular composition of *Bombyx mori* larva was reported by Morowitz [25]. *Bombyx mori*, the domestic silk moth, belongs to the moth family (Bombycidae). Its closest relative is the wild silk moth (*Bombyx mandarina*). The larva of *B. mori* is the silkworm. It has been very important for millennia, since it is used to produce silk. *Bombyx mori* larva feed on the leaves of mulberry (*Morus alba*), which are rich in protein [108].

B. mori represents a biothermodynamic system, which contains a highly organized amount of substance, clearly separated from its environment. It exchanges matter and energy with its environment and exhibits growth, which appears as a consequence of organization and accumulation of matter. Thus, *Bombyx mori* performs catabolic and anabolic processes.

The goal of this paper is to perform chemical and thermodynamic characterization of *B. mori*. Moreover, the goal is to determine thermodynamic properties of catabolism, anabolism and entire metabolism, as well as to formulate catabolism, biosynthesis and total metabolism reactions.

2. Methods

2.1. Data sources

Molecular composition of *Bombyx mori* larvae was taken from [25]. The molecular composition of *B. mori* larvae is 55.5% protein, 13.3% lipid and 1.8% carbohydrate (in mass fractions) [25].

The empirical formulas of protein and lipids was taken from [109]. The empirical formula of protein is $CH_{1.59}O_{0.32}N_{0.26}S_{0.007}$ (or $C_{100}H_{159}N_{26}O_{32}S_{0.7}$) [109]. The empirical formula of lipids is $CH_{1.92}O_{0.12}$ (or $C_{51}H_{98}O_6$) [109]. The empirical formula of carbohydrates is CH_2O [39,40]. Standard specific enthalpies of combustion of proteins, lipids and carbohydrates were taken from [110]. Standard specific enthalpy of combustion of protein is 22.2 MJ/kg [110]. Standard specific enthalpy of combustion of lipids is 39.8 MJ/kg [110]. Standard specific enthalpy of combustion of lipids is 18.0 MJ/kg [110].

Biosynthesis rate and metabolic heat rate of *B. mori* were taken from [111] and [112], respectively. The biosynthesis rate of *B. mori* larvae is $r_{bs} = 2.53\times10^{-6}$ C-mol/C-mol s (153%-wt/week) [111]. The metabolic heat rate of *B. mori* is $\dot{q}_{met} = -1.49\times10^{-4}$ kW/s [112].

2.2. Empirical formulas

Elemental composition of live matter was calculated using the molecular composition method [113]. The molecular composition method uses macromolecular composition of live matter as input [113]. The macromolecular composition comes in form of mass fractions of the macromolecular components of live matter [113]. These are first converted into mole fractions of macromolecular components, through the equation

$$x_X = \frac{w_X/M_r(X)}{\sum_Y w_Y/M_r(Y)} \tag{1}$$

where x_X is the mole fraction of macromolecular component X, w_X and w_Y represent the mass fractions of macromolecular components X and Y, respectively, while $M_r(X)$ and $M_r(Y)$ represent molar masses of empirical formulas of molecular components X and Y, respectively [113]. The summation is over all Y macromolecular components that constitute live matter [113]. The mole fractions of macromolecular components are used to find the empirical formula of live matter, using the equation

$$n_J = \sum_X x_X n_J(X) \tag{2}$$

where n_J is the number of atoms of element J in live matter and $n_J(X)$ is the number of atoms of element J in the empirical formula of macromolecular component X [113]. The summation is over all X macromolecular components that constitute the live matter [113].

2.3. Thermodynamic properties of live matter

Empirical formulas were used to predict standard thermodynamic properties of live matter, through the Patel-Erickson model [52,114], Battley model [50,59] and Hurst-Harrison model [115,116]. The Patel-Erickson model was used to find enthalpy, while the Battley model was used to find entropy. Enthalpy and entropy were combined to find Gibbs energy. Heat capacity was calculated through the Hurst-Harrison model.

2.3.1 Enthalpy of live matter

The Patel-Erickson model gives standard enthalpy of combustion of live matter, based on its empirical formula [52,114].

$$\Delta_C H_m^0 = -111.14 \frac{kJ}{C\text{-}mol} \cdot E \tag{3}$$

where $\Delta_C H_m{}^0$ is standard enthalpy of combustion of live matter and E is the number of electrons transferred to oxygen during oxidation [52,114]. E can be found from the empirical formula, through the equation

$$E = 4n_C + n_H - 2n_O - 0n_N + 5n_P + 6n_S \tag{4}$$

where n_J is the number of atoms of element J in the empirical formula of live matter [52,114].

