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INSTITUT FÜR BIOLOGIE

Bachelorarbeit

ZUM ERWERB DES AKADEMISCHEN GRADES
BACHELOR OF SCIENCE

"Arsenate Replacing Phosphate in Yeast Glycolysis"

"Der Austausch von Phosphat durch Arsenat in der Glykolyse von Saccharomyces Cerevisiae"

vorgelegt von

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Contents

1. Zusammenfassung

Vergleicht man die physikalischen und chemischen Eigenschaften von Arsenat (AsO_4^{3-}) und Phosphat (PO_4^{3-}), stellt man eine verblüffende Ähnlichkeit dieser beiden, als so gegensätzlich angenommenen Moleküle fest. Phosphat ist aus biologischen Organismen grundsätzlich nicht weg zu denken, während Arsenat stets als toxisch und lebensfeindlich betrachtet wird. In den letzten Jahren wurden vermehrt Arbeiten verfasst, die sich mit dem Austausch dieser beiden Moleküle auseinandersetzen, nicht zuletzt angeregt durch die vermeintlich spektakuläre Entdeckung eines Organismus (GFAJ-1), von dem proklamiert wurde, er sei in der Lage, Phosphat durch Arsenat, sowohl im anabolen, als auch innerhalb des katabolen Stoffwechsels zu ersetzen[23].

Das mathematische Modell dieser Arbeit besteht aus einem System von Differentialgleichungen, mit Hilfe derer die Änderung der Konzentrationen einzelner Metabolite im Stoffwechselweg der Glykolyse simuliert werden können. Aufbauend auf einem mathematischen Model der anaeroben Glykolyse von *Saccharomyces cerevisiae*[22][1], soll hier untersucht werden, welche Folgen dieses Austausches für den Organismus festzustellen sind, insbesondere dessen Energiehaushalt betreffend. Hierzu wurde innerhalb des Modells Phosphat vollständig durch Arsenat ersetzt, was Veränderungen der Enzymaktivitäten und vor allem die Ergänzung von Hydrolyse-Reaktionen im Modell erforderlich machte.

Die Lebenszeiten, der in der Glykolyse gebildeten Moleküle, wurden als neue Modellparameter eingeführt und das Verhalten des Modells, in Abhängigkeit zu diesen, untersucht. Weiterhin wurden ausserdem Veränderungen in den Enzymaktivitäten betrachtet und etwaigen Möglichkeiten nachgespürt, welche der verringerten Lebenszeit der Metabolite, auf dieser Ebene entgegen wirken könnten.

Als Maß für den Energiegehalt der Zelle wurde die Konzentration des ATP-Substituts ATAs (Adenosin 5'-triarsenat) verwendet. Das Model zeigte, dass die betroffenen Metabolite zwar entstehen, deren Lebenszeiten aber zu kurz sind, um einen ausreichenden Energiebetrag für den katabolen Stoffwechsel und damit das Überleben des Organismus zu gewährleisten. In einer näheren Betrachtung der biochemischen Zusammenhänge zeigte sich, dass vermutlich die Reduktion von Arsenat als Grund für die toxische Wirkung sowie das dabei entstehende Arsenit (AsO_3^{3-}) für die irreversible Schädigung der Proteine und Enzyme auszumachen ist und dieses möglicherweise eine zusätzliche Herausforderung für den Organismus darstellt.

[1] Hynne et al. aus dem Jahre 2001

2. Abstract

The comparison of the physical and chemical properties of arsenate (AsO_4^{3-}) and phosphate (PO_4^{3-}) shows an astonishing similarity between these two molecules. It is surprising, especially because these two molecules are seen as completely contrary. Phosphate is one of the most important compounds in biological organisms, whereas arsenate is thought to be toxic and life- threatening. In recent years, however, some scientists have worked on the topic of replacing phosphate by arsenate. These works were also encouraged by the supposed discovery of a bacteria strain (GFAJ-1) which seems to be able to use phosphate and arsenate in catabolic and anabolic metabolism[23] simultaneously.

This work comprise a system of differential equations, which are capable of simulating the concentration changes of the different metabolites in a metabolic pathway. Based on a mathematical model of the anaerobic glycolysis of *Saccharomyces cerevisiae*[22]from Hynne et al., this work is intended to investigate the influence of this replacement in regard of the energy level in the biological system. Therefore, a complete replacement of phosphate by arsenate was implemented, including changes of enzyme activities and the introduction of hydrolysis reactions into the model.

The lifetime of the metabolites were taken as new model parameters and their influence on the behaviour of the model was observed. A second step was the investigation of alterations in the enzyme activities and the possibility of the cell to counteract the instability of the metabolites by increasing the rate constants of the enzymes.

The ATAs (adenosine 5'-triarsenate) concentration was used as an indicator of the energy level in the cell. The model was able to show that the system was incapable to provide a sufficient amount of energy to sustain growth or even further metabolic reactions due to the instability of the formed metabolites. Furthermore, a review of the biochemical conditions leads to the suggestion that the reduction of arsenate to arsenite (AsO_3^{3-}) is the real reason for the irreversible damaging of proteins and enzymes. This aspect is assumed to be an additional challenge for the organism to replace phosphate by arsenate.

3. Introduction

"The Inheritance Powder", "King of Poison" and "Poison of Kings", that were the common names of arsenic (As) during a long period. It was one of the most frequent poisons used as homicidal agent[39]. It was popular, because the symptoms of the victims were not distinguishable from the common disease cholera. This had an end in 1836, when James Marsh was able to develop a highly sensitive test for the detection of arsenic, leading to the first uses of forensic toxicology in homicide investigations. But arsenic was also used in medical treatment of syphilis and cancer[39]. Later it was used in dyes, chemical weapons, herbicides[55] and doped semiconducters[18]. Today arsenic contamination of ground water is a challenging problem especially in developing countries and newly industrialised countries[46], but also in some regions in the US and Europe[46].

The history of and prejudices against arsenic made the discovery of the bacteria strain GFAJ-1 in December 2010 to an even more sensational discovery. It was claimed, that this bacterium is able to replace phosphate (PO_4^{3-}) by arsenate (AsO_4^{3-}) and sustain growth[23]. The organism was found in Mono Lake, a hypersaline sea (salinity 90 g L^{-1}[56]) with a high arsenic concentration (200 μM [56]), in California.
The discussion, if the results of the astrobiological institute of the NASA are true or not is still ongoing, but the main idea behind is much more important than that. The investigation of the possibilities to replace compounds in biological systems can lead to a better understanding of the mechanisms and chemical functions of these compounds in organisms and the evolution of life as a whole.

The replacement of phosphate by arsenate is an obvious topic in this field[15]. Firstly because arsenic and phosphorus are very similar elements in regard of their valence electrons, electronegativity and electron affinity[2], secondly because phosphorus, in form of phosphate, is a small and wide spread metabolite in biological systems, as a stabilising element in macromolecules as well as a substrate in metabolic reactions. Arsenate is the stable arsenic species in oxidising environments[5, 9, 55]. Many bacteria are challenged with an increased arsenic concentration in soil and water, leading to the need of a detoxification mechanism. Others are specialised in changing the oxidation state of arsenic species in respiration processes. They are a main factor in the distribution of arsenic species in natural environments[12, 26, 48, 49, 54, 59]. Nevertheless, only little is known about the precise mechanisms and processes which are taking place in these organisms, especially in regard of the avoidance of an intoxication with arenic.

The main interest of the author of this work is the catabolism in general and fundamental research of biological similarities. Due to that point the biological environment for the replacement of phosphate in this work was chosen as general and simultaneously as precise as possible. It was necessary to take a wide spread metabolic pathway such as the glycolysis, because it allows a better insight into the mechanisms in general and provides results with an universal validity. But it also has the advantage of providing access to possible data sources and to a great variety of knowledge. This point is especially important since this work is completely theoretical and dependent on experimental data.

[2] CRC Handbook of Chemistry and Physics

The main part of this work, chapter 6, is divided into three parts. The first part (section 6.1) is a short introduction into the physicochemical similarities and differences of arsenic and phosphorus or arsenate and phosphate, respectively. It also discusses possible reasons for the instability of the arsenylated compounds and the relation between arsenic species distribution and the redox potential of compounds in the cytosol. Furthermore, it shows possibilities to stabilise the arsenylated compounds based on physicochemical knowledge. The following section 6.2 gives a view on the current knowledge of arsenate detoxification processes in yeast and introduces biological aspects of arsenic species in the cytosol.

The results of the mathematical model in form of the observed behaviour of the simulated metabolic pathway is presented in section 6.3. Dependencies and influences of the model parameters are discussed and also possible compensations of the energy loss are presented. The model itself is discussed in chapter 5, showing all alterations on the original model, newly implemented reactions and changes in enzyme activities.

The final chapters 7 and 8, respectively, try to figure out the reliability of this work, in regard to made assumptions and lack of experimental data, this work cannot and does not claim to completeness. The chapters show the assumptions had to be made during this work and that the result should be taken as a tendency and a suggestion for further experiments.

4. Goals

The work is structured under three main parts, providing different informations about the topic.

The first part of the work is a general comparison of phosphorus (P) and arsenic (As), or phosphate (PO_4^{3-}) and arsenate (AsO_4^{3-}), respectively. This section will try to relate quantum chemical data to the atomic/molecular properties. It will be questioned why it is feasible to try the exchange of these molecules. When talking about similar elements it will be useful to compare other elements from the periodic table to be able to obtain a measure for these similarities. At last it will be necessary to take a look on the differences between the two of them, to see the reasons for the apparent differences of these elements. Different possible aspects effecting the stability of the ester and anhydride bonds and the arsenate itself will be compared and connected to the possibilities of the replacement.

The second part is a research about the biological facts, involving natural detoxification processes and the mechanisms of a possible conversion of arsenate in the cell. The methylation of the arsenate is often cited with regard to the heavy metal resistance, this and other possible mechanisms will be discussed. When talking about the stability of arsenate, the most obvious problem seems to be the reduction to arsenite (AsO_3^{3-}). This chemical compound has different chemical and physical properties affecting not only the metabolism but the whole cell. The possibly involved intrinsic molecules and the role of their redox potential will also be discussed in regard to this reaction.

Finally, the main part is the mathematical model of the replacement of phosphate by arsenate in the glycolysis of *Saccharomyces cerevisiae*. The obvious aim is to make statements on the dynamic behaviour of the whole pathway and the question if this replacement still provides a sufficient energy level for the cell to sustain growth. The relations between model parameters, like molecule lifetimes and enzyme activities will be discussed, also in regard to possible solutions counteracting the energy loss due the increased instability. Furthermore, the model could facilitate a clear sight on the critical and rate limiting steps in the altered pathway and could show, which are the most important compounds in regard to the influence of their stability on the model.

The first two parts of the work should be taken as a additional collection of information to allow the characterisation of the reliability and classification of the work. These informations are necessary to take notice of the assumptions made for and the validity of the model in regard of the scientific benefit.

5. Mathematical model

The foundation of the work is a deterministic-continuous model of the glycolysis of *Saccharomyces cerevisiae*. The model comprise differential equations to simulate the concentrations of the main molecules which are part of the glycolysis under anaerobic conditions. The model is simulating the anaerobic metabolic pathway from the uptake of glucose ($C_6H_{12}O_6$) from the medium until the formation of ethanol (C_2H_5OH) and the efflux from the cell. It also contains three branches, firstly the storage of glucose 6-phosphate in glycogen and secondly the conversion of dihydroxyacetone phosphate in glycerol. The third branch is the efflux of acetaldehyde from the cell. The first model equations, initial values and constants were taken from F. Hynne et al.[3][22]. All alterations and extensions of the model are discussed in this section. The calculations and graphs of the model were made with Mathematica 7.0.0. using the method *NDSolve*.

5.1. Complete substitution of phosphate by arsenate

This first attempt to simulate the possibility of arsenate (AsO_4^{3-}) to replace phosphate (PO_4^{3-}) in the glycolysis is based on a complete replacement. Therefore, the first step was to replace the phosphorylated metabolites by their arsenylated analogues, the replacement and the abbreviations used in this model are shown in table 1 and 2, respectively.

