Полученные данные подвергнуты регрессионному анализу для определения линейных и квадратичных коэффициентов регрессии. На основании полученных значений при помощи математического моделирования рассчитывался экстремум по критерию оптимальности и определялись значения концентраций исследуемых компонентов среды, соответствующие данной точке функции.

В ходе работ составлена питательная среда МД-2 соответствующая следующей прописи: этанол — 20 г/л; калий фосфорнокислый однозамещенный — 13,5 г/л; аммоний сернокислый — 3,8 г/л; магний сернокислый — 1,0 г/л; тиамин — 0,004 г/л; биотин — 0,01г/л; кальций хлористый — 0,12 г/л; дрожжевой автолизат — 25,0 мл/л.

Для проверки степени оптимизации среды был проведен ряд режимов культивирования дрожжей *Candida tropicalis* шт. СК-4 в лабораторном ферментере «Biostat» B 10 L на средах МД и МД-2. Режим ферментации: температура 28-29 °С; аэрация — 1:1; скорость вращения мешалки — 300 мин$^{-1}$; поддержание уровня pH проводилось в пределах 4,5 — 5,0. Культивирование продолжалось до полного исчерпания углеродного субстрата.

В ходе работ установлено, что на среде МД средняя максимальная концентрация сухой биомассы — 12,07 г/л достигается к 65 ч культивирования. При культивировании дрожжей на среде МД-2 максимальное значение по концентрации биомассы достигается на 36 ч роста и составляет в среднем 12,12 г/л.

Таким образом, используя математический подход к оптимизации состава питательной среды для культивирования дрожжей *Candida tropicalis* шт. СК-4, удалось за короткий срок получить среду, позволяющую в процессе культивирования в лабораторном биореакторе при прочих равных условиях сократить продолжительность культивирования с 65 до 36 ч.

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Түйін

2^4 толық факторлы тәжірибеде *Candida tropicalis* жем ашытқыларды өсіру үшін минералды-ашытқылық қоректендіргіш ортаның құрамының оңтайландыруы өткізілді.

Resume

The optimization of mineral-yeast medium for the cultivation of fodder yeast *Candida tropicalis* in a full factorial experiment 2^4.

Bassam R[1]., Artmann G.M[1]., Hescheler J[2]., Graef T[1]., Temiz Artmann[1] A., Porst D[1]., Linder P[1]., Kayser P[1]., Arinkin V[1]., Gossmann M[1]., Digel I[1].

ALTERATIONS IN HUMAN HEMOGLOBIN STRUCTURE RELATED TO RED BLOOD CELL STORAGE

[1] *Institute of Bioengineering (IFB), FH Aachen University of Applied Sciences, Germany,*
bassam@fh-aachen.de,
[2] *Institute of Neurophysiology, University of Cologne, Germany*

The importance of the availability of stored blood or blood cells, respectively, for urgent transfusion cannot be overestimated. Nowadays, blood storage becomes even more important since blood products are used for epidemiological studies, bio-technical research or banked for transfusion purposes. Thus blood samples must not only be processed, stored, and shipped to preserve their efficacy and safety, but also all parameters of storage must be recorded and reported for Quality Assurance. Therefore, blood banks and clinical research facilities are seeking more accurate, automated means for blood storage and blood processing.

Routine blood storage is limited to 21 days at 1°-6°C when treated with acid-citrate-dextrose (ACD), heparin, etc. and 35 days when treated with citrate-phosphate-dextrose-adenine (CPDA1) and involves refrigeration but usually not freezing. There has been increasing controversy about whether the age of blood is a factor in transfusion efficacy, specifically on whether older blood directly or indirectly increases risks of complications. Obviously, during storage the composition of blood samples is subjected to various changes and, without any doubt, deeper understanding of such processes is of critical importance for future improvements in biomedical practice. In this work we have made an attempt to address some molecular processes related to the ageing of stored blood samples.