$\Delta_C H_m{}^0$ is then converted into standard enthalpy of formation, $\Delta_f H_m{}^0$, of live matter, through the Hess's law [52,95]. $\Delta_C H_m{}^0$ is defined as the enthalpy change for the reaction of complete oxidation of organic matter by oxygen.

$$C_{nC}H_{nH}O_{nO}N_{nN}P_{nP}S_{nS} + (n_C + \tfrac{1}{4}\, n_H + 1\tfrac{1}{4}\, n_P + 1\tfrac{1}{2}\, n_S - \tfrac{1}{2}\, n_O)\, O_2 \rightarrow n_C\, CO_2 + \tfrac{1}{2}\, n_H\, H_2O + \tfrac{1}{2}\, n_N\, N_2 + \tfrac{1}{4}\, n_P\, P_4O_{10} + n_S\, SO_3 \tag{5}$$

Standard enthalpies of formation of all the inorganic compounds in this reaction are known [117,118]. Thus, the Hess's law can be applied to calculate $\Delta_f H_m^0$, of live matter, through the equation [95,117,118]

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

2.3.2 *Entropy of live matter*

The Battley model is a predictive biothermodynamic model that can be used to find standard molar entropy of live matter, S_m^0, from its empirical formula [50,59].

$$S_m^0(bio) = 0.187 \sum_J \frac{S_m^0(J)}{a_J} n_J \quad (7)$$

where $S_m^0(bio)$ is standard molar entropy of live matter, $S_m^0(J)$ standard molar entropy of element J in its standard state, a_J number of atoms of element J in its standard state, and n_J number of atoms of element J in the empirical formula of live matter [50,59]. The summation is over all J elements constituting the live matter [50,59]. For example, the standard state of hydrogen is H_2, with an entropy of 130.684 J/mol K and $a_H = 2$ [117].

The Battley model can also give standard entropy of formation, $\Delta_f S^0$, of live matter [50,95]. In that case it takes the form

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

$\Delta_f S_m^0$ is then combined with $\Delta_f H_m^0$ to find standard Gibbs energy of formation, $\Delta_f G_m^0$, of live matter, using the equation

$$\Delta_f G_m^0 = \Delta_f H_m^0 - T\Delta_f S_m^0 \quad (9)$$

where T is temperature.

2.3.3 *Heat capacity of live matter*

Heat capacity of live matter can be found from its empirical formula through the Hurst-Harrison model [115,116]. The Hurst-Harrison model gives standard molar heat capacity at constant pressure, $C_{p,m}^0$, for live matter

$$C_{p,m}^0(bio) = \sum_J c_J n_J \quad (10)$$

where c_J is the contribution of element J to the heat capacity, while n_J is the number of atoms of element J in the empirical formula of live matter [115,116]. The values of c_J for different elements can be found in [115].

2.3.4 *Specific thermodynamic properties of live matter*

Thermodynamic properties of live matter discussed above are all in molar form (per C-mole of live matter). Sometimes, it is more convenient to have them expressed in specific form (per gram of live matter). Thus, standard thermodynamic properties of live matter were also reported in specific form. Specific thermodynamic properties can be calculated from molar properties, through the equation

$$X_g = \frac{X_g}{M_r} \quad (11)$$

where X_g is the property in specific form, X_m the corresponding property in molar form, and M_r molar mass of live matter. This equation was applied to $\Delta_f H^0$, S^0, $\Delta_f G^0$, $C_p^{\ 0}$, $\Delta_C H^0$ and $\Delta_f S^0$.

2.4. Biosynthesis, catabolism and metabolism reactions

Based on empirical formulas, macrochemical equations were constructed that describe biosynthesis, catabolism and metabolism. These reactions, which describe the overall stoichiometry of major metabolic processes, have been applied with great success in bioengineering [39,40,43,45], microbiology [49,51,52,57,58,92,93,102,103], plant science [100] and soil research [74].

2.4.1 Biosynthesis reactions

Biosynthesis reactions are macromolecular equations that describe conversion of nutrients by organisms into new live matter [39,40,49,119]. They are sometimes called anabolic reactions [39,40,119]. Each biosynthesis reaction consists of nutrients as reactants, live matter as the main product of biosynthesis and additional biosynthesis products. Every nutrient taken by the organism from the environment contributes one or more elements to the formation of new live matter [120]. The biosynthesis reaction for *Bombyx mori* has the general form

$$\nu_{pr}\ CH_{1.59}O_{0.32}N_{0.26} + \nu_{H2O}\ H_2O + \nu_{CO2}\ CO_2 \rightarrow \nu_{bio}\ C_{nC}H_{nH}O_{nO}N_{nN} + \nu_{CH4N2O}\ CH_4N_2O + \nu_{O2}\ O_2 \qquad (12)$$

where ν_{pr}, ν_{H2O}, ν_{CO2}, ν_{bio}, ν_{NH3} and ν_{O2} represent the stoichiometric coefficients of proteins, water, CO_2, live matter, ammonia and oxygen, respectively. In this work the convention with positive stoichiometric coefficients for products and negative stoichiometric coefficients for reactants was used [117,118]. $CH_{1.59}O_{0.32}N_{0.26}$ is the empirical formula of proteins [109] and $C_{nC}H_{nH}O_{nO}N_{nN}$ is the empirical formula of live matter.

Proteins are the source of carbon and nitrogen. Water is a source of hydrogen atoms needed for production of live matter. The main product is new live matter with an empirical formula $C_{nC}H_{nH}O_{nO}N_{nN}$. Excess nitrogen is released as urea (CH_4N_2O), which is the end product of nitrogen metabolism. O_2 takes excess electrons produced by oxidation of nutrients. It will disappear from the product side, when the catabolic reaction is added.