Table 1: Metabolite replacements in the model

glucose 6-phosphate	$\Longrightarrow$	glucose 6-arsenate
1,3-bisphosphoglycerate	$\Longrightarrow$	1,3-bisarsenoglycerate
fructose 6-phosphate	$\Longrightarrow$	fructose 6-arsenate
phosphoenolpyruvate	$\Longrightarrow$	arsenoenolpyruvate
fructose 1,6-bisphosphate	$\Longrightarrow$	fructose 1,6-bisarsenate
adenosine 5'-triphosphate	$\Longrightarrow$	adenosine 5'-triarsenate
glyceraldehyde 3-phosphate	$\Longrightarrow$	glyceraldehyde 3-arsenate
adenosine 5'-diphosphate	$\Longrightarrow$	adenosine 5'-diarsenate
dihydroxyacetonephosphate	$\Longrightarrow$	dihydroxyacetonearsenate
adenosine 5'-monophosphate	$\Longrightarrow$	adenosine 5'-monoarsenate

Table 2: Abbreviations in the model

extracellular glucose	Glcx	intracellular ethanol	EtOH
intracellular glucose	Glc	extracellular ethanol	EtOHx
glucose 6-As	G6As	intracellular glycerol	Glyc
fructose 6-As	F6As	extracellular glycerol	Glycx
fructose 1,6-bisarsenate	FBAs	extracellular acetaldehyde	ACAx
glyceraldehyde 3-arsenate	GAAs	extracellular cyanide	CNx^-
dihydroxyacetonearsenate	DHAAs	adenosine 5'-triarsenate	ATAs
1,3-bisarsenoglycerate	BAsG	adenosine 5'-diarsenate	ADAs
arsenoenolpyruvate	AsEP	adenosine 5'-monoarsenate	AMAs
pyruvate	Pyr	nicotinamide adenine dinucleotide (red)	NADH
intracellular acetaldehyde	ACA	nicotinamide adenine dinucleotide (ox)	NAD^+

[3]Full-scale model of glycolysis in *Saccharomyces cerevisiae*

To get a better overview on the arsenate pool, arsenate was implemented as another compound into the model. The concentration was assumed to 50 mM, an approximate value for free phosphate in the cytosol of *S. cerevisiae*.[4]

The model, shown on figure 2, consists of a system of 23 ordinary differential equations, one for each chemical compound and 37 reactions, 24 enzyme reactions (written in blue), 11 hydrolysis reactions (red arrows) and 2 formation reactions (green arrows). These reactions are shown in Table 7. The formed metabolites which are not part of the model are written in italics, these compounds are no longer available for the pathway and further calculations.

In the original model from Hynne the concentration of glucose in the mixed flow medium (*Glcx0*) was the model parameter. Depending on this parameter he was able to show, that the system oscillates for parameter values higher than 18.5 mM glucose. For this value, the system is in a stable steady state and for that reason, it was taken as the concentration in the mixed flow medium in this model.
As in the original model, the total sum of ATAs, ADAs and AMAs was set constant, while AMAs additional was stable and not affected by hydrolysis, the initial concentration of these compounds were not changed.

Other alteration and changes are presented in the following sections, all other values, initial concentrations and equilibrium and rate constants were taken from the original model as seen in appendix A.1.

5.2. Derivation of rate expressions for hydrolysis of arsenylated compounds

It is known[1][5], that ester and anhydride bonds involving arsenate are extreme unstable in reducing aqueous environments like the cytosol. Every arsenylated metabolite despite from AMAs (see *Discussion*, chapter 7) in the model is affected by a strong hydrolysis. Metabolites with two bonded arsenates are even more unstable, this fact is taken into account with an independent rate equation for every bonded arsenate and the same rate equation for the extreme unstable diester bonds in ADAs and ATAs and the arsenate bonded in 1-position in fructose 1,6-bisarsenate and 1,3-bisarsenoglycerate.

The data for the spontaneous hydrolysis of the arsenylated compounds were taken from R. Lagunas[6][52]. Since the data shows a catalytic effect of the arsenate concentration on the hydrolysis, a second-order rate equation was used to simulate the catalytic effect of arsenate leading to a concentration dependent rate coefficient in the original first-order rate. The rate constants in the mentioned publication were measured for arsenate concentrations of 110, 220 and 660 mM, respectively. Since the arsenate concentrations are several times higher than the concentration in the model, the suggested values for the rate constant were not acceptable in the model. Since the values exhibit a perfect linear character of the dependency, the utilisation of the linear regression as rate coefficient was suggested, shown in figure 1.

[4] http://bionumbers.hms.harvard.edu - "Intracellular pH is a tightly controlled signal in yeast" - Orij et al.
[5] [4, 10, 11, 16, 21, 25, 34, 36, 40, 41, 52, 60]
[6] Sugar-Arsenate Esters: Thermodynamics and Biochemical Behaviour

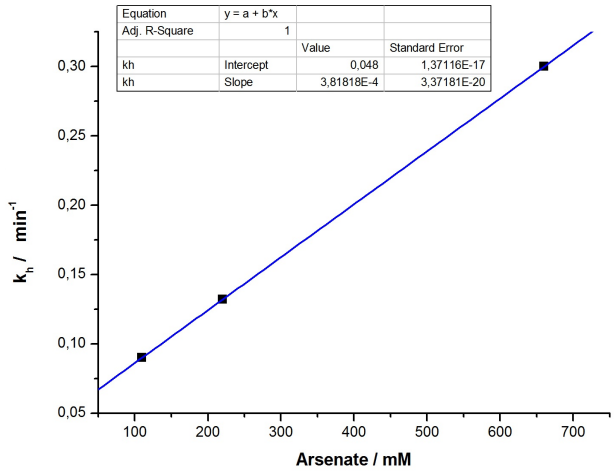


Figure 1: Linear regression of the rate constants taken from Lagunas et al.

- Used to obtain the hydrolysis rate for arsenylated compounds for an arsenate concentration of 50 mM.

Rate equation, second-order rate[7]:

$$\frac{d[X\text{-}As]}{dt} = v = k \cdot [As] \cdot [X\text{-}As]$$

Linear equation:

$$k([As]) = 4 \cdot 10^{-4} \cdot [As] + 4.8 \cdot 10^{-2} = (4 \cdot 10^{-4} + \frac{4.8 \cdot 10^{-2}}{[As]}) \cdot [As]$$

Resulting rate equation:

$$\Longrightarrow \qquad \frac{d[X\text{-}As]}{dt} = (4 \cdot 10^{-4} + \frac{4.8 \cdot 10^{-2}}{[As]}) \cdot [As] \cdot [X\text{-}As] \tag{1}$$

To take into account the much faster hydrolysis of diester bonds in ATAs and ADAs and to underline the instability of the arsenate bonded in 1-position the rate equations were derived from exponential decay, e.g. radioactive decay. The derivation of this decay equation is used as a first-order rate equation.

Rate equation, first-order rate:

$$\frac{d[X\text{-}As]}{dt} = v = k \cdot [X\text{-}As]$$

Equation exponential decay[8]:

$$N = N_0 \cdot \left(\frac{1}{2}\right)^{\frac{t}{T_{\frac{1}{2}}}}$$

[7] [As] - arsenate, [X-As] - arsenylated compound
[8] N - arbitrary entity

Derivation:

$$\frac{dN}{dt} = -\frac{2^{-\frac{t}{T_{\frac{1}{2}}}} \cdot ln(2)}{T_{\frac{1}{2}}} \cdot N_0$$

Resulting rate equation:

$$\frac{d[X\text{-}As]}{dt} = -\frac{2^{-\frac{t}{T_{\frac{1}{2}}}} \cdot ln(2)}{T_{\frac{1}{2}}} \cdot [X\text{-}As] \tag{2}$$

The use of a equation for discrete entities in a continuous model is a problematic issue. The term $2^{-\frac{t}{T_{1/2}}}$ decreases the kinetic rate in regard of the model runtime t, and not to the life-time of the compounds. To avoid this time-dependency to the runtime of the model, t was set to 60 ms, the maximum calculation step size of the model. Hence, the resulting hydrolysis rate is set constant, the use of this formula is only a matter of convenience in regard of literature values and allows the use of the mean regeneration time as variable t. The maximum half-life of the arsenate in 1-position was here assumed as 1 s, the half-life of the ester bond to the outer arsenate in ATAs and ADAs was assumed as 240 ms and 10 s, respectively.

5.3. The effect of P, As exchange on enzyme activities

The replacement of phosphate by arsenate is possible since the enzyme are not capable of distinguishing between these two similar molecules. Nevertheless, small changes in enzyme activities and affinities could be observed[16, 57, 58] in regard to the new substrate arsenate. Because it was not possible to receive any values for the probable different enzyme activities the modifications were made following a tendency. The K_m values were increased, the V_m values and rate constants were decreased. Since the exact values were not known and every enzyme could possibly have its own activity changes, the values were altered in percentage of the original value. The alteration was set to about 40 %. The effect of these changes on the model with and without arsenate are presented in Chapter 6.3.1.

Furthermore, the ATAs consume was set to a constant value to avoid the decreasing of the consume rate with a decreasing ATAs concentration under a vital level. The rate was set to 20 % of the steady state consume in the original model.

5.4. Rate expression for spontaneous formation of arsenylated compounds

The increased kinetics of spontaneous reactions with arsenate leads not only to the necessity of implementing hydrolysis reactions into the model but also to the addition of rate expressions for spontaneous formation of arsenylated compounds. These reactions were also added to the model based on the work of R. Lagunas[52] from the year 1980, again the rate constants were measured for the arsenate concentrations 110, 220 and 660 mM, respectively. The dependency of the rate constant on the arsenate concentration was following an exponential growth, since no satisfying regression could be obtained, the rate constant of the second-order rate in this model was set to $3.297 \cdot 10^{-7}$ mM^{-1} min^{-1}. This value was obtained by a reduction of the measured value for 110 mM arsenate by about 10 % (data not shown). In the model only the formation of glucose 6-arsenate and ADAs are considered.

Table 3: Reactions in the model

enyzme reactions				
	inGlc		$\rightleftharpoons$	Glc_x
	GlcTrans	Glc_x	$\rightleftharpoons$	Glc
	HK	$Glc + ATAs$	$\rightarrow$	$G6As + ADAs$
	PGI	$G6As$	$\rightleftharpoons$	$F6As$
	PFK	$F6As + ATAs$	$\rightleftharpoons$	$FBAs + ADAs$
	ALD	$FBAs$	$\rightleftharpoons$	$GAAs + DHAAs$
	TIM	$DHAAs$	$\rightleftharpoons$	$GAAs$
	GAPDH	$GAAs + NAD^+ + As$	$\rightleftharpoons$	$BAsG + NADH$
	lpPEP	$BAsG + ADAs$	$\rightleftharpoons$	$AsEP + ATAs$
	PK	$AsEP + ADAs$	$\rightarrow$	$Pyr + ATAs$
	PDC	Pyr	$\rightarrow$	ACA
	ADH	$ACA + NADH$	$\rightarrow$	$EtOH + NAD^+$
	difEtOH	$EtOH$	$\rightleftharpoons$	$EtOH_x$
	outEtOH	$EtOH_x$	$\rightarrow$	
	lpGlyc	$DHAAs + NADH$	$\rightarrow$	$Glyc + NAD^+ + As$
	difGlyc	$Glyc$	$\rightleftharpoons$	$Glyc_x$
	outGlyc	$Glyc_x$	$\rightarrow$	
	difACA	ACA	$\rightleftharpoons$	ACA_x
	outACA	ACA_x	$\rightarrow$	
	lacto	$ACA_x + CN_x^-$	$\rightarrow$	
	inCN	CN_x^-	$\rightleftharpoons$	
	storage	$G6As + ATAs$	$\rightarrow$	$ADAs + 2As$
	consume	$ATAs$	$\rightarrow$	$ADAs + As$
	AK	$ATAs + AMAs$	$\rightleftharpoons$	$2\ ADAs$
hydrolysis reaction				
	1	$G6As$	$\rightarrow$	$Glc + As$
	2	$F6As$	$\rightarrow$	$fructose + As$
	3.1	$FBAs$	$\rightarrow$	$F6As + As$
	3.6	$FBAs$	$\rightarrow$	$fructose\ 1\text{-}As + As$
	4	$DHAAs$	$\rightarrow$	$dihydroxyacetone + As$
	5	$GAAs$	$\rightarrow$	$glyceraldehyde + As$
	6.1	$BAsG$	$\rightarrow$	$GAAs + As$
	6.3	$BAsG$	$\rightarrow$	$1\text{-}arsenoglycerate + As$
	7	$AsEP$	$\rightarrow$	$enol\ pyruvate + As$
	$hADAs$	$ADAs$	$\rightarrow$	$AMAs + As$
	$hATAs$	$ATAs$	$\rightarrow$	$ADAs + As$
formation reactions	$fG6As$	$Glc + As$	$\rightarrow$	$G6As$
	$fADAs$	$AMAs + As$	$\rightarrow$	$ATAs$

