3. SUMMARY

RF (vitamin B_2) is an essential component of the diet of humans and animals, serves as a precursor to coenzymes: flavin mononucleotide (FMN) and adenine dinucleotide (FAD), which are involved in the metabolism and other processes in cells. Commercially produced vitamin B_2 is used in animal feed as a dietary supplement and feed ingredient, a food additive in the food industry and for the treatment and prophylaxis of a variety of diseases. The global market for RF production stands at 9 000 tonnes per year with turnover of 150 - 500 million depending on the price of RF per kilogram. Almost to the end of the twentieth century. RF were obtained mainly by chemical synthesis. In the last 25 years there has been strong development of microbial synthesis of RF. The main microorganisms used for industrial production of RF bacteria *B. subtilis*, fungus *A. gossypii* and the yeast *C. famata*. . Dep8 strain of yeast *C. famata* was used for the industrial production of RF by ADM (USA), but due to the instability of the RF synthesis abandoned his participation. The aim of this study was to optimize the RF synthesis by recombinant strains of the yeast *C. famata* it included: construction and selection of the best producer of RF from the resulting transformants, developing optimal production and cultivation conditions for selected strains, evaluation of RF synthesis rate by yeast *C. famata* in different substrates and variable culture conditions, perform batch culture of "batch" and "fed-batch" in flasks and bioreactors with a volume of 1.3 L and 7.0 L. In this study used two strains of yeast *C. famata*: strain # 3 (Dmytruk *et al.*, 2011) and constructed during this work strain # 91. The results showed that both strains contain two copies of the gene *SEF1* derived from yeasts *Debaryomyes hansenii*, but they different in specific activity of enzymes RF synthesis. The specific activity of GTP cyclohydrolase II and RF synthase strain # 91 was about 1.3 times higher than in strain # 3 (Dmytruk et al., 2011). These results confirm the hypothesis that the level of synthesized RF is associated with the specific enzymes involved in the synthesis of RF. Through statistical analysis Placket-Burman design, and performed experiments it was found that the most important for the RF synthesis components was: glucose, yeast extract, $MnCl_2$, $CaCl_2$ and $K_2Cr_2O_7$. The study showed that the resulting optimal culture medium Op5m for yeast *C. famata* significantly increases the level of RF synthesis. For the next experiments selected strain # 91 yeast *C. famata.* For achieve the maximum synthesis of RF using the "fed-batch" cultivation by strain # 91 in 1.3 L and 7.0 L fermenters in the Op5m medium and optimize culture conditions. After 48 hours added to the cultures 10 - fold concentrate Op5m medium and 50% glucose solution to cultivation medium and an aerated substrate with a mixture of air enriched with pure oxygen. # 91 yeast strain *C. famata* reached

the highest RF synthesis parameters during the culture in a bioreactor capacity. 7.0 L. The concentration of the RF in the culture medium was 16.8 g / L after 136 h process yield synthesis RF level RF 185.3 mg / g biomass, the maximum biomass was 90.8 g / L after 124 h of incubation. With respect to the culture in a fermenter with capacity. 1.3 L concentration RF synthesis increased 60%. During the same cultivation in 1.3 L bioreactor the concentration of the RF was 10.2 g / L with a maximum biomass to 78.1 g / L, the yield of the RF synthesis was 130.1 mg / g biomass. The strategy to optimize culture conditions developed in this study resulted in increased production of RF to the level of 16.8 g / L, as compared to the parental strain AF-4 (Dmytruk *et al.*, 2011) during fed-batch fermentation in the 7.0 L bioreactor. During the fermentation of 1.3 L and 7.0 L bioreactors by fed-batch RF yield of the synthesis was 2.2 and 1.6-fold lower than in the batch culture in shake flasks. The results indicate that further optimization of the composition of the media, fermentation conditions, as well as a well-controlled power system will be relevant to further increase the synthesis of RF by the strain # 91. The level of saturation of the substrate oxygen (DO) is a factor that could have a significant effect on cell growth and the production of desired metabolites. In this study demonstrated that the concentration of oxygen in the substrate had a significant impact on the synthesis of RF strain # 91 yeast *C. famata*, as it appears advisable to further optimization of culture conditions. The determination of the amount of pure oxygen aeration added to a mixture of oxygen / air and its influences on the synthesis of RF strain # 91. Developed in this paper # 91 yeast strain *C. famata*, may prove to be efficient industrial manufacturer of RF and contribute to the development of biotechnology vitamins in the world. The results indicate the advisability of further continuation of research into production RF by the yeast *Candida famata*, particularly in terms of methods for the genetic improvement of strains, testing the activity of key enzymes responsible for this biosynthesis, breeding systems optimization and scale up production.