2.4.2 Catabolism reactions

Catabolism reactions describe degradation of nutrients by organisms into simple catabolic products, to provide energy that drives the metabolism [39,40,49,119]. A catabolism reaction contains nutrients and the electron acceptor as reactants, and on the products side catabolic waste products [39,40,49]. The general catabolism reaction for *B. mori* has the form

$$\nu_{lp}\ CH_{1.92}O_{0.12} + \nu_{ch}\ CH_2O + \nu_{O2}\ O_2 \rightarrow \nu_{H2O}\ H_2O + \nu_{CO2}\ CO_2 \qquad (13)$$

where ν_{lp}, ν_{ch}, ν_{O2}, ν_{H2O} and ν_{CO2}, represent the stoichiometric coefficients of lipids, carbohydrates, oxygen, water and carbon dioxide, respectively. No new live matter is produced in the catabolism reaction [39,40,49]. $CH_{1.92}O_{0.12}$ is the empirical formula of lipids [109] and CH_2O is empirical formula of carbohydrates. Lipids and carbohydrates represent the main sources of energy. The ratio of lipids and carbohydrates in *Morus alba* (mulberry) leaves, which represent the main food for *B. mori* larvae [111], was taken from [108]. Oxygen is the electron acceptor, which takes electrons from the nutrients to release energy [40]. The catabolic waste products are CO_2 and H_2O. CO_2 takes the oxidized carbon atoms. H_2O takes excess hydrogen.

2.4.3 Metabolism reactions

Biosynthesis and catabolism are the main components of metabolism [40,119]. Thus, the biosynthesis and catabolism reactions are added to obtain the metabolism reaction. The catabolism reaction is multiplied with the reciprocal of the biomass yield, Y (the method for finding Y will be considered below) and then added to the biosynthesis reaction [40,49]. Thus, the stoichiometric coefficient of molecule X in the metabolism reaction, $\nu_{X,met}$, can be found through the equation

$$\nu_{X,met} = \frac{1}{Y}\nu_{X,cat} + \nu_{X,bs} \tag{14}$$

where Y is the biomass yield, while $\nu_{X,cat}$ and $\nu_{X,bs}$ represent the stoichiometric coefficients of molecule X in the catabolism and biosynthesis reactions, respectively [40]. Thus, the metabolism reaction has the general form

$$\nu_{pr}\ CH_{1.59}O_{0.32}N_{0.26} + \nu_{lp}\ CH_{1.92}O_{0.12} + \nu_{ch}\ CH_2O + \nu_{O2}\ O_2 \rightarrow \nu_{bio}\ C_{nC}H_{nH}O_{nO}N_{nN} + \nu_{CH4N2O}\ CH_4N_2O + \nu_{H2O}\ H_2O + \nu_{CO2}\ CO_2 \tag{15}$$

2.5. Thermodynamic properties of biosynthesis, catabolism and metabolism

Once the biosynthesis, catabolism and metabolism reactions and stoichiometric coefficients are known, their thermodynamic properties can be determined. This is done through the Hess's law [117,118]. Standard thermodynamic properties of reaction r are given by the equations

$$\Delta_r H^0 = \sum_X \nu_X\, \Delta_f H^0(X) \tag{16}$$

$$\Delta_r S^0 = \sum_X \nu_X\, S_m^o(X) \tag{17}$$

$$\Delta_r G^0 = \sum_X \nu_X\, \Delta_f G^0(X) \tag{18}$$

$$\Delta_r C_{p,m}^0 = \sum_X \nu_X C_{p,m}^o(X) \tag{19}$$

where $\Delta_r H^o$, $\Delta_r S^o$, $\Delta_r G^o$ and $\Delta_r C_{p,m}{}^o$ are standard enthalpy, entropy, Gibbs energy and heat capacity of reaction r, respectively, ν_X is the stoichiometric coefficient of substance X in reaction r, $\Delta_f H^o(X)$ standard enthalpy of formation of substance X, $S_m{}^o(X)$ standard molar entropy of substance X, $\Delta_f G^o(X)$ standard Gibbs energy of substance X, and $C_{p,m}{}^o(X)$ standard molar heat capacity at constant pressure of substance X [117,118]. The summation is over all X substances that react in reaction r [117,118]. The convention with positive stoichiometric coefficients for products and negative stoichiometric coefficients for reactants was used [117,118]. Except for thermodynamic parameters, this work also analyzes the kinetic parameters of metabolism, which are described in the next subsection.