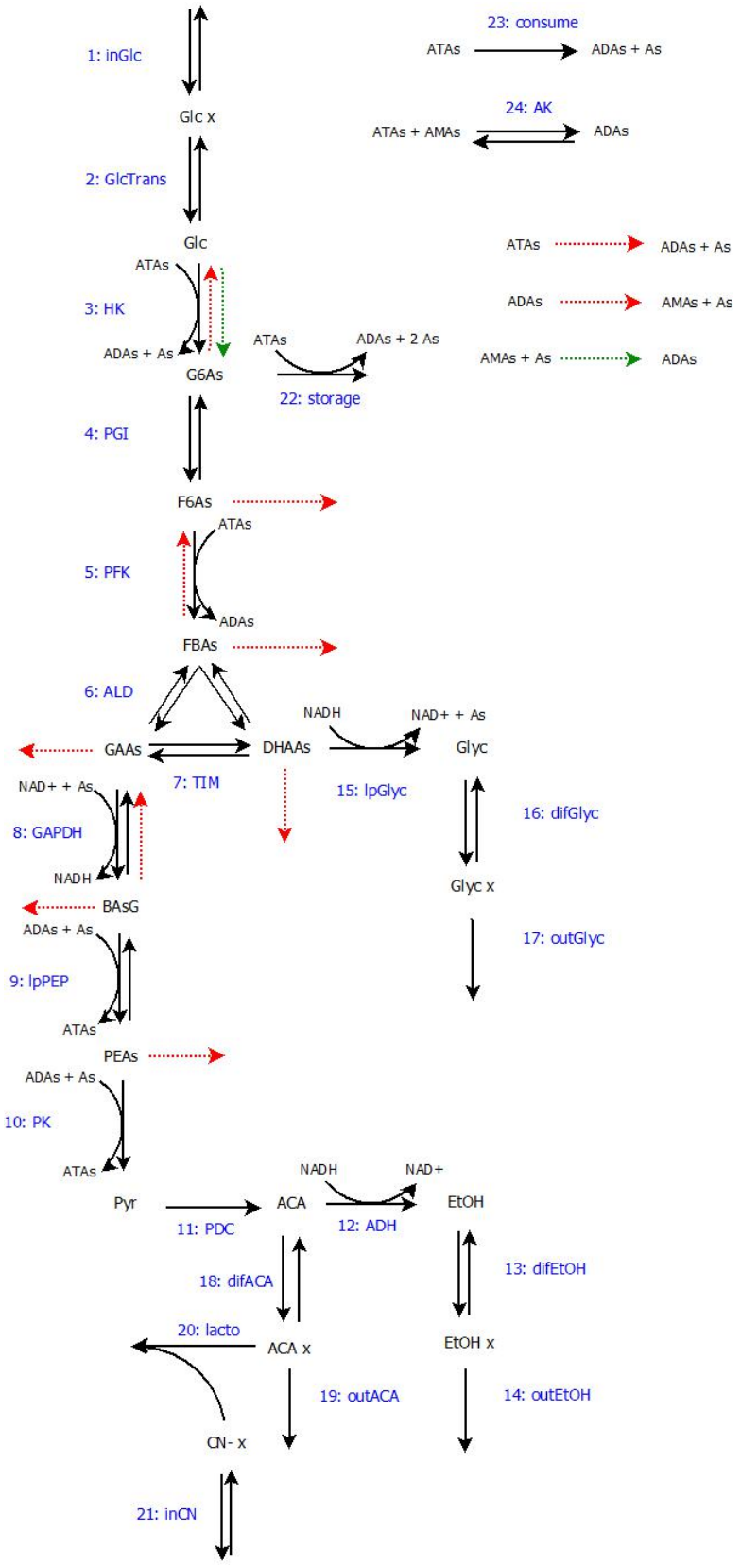


Figure 2: Reaction network of the model

– Red arrows represent spontaneous hydrolysis reactions, green arrow represent spontaneous formation reactions.

6. Results

6.1. Physicochemical similarities between P and As

We first want to understand, how similar or how different P and As are for a cell and what an appropriate measure for these similarities/differences is. The search for two similar elements is in the first step a search for identical valence electron distributions, because these are responsible for the possible bindings of the atom. Consequently, two similar elements will be found in the same group in the periodic table, they have the same occupation of the outer electron orbitals. By looking further, many elements show a similarity in the main properties ionisation energy, electronegativity, electron affinity or atomic radius. Only very few of them show significant similarities in all these properties, especially including at least one biological relevant element. Phosphorus (P) and Arsenic (As) are a pair with high similarities, others are for example the pairs calcium (Ca) - strontium (Sr) and sulphur (S) - selenium (Se).
The mean difference between the electronegativity of main group elements in the second and the third period is 8.8 %, compared to 0.5 % between phosphorus and arsenic. The biggest discrepancy between the latter ones are of course in the atom weight, due to the increased number of subatomic particles in the nucleus. These and other values are shown in table 4.

Table 4: Comparison between the main physicochemical properties of P and As

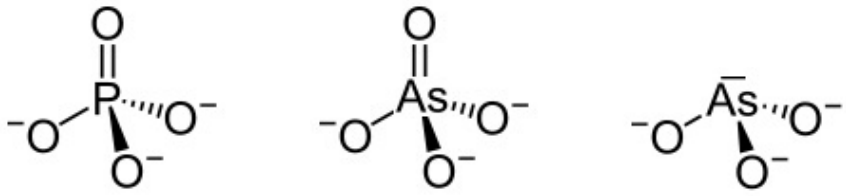
	P	As	Difference
Atomic number	15	33	
Atomic weight[18]	30.974	74.92	+141.9%
Ionisation energy	10.4867 eV	9.7886 eV	−6.7%
Electronegativity (Pauling scale)	2.19	2.18	−0.5%
Electron affinity[a]	$0.7465 \pm 3 \cdot 10^{-4}$ eV	$0.804 \pm 2 \cdot 10^{-3}$ eV	+7.3%
Atomic radius (VdW)	180 pm	185 pm	+2.8%
Atomic radius (covalent)	109 pm	120 pm	+10.1%
Ground-state configuration	$[Ne]3s^2 3p^3$	$[Ar]3d^{10}4s^2 4p^3$	

a – Different methods for measurement

Source – CRC Handbook of Chemistry and Physics (http://www.hbcpnetbase.com/)

Although the arsenic atom is more than twice as heavier than the phosphorus atom, the increase of the atomic radius is only about 10 %. One reason for the similarity in this and other properties is the contraction of the electron orbitals[28]. The effective nuclear charge (Z_{eff}) is higher, due to the increased number of protons in the nucleus of the atom. This leads to the contraction of the s- and p-orbitals, enhanced by the only weak screening of "the directionality and diffuse nature of the d-orbital"[28]. Consequently, the most similar elements will be found in adjacent periods, except for the first period.

The compounds of interest in this work are the oxyacid of arsenic in the oxidation state V, orthoarsenic acid or commonly arsenate (H_3AsO_4) and the oxyacid of phosphorus[6] in the oxidation state V, orthophosphoric acid or phosphate (H_3PO_4). Another related chemical compound is the reduced form of the arsenic acid, arsenous acid or commonly arsenite (H_3AsO_3). Since arsenate, in contrary to phosphate, is an oxidising compound[18] and can be easily reduced to arsenite, especially in reaction with sulphur[24, 55], this will be discussed in the subsequent sections. Arsenite inherits different physicochemical properties and is therefore not capable of substituting for arsenate or phosphate, respectively. The structural formulas of all three shown in figure 3.
One major aspect in the interaction with enzymes and other chemical compounds in the biological system is the charge of a molecule. The charge of oxyacids is subject to the pH of the environmental solution and the pK_a-values of the acid. Table 5 shows the different pK_a-values for phosphate and arsenate. It shows also the different bond lengths between an oxygen atom and the phosphorus or arsenic atom, respectively. It can be seen, that the covalent bond between arsenate and oxygen is more exposed to the molecules of the surrounding medium due to the increased length. Since the molecules are small and have a tetrahedral shape[6], the length of these bonds can also be used for an estimation of the radius of the entire molecule[34].



(a) Phosphate-Ion (b) Arsenate-Ion (c) Arsenite-Ion

Figure 3: Structural formula of phosphate (PO_4^{3-}), arsenate (AsO_4^{3-}) and arsenite (AsO_3^{3-})

Table 5: Dissociation constants (25^o C) and bond properties of phosphate and arsenate

	H_3PO_4	H_3AsO_4
pK_1	2.16	2.26
pK_2	7.21	6.76
pK_3	12.32	11.29
Bond length to O[16]	152 – 154 pm	168 – 171 pm
Dissociation energy, bond to O atom[a]	589 kJ	484 ± 8 kJ

a – 298 K, measured in diatomic molecules

Source – CRC Handbook of Chemistry and Physics (http://www.hbcpnetbase.com/)

The observation of the physicochemical properties of the atoms and their related oxyacids allows some preliminary statements about the possibility of the replacement.

The enzyme ability to distinguish between these two molecules is dependent to the accuracy of the mechanism used to identify the molecules. Despite of the higher weight and an slightly increased radius of approximately 10 % the arsenate is indeed very homologous to

the phosphate molecule. The similarity reaches further in the mentioned physicochemical properties, up to their shape, concerning angles and charge distribution[4]. The accuracy of the glycolytic enzymes to identify these compounds seems to be too low to distinguish between these two substrates. This leads to the suggestion that a very similar property of phosphate and arsenate is the crucial property for the distinction by these enzymes.[16]

The different behaviour of arsenate, acting as an oxidizing compound along with the reduction to arsenite could, however, be a challenging difference between these two molecules. Besides that, the increased length of the covalent bonds between the arsenic and the oxygen atom seems to give rise to hydrolysis through the surrounding medium[11]. It could be observed, that arsenate diesters hydrolyse with an even higher rate than triesters[21] and that the conformation of arsenylated compounds can also influence the hydrolysis rate[10]. These observations strengthen the hypothesis that the accessibility of the bonds to the surrounding solvent is a main aspect in the stability of the arsenylated compounds. Since the hydrolysis is even increased in alkaline mediums, a nucleophilic attack of hydroxide ions (OH^-) is suggested[25, 58] when using water as solvent.

Concerning stabilising mechanisms of arsenylated compounds on a foundation of physicochemical properties, the use of a more unpolar medium[11] or decreasing of the pH is suggested[36, 52, 64], also the decrease of the temperature to slow down the hydrolysis rate is thinkable[32, 63]. Another possibility is the use of a methyl group instead of an oxygen atom to bind the arsenate to a chemical compound[2, 17]. This mechanisms are thinkable, but the physiological relevance need to be discussed and proved in experimental works, for this model they are not feasible due to a lack of experimental data (see *Discussion*, chapter 7).

The second crucial aspect is the redox potential of compounds in the solvent in regard of the reduction of arsenate. As mentioned above, arsenate is the most stable arsenic species in oxidising environments (As(V)/As(III) appr. between +100 and +200 mV[5, 48, 55]). Especially sulphur and thiol groups are capable of reducing arsenate very easily[20, 45]. This issue will be discussed in the subsequent section, since glutathione and other sulphur containing antioxidants are used in *S. cerevisiae* and many others for redox regulation in the cytosol with a redox potential of about -250 mV[27] between the oxidised and reduced species.

