Enterobacter cloacae SLD1a-1 bacteria to elemental selenium. The whole process occurred near the cell plasma membrane. ~~In terms of~~According to the biochemical studies presented by Yee et al. [2007], selenate reductase closely bound to the cytoplasmic membrane~~,~~ is responsible for the reduction of selenium. The obtained results demonstrate the possibility of ~~application of~~using bacteria to perform the bioremediation process of selenate ions from contaminated soils, sediments, and industrial waste [Sarret et al., 2005; Ridley et al., 2006].

Čertík et al. [2013] demonstrated that the addition of selenium to the culture medium caused a change in the fatty acid profile ~~occurring in~~of the cytoplasmic membrane of yeast~~.~~; ~~One~~they observed an increase in the content of C-18 fatty acids~~,~~ Whereas, in yeast enriched in carotenoids, they noticed ~~and~~ a decomposition of unsaturated fatty acids (linoleic and linolenic acids). ~~in the lipid membranes of yeast enriched in carotenoids.~~

Using electron microscopy techniques, t~~T~~he authors of other publications [Gerrard et al., 1974] ~~with the use of electron microscopy techniques~~ demonstrated~~,~~ that *Escherichia coli* cultured in a medium containing 340 mg/L of selenite solution (IV) was able to bind selenium in the outer membrane and in the cytoplasmic membrane of bacteria, as well as in between them. It proved that the activation of the defense mechanism is based on the accumulation of selenium in the cell membranes, thereby preventing the penetration of SeO_3^{2-} ions into the cytosol [Chmielowski and Tyflewska, 2007].

3. Intracellular accumulation of selenium

The process of intracellular accumulation of selenium occurs through active transport inside the cell interior of yeasts. To o~~O~~vercome~~ing~~ the~~of~~ membrane assembly requires the presence of specific transport mechanisms. So far, only a few reports describing this process in yeast cells have been published. In addition, there ~~is~~are no studies on identification of selenium carriers at the molecular level [Rosen and Liu, 2009]. Analysis of the available research [Ponce de Leon et al. 2002; Gharieb and Gadd, 2004] indicates that the majority of publications devoted to the study of intracellular accumulation of selenium relates mainly to yeast and bacteria.

Studies conducted by Sirko et al. [1990] and Turner et al. [1998] demonstrated that selenium is absorbed by *Escherichia coli* through sulfur ABC membrane transporters which are encoded by *cysAWTP* operon [Shaw et al., 2012]. The transport complex is composed of two CysA molecules which bind ATP nucleotides, two integral membrane proteins~~:~~ (CysT~~,~~ and CysW)~~,~~ and CysP periplasmic sulphate-binding protein [Rosen and Liu, 2009]. This is an example of ~~an~~ active transport, which occurs with the contribution of specialized, integral proteins.

ABC pumps for the transport of selenium ions use the energy derived from hydrolysis of ATP binding. A distinct selenium transport system based on sulphate permease has also been reported. This correlation was confirmed by Cherest et al. [1997] in experiments involving *Saccharomyces cerevisiae* yeasts, wherein Sul1p and Sul2p specific transport systems exhibiting a high affinity toward~~s~~ sulfur~~,~~ were used to transfer SeO_3^{2-} ions~~,~~ In contrast, Zhiltsov et al. [1996] found that *Candida utilis* VSB-651 strains, demonstrating the high activity of reductase enzymes and glutathione peroxidase, were able to bind two times more selenium ~~in comparison~~compared to *Candida ethanolica* VSB-814 yeasts with reduced activity of these enzymes. Most likely, in this case, selenium accumulation was associated with activity of redox enzymes.

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Danch and Chmielowski [1985] studied the binding process of selenium (IV) by *Saccharomyces cerevisiae* D37 yeast strain. It was found, that the content of selenium in yeast cells grown in the presence of glucose and fructose reached the level of selenium equal to 14 and 11 mg/g, respectively. It was also found that the content of selenium in the nuclear fraction isolated from the cells after cultivation enriched with fructose~~,~~ was significantly higher (86%) as compared to the fraction isolated from cells cultured in medium containing glucose (59%).