2.6. Kinetic properties of metabolism

Kinetic properties of metabolism determined in this research include biosynthesis heat rate, $\dot{q}_{bs}$, catabolism heat rate, $\dot{q}_{cat}$, catabolism rate, r_{cat}, and biomass yield, Y. The input are literature values of total metabolic heat rate, $\dot{q}_{met}$, and biosynthesis rate, r_{bs}. In the first step, the standard enthalpy of biosynthesis, $\Delta_{bs}H^o$, was multiplied with the biosynthesis rate, r_{bs}, to find the biosynthesis heat rate, $\dot{q}_{bs}$, through the equation [110,121,122]

$$\dot{q}_{bs} = r_{bs} \cdot \Delta_{bs} H^0 \tag{20}$$

Biosynthesis and catabolism are the two main components of metabolism [39,40,119]. Thus, according to the Hess's law [117,118], the metabolic heat rate, $\dot{q}_{met}$, is the sum of the biosynthesis heat rate and catabolism heat rate, $\dot{q}_{cat}$.

$$\dot{q}_{met} = \dot{q}_{bs} + \dot{q}_{cat} \tag{21}$$

This means that the catabolic heat rate is

$$\dot{q}_{cat} = \dot{q}_{met} - \dot{q}_{bs} \tag{22}$$

This equation was used to find $\dot{q}_{cat}$.

The catabolism heat rate was then used to find the catabolism reaction rate. Catabolism reaction rate can be found by dividing the catabolism heat rate, $\dot{q}_{cat}$, with standard enthalpy of catabolism, $\Delta_{cat}H^{0}$[110,121,122]

$$r_{cat} = \frac{\dot{q}_{cat}}{\Delta_{cat}H^{0}} \tag{23}$$

Finally, rates of biosynthesis and catabolism were used to find the biomass yield. The ratio of r_{bs} and r_{cat} is equal to the biomass yield, Y [40].

$$Y = \frac{r_{bs}}{r_{cat}} \tag{24}$$

The biomass yield Y is then used to combine the biosynthesis and catabolism reactions into the metabolism reaction with equation (14), as described above.

2.7. Phenomenological equations and coefficients

The kinetic and thermodynamic parameters are united through the framework of nonequilibrium thermodynamics. This is done by using phenomenological equations. Phenomenological equations give rates of processes based on their physical driving forces [39,110,123]. The physical driving force of chemical reactions is Gibbs energy [39,110,123]. Gibbs energy of a chemical reaction can be used to find its affinity [123]. This is done through the equation

$$A_i = -\frac{\Delta_i G}{T} \tag{25}$$

where T is temperature, while A_i and $\Delta_i G$ are affinity and Gibbs energy of reaction i, respectively [123]. This equation was used to find biosynthesis affinity, A_{bs}, and catabolism affinity, A_{cat}, based on their Gibbs energies.

If a system performs multiple chemical reactions simultaneously, the rate of every reaction can be found through the phenomenological equation

$$r_i = \sum_j L_{ij} A_j \tag{26}$$

where r_i is the rate of reaction i, L_{ij} the phenomenological coefficient between reactions i and j, while A_j is affinity of reaction j [123]. The summation is over all j chemical reactions that the system performs [123].

In our case, we have a system with two reactions – biosynthesis and catabolism. Thus, the phenomenological equations take the form

$$r_{bs} = L_{BB} A_{bs} + L_{BC} A_{cat} \tag{27}$$

$$r_{cat} = L_{BC} A_{bs} + L_{CC} A_{cat} \tag{28}$$

where r_{bs} and r_{cat} are the rates of the biosynthesis and catabolism reactions, respectively, while A_{bs} and A_{cat} the affinities (driving forces) of biosynthesis and catabolism reactions, respectively [39,123]. L_{BB}, L_{CC} and L_{BC} are the phenomenological coefficients [39,123]. L_{BB} describes the influence of the biosynthesis driving force on biosynthesis rate [39,123]. L_{CC} describes the influence of catabolism driving force on catabolism rate [39,123]. L_{BC} is called the coupling coefficient, since it describes the influence of catabolism on biosynthesis and vice versa [39,123].

Under complete coupling between biosynthesis and catabolism, the coupling coefficient can be found through the equation [39]

$$L_{BC} = \sqrt{L_{BB} L_{CC}} \tag{29}$$

Finally, when equations (27), (28) and (29) can be combined and solved for L_{BB} and L_{CC}. This gives

$$L_{BB} = \frac{r_{bs}^2}{r_{cat}A_{cat} + r_{bs}A_{bs}} \tag{30}$$

$$L_{CC} = \frac{r_{cat}^2}{r_{cat}A_{cat}+r_{bs}A_{bs}} \tag{31}$$

Equations (29), (30) and (31) were used to find the L_{BB}, L_{CC} and L_{BC}.

3. Results

This paper reports for the first time the empirical formula of an insect - *Bombyx mori* (silkworm). It was determined through the molecular composition method [113]. It is given in Table 1. The empirical formula of the *Bombyx mori* larva is $CH_{1.6803}O_{0.2815}N_{0.1903}S_{0.005122}$, which has a molar mass of 21.04 g/C-mol.