6.2. Biological background

In spite of the fact that arsenic intoxications and their direct consequences are well known[29, 31, 38], only little is known about the mechanisms on a molecular level. Many experiments were made in the 20. century in regard of arsenic[44, 51], even the influence of arsenate on the glycolysis of yeast was investigated as early as 1911[3]. Nevertheless, the actual knowledge about the biochemical influence of arsenic on biological systems is mainly an effort of the last 20 years. Primarily powered by the searching for a solution for the problem of arsenic contaminated groundwater (e.g. [26, 32, 46]) in many countries on the one hand and fundamental research of microbiologists (e.g. [49, 54, 56]) on the other hand.
It is known that apparently almost all tested organisms have a suggested detoxification mechanism for an arsenic intoxication[8]. The most famous one is the methylation of the arsenic compounds and the subsequent excretion or accumulation[14, 30, 33]. After an uptake of arsenate[50] the following step in this mechanism is always the reduction to arsenite[19] and finally one or more methylation steps[14, 15]. Also humans and other mammals are suggested to have such an mechanism[64]. Commonly formed methylated arsenic compounds are monomethylarsonic acid MMA(V) ($H_2AsO_3CH_3$), monomethylarsonous acid MMA(III) ($H_2AsO_2CH_3$) or arsenobetaine ($As(CH_3)_3CH_2COOH$)[30, 43], since some of these formed compounds are still as toxic as the trivalent arsenic species[42, 43] the purpose of some detoxification processes is still questioned. The most cited pathway of detoxification via methylation was suggested by F. Challenger et al. in 1945[15, 33].
This pathway shows, that the detoxification of arsenate implies always the reduction to arsenite in the first step, after the reoxidation and methylation every further methylation step needs also an previous reduction. Three different types of specific arsenate reductases could be identified so far[8, 35, 54]. Each of them are using glutathione, glutaredoxin or thioredoxin as reductant, sulphur containing compounds.
The suggested detoxification process[42, 54] in *S. cerevisiae* is shown in figure 4[54]. The gene cluster ARR is responsible for the active arsenate resistance. After the unintended uptake of arsenate through the phosphate transporter Pho87p, the ARR1 gene is responsible for the yeast transcriptional regulator ArsR. The ARR2 gene transcripts for the arsenate reductase Arr2p, which shows similarities to a PTPase. Finally the arsenite is excreted through the Arr3p arsenite efflux pump, which is transcripted by the ARR3 gene, or sequestrated as a glutathione-conjugated substrate in a vacuole by the transporter Ycf1p. In some publications, the arsenate reductase of yeast Arr2p is also named as Acr2p[13].

The different methods of arsenic detoxification or resistance are agreeing in the reduction of arsenate to arsenite with a sulphur containing reductant. This mechanism occurs extraordinary since arsenite is proved to be far more toxic than arsenate[31]. This toxicity results in the increased reactivity of arsenite especially in regard of thiol groups of proteins and the ability to inhibit some essential of them, e.g. the pyruvate dehydrogenase[31]. It could be observed also an decreased level of glutathione and an related increased concentration of reactive oxygen species (ROS) in the cytosol[29, 61]. In contrast, an active inhibition of enzymes could not be verified for arsenate, also interactions with thiol groups of proteins were not observed[20]. All enzymes tested so far in vitro with arsenate as phosphate substitute were willingly using it as a substrate[4, 16, 24], even though with an altered affinity and reaction rate. The tendency of an increased rate constant and decreased equilibrium constant (K_M) could be observed[57, 58]. The inability of the organism to distinguish between phosphate and arsenate occurs as the only reason to reduce arsenate to its reduced analogue.

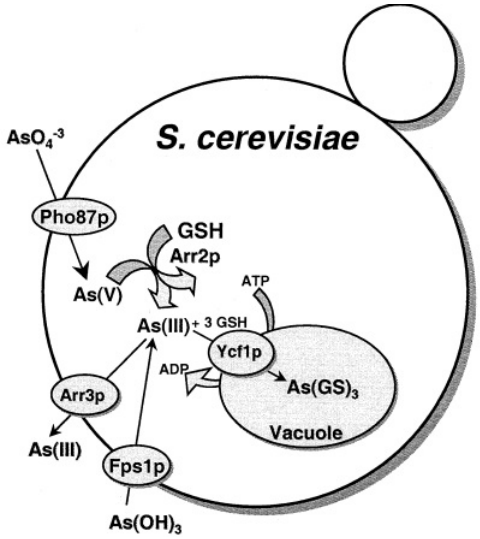


Figure 4: Diagram of arsenate detoxification mechanisms in S. cerevisiae

Source – Figure taken from [54] Mukhopadhyay et al. "Microbial arsenic: from geocycles to genes and enzymes", FEMS Microbiology Reviews 26, 2002

The recent work of Németi et al.[7] and Gregus et al. [65] describes furthermore the ability of glutathione to act independently as a reductant for arsenate[37]. They were able to show that glutathione can not only reduce free arsenate in the cytosol but moreover reduce arsenates in ester and anhydride bonds even more easily. Some of the enzymes of the glycolysis would act therefore as a helping agent in the reduction process of arsenate[7]. Since glutathione is a common redox regulator in the cytosol of biological organisms[27] the possible function of arsenate as a phosphate substitute has to be even more questioned.

Finally the often cited instability of ester and anhydride bonds is to be taken into account. Probably the sightly increased bond length of the As - O bond is the main reason why these bonds are open for a fast hydrolysis. The calculated Gibbs energy of hydrolysis for the ester bond in glucose 6-phosphate and glucose 6-arsenate were given as $\Delta G^{o'}$ = -13.81 kJ mol^{-1} and $\Delta G^{o'}$ = -14.23 kJ mol^{-1}[36][9], respectively. These similar values indicate the hydrolysis founded to be based on an increased kinetic instead of a thermodynamically reason. This increased kinetic behaviour can also be observed in regard of the spontaneous formation of these bonds. Also phosphate is capable of forming ester and anhydride bonds independently, but with a rate smaller in orders of 10^5[11, 57].

In conclusion, the replacement of phosphate by arsenate is afflicted by two different main issues. On the one hand the arsenate resistance, detoxification mechanisms and spontaneous reduction are leading to the decrease of the arsenate concentration and simultaneously to a toxic stress due to the evolving arsenite, on the other hand the instability of the ester and anhydride bonds of arsenate is leading to a decreased life-time of the products in orders of magnitude.

[9] 298 K, pH 7.0

6.3. Mathematical model

Based on the glycolysis model from Hynne et al. [22] it was possible to simulate this metabolic pathway with the theoretical replacement of phosphate (H_3PO_4) by arsenate (H_3AsO_4). This model allows observations of the dependencies of the different compound concentrations to the variables and parameters of the model. By reading the subsequent sections it is important to consider that this model simulates a hypothetical complete replacement of the phosphate by arsenate, the previous discussed reduction of arsenate to arsenite is not part of the model. Instead, in the beginning of this section the alterations of the enzyme activities will be discussed in regard of using arsenate as a substrate. In the next section 6.3.2, the view will be taken on the instability of the arsenate containing compounds, their life-time and behaviour of the model in regard of the energy level of the simulated biological system. The last section 6.3.3. shows possible countermeasures of the system to counteract the instability and hydrolysis of the involved compounds. All equations, constants and kinetic rates, also all alterations and changes which have been made on the original model are discussed in section 5 or shown in the appendix, respectively.

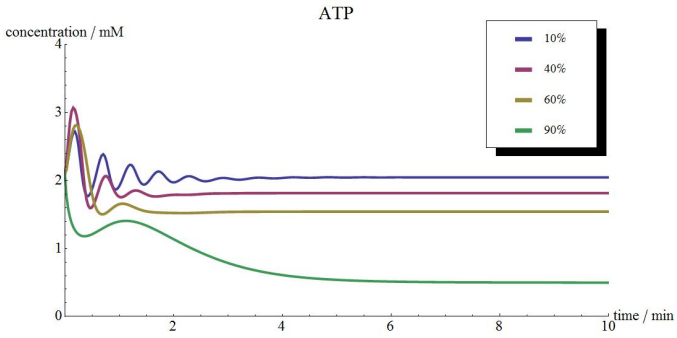
6.3.1. Changes of the enzyme activities due to lower affinity to arsenate

All experimentally tested enzymes were able to use arsenate as a substrate, although with a changed affinity and activity[57, 58]. The influence of these changes will be discussed in this section. All rate (k and V_M) of the enzymes with arsenate or arsenylated compounds as substrate were decreased, all equilibrium constants K_M of these enzymes were increased. As measure for these changes a percentage of the original values were used. To be able to compare the influence on the model, the same changes were made on the original model of Hynne et al.[22].

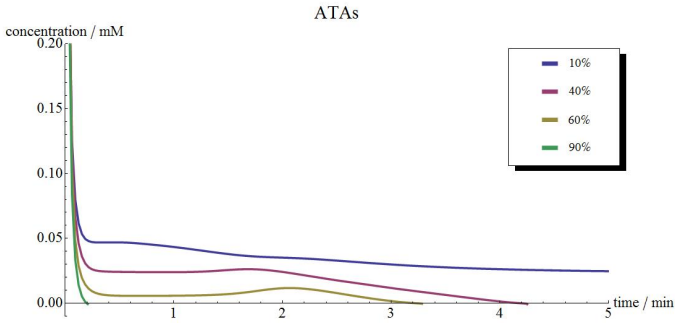
As shown in figure 5, the original model reaches a steady state for all values of changed enzyme activity, the arsenate model only for changes smaller than 20 %.

The graphs show that these changes can lead to a large decrease in the energy level of the cell. The steady state concentration of ATAs falls with the rising percentage of activity until 0.5 mM for a 90 % change. Also the concentration of NADH falls to a crucial level of 0.04 mM for that value. Only the concentration of intracellular glucose (Glc) increases, it reaches a steady state concentration of 15.6 mM instead of 0.6 mM without a change in the enzyme activity. These values suggest that the changed enzyme activity has possibly a great influence on the energy loss of the system due to the exchange of phosphate by arsenate.

In further calculations a change in the enzyme activities of 40 % is assumed.



(a) Changes in enzyme activities of all enzymes and the resulting ATP concentration



(b) Changes in enzyme activities of all enzymes and the resulting ATAs concentration

Figure 5: Simulation of the concentrations in dependency to changes in enzyme activities of all enzymes. Comparison between the original model and the replacement model

6.3.2. Influence of the new parameters on the model

The progression of the concentrations of a selected number of representative compounds can be seen in figures 8, 9 and 10.
Most curves show an interval of a constant concentration between 12 sec and 75 sec, it is suggested that during this period, the system is in a quasi equilibrium, due to the initial concentrations. This state lasts only one minute, after that, the system can no longer sustain the effort to compensate the energy loss through the hydrolysis reactions.
The intracellular glucose concentration (figure 8(a)) and the extracellular glucose concentration (graph not shown) are increasing similarly during the whole process. The enzymatic formation of glucose 6-arsenate is dependent on the ATAs concentration, since the arsenate substrate is taken from ATAs. The formation is supported by the spontaneous formation of this compound using free arsenate, but this reaction has a much smaller rate than the spontaneous hydrolysis. Hence, the level of the glucose 6-arsenate concentration is decreasing with a very fast kinetic 8(b) and lead to the accumulation of intracellular glucose.
The weak formation of glucose 6-arsenate is affecting the whole pathway and this effect can also be observed on the progression of the compound concentrations at the end of the system, e.g. the concentrations of pyruvate (figure 9(a)) or acetaldehyde (figure 9(b)), which is directly related to the latter, as seen on figure 2.
Since the reduction of the coenzyme NAD^+ is implemented just in the glycolysis and other

ways of reduction are not considered, the instability of arsenate is directly affecting the NADH concentration (figure 10(d)). The lack of NADH ceases two of the branching reactions and ethanol (figure 9(c)) and glycerol (figure 9(d)) are no longer formed. This is the reason why the deletion of the branching is not improving the life-time of the system notable. Without the reactions of storage (22) and forming of glycerol, the life-time is prolonged for less than 1 minute.

Apart from the concentrations of AMAs and Glc (figures 10(c) and 8(a)) all shown curves are decreasing to zero. After the simulation all energy carriers are present in the form of AMAs, with the initial concentrations of 0.33 mM for AMAs, 1.5 mM for ADAs and 2.1 mM for ATAs, the end concentration of AMAs is 3.93 mM.

As already mentioned in section 5, the main parameter of the original model was the concentration of glucose in the mixed flow medium (Glcx0). This parameter has only very little effect on the here present model. Table 6 shows different selected parameter values and their influence on the runtime until the main concentrations are dropped to zero.