One primary function of red blood cells (RBCs) is to transport oxygen from the lungs to metabolizing tissues /1, 2/. To maintain adequate perfusion of microvascular networks, RBCs must be able to adequately deform at the physiological hematocrits, within a wide range of pressures, flow conditions and in vessel diameters ranging from 3-8 μm (capillaries) to 50-100 μm (arterioles and venules). Maintaining an appropriate "deformability" is, therefore, crucial for RBC physiological function /3, 4, 7/. As we have demonstrated elsewhere /8, 29/ hemoglobin contributes to this process significantly.

In addition to transporting O_2, RBCs can sense the state of local tissue oxygenation and adjust the rate of O_2 delivery by regulating the local blood flow in microvascular networks /5, 6, 9, 10/ via three possible nitric oxide-mediated mechanisms: (i) release of adenosine triphosphate (ATP) to stimulate production of NO by the endothelial cells lining the walls of the vessels /11/; (ii) release of NO from S-nitroso- (SNO) Hb upon deoxygenation of Hb /12/; and (iii) reduction of nitrite (NO2-) present in the blood stream to NO by deoxyhemoglobin /13, 14/. Moreover, there is a large group of specialized enzymes, peroxiredoxins (Prxs), working together with hemoglobin in an RBC, but their particular role yet needs to be understood /15/.

It is, in fact, the hemoglobin molecule that is mostly responsible for RBC functions as well as for its mechanical and chemical characteristics. Biochemically, hemoglobin consists of a protein component comprising two α- and β-polypeptide chains, and a prosthetic heme group that reversibly binds one oxygen molecule. The redox potential of Hb, its affinity, its dynamics are all sensitive to the globin alterations surrounding the heme /20/. Such alterations usually occur in the Hb molecule as a result of its binding with a broad class of low-molecular weight modifiers usually present in erythrocytes or somewhere else in the blood. Such molecules, indubitably or potentially able of controlling Hb (and hence RBCs) function are for example ATP, 2, 3-diphosphoglycerate (2,3-DPG), phosphatidylinositol 4,5-bisphosphate (PIP2), NO, CO_2 and probably many others.

The exact nature and mechanisms of such interactions remain still obscure in spite of intensive studies of hemoglobin and its derivates performed in the past. Many existing facts are still waiting to be systematized and interpreted in terms of molecular biophysics.

During RBC storage in additive solutions, distinct changes in the cytosolic concentration of Hb-mediator molecules occur. For instance, the level of ATP increases early in storage, peaks after about two weeks, but then gradually declines to below 50% by week 6 of storage /15/. In turn, the 2, 3-diphosphoglycerate (2, 3-DPG) levels decline rapidly over the first week of storage, falling to undetectable levels by the end of the week /18/. The importance of organic phosphates, such as 2, 3-diphosphoglycerate /21, 22/ and adenosine- 5'-triphosphate (ATP) /22/, in allosteric control of Hb is well recognized. Adenosine- 5'-triphosphate (ATP) is an important intra-erythrocytic organic phosphate in vivo.

In red blood cells, the concentration of ATP is around 0.2-2 mM and its variations induce a pH-dependent tetramerization of deoxyHb in vertebrates /25/. The change in the concentration of ATP in red blood cell results in modulation of Hb oxygen affinity /24/. The loss of ATP may also diminish the ability of transfused RBC to affect NO-mediated arteriole vasodilatation in response to hypoxia /17/. Diphosphoglycerate and its derivates are also important players in RBCs metabolism. Because of the loss of 2, 3-DPG, stored RBC release O_2 to the tissues less readily than normal cells. After transfusion, however, 2, 3-DPG is rapidly resynthesised to 50% of the normal level in as little as 7 hours, and to 95% of the normal level in 2-3 days /19/.

In addition to organic phosphates, some other heterotropic effectors, such nitric oxide, carbon dioxide and others, although bound spatially at remote sites, are capable of influencing the oxygenation process and have been shown to affect the oxidation process as well /23/ but the exact pathways involved are not known so far.