Table 1: Empirical formula and molar mass of live matter of *Bombyx mori* (silkworm) larva. The empirical formula has the general form $C_{nC}H_{nH}O_{nO}N_{nN}S_{nS}$.

c	n_H	n_O	n_N	n_S	Mr (g/C-mol)
	1.6803	0.2815	0.1903	0.005122	21.04

Thermodynamic properties of live matter are reported for the first time in this paper for an insect. They are given in Table 2. They were determined based on the empirical formula, through the Patel-Erickson [52,114] and Battley models [50,59]. These include standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, S^0_m, standard Gibbs energy of formation, $\Delta_f G^0$, standard molar heat capacity at constant pressure, $C_{p,m}{}^0$, standard enthalpy of combustion, $\Delta_C H^0$, and standard entropy of formation, $\Delta_f S^0$. All the properties were reported per C-mole, X_m, and per gram, X_g. For the live matter of *Bombyx mori* larva, standard enthalpy of formation is -63.52 kJ/C-mol (-3.020 kJ/g), standard molar entropy is 30.40 J/C-mol K (1.445 J/g K), standard Gibbs energy of formation is -24.12 kJ/C-mol (-1.146 kJ/g), standard molar heat capacity at constant pressure is 31.00 J/C-mol K (1.474 J/g K), standard enthalpy of combustion is -572.16 kJ/C-mol (-27.197 kJ/g) and standard entropy of formation is -132.17 J/C-mol K (-6.283 J/g K).

Table 2: Thermodynamic properties of live matter of Bombyx mori (silkworm) larva: standard enthalpy of formation, $\Delta_f H^0$, standard molar entropy, S^0_m, standard Gibbs energy of formation, $\Delta_f G^0$, standard molar heat capacity at constant pressure, $C_{p,m}{}^0$, standard enthalpy of combustion, $\Delta_C H^0$, standard entropy of formation, $\Delta_f S^0$. All the properties are given per C-mole, X_m, and per gram, X_g.

Per C-mole	$\Delta_f H_m{}^0$ (kJ/C-mol)	$S_m{}^0$ (J/C-mol K)	$\Delta_f G_m{}^0$ (kJ/C-mol)	$C_{p,m}{}^0$ (J/C-mol K)	$\Delta_c H_m{}^0$ (kJ/C-mol)	$\Delta_f S_m{}^0$ (J/C-mol K)
	-63.52	30.40	-24.12	31.00	-572.16	-132.17

Per gram	$\Delta_f H_g{}^0$ (kJ/g)	$S_g{}^0$ (J/g K)	$\Delta_f G_g{}^0$ (kJ/g)	$C_{p,g}{}^0$ (J/g K)	$\Delta_c H_g{}^0$ (kJ/g)	$\Delta_f S_g{}^0$ (J/g K)
	-3.020	1.445	-1.146	1.474	-27.197	-6.283

Biosynthesis, catabolism and entire metabolism reactions were formulated for the first time for an insect, based on the empirical formula. They are given in Table 3. The biosynthesis reaction for the *Bombyx mori* larva is

$$CH_{1.59}O_{0.32}N_{0.26} + 0.1149\ H_2O + 0.0349\ CO_2 \rightarrow CH_{1.6803}O_{0.2815}N_{0.1903} + 0.0349\ CH_4N_2O + 0.0941\ O_2 \quad (32)$$

where $CH_{1.59}O_{0.32}N_{0.26}$ is the empirical formula of protein, $CH_{1.6803}O_{0.2815}N_{0.1903}$ is the empirical formula of *B. mori* live matter (Table 1) and CH_4N_2O is the formula of urea. The catabolic reaction for *B. mori* larva is

$$0.16\ CH_{1.92}O_{0.12} + 0.84\ CH_2O + 1.0674\ O_2 \rightarrow CO_2 + 0.9937\ H_2O \quad (33)$$

where $CH_{1.92}O_{0.12}$ is the empirical formula of lipids and CH_2O the empirical formula of carbohydrates. The total metabolic reaction for *B. mori* larva is

$$CH_{1.59}O_{0.32}N_{0.26} + 0.0441\ CH_{1.92}O_{0.12} + 0.2315\ CH_2O + 0.2\ O_2 \rightarrow CH_{1.6803}O_{0.2815}N_{0.1903} + 0.0349\ CH_4N_2O + 0.2407\ CO_2 + 0.1589\ H_2O \quad (34)$$

Table 3: Stoichiometry of biosynthesis, catabolism and entire metabolism: ν_{pr} is stoichiometric coefficient of proteins, ν_{lp} stoichiometric coefficient of lipids, ν_{ch} stoichiometric coefficient of carbohydrates, ν_{bio} stoichiometric coefficient of live matter. The stoichiometric coefficients are positive for products and negative for reactants.

Process	ν_{pr}	ν_{lp}	ν_{ch}	ν_{bio}	$\nu_{CH_4N_2O}$	ν_{H_2O}	ν_{O_2}	ν_{CO_2}
Biosynthesis	-1.0000	0.0000	0.0000	1.0000	0.0349	-0.1149	0.0941	-0.0349
Catabolism	0.0000	-0.1600	-0.8400	0	0.0000	0.9937	-1.0674	1.0000
Entire metabolism	-1.0000	-0.0441	-0.2315	1.0000	0.0349	0.1589	-0.2000	0.2407