Table 6: Influence of parameter alterations on the runtime of the simulation

Parameter	value[c]	runtime	Parameter	value[c]	runtime
Glcx0:[a]	5 mM	3.9 min	Half-life of ADAs:	10 s	4.3 min
	18.5 mM	4.3 min		30 s	7.0 min
	30 mM	4.3 min		1 min	9.5 min
	50 mM	4.3 min		1000 min	39.0 min
ATAs consume rate:	100%[b]	1.8 min	Half-life of ATAs:	240 ms	4.3 min
	60%[b]	2.4 min		1 s	11.0 min
	40%[b]	3.0 min		1.5 s	steady state
	20%[b]	4.3 min		2 s	steady state
change in enzyme activity:	20%	steady state	Half-life of FBAs (1-pos.)	1s	4.3 min
	40%	4.3min		2s	6.5 min
	60%	3.3min		5s	44.4 min
	80%	15s		10s	steady state

a – Concentration of glucose in the mixed flow medium

b – Per cent of steady state consume in the model of Hynne[22]

c – The values are chosen from a theoretical not a physiological point of view

The life-times of the ATAs molecule or the bonded arsenate on the 1-position on fructose 1,6-bisarsenate are suggested to be two possibilities to stabilise the system and provide enough energy to sustain at least a minimal energy level. The figures 6 and 7 show the influence of these life-times on the steady state concentration of ATAs. It can be seen that the life-time of fructose 1,6-bisarsenate has even more effect on the model and the ATAs concentration than the life-time of the latter.

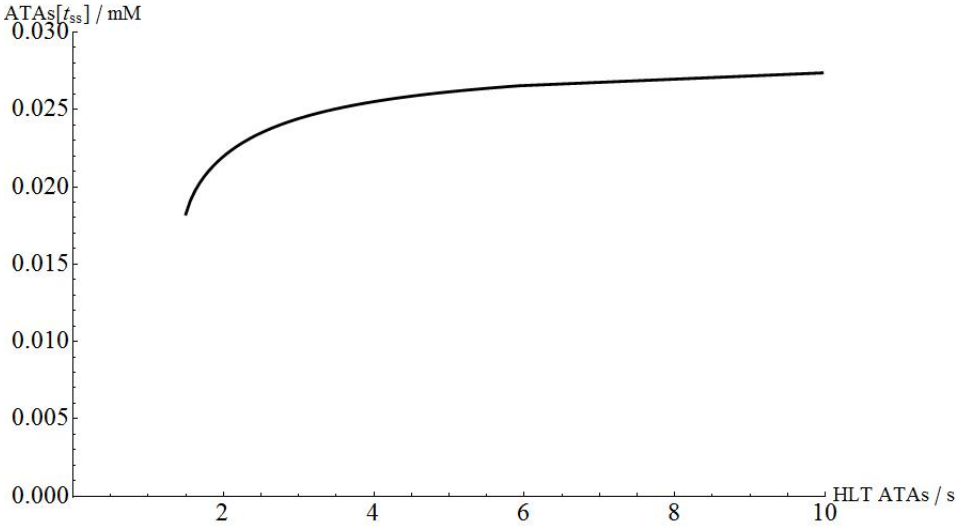


Figure 6: Dependency of the half-life of ATAs on steady state concentration of this molecule

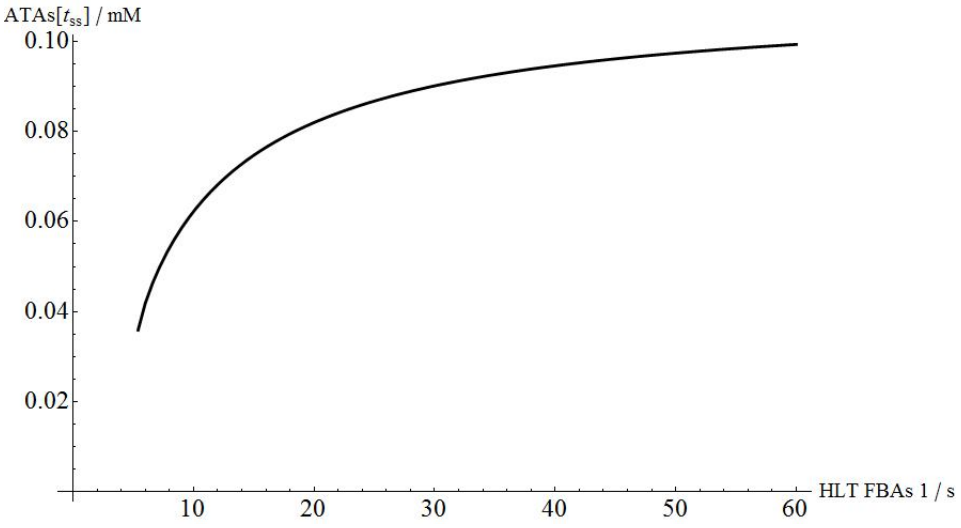
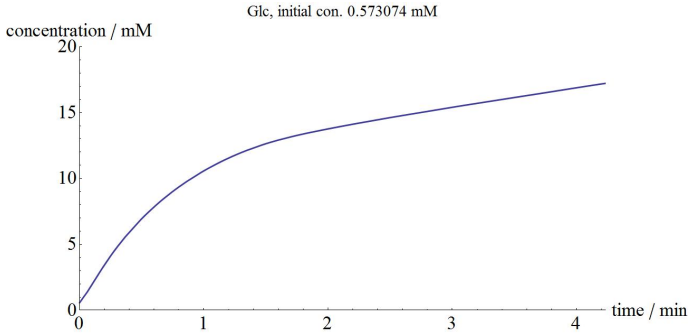
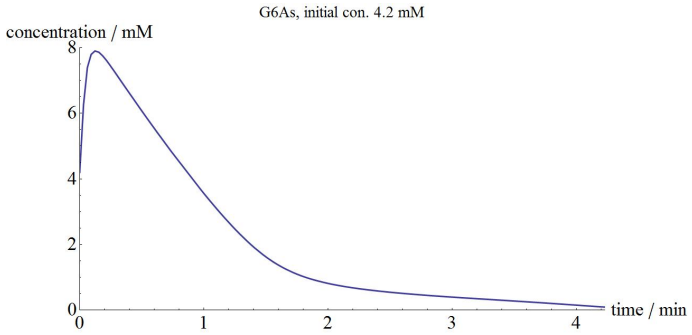


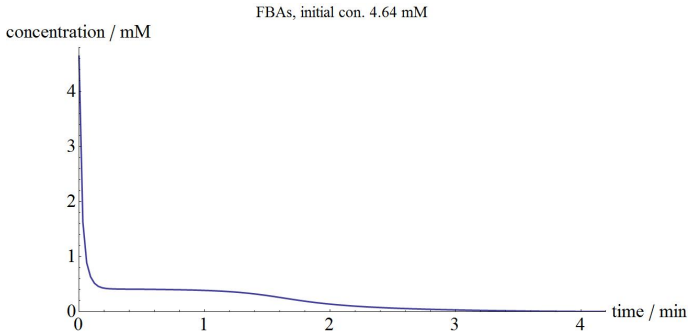
Figure 7: Dependency of the half-life of the bond between arsenate and fructose 1,6-bisarsenate at 1-position on the steady state concentration of ATAs



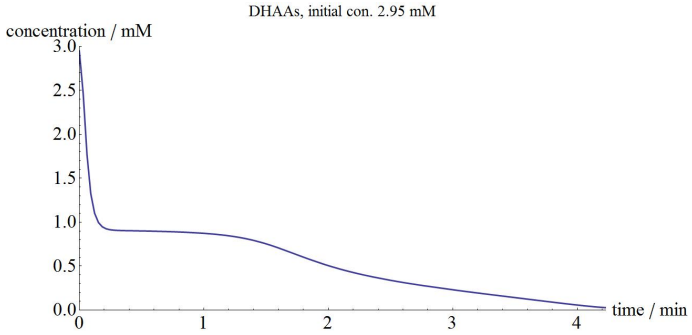
(a) Simulateion of intracellular glucose concentration



(b) Simulation of glucose 6-arsenate concentration

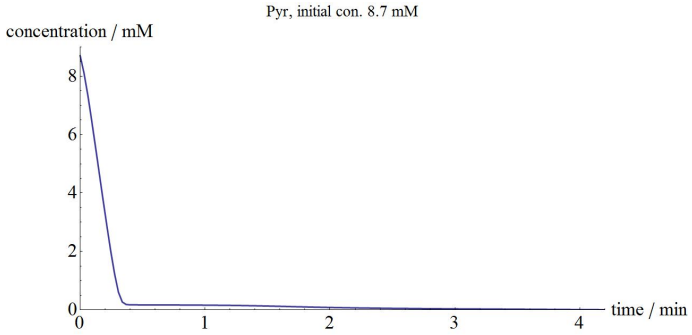


(c) Simulation of fructose 1,6-bisarsenate concentration

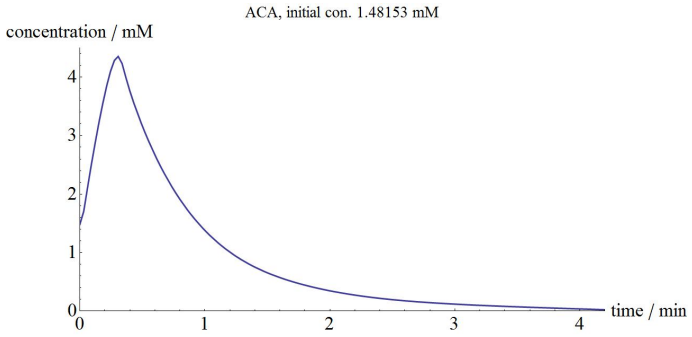


(d) Simulation of dihydroxyacetonearsenate concentration

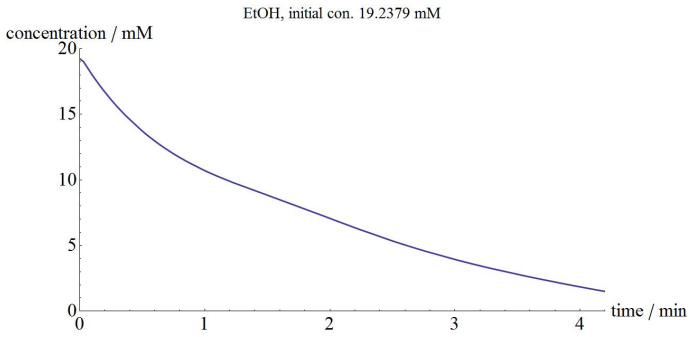
Figure 8: Simulation of the concentrations of the compounds intercellular glucose, glucose 6-arsenate, fructose 1,6-bisarsenate and dihydroxyacetonearsenate. Model runtime 4.2 min, breakdown due to decreased concentrations



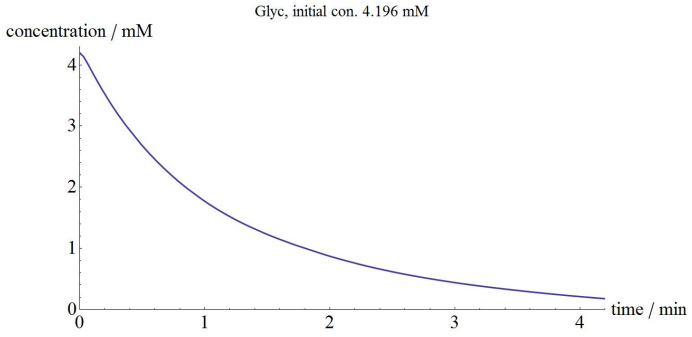
(a) Simulateion of pyruvate concentration



(b) Simulation of acetaldehyde concentration

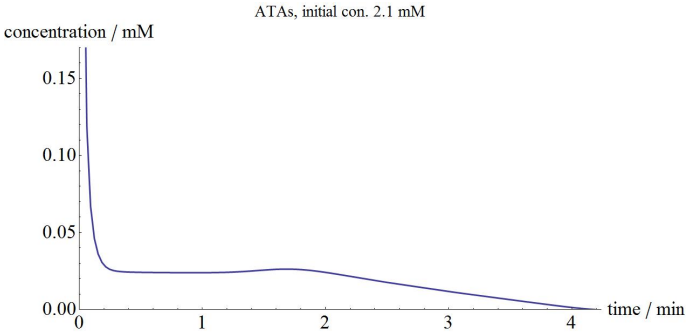


(c) Simulation of ethanol concentration

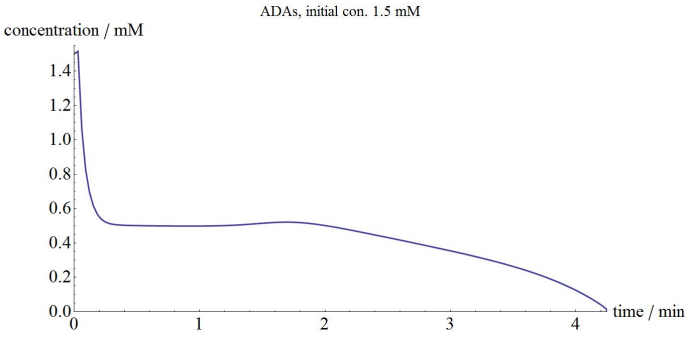


(d) Simulation of glycerol concentration

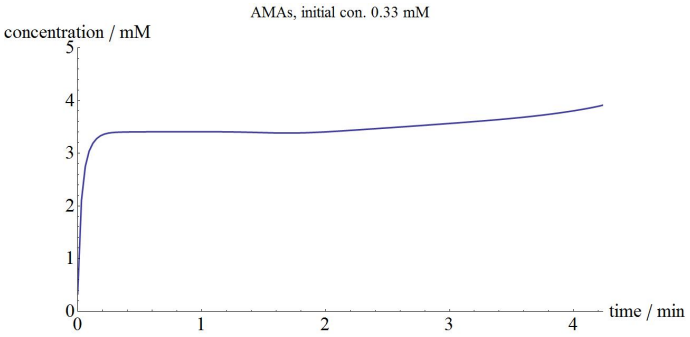
Figure 9: Simulation of the concentrations of final products and storage molecules pyruvate, acetaldehyde, ethanol and glycerol. Model runtime 4.2 min, breakdown due to decreased concentrations



