SOLUBILIZER OF BACTERIAL ORIGIN SURFACTIN INCREASES THE BIOLOGICAL ACTIVITY OF C60 FULLERENE

Sergey Emelyantsev [1*], Evgeniya Prazdnova[1], Vladimir Chistyakov[1]

[1] Academy of Biology and Biotechnologies, Southern Federal University, 344090 Rostov-on-Don, Russia

*To whom correspondence should be addressed. Email: emelyancev@sfedu.ru

Abstract

Currently, there exists conflicting data regarding the biological activity of unmodified fullerene C_{60}. Various sources report its toxicity, geroprotective activity, and potential interaction with DNA. Contradictory findings regarding the toxicity of C_{60} may arise from the use of toxic solvents, as well as the influence of bioavailability and bioactivity on the preparation conditions of C_{60} suspensions. Furthermore, the microbiota of experimental animals can impact geroprotective activity results by releasing surfactants that facilitate substance penetration through the cell membrane.

In this study, we selected conditions for solubilizing fullerene C_{60} in a solution of surfactin, a surfactant of bacterial origin, as well as in a 2% aqueous solution of TWEEN 80, employing ultrasound. Through bioluminescent analysis using lux biosensors in E. coli MG1655, we observed that C_{60} in surfactin reduced induced genotoxic and oxidative stress. Given that surfactin enhances membrane permeability to fullerene C_{60}, suspensions of fullerene in designated concentrations of surfactin can be regarded as a DNA protector and antioxidant, warranting further investigation as a promising component of novel drugs.

Keywords: fullerene C60, surfactin, antioxidant effects, genotoxicity, *Escherichia coli*, bioluminescence assay.

1. Introduction

Fullerenes represent the fourth allotropic modification of carbon, following diamond, graphite, and carbyne. Among the various types, the most stable and symmetrically synthesized fullerene is C_{60}, commonly referred to as buckminsterfullerene. This fullerene adopts a polyhedron structure, resembling a closed sphere with 12 pentagons and 20 hexagons, with carbon atoms occupying the vertices.

In solutions, fullerenes tend to form clusters composed of multiple molecules. While $C6_0$ is insoluble in polar solvents, it exhibits slight solubility in acyclic hydrocarbons. The highest solubility has been observed in aromatic solvents and their derivatives such as benzene, toluene, chlorobenzene, dichlorobenzene, as well as in carbon disulfide (1). However, the listed substances are slightly soluble in water and toxic to living cells. Thus, to prepare biocompatible aqueous suspensions of fullerenes, it is necessary to use non-toxic surfactants.

An important chemical property of the C_{60} molecule is the presence of 30 double bonds, which ensures reactivity towards free radicals. In particular, 1 molecule of $_{C60}$ can attach up to 34 photochemically formed methyl radicals (2).

C_{60} genotoxicity studies show conflicting results and have methodological limitations due to the use of toxic solvents. According to (3), water-soluble nano-C_{60}, obtained by dissolution in tetrahydrofuran (THF) followed by evaporation, is cytotoxic to cell cultures of human dermal fibroblasts, liver carcinoma and neuronal astrocytes due to ROS-mediated lipid peroxidation, without affecting DNA concentration and mitochondrial activity. However, in study (4) oral administration of a C_{60} derivative, carboxyfullerene $C_{60}(C(COOH_2))_3$, to mice from 12 months of age increased their lifespan by 11% and prevented the age-associated increase in superoxide radical generation in 24-month-old mice. mitochondria of the brain.

In (5) intraperitoneal injections of C_{60} reduced ROS formation and concentrations of antioxidant enzymes (GSH , CAT , and GPx) in rats after muscle fatigue.

Results of a study (6) on biosensors *E. coli* and *S. cerevisiae* with green fluorescent protein (GFP) – a reporter of gene expression, as well as measurement of the expression of genes associated with DNA repair in human lung cell culture A549 using RT-qPCR, micronuclear test and DNA comet method showed C_{60} genotoxicity , while the Ames test gave negative results. According to the results (7) C_{60} did not affect the frequency of mutagenesis in the cII locus of mouse pulmonary epithelial cells of the FE1-MutaTM Mouse line, the number of DNA strand breaks, but increased the level of (FPG)-sensitive sites by 1.22 times. According to (8), a mixture of fullerenes C_{60} and C_{70} does not have a mutagenic effect in the Ames test, does not induce chromosomal aberrations in Chinese hamster cell culture, and does not cause acute toxicity (dose 2000 mg/kg) in vivo in rats.

According to the results of the article (9) genotoxicity assessment using the DNA comet method showed that concentrations of 10-100 μM polyhydroxylated fullerene $C_{60}(OH)_{24}$ have a moderate dose-dependent genotoxic effect.