Depending on the type of sugar in the culture medium, the increased content of selenium in the nuclear fraction and the total selenium accumulation~~,~~ denoted the presence of different transport mechanisms. On this basis, it~~s~~ was assumed~~,~~ that complexed selenium ions with an absorbed sugar substrate~~,~~ were transported to the cell cytosol. Kinetics of intracellular binding of selenium in *Saccharomyces cerevisiae* yeasts indicated the existence of two transport systems~~: —~~one with high and the other with low affinity toward~~s~~ selenium. Both types of transports were dependent on the presence of glucose in the culture medium, significantly increasing the rate of sorption of selenium by yeast cells [Gharieb and Gadd, 2004; Rosen and Liu, 2009].

A similar correlation between the content of selenium and the assimilation of glucose in the culture medium of *Candida albicans* 806 was observed by Falcone and Nickerson [1963]. The authors reported that sodium selenate (IV) inhibited the anaerobic process of assimilation of glucose by yeast. The use of oxygen in the medium was reduced by 84%, while the carbon dioxide emission during fermentation decreased only by 63%. This phenomenon was explained by the occurrence of strong inhibition of glucose oxidation by sodium selenate (IV). Simultaneously, for *Candida albicans* and *Saccharomyces cerevisiae*, it was demonstrated that phosphate ions compete with selenite (IV) ions for binding sites in transport systems. Inhibitory effect of 2,4-dinitrophenol (DNP) was also observed [Falcone and Nickerson, 1963].

Studies presented by Kłapcińska et al. [1993] showed that the presence of phosphate (V) and sulfate (IV) ions in the culture medium had an inhibitory effect on the binding affinity of selenium (IV) by *Saccharomyces cerevisiae* yeasts.~~, was demonstrated by the presence of phosphate (V) and sulfate (IV) ions in the culture medium.~~ It was also reported that i~~I~~n the presence of sulphate (IV) ions, the ~~ability of~~ yeast~~s~~ cells had the ability to carry out the reduction of selenate (IV) ions to elemental selenium.~~, was reported.~~ In addition, the presence of metabolic inhibitors, 2,4-dinitrophenol, potassium cyanide, sodium azide, N-ethylmaleimide, and CCCP (carbonyl cyanide chlorophenylhydrazone), influenced the reduction of selenium biosorption by yeast cells [Gharieb and Gadd, 2004; Chmielowski and Tyflewska, 2007].

The presence of different substances in the culture medium has led to ~~Increasing importance in~~ the theoretical considerations on the accumulation of selenium by yeast gaining more importance.~~ is gained by the presence of different substances in the culture medium.~~ Studies conducted by Gharieb and Gadd [2004] have shown that selenium transport was inhibited by the presence of sulfur in the molecules of exogenous amino acids~~:~~ like methionine, cysteine, and cystine~~ containing sulfur in their molecules~~. The presence of high concentrations of sulfate (IV) and (VI) in the culture medium~~,~~ did not affect the biosorption of selenium by *Saccharomyces cerevisiae* yeasts.

Observations of other authors [Golubev and Golubev 2002] confirmed that the tolerance of yeast to the presence of selenium in the culture medium depends on the medium composition

and the presence of sulfur amino acids. According to Demirci and Pometto [1999], Se/S ratio estimated at 4: 1 in the culture medium is optimal for efficient bioaccumulation of inorganic selenium and its transformation into organic forms by yeast cell biomass. In conclusion, the authors showed a correlation between selenium binding and the occurrence of metabolic products of sulfur [Gharieb and Gadd, 2004].

The studies conducted by Lazard et al. [2010] showed that accumulation of selenate ions by *Saccharomyces cerevisiae* yeasts was determined by the presence of phosphate ions in the culture medium. Based on this experiment, it was found that Pho84p and Pho89p transporters were major factors contributing to the binding of selenate (IV) ions by yeast in a culture medium with low phosphate content ~~in the culture medium~~. In terms of a higher concentration of phosphate in the medium, selenium transport was gradually replaced by low affinity transporters (Pho87p, Pho90p~~,~~ and Pho91p). In consequence, absorption of selenium was reduced, and cell resistance toward~~s~~ increased doses of selenium increased.