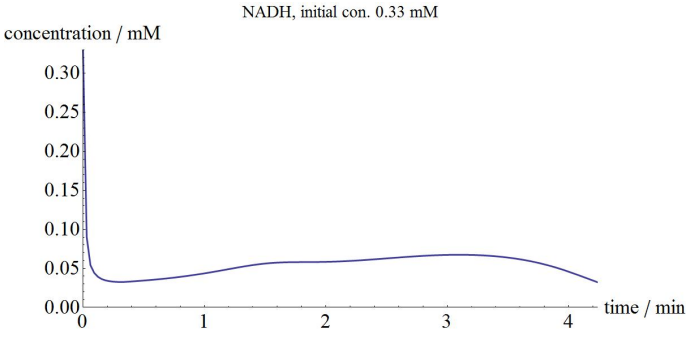
(a) Simulateion of ATAs concentration



(b) Simulation of ADAs concentration



(c) Simulation of AMAs concentration



(d) Simulation of NADH concentration

Figure 10: Simulation of the concentrations of the energy carriers ATAs, ADAs, AMAs and the coenzyme NADH. Model runtime 4.2 min, breakdown due to decreased concentrations

6.3.3. Possibilities to counteract the energy loss

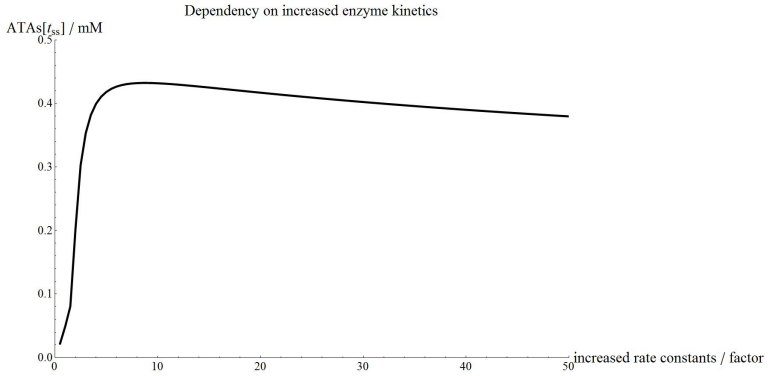
In this last section it will be investigated, whether it is possible to counteract the energy loss of the system by increasing the enzyme activities.
The shorten life-time of the arsenylated compounds lead to a decreased concentration of ATAs and therefore to a severe energy loss in the cell. This aspect was shown in the last two sections. The question is if the system is theoretically able to compensate this challenging problem by an activation of the involved enzymes. Thinkable are the use of different enzymes with altered affinities or activities, but also an increase of the enzyme concentrations.

The publication of Harden and Young from the year 1911[3] describes a short but strong fermentation of glucose by yeast when introducing sodium arsenate into the medium. This description leads to the hypothesis that due to the energy loss, the cell increases the enzyme activities to obtain more energy.

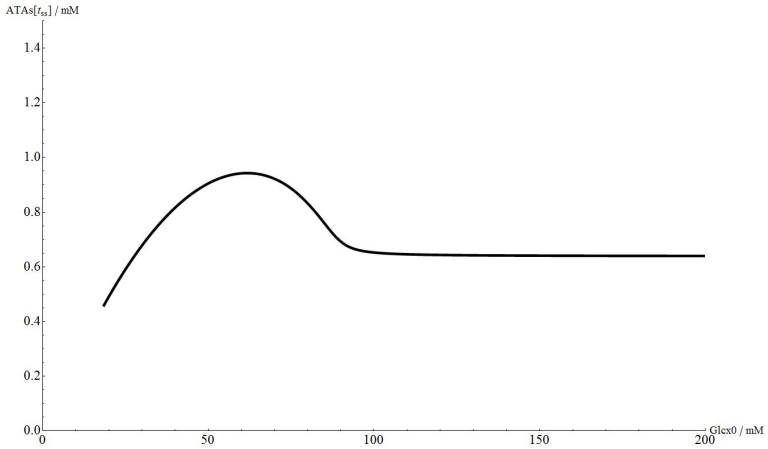
The increase is implemented as a rise of the rate constants and V_M values of all enzymes in the model by a factor between 2 and 100. All other values were left as described below. This increase is only a theoretical attempt to show the possible influence and can also be used as a measure of the energy loss due the instability of the ester and anhydride bonds between arsenate and the compounds in the glycolysis. Figure 11(a) shows the ATAs concentration in dependence to the factor of increase. It shows a maximum ATAs concentration at an increase of the enzyme rates approximately by the factor 5. This maximum evolves due to the accessible glucose in the outer medium (Glcx0). Figure 11(b) shows the dependency of the ATAs concentration on this glucose concentration when the enzyme rates are increased by the factor 10. This curve shows a clear maxima and therefore the maximum ATAs concentration which is possible with this factor of increase.

To show the dependencies in one graph, a 3D-graph was used, figure 11(c). An increase by the factor 10 and the increasing of the glucose concentration to about 50 mM can possibly compensate the energy loss and increases the ATAs concentration to half of the initial value. It has although to be mentioned that the NADH concentration is not affected by these increase of the enzyme activity and still stays at a very low concentration of about 0.07 mM. The same graph for these investigated aspects in the original model are shown in figure 12. The maximum of the ATAs concentration shown in figure 11(b) can also be observed in this graph although with a smaller extent. The only difference in the calculations is the addition of the hydrolysis and formation rates in figure 11.

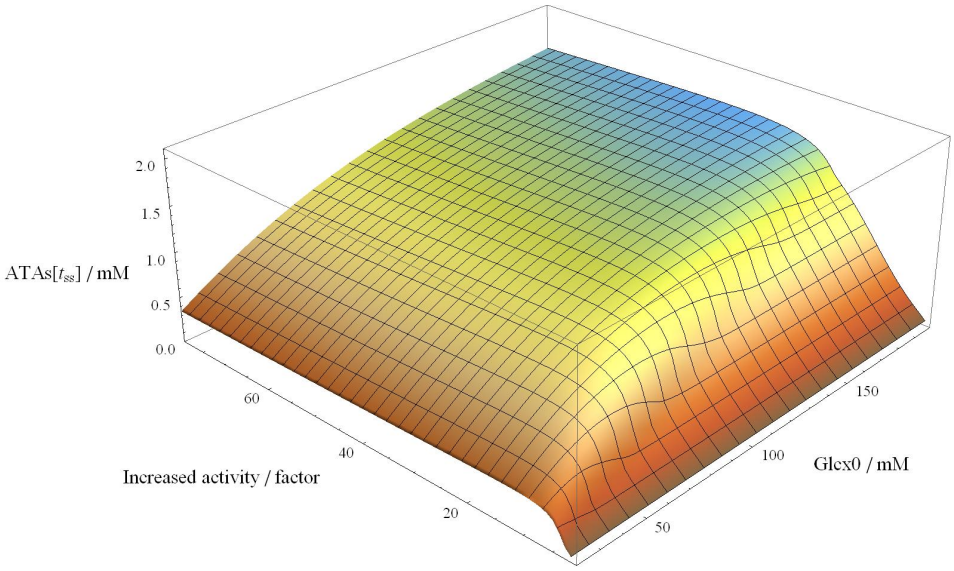
This graph and the resulting statements are based on a theoretical point of view, physiological aspects such as osmolarity and possible enzyme activities were not considered so far.



(a) Influence of increased enzyme activities on the ATAs steady state concentration. Increasing by factor (multiplier), Glcx0 = 18.5



(b) Influence of the Glcx0 concentration on the ATAs steady state concentration, enzyme activity increased by factor 10



(c) Influence of enzyme activity and Glcx0 concentration on ATAs steady state concentration

Figure 11: Possible compensation of the energy loss with increased enzyme activities of all enzymes. Measure for the energy is the steady state concentration of ATAs

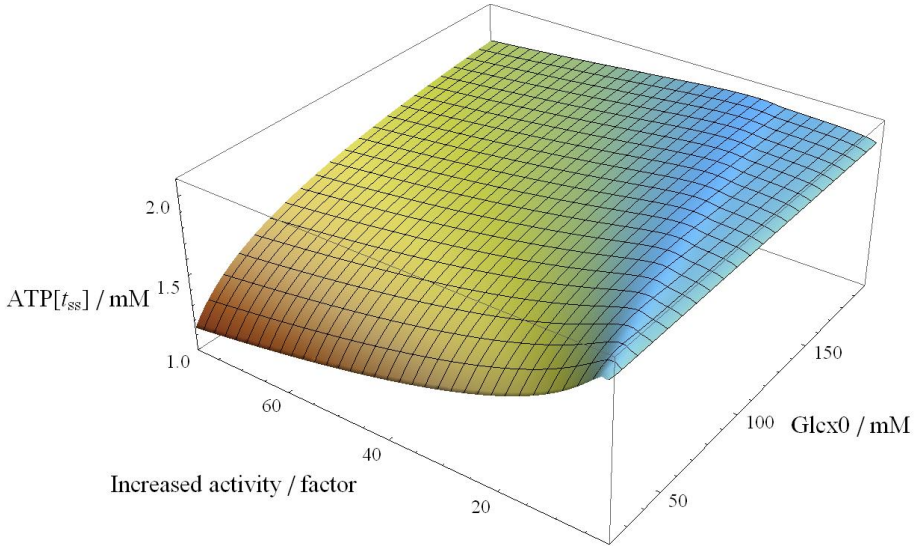


Figure 12: Influence of enzyme activity and Glcx0 concentration on ATP steady state concentration

– Shows the same simulation as figure 11(c) for the original model. Different behaviour probable due to hydrolysis reactions of arsenylated compounds

7. Discussion

As mentioned before, the question of a possible replacement of phosphate by arsenate underlies many different aspects. Sections 6.1 and 6.2 show that the reduction of arsenate and the subsequent probable inhibition of the enzymes is part of the main issue. This reduction was not implemented in the model, on the one hand because of a lack in information about the reducing mechanisms and their kinetics, on the other hand because the influence of arsenite on enzyme activities and other probable effect on the metabolic system are not known so far. One known issue is the inhibition of the pyruvate dehydrogenase through arsenite or ROS[61], which evolves at higher rates due to arsenite[62], this issue and the consequently cease of an aerobic metabolism was circumvented by using a model of the anaerobic metabolism. Also still unknown is the precise relationship between arsenite, glutathione and reactive oxygen species like superoxide anion radicals ($\cdot O^{2-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH^{-}$). Wheather the rise of ROS is due to the oxidising of glutathione during the reduction of arsenate or if its based on another mechanisms stays still unknown. Some hypothesis suggest even a reoxidising of arsenite by ROS[62] which would lead to a even more complicated relation between these compounds.

This first model was therefore intended to take into account only the instability and the spontaneous hydrolysis of the arsenate ester and anhydride bonds, also a simple characterisation of possible altered enzyme activities. The attempt was to introduce a model based on a proved and secure knowledge about the mechanisms of this replacement which simulates the most possible realistic behaviour of the enzymes and compound concentrations. The model as discussed in section 5 shows a very short life-time of the metabolic pathway. After 4.2 minutes 99.4 % of the arsenate is present as free arsenate and nearly all metabolite concentrations are below physiological level. But also the reach of a steady state is not

synonymous with the survival of a theoretical organism, firstly because the concentrations are still very low, secondly because the necessary stability is possibly not available under physiological conditions and thirdly because the system is reduced to a single metabolic pathway.