Table 4 shows thermodynamic properties of biosynthesis, catabolism and entire metabolism of *B. mori* larva. They were calculated with the Hess's law based on the biosynthesis, catabolism and total metabolism reactions (Table 4) and thermodynamic properties of live matter (Table 2). These include standard reaction enthalpies, $\Delta_r H^0$, entropies, $\Delta_r S^0$, Gibbs energies, $\Delta_r G^0$, and heat capacities, $\Delta_r C_{p,m}{}^0$. Standard enthalpy of biosynthesis is 94.06 kJ/C-mol, standard entropy of biosynthesis is 6.59 J/C-mol K, standard Gibbs energy of biosynthesis is 92.08 kJ/C-mol and standard heat capacity of biosynthesis is -5.07 J/C-mol K. Standard enthalpy of catabolism is -554.79 kJ/C-mol, standard entropy of catabolism is 22.45 J/C-mol K, standard Gibbs energy of catabolism is -561.41 kJ/C-mol and standard heat capacity of catabolism is 43.15 J/C-mol K. Standard enthalpy of metabolism is -58.80 kJ/C-mol, standard entropy of metabolism is 12.78 J/C-mol K, standard Gibbs energy of metabolism is -62.60 kJ/C-mol and standard heat capacity of metabolism is 6.82 J/C-mol K.

Table 4: Thermodynamic properties of biosynthesis, catabolism and entire metabolism: $\Delta_r H^0$ standard reaction enthalpy, $\Delta_r S^0$ standard reaction entropy, $\Delta_r G^0$ standard reaction Gibbs energy, $\Delta_r C_{p,m}{}^0$ standard reaction heat capacity.

Name	**$\Delta_r H^0$ (kJ/C-mol)**	**$\Delta_r S^0$ (J/C-mol K)**	**$\Delta_r G^0$ (kJ/C-mol)**	**$\Delta_r C_{p,m}{}^0$ (J/C-mol K)**
Biosynthesis	94.06	6.59	92.08	-5.07
Catabolism	-554.79	22.45	-561.41	43.15
Entire metabolism	-58.80	12.78	-62.60	6.82

Table 5 gives kinetic properties of biosynthesis, catabolism and metabolism of *B. mori* larva: $\dot{q}_{bs}$ biosynthesis heat rate, $\dot{q}_{cat}$ catabolism heat rate, r_{cat} catabolism reaction rate, Y biomass yield. The biosynthesis heat rate is 2.38×10^{-4} kW/C-mol, the catabolism heat rate is -3.87×10^{-4} kW/C-mol, the catabolism reaction rate is 6.97×10^{-7} C-mol/C-mol s, and the biomass yield is 3.63.

Table 5: Kinetic properties of biosynthesis, catabolism and entire metabolism: $\dot{q}_{met}$ metabolic heat rate, $\dot{q}_{bs}$ biosynthesis heat rate, $\dot{q}_{cat}$ catabolism heat rate, r_{bs} biosynthesis reaction rate, r_{cat} catabolism reaction rate, *Y* biomass yield. The r_{bs} and $\dot{q}_{met}$ values were taken from [111] and [112], respectively.

$\dot{q}_{met}$ (kW/C-mol)	$\dot{q}_{bs}$ (kW/C-mol)	$\dot{q}_{cat}$ (kW/C-mol)	r_{bs} (C-mol/C-mol s)	r_{cat} (C-mol/C-mol s)	Y
-1.49E-04	2.38E-04	-3.87E-04	2.53E-06	6.97E-07	3.63

Table 6 gives reaction affinities and phenomenological coefficients for *B. mori* larva: A_{cat} catabolism affinity, A_{bs} biosynthesis affinity, L_{CC} catabolism-catabolism phenomenological coefficient, L_{BB} biosynthesis-biosynthesis phenomenological coefficient, L_{BC} biosynthesis-catabolism (coupling) phenomenological coefficient. They were calculated based on the phenomenological equations [39,110,123]. The catabolism affinity is 1.8830 kJ/C-mol K, biosynthesis affinity is -0.3088 kJ/C-mol K, catabolism-catabolism phenomenological coefficient is 9.1463×10^{-7} C-mol K/kJ s, biosynthesis-biosynthesis phenomenological coefficient is 1.2048×10^{-5} C-mol K/kJ s and biosynthesis-catabolism phenomenological coefficient is 3.3196×10^{-6} C-mol K/kJ s.

Table 6: Reaction affinities (driving forces) and phenomenological coefficients: A_{cat} catabolism affinity, A_{bs} biosynthesis affinity, L_{CC} catabolism-catabolism phenomenological coefficient, L_{BB} biosynthesis-biosynthesis phenomenological coefficient, L_{BC} biosynthesis-catabolism (coupling) phenomenological coefficient.

A_{cat} (kJ/C-mol K)	A_{bs} (kJ/C-mol K)	L_{CC} (C-mol K/ kJ s)	L_{BB} (C-mol K/ kJ s)	L_{BC} (C-mol K/ kJ s)
1.8830	-0.3088	9.1463E-07	1.2048E-05	3.3196E-06

4. Discussion

All organisms originate from the last universal common ancestor (LUCA). Therefore, all organisms interact with their environment and therefore obey the same laws of chemistry and physics. *Bombyx mori* represents an open thermodynamic system with the property of growth, which interacts with its surroundings. From the surroundings it takes nutrients and metabolizes them. A part of the substances taken are incorporated into new live matter produced by the organism, which thus represents a growing system. The rest is exported into the environment as CO_2, H_2O and urea. This is why *B. mori* performs catabolic and anabolic reactions that together make the metabolism.