Studies published by McDermott et al. [2010] demonstrated that symporter Jen1p monocarboxylic conveyor is responsible for the transport of selenite (IV) into the yeast cell interior. It is involved in the transport of pyruvic acid, lactic acid, acetic acid, and propionic acid [Paiva et al., 2013]. Transport of selenite (IV) is based on the structural similarity of selenium anions and the anions of carboxylic acids. In addition, these molecules have similar dissociation constants, while within physiological pH~~,~~ they are mono-negative anions. Under anaerobic environment in the absence of fermentable substrates, increased accumulation of selenium via Jen1p conveyor was observed. This phenomenon was explained by the use of carboxylic acids in the absence of preferred carbon sources, e.g. glucose.
Suhajda et al. [2000] conducted experiments in which the influence of culture conditions on the bioavailability of selenium of *Saccharomyces cerevisiae*~~,~~ was evaluated. Based on the obtained results, it was found that the active acidity of the culture environment and the level of dissolved oxygen in the medium~~,~~ were the most important influence factors. In addition, the dynamics of biosorption of selenium by yeasts were~~as~~ influenced by the concentration and the type of this element [Pérez-Corona et al., 2011].

Selenium in an organic form exhibits improved properties in terms of biosorption by yeast cells and is less toxic than its inorganic form [Zhan et al., 2011]. It should be stressed that binding of selenium by yeasts decreases with high contents of sulfur and heavy metals in the culture media. Furthermore, the presence of glucose may cause a reduction of selenium occurring in the form of SeO_3^{2-} ions, which results in the formation of red elemental selenium [Mapelli et al., 2011].
Ponce de Leon et al. [2002] conducted studies where different methods of selenium dosage were used for the experimental culture of *Saccharomyces cerevisiae*. It was shown that the best method to obtain selenium yeasts rich in one of the most favorable organic forms of this element (L-selenomethionine) was to add lower doses of sodium selenite (IV) (from 10 to 50 mg/L) in the early logarithmic growth phase of yeasts [Gharieb and Gadd, 2004]. The highest content of selenium in yeast cells (2354 µg/g) was obtained in the experimental medium supplemented with sodium selenate (IV) at a dose of 50 mg/L [Ponce de Leon et al., 2002].
Yin et al. [2010] showed that optimal parameters for the enrichment of *Saccharomyces cerevisiae* with selenium~~,~~ were~~:~~ temperature of 27.4°C and active acidity (pH) at the level of 5.8.
The process of absorption of selenium by *Saccharomyces uvarum* yeasts was described by Marinescu et al. [2011]. It was found that yeast cultured for 24 hours in malt wort with sodium selenate (IV) at a concentration between 30 and~~-~~ 180 µg/mL~~,~~ bound large amounts of

selenium (625—2215 µg/g). When waste water from the brewing industry wasere used as a culture medium, it was observed that yeasts bound much less selenium (412—1624 µg/g). The authors noted that the significant increase in the accumulation of selenium by yeast biomass was affected by temperature (30°C) and by the addition of selenium to the culture medium during the initial phase of yeast growth.

Demirci and Pometto [1999] reported, that in continuous culture of *Saccharomyces cerevisiae*, the use of sodium selenate (VI) at a dose of 280 mg/L as a source of selenium in the culture medium, caused a reduction in cellular biomass productivity (0.7 g/L). The content of selenium in yeast cell biomass was 687µg/g. As compared to the culture, in which sodium selenate (IV) (690 mg/L) was used, one reported an increase in the content of biomass of yeast cells (1904 µg/g). In addition, the production efficiency of yeast cell biomass was 1.8 g/L.

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In conclusion, it should be stressed that binding of selenium by microbial cells largely depends on the culture conditions, the concentration of selenium in experimental medium and the organisms used. It has an impact on the yield of the biomass and the content of selenium in the cell biomass. The mechanism of transport and bioaccumulation of selenium is associated with the presence of different transport conveyors or the existence of non-specific transport of ions complexed with sugar substrates [Chmielowski and Tyflewska, 2007].

4. Metabolism of selenium by yeast cells

Due to the chemical similarity of such elements as sulfur and selenium [Fujs et al., 2009], microorganisms absorb selenium inside the cell interior most likely by using enzymatic conveyors, i.e. Sul1 and Sul2 sulfate permeases [Mapelli et al., 2012]. It suggests that transport of selenate (VI) is strictly dependent on the presence of sulfate (VI) in the culture medium. In terms of sulfur deficiency, the ability of selenium absorption by microorganisms increases. Studies on the presence of other transport systems, specific for selenium absorption [McDermott et al., 2010], in which phosphate or monocarboxylic acids transport is used, have also been conducted [Lazard et al., 2011]. An enzymatic reduction is observed iIn the first stage of selenium metabolism in yeast cells, its enzymatic reduction is observed (Fig. 2).