Thus, there are still many unanswered questions with regard to how exactly ATP, 2,3-DPG and other modulators affect Hb. Some proteins bind ATP by a characteristic protein fold known as the Rossmann fold, which is a general nucleotide-binding structural domain that can also bind NADH /27/. The largest kinase super family, protein kinases, are most common ATP-binding proteins sharing common structural features specialized for ATP binding and phosphate transfer /28/. Yet, recent observations have suggested that even the proteins presumably not reacting with ATP can change their properties if ATP is present /28/. For example, the change in the concentration of ATP in RBCs results in modulation of Hb oxygen affinity, where Hb become able to accomplish oxygen transport, oxygen storage and electron transfer reaction in polar environment.

Our dynamic light scattering (DLS) studies on change of hemoglobin dynamics at different concentrations of ATP have shown that the hydrodynamic radii of Hb samples prepared in ATP-containing phosphate buffer saline (PBS) and ATP- potassium- based analog buffer (also referred to as CD-buffer), varied in the range 3 ± 1 nm and 4 ± 1 nm respectively, whereas the hydrodynamic radii of the samples prepared in PBS and CD buffers without ATP amounted in 6 ± 1 nm and 5 ± 1 nm respectively. These data suggest that in both cases ATP induces either slight unfolding or facilitated aggregation of Hb molecules. Further circular dichroism (CD) measurements have supported this point of view by demonstrating that ATP causes distinct sodium-dependent unfolding of the Hb secondary structure by 15 mDeg at room temperature and 222 nm wavelength.

Another experiment to mention here that seems to complement and extend our results was performed by protein film voltammetry technique (PFV) on the introduction of ATP to Hb. In that case, a noticeable positive shift of the cathode peak was observed. A consequent addition of ATP up to 3 mM concentration lead to the continuous positive peak shift. The reported effect of DPG is much stronger than that of ATP. These data can be interpreted as ATP's allosteric effect on Hb with its phosphate part as an effector leading to stabilization of the reduced state of Hb in the physiological concentration range /19/.

Additional measurements with temperature variation also showed evident effects of ATP on the denaturation temperature of hemoglobin samples. The samples prepared in PBS and CD buffer with ATP showed first signs of denaturation at 58°C and 57°C respectively, whereas those prepared without ATP in both buffers appeared to aggregate at 54 °C. The unifying explanation of the particular character of the changes observed in our experiments is still to be developed. One possible direction can be that at physiological pH, ATP possesses four negative charges, which implicates that the effect of ATP on Hb may be similar to that of anionic heterotropic effectors. It has been traditionally explained that anionic heterotropic effectors operate on Hb as inhibitors with the formation of salt bridge between effectors and Hb, and preferentially bind to the low oxygen affinity quaternary conformation /24/, although a recent study has shown no significant binding sites of Hb to some anions /26/. Future studies using differential scanning calorimetry and NMR spectroscopy will probably shed more light on the thermodynamic and kinetic nature of the changes in Hb molecules related to RBC storage.

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Preiß C[1]., LinderP[1]., Wendt K[1]., Krystek M[1]., Digel I[2]., Gossmann M[3]., Artmann[3]A.T., Porst D[3]., Kayser P[3]., Bassam R[2]., Artmann G.M[1].

ENGINEERING TECHNOLOGY FOR PLANT PHYSIOLOGY AND PLANT STRESS RESEARCH

Institute of Bioengineering (IFB), FH Aachen University of Applied Sciences, [1]Laboratory for Cellular Biophysics, [2]Laboratory of Cell- and Microbiology, and [3] Laboratory of Medical & Molecular Biology, Juelich, Germany, Linder@fh-aachen.de

Plant physiology and plant stress: Plant physiology will be much more important for human mankind because of yield and cultivation limits of crops determined by their resistance to stress. To assess and counteract various stress factors it is necessary to conduct plant research to gain information and results on plant physiology. Especially for agriculture this is of great significance, because stress is very harmful to plants resulting in reduction of biomass production of crops. Stress