The empirical formula of *B. mori* is given in Table 1. The empirical formula of *B. mori* is $CH_{1.6803}O_{0.2815}N_{0.1903}S_{0.005122}$. The empirical formula of the human organism is $CH_{1.7131}O_{0.2674}N_{0.0965}P_{0.0189}S_{0.0033}Na_{0.0033}K_{0.0027}Mg_{0.0006}Ca_{0.0187}Fe_{0.0042}Cl_{0.0020}I_{7.69\times10^{-8}}$ [101]. The empirical formula of the *Saccharomyces cerevisiae*, which belongs to eukaryotic microorganisms (fungi), is $CH_{1.613}O_{0.557}N_{0.158}P_{0.012}S_{0.003}K_{0.022}Mg_{0.003}Ca_{0.001}$ [50]. The empirical formula of the plant *Zea mays* (corn) is $CH_{1.71}O_{0.77}N_{0.029}P_{0.0018}S_{0.0015}K_{0.0065}Mg_{0.0021}Ca_{0.0016}Al_{0.0011}Si_{0.0125}Mn_{0.00018}Fe_{0.0004}Cl_{0.0011}$ [100]. The empirical formula of the bacterium *Escherichia coli* is $CH_{1.74}O_{0.34}N_{0.22}$ [124]. The empirical formula of *Chlorella* microalgae is $CH_{1.719}O_{0.404}N_{0.175}P_{0.0105}$ [125]. The empirical formula of the SARS-CoV-2 virus XBB.1.5 variant nucleocapsid is $CH_{1.573540}O_{0.342703}N_{0.312374}P_{0.00603}S_{0.00336}$ [92]. The empirical formula of the Ebola virus nucleocapsid is $CH_{1.569}O_{0.3281}N_{0.2786}P_{0.00173}S_{0.00258}$ [126]. The differences in elemental composition are a consequence of changes that appeared during evolution, from LUCA to the different species of organisms present today. This paper reports for the first time the empirical formula of an insect. Thus for now it is not possible to make a comparison with other insects.

Knowing empirical formulas enables to define biosynthesis, catabolism and entire metabolism reactions for organisms [39,40,51,52,127,128]. The reactions were formulated for the larval stage of *B. mori*, since growth is the most intense in this stage. The biosynthetic (anabolic) reaction needed for growth of *B. mori* larva is

$$CH_{1.59}O_{0.32}N_{0.26} + 0.1149\ H_2O + 0.0349\ CO_2 \rightarrow CH_{1.6803}O_{0.2815}N_{0.1903} + 0.0349\ CH_4N_2O + 0.0941\ O_2 \tag{35}$$

where $CH_{1.59}O_{0.32}N_{0.26}$ is the empirical formula of protein and CH_4N_2O is the formula of urea. In this reaction, proteins are transformed into new live matter, represented by its empirical formula, $CH_{1.6803}O_{0.2815}N_{0.1903}$. The excess nitrogen is released as urea.

The catabolic reactions for *B. mori* larva start from lipids and carbohydrates as reactants, which are degraded into CO_2 and H_2O.

$$0.16\ CH_{1.92}O_{0.12} + 0.84\ CH_2O + 1.0674\ O_2 \rightarrow CO_2 + 0.9937\ H_2O \tag{36}$$

where $CH_{1.92}O_{0.12}$ is the empirical formula of lipids and CH_2O is the empirical formula of carbohydrates. The catabolic processes release energy, which drives the metabolism.

The entire metabolism of *B. mori* is described by the total metabolic reaction. In this reaction the nutrients (proteins, lipids and carbohydrates) are converted into new live matter ($CH_{1.6803}O_{0.2815}N_{0.1903}$), additional product (urea) and catabolic products (CO_2 and H_2O).

$$CH_{1.59}O_{0.32}N_{0.26} + 0.0441\ CH_{1.92}O_{0.12} + 0.2315\ CH_2O + 0.2\ O_2 \rightarrow CH_{1.6803}O_{0.2815}N_{0.1903} + 0.0349\ CH_4N_2O + 0.2407\ CO_2 + 0.1589\ H_2O \tag{37}$$

The driving force for all chemical reactions is Gibbs energy [39,110,123]. The driving force (Gibbs energy) of biosynthesis is 92.08 kJ/C-mol (Table 4). Gibbs energy of biosynthesis of *B. mori* is positive. This means that the process of biosynthesis on its own is an unfavorable process. In order for biosynthesis to proceed, it must be coupled with a thermodynamically favorable process, to provide the necessary driving force in form of negative Gibbs energy [39,110,123]. This process is the catabolism. The driving force for catabolism of *B. mori* is its highly negative Gibbs energy: -554.79 kJ/C-mol (Table 4). This means that catabolism is highly favorable and provides enough Gibbs energy for both itself and biosynthesis. Therefore, the driving force for the entire metabolism is -62.60 kJ/C-mol. The negative Gibbs energy value means that metabolism is a favorable process and can occur spontaneously in nature.