Initially, selenate (VI) is converted in an enzymatic process to (APSe) in a reaction catalyzed by ATP sulfurylase. The product formed in the reaction catalyzed by PAPSe reductase is converted into selenate (IV). In the described reaction, NADPH is a reducing agent [Bánszky et al., 2003; Fujs et al., 2009]. In a further step, inside yeast cells, selenate (IV) may be converted into hydrogen selenide via two routes. At first, reduction of selenite (IV) is catalyzed by sulfate reductase using NADPH as a reducing agent.

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The second transformation process of SeO_3^{2-} with the participation of glutathione is slightly more complicated, as selenate (IV) reacts spontaneously with the reduced form of glutathione (GSH). As a result, selenodiglutathione (GS-Se-SG) and the oxidized form of glutathione (GSSG) (Rreaction 1) are formed. The oxidized form of GSSG as a hazardous compound (forms disulfides with thiol-containing proteins and oxidizes them) is transported to the vacuole or converted into a reduced form (GSH) by glutathione reductase [Bukowsko, 2005]. In a further step, intracellular selenodiglutathione is converted into glutathionyselenol (GS-Se-H), and then to hydrogen selenide (H_2Se/HSe^-), with simultaneous formation of the oxidized form of glutathione (glutathione disulfide, GSSG) (reactions 2 and 3).

Volatile compounds of hydrogen selenide can freely pass through the vacuolar membrane into the cytoplasm of the cell via passive transport, according to concentration gradient. As a result of such series of reactions, transport of glutathione disulfide to the vacuole without the

accumulation of selenium is observed [Tarze et al., 2007; Lazard et al., 2011; Mapelli et al., 2011].
Glutathionylselenol can undergo further transformations forming elemental selenium and glutathione in the presence of superoxide dismutase (reaction 4) [Tarze et al., 2007].

The resulting hydrogen selenide is the major intermediate metabolite involved in the synthesis pathway of all forms of selenium occurring in microbial cells (Fig. 3). It is further metabolized forming organic compounds, including seleno amino acids. The first step of the reaction is the biosynthesis of homoselenocysteine. Hydrogen selenide is bound to O-acetylhomoserine involving homocysteine synthase, and in consequence, the formation of selenohomocysteine and acetic acid is observed.
In subsequent steps, selenohomocysteine may be converted into selenocystathionine or selenomethionine. In the first case, selenohomocysteine is bound to serine involving cystathionine β-synthase forming selenocystathionine and water. In the second case, homoselenocysteine is subjected to a methylation process, resulting in ~~a~~the formation of selenomethionine. The reaction is catalyzed by homocysteine methyltransferase [Mapelli et al., 2011; Kitajima and Chiba, 2013].

The resulting selenomethionine, in the presence of oxygen, can be converted to its oxidized form [Pedrero et al., 2007; Schrauzer, 2006]. In the reaction catalyzed by S-*adenozylomethionine* synthase, selenomethionine is converted to Se-*adenosyl-selenomethionine* (SeAM). In the subsequent reaction, SeAM is subjected to enzymatic methylation process, as a result of which, *adenosyl homo-seleno cysteine* (SeAHCys) is released [Arnaudguilhem et al., 2012]. Consequently, the resulting compound undergoes hydrolysis, which results in the formation of selenohomocysteine [Kitajima and Chiba, 2013].
Selenocysteine is formed as a result of the transformation of selenomethionine using cystathionine γ-lyase enzyme. In subsequent reactions, selenocysteine reacts with S-adenosylmethionine (SAM) and may be converted into seleno-methyl-selenocysteine (SeMeCys) and S-adenosil-homo-selenocysteine by selenomethyltransferase (SMT). In the next step of the described transformations, Se-methylselenocysteine is converted into γ-glutamyl-Se-methyl cysteine [Mapelli et al., 2011]. The next stage of the transformation of selenium in yeast cells is the incorporation of selenocysteine into proteins (Fig. 4). Incorporation process is possible through a specific Sec-tRNA^{Sec} complex.
During the first stage, tRNA^{Sec} is an aminoacylated serine, which provides the carbon skeleton for selenocysteine, and thus, Ser-tRNA^{Sec} is formed [Xu et al., 2005]. The reaction requires the energy input supplied by ATP and is catalyzed with the contribution of conventional seryl-tRNA synthetase (SerRS). Next, the entire complex is phosphorylated by O-phosphoseryl-*tRNASec kinase* (PSTK). The next step is to convert Ser-tRNA^{Sec} into Sec-tRNA^{Sec} using monoselenophosphate (H_2PO_3SeH) as a donor of activated selenium.