The decision to use glycolysis as the model pathway was made because this pathway is the most common. Therefore it was possible to have access to a lot of experimental data about enzyme affinities and even about the reaction of some of enzymes with arsenate. The same reason is to refer when discussing the use of *S. cerevisiae*. This organisms is comparably well known and especially the glycolysis or fermentation is observed since a long time. Nevertheless, a general validity of this model cannot be given. Enzyme activities and reaction rates are very divergent between different organisms, also branching points and final products of the glycolysis are in a great variety.

Concerning the model itself the used rate equations of Hynne have also to be questioned, whether they are capable of simulating a healthy steady state of yeast under natural physiological conditions, it is not sure that these equations are able to simulate also a difficult and challenging condition like the replacement of phosphate.
The hydrolysis rate of the arsenylated compounds is $6.8 \cdot 10^{-2}$ min^{-1} for an arsenate concentration of 50 mM and therefore in the range of the literature values between 0.3 min^{-1}[52] and $2.52 \cdot 10^{-2}$ min^{-1}[36]. The rates of the hydrolysis or life-times of ADAs and ATAs were estimated based on values for the hydrolysis of AMAs[53], ADPAs[1] (adenosine 5'-diphosphate arsenate) and pyroarsenate[60]. These values represent an estimation and are able to effect the results of the model intensely. Especially the half-life of the arsenate bonded in 1-position on fructose 1,6-bisarsenate has great influence on the runtime of the model. More experimental data is needed to verify these values and to allow statements about the precise framework conditions of the replacement of phosphate. Because of the decreasing energy yield it has to be assumed that the enzymes will be regulated to a higher rate, as seen in [52], the concentration may be increased or the enzymes are maybe even replaced by similar enzymes with higher affinities. The results in section 6.3.3. are very questionable, firstly because of the global change of the enzyme activities by the same factor, secondly because the alterations may be far away from physiological conditions. The original model has furthermore shown a different behaviour (see figure 12) when increasing the enzyme activity and the Glcx0 concentration. The precise reason for the different behaviour of the model remains unclear but it seems to be based on the hydrolysis reactions. Whether it is a realistic behaviour or just an misleading of the model stays unclear so far. Nevertheless, the idea of counteracting the instability of the compounds by increasing the enzyme activity seems to be possible, even though just for small arsenate concentrations. Other cell reactions affecting the metabolite concentrations or the reaction rates itself, e.g. the degradation of stored energy carriers like glycogen.

Alterations of the physicochemical conditions in the cytosol are another possible influences in the possibility of the replacement. Especially the change of the pH seems a reliable possibility to enhance the stability of bindings involving arsenate, even though the stability of bindings involving phosphate are decreased simultaneously[36]. The use of methyl groups for the binding is another often cited possibility and was used for some experiments because of the increased stability of the binding[2]. The change in the size of the molecule would nevertheless lead to changes in the affinity and activity of the enzymes. All the cited ways to counteract the energy loss of the organism are possible in principle. However, every

change in these conditions leads to the necessity of adaptation on another level.

The alterations been made were based on experimental data, but often obtained under different conditions and mostly in vitro, which can be very different to the behaviour in vivo. Especially the change of the rate and equilibrium constants was difficult and highly hypothetical. Only few enzymes were tested in regard of their rates and affinities when using arsenate as substrate and the results were ambiguous, between orders of magnitude and only a few per cent. The use of 40 % was an essay to follow the tendency, since most of the tested glycolytic enzymes have showed a similar changes in their behaviour. The increase or decrease of all involved enzymes by the same value is nonetheless just an inaccurate assumption.
The spontaneous formation of relevant arsenylated compounds was only implemented as the formation of glucose 6-arsenate and ADAs, both reactions are trying to take into account the formation at all. The kinetics are still too small to sustain a sufficient rate, but they are completing the view on the mechanisms taken place in the cytosol. It should be mentioned, that not only glucose 6-arsenate and ADAs would be formed spontaneously but even the rate of the ADAs formation was just a suggestion and all further implemented reaction would lead to a even more hypothetical model. Keeping the model as simple as possible was the reason to avoid these additional reactions, theoretically the arsenate would build up bindings to every possible compound, for example not only glucose 6-arsenate but also glucose 1-arsenate, etc.. Neglecting the compounds evolving through the spontaneous hydrolysis are based on the same reason, the effect on the model is very small and it would lead to the addition of at least 4 new compound pools with unknown kinetics for the returning into the pathway.

Another arguable assumption was the stability of AMAs, this compound would of course also be affected by a hydrolysis reaction. The absence of this reaction is mainly based on the original model from Hynne, a hydrolysis would lead to the need of a adenine-nucleotide pool and the reactions of forming AMAs. Since the model is showing already an insufficient energy level and the half-life was measured by [53] as 30 - 45 min, the addition of these compounds and reactions seem to be negligible.

The organisms suggested to be capable of replace phosphate by arsenate GFAJ-1 is member of the family *Halomonadaceae*, a *Gammaproteobacteria*. It is still not known which metabolic pathways are used from this organism, even when its probable that glycolysis is one of them[23]. Nevertheless, the comparison of a halophilic *Proteobacteria* with yeast is misleading. Arsenate resistant and arsenate respiratory bacteria are demonstrate, that it has to be possible to distinguish between phosphate and arsenate. It could also be possible that mechanisms exist to secure the ester and anhydride bonds from unintended hydrolysis, the previously stated ones are just a few of the thinkable mechanisms.

This work shows the main problems of biological systems to replace phosphate by arsenate. The main difference of these molecules is the variation in the kinetic behaviour of forming and hydrolyse spontaneously bindings to other molecules in the order of 10^5[11, 57]. The results of the mathematical model are in good agreement with experimental data, of course based on the assumption that some of the observed effects are based on the evolution of arsenite. Despite from that, the instability is still a difficult problem and at least for *S. cerevisiae* the possibility of replacing phosphate by arsenate seems to be unlikely.

8. Perspective

The natural next step concerning a model of the glycolytic pathway and arsenate would be the implementation of an arsenate and a phosphate pool into the model at the same time. This lead to a far more complicated model not only because of the competitive substrates, different enzyme rates and regulations but also because the model would need far more metabolites. Compounds with two or even more bounded phosphate or arsenate can occur in many different manners. For example the fructose 1,6-bisphosphate can occur in four different ways since arsenate could be bonded at 1-position, 6- position or at both positions. At the moment no information is available how these different compounds behave, whether there are favoured positions for arsenate with a different stability and whether these compounds have altered equilibrium constants and influences on the enzymes. Quantum chemistry will possibly be able to explain the relations and properties of the arsenate and the arsenate containing bonds. This would be a large step forward in the calculation and simulation of biological mechanisms.
Another aspect is the focus on the energy carriers ATAs in this work. A further investigation could involve the influence of these replacement on the NADH concentration encouraged by the differences in the development of the concentrations during the simulations.

Furthermore, if it is possible to obtain more informations about the arsenate reducing mechanisms in biological cells, about the relationship of ROS and arsenate and about the influence of arsenite on biological systems, this would also be a promising issue of research. Especially for the use of arsenic containing pharmaceuticals and the treatment of long-term arsenic intoxications. The methylation of arsenate is suggested to be the most famous way of a detoxification or resistance process. Difficult to understand are the fact, that some of the formed methylated compounds are even more reactive and therefore toxic than the original ones. However, almost all investigated organisms are showing the ability to generate an detoxification process via reducing arsenate, in section 6.2 the suggested mechanism of *S. cerevisiae* is discussed. Two other mechanisms were mentioned in the literature, the frequency of these three similar arsenate specific detoxification processes give way to speculations and hypothesis. Especially about the origin of these mechanisms and taxonomical relations between the different organisms. These speculations can only be proved by more experiments and more effort will be needed to obtain a deeper understanding of these mechanisms and their particular function in the biological systems.

The use of arsenate for experimental research could also lead to more precise informations about enzymes. The inability of glycolytic enzymes to distinguish between phosphate and arsenate allows the suggestion, that shape and charge are more important than size and weight of the used substrates. Some authors assumed here an evolutionary reason, saying it was not necessary to distinguish between the two of them[16]. On the other hand there are obviously enzyme which are capable of distinguishing between these similar compounds, maybe using the willingly reduction of arsenate to arsenite or the affinity of arsenate to sulphur. Investigation of different enzymes with phosphate and arsenate could lead to a deeper understanding of the enzymes itself and would possibly allow to make predictions of enzyme affinities and kinetic rates.

If it is possible to avoid the reduction of arsenate, this compound could be used to measure maximum enzyme rates. The lack of energy due to the spontaneous hydrolysis of the formed metabolites could lead to an increased catabolic metabolism probably to a complete cease of the anabolic pathways. The cells could be investigated under these conditions which are forcing the cell to change their behaviour completely to an more energy based metabolism. This could be used to obtain further informations about the minimum energy level of cells and possibilities of biological systems to regulate their metabolic pathways.

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A. Appendix

A.1. Original values of the model by Hynne et al., tables taken from [22]

Table 7: Reactions of the model from Hynne et al.

r	reaction			
1	inGlc		$\rightleftharpoons$	Glc_x
2	GlcTrans	Glc_x	$\rightleftharpoons$	Glc
3	HK	$Glc + ATP$	$\rightarrow$	$G6P + ADP$
4	PGI	G6P	$\rightleftharpoons$	F6P
5	PFK	$F6P + ATP$	$\rightleftharpoons$	$FBP + ADP$
6	ALD	FBP	$\rightleftharpoons$	$GAP + DHAP$
7	TIM	DHAP	$\rightleftharpoons$	GAP
8	GAPDH	$GAP + NAD^+$	$\rightleftharpoons$	$BPG + NADH$
9	lpPEP	$BPG + ADP$	$\rightleftharpoons$	$PEP + ATP$
10	PK	$PEP + ADP$	$\rightarrow$	$Pyr + ATP$
11	PDC	Pyr	$\rightarrow$	ACA
12	ADH	$ACA + NADH$	$\rightarrow$	$EtOH + NAD^+$
13	difEtOH	EtOH	$\rightleftharpoons$	$EtOH_x$
14	outEtOH	$EtOH_x$	$\rightarrow$	
15	lpGlyc	$DHAP + NADH$	$\rightarrow$	$Glyc + NAD^+$
16	difGlyc	Glyc	$\rightleftharpoons$	$Glyc_x$
17	outGlyc	$Glyc_x$	$\rightarrow$	
18	difACA	ACA	$\rightleftharpoons$	ACA_x
19	outACA	ACA_x	$\rightarrow$	
20	lacto	$ACA_x + CN_x^-$	$\rightarrow$	
21	inCN	CN_x^-	$\rightleftharpoons$	
22	storage	$G6P + ATP$	$\rightarrow$	ADP
23	consume	ATP	$\rightarrow$	ADP
24	AK	$ATP + AMP$	$\rightleftharpoons$	2 ADP

$[NAD^+] + [NADH] = \text{constant}$

$[ATP] + [ADP] + [AMP] = \text{constant}$

Table 8: Parameters in the model of Hynne et al.