The driving force for the metabolism of *B. mori* is -62.60 kJ/C-mol. On the other hand, the driving force for metabolism of microorganisms (bacteria) is -500 kJ/C-mol [40,45,129]. The driving force of metabolism, $\Delta_{met}G$, is important since it is proportional to growth rate, r, according to the growth phenomenological equation

$$r = -\frac{L_g}{T}\Delta_{met}G \tag{38}$$

(where L_g is the growth phenomenological coefficient) [39,94,123]. This means that since the driving force of microorganisms is much greater than that of *B. mori*, microorganisms should have a greater multiplication rate. Indeed, microorganisms can divide every 20 minutes [120]. On the other hand, *Bombyx mori* larvae take days to grow [111].

This research also reports kinetic properties of biosynthesis, catabolism and entire metabolism of *B. mori* (Table 5). The biosynthesis heat rate is 2.38×10^{-4} kW/C-mol, while the catabolism heat rate is -3.87×10^{-4} kW/C-mol. This means that catabolism can provide enough energy to make entire metabolic process exothermic (-1.49×10^{-4} kW/C-mol [112]).

Table 6 gives reaction affinities and phenomenological coefficients for catabolism and biosynthesis of *B. mori*. The phenomenological coefficients allow us to analyze how energy of catabolism is used to make biosynthesis feasible. The rate of catabolism is given by the catabolism phenomenological equation

$$r_{cat} = \left(-\frac{L_{CC}}{T}\Delta_{cat}G\right) + \left(-\frac{L_{BC}}{T}\Delta_{bs}G\right) \qquad (39)$$

where L_{CC} is the catabolism-catabolism phenomenological coefficient, L_{BC} the biosynthesis-catabolism phenomenological coefficient, $\Delta_{cat}G$ Gibbs energy of catabolism and $\Delta_{bs}G$ Gibbs energy of biosynthesis [39,123]. Similarly, the rate of biosynthesis is given by the biosynthesis phenomenological equation

$$r_{bs} = \left(-\frac{L_{BC}}{T}\Delta_{cat}G\right) + \left(-\frac{L_{BB}}{T}\Delta_{bs}G\right) \qquad (40)$$

where L_{BB} is the biosynthesis-biosynthesis phenomenological coefficient [39,123]. The biosynthesis reaction of *B. mori* has a positive Gibbs energy change of +92.08 kJ/C-mol. The biosynthesis-biosynthesis phenomenological coefficient is 1.2048×10^{-5} C-mol K/kJ s. Due to the negative sign in the biosynthesis phenomenological equation, this means that the biosynthesis reaction is not a favorable process. However, biosynthesis is coupled to catabolism, which is described through the biosynthesis-catabolism phenomenological coefficient, which is 3.3196×10^{-6} C-mol K/kJ s. Gibbs energy of catabolism is -561.41 kJ/C-mol. This energy hence makes biosynthesis possible. The biosynthesis-catabolism coupling also regulates the rate of catabolism. The catabolism-catabolism phenomenological coefficient is 9.1463×10^{-7} C-mol K/kJ s and Gibbs energy of catabolism is -561.41 kJ/C-mol. This means that the catabolic processes should proceed very fast. However, since Gibbs energy of biosynthesis is positive (+92.08 kJ/C-mol), it decreases the catabolism rate according to the catabolism phenomenological equation. Therefore, catabolism makes biosynthesis feasible and biosynthesis regulates the rate of catabolism.

5. Conclusions

The empirical formula is reported for the first time for an insect: *Bombyx mori* (domestic silk moth). The empirical formula of *Bombyx mori* is $CH_{1.6803}O_{0.2815}N_{0.1903}$. Biosynthesis, catabolic and total metabolic reactions were formulated for *B. mori*. They show how nutrients that provide energy and elements are transformed into new live matter and other metabolic products.

Driving force (Gibbs energy) of metabolism is reported for the first time for an insect. Gibbs energy of metabolism of *B. mori* is -62.60 kJ/C-mol. The negative Gibbs energy of metabolism means that metabolism and growth of *B. mori* are thermodynamically favorable processes that can occur spontaneously in nature. Furthermore, driving force (Gibbs energy) of metabolism of *B. mori* is much less negative than that of microorganisms (-500 kJ/C-mol). This can explain from the bioenergetic perspective why the growth rate of insects is much lower than that of microorganisms.

Driving forces (Gibbs energies) of biosynthesis and catabolism are reported for the first time for an insect. Gibbs energy of biosynthesis of *B. mori* is +92.08 kJ/C-mol, while Gibbs energy of catabolism is -561.41 kJ/C-mol. Therefore, due to the positive Gibbs energy, biosynthesis is not a favorable process. However, the highly negative Gibbs energy of catabolism provides enough driving force to make biosynthesis feasible, according to the biosynthesis phenomenological equation.

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Author statement

Marko E. Popović: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing.
Marijana Pantović Pavlović: Validation, Resources, Writing - Review & Editing, Funding acquisition.
Gavrilo Šekularac: Validation, Resources, Writing - Review & Editing, Funding acquisition.

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