Next, the resulting Sec-tRNA^{Sec} complex is transported by Secp43 factor to the nucleus, where incorporation of selenocysteine into proteins occurs [Allmang et al., 2009; Turanow et al., 2011]. Selenocysteine is encoded post-transnationally by a specific UGA codon [Rayman, 2004], which also constitutes as a terminal codon [Papp et al., 2007]. During the synthesis of the polypeptide chain, the translational complex recognizes UGA codon thanks to the interaction of *trans* elements~~:~~ —SBP2-binding protein, acting as an elongation factor of EFSec protein, and cis element that is a specific mRNA secondary structure of a characteristic nucleotide sequence known as SECIS.
This sequence is located within a non-coding sequence at 3'-end of mRNA (3'UTR) [Fagegaltier et al., 2000; Suzuki, 2005; Bubenik et al., 2013]. In a further stage, binding of

EFSec-Sec-tRNA^{Sec} to ribosomal initiation complex via interaction with SBP2-binding protein, L30 and the SECIS structure is observed [Papp et al., 2007]. Secp43 is responsible for the formation and stabilization of the entire protein complex [Papp et al., 2007; Squires and Berry, 2008; Bifano et al., 2013].
The t~~T~~ranslation complex, formed ~~during~~while reading the information contained in mRNA, reads the nucleotide sequence, and then translates it into an amino acid sequence (Fig. 5). After reading UGA codon, Sec-tRNA^{Sec} is directed to the acceptor site on a ribosome and thus a process of synthesizing selenium proteins occurs [Driscoll and Copeland, 2003].

Enrichment of yeast with selenium is a consequence of the formation of many different selenium proteins. Selenomethionine is the basic form of selenium in yeast cells. Many authors, in their publications on selenomethionine determination [Schrauzer, 2000; Tapiero et al., 2003; Gharieb and Gadd, 2004; Perucchietti et al., 2012; Rayman, 2012], emphasize~~,~~ that it may constitute up to 90% of the total content of selenium in yeast cells. Selenomethionine is the most absorbable form of selenium in human and animal organisms. It exhibits antioxidant properties, improves the immunity of an organism~~,~~ and stimulates the activity of DNA repair enzymes [Laffon et al., 2010].

Selenomethionine is non~~-~~specifically incorporated into proteins instead of methionine [Dumont et al., 2006; Letavayová et al., 2006]. Moreover, it is effectively stored in tissues [Tapiero et al., 2003; Rayman, 2008]. Among other selenium compounds found in yeast cells, one can include ~~among others :~~ selenocysteine, selenocystationine, Se-methylselenocysteine~~,~~ and γ-glutamyl-Se-methylselenocysteine [Schrauzer, 2000]. During ~~the progress of~~ research ~~in~~on ~~terms of~~ the identification of selenium proteins occurring in yeast cell biomass, more and more attention is being paid to ~~the~~ anti~~-~~cancer compounds, ~~including~~ e.g. Se-methylselenocysteine.

In comparison to the other forms of selenium occurring in yeast cells, Se-methylselenocysteine exhibits strong anti~~-~~cancer properties. In humans and animals, Se-methylselenocysteine is converted into a compound methylselenol (CH_3SeH) characterized by the highest anti~~-~~cancer activity~~ methylselenol (CH_3SeH)~~. Another example of an organic selenium compound occurring in yeast~~,~~ is selenocysteine. It is widely considered as the 21st natural amino acid [Rayman, 2008; Bubenik et al., 2013]. It is involved in the biosynthesis of selenium proteins [Allmang and King, 2012].

Metabolism of selenium in yeast cells is a very complex process~~es~~. A careful analysis of the forms and transformations ~~to~~ which selenium compounds are subjected to in yeast~~,~~ will allow for a better understanding of its bioaccumulation and speciation. There is also ~~a~~the possibility ~~to use~~of using selenium-enriched yeast biomass to ~~obtain~~produce protein and mineral preparations ~~in order to fulfill~~to be used as supplements to overcome deficiencies of this element in the diet.