	Parameter	Value
Volume ratio	y_{vol}	59
Specific flow rate at bifurcation	$k_0(min^{-1})$	0.048
Mixed flow concentration, glucose	$[Glc_x]_0(mM)$	18.5
Mixed flow concentration, cyanide	$[CN_x^-]_0(mM)$	5.60

Compound	c_s
Glc_x	1.55307
Glc	0.573074
G6P	4.2
F6P	0.49
FBP	4.64
GAP	0.115
DHAP	2.95
BPG	0.00027
PEP	0.04
Pyr	8.7
ACA	1.48153
EtOH	19.2379
$EtOH_x$	16.4514
Glyc	4.196
$Glyc_x$	1.68478
ACA_x	1.28836
CN_x^-	5.20358
ATP	2.1
ADP	1.5
AMP	0.33
NADH	0.33
NAD^+	0.65

Table 9: Initial values

Reaction	Parameter	Value
GlcTrans	K_{2Glc}	1.7
	K_{2IG6P}	1.2
	K_{2IIG6P}	7.2
	P_2	1
HK	K_{ATP}	0.1
	K_{3Glc}	0
	K_{3dGlc}	0.37
PGI	K_{4G6P}	0.8
	K_{4F6P}	0.15
	K_{4eq}	0.13
PFK	K_5	0.021
	κ_5	0.15
ALD	V_{6r}/V_{6f}	5
	K_{6eq}	0.081
	K_{6FBP}	0.3
	K_{6GAP}	4.0
	K_{6DHAP}	2.0
	K_{6IGAP}	10.0
TIM	K_{7DHAP}	1.23
	K_{7GAP}	1.27
	K_{7eq}	0.055
GAPDH	K_{8GAP}	0.6
	K_{BPG}	0.01
	K_{NAD}	0.1
	K_{NADH}	0.06
	K_{8eq}	0.0055
PK	K_{10ADP}	0.17
	K_{10PEP}	0.2
PDC	K_{11}	0.3
ADH	K_{12ACA}	0.71
	K_{12NADH}	0.1
difEtOH	k_{13}	$k_{16}\cdot$ 8.8
G3PDH	K_{15NADH}	0.13
	K_{15DHAP}	25
	$K_{15INADH}$	0.034
	K_{15INAD}	0.13
difGlyc	k_{16}	1.9
difACA	k_{18}	$k_{16}\cdot$ 13

Table 10: Constants

Table 11: Rate constants in the model of Hynne et al.

r	Reaction	Parameter	Forward	Reverse
1	inGlc	$k_0(min^{-1})$	0.048	0.048
2	GlcTrans	$V_{2m}(mM\ min^{-1})$	$1.01496 \cdot 10^3$	$1.01496 \cdot 10^3$
3	HK	$V_{3m}(mM\ min^{-1})$	$5.17547 \cdot 10^1$	
4	PGI	$V_{4m}(mM\ min^{-1})$	$4.96042 \cdot 10^2$	$4.96042 \cdot 10^2$
5	PFK	$V_{5m}(mM\ min^{-1})$	$4.54327 \cdot 10^1$	
6	ALD	$V_{6m}(mM\ min^{-1})$	$2.20782 \cdot 10^3$	$1.10391 \cdot 10^4$
7	TIM	$V_{7m}(mM\ min^{-1})$	$1.16365 \cdot 10^2$	$1.16365 \cdot 10^2$
8	GAPDH	$V_{8m}(mM\ min^{-1})$	$8.33858 \cdot 10^2$	$8.33858 \cdot 10^2$
9	lpPEP	$k_9(mM^{-1}min^{-1})$	$4.43866 \cdot 10^5$	$1.52862 \cdot 10^3$
10	PK	$V_{10m}(mM\ min^{-1})$	$3.43096 \cdot 10^2$	
11	PDC	$V_{11m}(mM\ min^{-1})$	$5.31328 \cdot 10^1$	
12	ADH	$V_{12m}(mM\ min^{-1})$	$8.98023 \cdot 10^1$	
13	difEtOH	$k_{13}(min^{-1})$	$1.67200 \cdot 10^1$	$1.67200 \cdot 10^1$
14	outEtOH	$k_0(min^{-1})$	0.048	
15	lpGlyc	$V_{15m}(mM\ min^{-1})$	$8.14797 \cdot 10^1$	
16	difGlyc	$k_{16}(min^{-1})$	1.9	1.9
17	outGlyc	$k_0(min^{-1})$	0.048	
18	difACA	$k_{18}(min^{-1})$	$2.47 \cdot 10^1$	$2.47 \cdot 10^1$
19	outACA	$k_0(min^{-1})$	0.048	
20	lacto	$k_{20}(mM^{-1}min^{-1})$	$2.83828 \cdot 10^{-3}$	
21	inCN	$k_0(min^{-1})$	0.048	0.048
22	storage	$k_{22}(mM^{-1}min^{-1})$	2.25932	
23	consume	$k_{23}(min^{-1})$	3.20760	
24	AK	$k_{24}(mMmin^{-1})$	$4.32900 \cdot 10^2$	$1.33333 \cdot 10^2$

r	Rate equation (v_r)
1	$v_{\rightarrow} = y_{vol} k_0 [Glc_x]_0$ $v_{\leftarrow} = y_{vol} k_0 [Glc_x]$
2	$v_{\rightarrow} = \dfrac{V_{2m} \dfrac{[Glc_x]}{K_{2Glc}}}{1 + \dfrac{[Glc_x]}{K_{2Glc}} + \dfrac{P_2 \dfrac{[Glc_x]}{K_{2Glc}} + 1}{P_2 \dfrac{[Glc]}{K_{2Glc}} + 1} \left(1 + \dfrac{[Glc]}{K_{2Glc}} + \dfrac{[G6P]}{K_{2IG6P}} + \dfrac{[Glc][G6P]}{K_{2Glc} K_{2IIG6P}} \right)}$ $v_{\leftarrow} = \dfrac{V_{2m} \dfrac{[Glc]}{K_{2Glc}}}{1 + \dfrac{[Glc]}{K_{2Glc}} + \dfrac{P_2 \dfrac{[Glc]}{K_{2Glc}} + 1}{P_2 \dfrac{[Glc_x]}{K_{2Glc}} + 1} \left(1 + \dfrac{[Glc_x]}{K_{2Glc}} \right) + \dfrac{[G6P]}{K_{2IG6P}} + \dfrac{[Glc][G6P]}{K_{2Glc} K_{2IIG6P}}}$
3	$v_{\rightarrow} = \dfrac{V_{3m}[ATP][Glc]}{K_{3DGlc} K_{3ATP} + K_{3Glc}[ATP] + K_{3ATP}[Glc] + [Glc][ATP]}$
4	$v_{\rightarrow} = \dfrac{V_{4m}[G6P]}{K_{4G6P} + [G6P] + \dfrac{K_{4G6P}}{K_{4F6P}}[F6P]}$ $v_{\leftarrow} = \dfrac{V_{4m} \dfrac{[F6P]}{K_{4eq}}}{K_{4G6P} + [G6P] + \dfrac{K_{4G6P}}{K_{4F6P}}[F6P]}$
5	$v_{\rightarrow} = \dfrac{V_{5m}[F6P]^2}{K_5 \left(1 + \kappa_5 \dfrac{[ATP]^2}{[AMP]^2} \right) + [F6P]^2}$
6	$v_{\rightarrow} = \dfrac{V_{6f}[FBP]}{K_{6FBP} + [FBP] + \dfrac{[GAP] K_{6DHAP} V_{6f}}{K_{6eq} V_{6r}} + \dfrac{[DHAP] K_{6GAP} V_{6f}}{K_{6eq} V_{6r}} + \dfrac{[FBP][GAP]}{K_{6IGAP}} + \dfrac{[GAP][DHAP] V_{6f}}{K_{6eq} V_{6r}}}$ $v_{\leftarrow} = \dfrac{V_{6f} \dfrac{[GAP][DHAP]}{K_{6eq}}}{K_{6FBP} + [FBP] + \dfrac{[GAP] K_{6DHAP} V_{6f}}{K_{6eq} V_{6r}} + \dfrac{[DHAP] K_{6GAP} V_{6f}}{K_{6eq} V_{6r}} + \dfrac{[FBP][GAP]}{K_{6IGAP}} + \dfrac{[GAP][DHAP] V_{6f}}{K_{6eq} V_{6r}}}$
7	$v_{\rightarrow} = \dfrac{V_{7m}[DHAP]}{K_{7DHAP} + [DHAP] + \dfrac{K_{7DHAP}}{K_{7GAP}}[GAP]}$ $v_{\leftarrow} = \dfrac{V_{7m} \dfrac{[GAP]}{K_{7eq}}}{K_{7DHAP} + [DHAP] + \dfrac{K_{7DHAP}}{K_{7GAP}}[GAP]}$

Figure 13: Equations 1 - 7 of the model from Hynne et al.

r	Rate equation (v_r)
8	$v_{\rightarrow} = \dfrac{V_{8m}[\mathrm{GAP}][\mathrm{NAD^+}]}{K_{8\mathrm{GAP}}K_{8\mathrm{NAD}}\left(1+\dfrac{[\mathrm{GAP}]}{K_{8\mathrm{GAP}}}+\dfrac{[\mathrm{BPG}]}{K_{8\mathrm{BPG}}}\right)\left(1+\dfrac{[\mathrm{NAD^+}]}{K_{8\mathrm{NAD}}}+\dfrac{[\mathrm{NADH}]}{K_{8\mathrm{NADH}}}\right)}$ $v_{\leftarrow} = \dfrac{V_{8m}\dfrac{[\mathrm{BPG}][\mathrm{NADH}]}{K_{8eq}}}{K_{8\mathrm{GAP}}K_{8\mathrm{NAD}}\left(1+\dfrac{[\mathrm{GAP}]}{K_{8\mathrm{GAP}}}+\dfrac{[\mathrm{BPG}]}{K_{8\mathrm{BPG}}}\right)\left(1+\dfrac{[\mathrm{NAD^+}]}{K_{8\mathrm{NAD}}}+\dfrac{[\mathrm{NADH}]}{K_{8\mathrm{NADH}}}\right)}$
9	$v_{\rightarrow} = k_{9f}[\mathrm{BPG}][\mathrm{ADP}]$ $v_{\leftarrow} = k_{9r}[\mathrm{PEP}][\mathrm{ATP}]$
10	$v_{\rightarrow} = \dfrac{V_{10m}[\mathrm{ADP}][\mathrm{PEP}]}{(K_{10\mathrm{PEP}}+[\mathrm{PEP}])(K_{10\mathrm{ADP}}+[\mathrm{ADP}])}$
11	$v_{\rightarrow} = \dfrac{V_{11m}[\mathrm{Pyr}]}{K_{11}+[\mathrm{Pyr}]}$
12	$v_{\rightarrow} = \dfrac{V_{12m}[\mathrm{ACA}][\mathrm{NADH}]}{(K_{12\mathrm{NADH}}+[\mathrm{NADH}])(K_{12\mathrm{ACA}}+[\mathrm{ACA}])}$
13	$v_{\rightarrow} = k_{13}[\mathrm{EtOH}]$ $v_{\leftarrow} = k_{13}[\mathrm{EtOH}_x]$
14	$v_{\rightarrow} = y_{\mathrm{vol}}k_0[\mathrm{EtOH}_x]$
15	$v_{\rightarrow} = \dfrac{V_{15m}[\mathrm{DHAP}]}{K_{15\mathrm{DHAP}}\left(1+\dfrac{K_{15\mathrm{INADH}}}{[\mathrm{NADH}]}\left(1+\dfrac{[\mathrm{NAD^+}]}{K_{15\mathrm{INAD}}}\right)\right)+[\mathrm{DHAP}]\left(1+\dfrac{K_{15\mathrm{NADH}}}{[\mathrm{NADH}]}\left(1+\dfrac{[\mathrm{NAD^+}]}{K_{15\mathrm{INAD}}}\right)\right)}$
16	$v_{\rightarrow} = k_{16}[\mathrm{Glyc}]$ $v_{\leftarrow} = k_{16}[\mathrm{Glyc}_x]$
17	$v_{\rightarrow} = y_{\mathrm{vol}}k_0[\mathrm{Glyc}_x]$
18	$v_{\rightarrow} = k_{18}[\mathrm{ACA}]$ $v_{\leftarrow} = k_{18}[\mathrm{ACA}_x]$
19	$v_{\rightarrow} = y_{\mathrm{vol}}k_0[\mathrm{ACA}_x]$
20	$v_{\rightarrow} = y_{\mathrm{vol}}k_{20}[\mathrm{ACA}_x][\mathrm{CN}_x^-]$
21	$v_{\rightarrow} = y_{\mathrm{vol}}k_0[\mathrm{CN}_x^-]_0$ $v_{\leftarrow} = y_{\mathrm{vol}}k_0[\mathrm{CN}_x^-]$
22	$v_{\rightarrow} = k_{22}[\mathrm{ATP}][\mathrm{G6P}]$
23	$v_{\rightarrow} = k_{23}[\mathrm{ATP}]$
24	$v_{\rightarrow} = k_{24f}[\mathrm{AMP}][\mathrm{ATP}]$ $v_{\leftarrow} = k_{24r}[\mathrm{ADP}]^2$

Figure 14: Equations 8 - 24 of the model from Hynne et al.

Eigenständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Bachelorarbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Alle Stellen, die den Quellen entnommen wurden, sind als solche kenntlich gemacht worden. Diese Arbeit hat in gleicher oder ähnlicher Form noch keiner Prüfungsbehörde vorgelegen.

(Ort, Datum) (Unterschrift)