The results of another study (10)of DNA damage in human lymphocytes using the DNA comet method showed that the compound $C_{60}[C(COOH)_2]_3$ is not genotoxic to *Homo sapiens* lymphocytes.

In 2013, we (11) put forward a hypothesis about the ability of fullerene to be a proton carrier. The hypothesis suggested that fullerene can absorb protons and acquire a positive charge, which allows it to penetrate mitochondria. Thus, the formation of superoxide anion radical is reduced due to the gentle uncoupling of respiration and phosphorylation. This mechanism has been shown for a number of other antioxidants (12). Theoretical modeling using the DFT method showed that such a mechanism has a right to exist (13).

In 2012, an article by Baati et al. (14), which described the geroprotective properties of fullerenes. The study shows that oral administration of C_{60} dissolved in olive oil (0.8 mg/ml) in regular doses (1.7 mg/kg body weight) to rats not only does not entail chronic intoxication, but it almost doubles their lifespan effects on lifespan are mainly due to the attenuation of age-related increases in oxidative stress. Pharmacokinetic studies have shown that dissolved C_{60} is absorbed in the gastrointestinal tract and excreted within several tens of hours. The effect of unmodified $C_{60,}$ dissolved without the use of toxic surfactants, on life expectancy emphasizes the lack of chronic toxicity.

However, in a study (15) conducted on mice, oral administration and intraperitoneal injections of $C_{60\ in}$ olive oil did not significantly increase lifespan. We believe that one of the reasons for the lack of effect of $C_{60\ in}$ olive oil on the life expectancy and health of mice in the study (15) may be differences in the microbiota and a lower level of production of surfactin (or other biosurfactants) compared to the intestinal microflora of rats in the experiment (14).

Surfactin (chemical formula $C_{53}H_{93}N_7O_{13}$) is a bacterial cyclic lipopeptide produced by a non-ribosomal peptide synthetase, surfactin synthetase. In particular, it is produced by the gram-positive bacteria *Bacillus subtilis* (16–18). The structure is a long chain of β-hydroxy fatty acid, the number of backbone carbon atoms ranging from 13 to 15, linked to a peptide loop of 7 amino acid residues. The most common sequence of this heptapeptide is Glu-Leu-Leu-Val-Asp-Leu-Leu, comprised of the hydrophilic amino acid residues aspartate (Asp) and glutamate (Glu), and the hydrophobic amino acids valine (Val) and leucine (Leu) (19). Surfactin exhibits antifungal, antiviral, antibacterial properties; for *E. coli* minimum inhibitory concentration is 3.125 g/l (20), is a strong surfactant (21)is able to reduce the surface tension of water from 72 to 27 mN/m at the critical micelle concentration (CMC) , defined as “about 10 mg/L” by the authors (22). Surfactin has a non-specific mechanism of biological action, increasing membrane permeability due to the detergent effect exerted in

concentrations from 12 to 50 μg/ml (23), which may be a consequence of the fact that the CMC of surfactin, depending on the method of its preparation, is in the range of 13-17 μg/ml (24).

In nanotechnology, surfactin has proven to be a powerful stabilizer of nanoparticle suspensions. In a study (25), it was found that silver nanoparticles, synthesized through the reduction of AgNO3 with sodium borohydride in the presence of surfactin at a concentration of 25 mg/L derived from B. subtilis (BBK006), exhibited prolonged stability for a period of 2 months. Conversely, the control experiment without surfactin witnessed rapid aggregation of the nanoparticles, resulting in the formation of large aggregates. The authors suggest that imide groups with a lone pair of electrons may play a role in the stabilization of nanoparticles, but do not exclude the stabilization of nanoparticles due to the micellar structure of the surfactin solution. Another study (26) reported the stabilization of nanoparticles through the presence of functional amino groups.

The authors (27) confirmed the possibility of using surfactin to increase the absorption of insulin in the intestine when taken orally. The use of surfactin as a surfactant replacement will reduce environmental pollution from toxic chemically synthesized solubilizers (19).

The aim of this research was to investigate the impact of C_{60} fullerene on the expression of genes linked with SOS response and antioxidant defense in an *E. coli* model, and to further examine the influence of the surfactin solubilizer on these effects.

2. Materials and methods

Unmodified C60 fullerene from Sigma-Aldrich, St. Louis, USA, was utilized in the experiments. Inducers of oxidative and genotoxic stress included paraquat (1,1'-dimethyl-4,4'-dipyridylium dichloride) supplied by Sigma-Aldrich, hydrogen peroxide (JSC "Ekos-1") with

special purity grade, and 1,4-dioxide of 2,3-quinoxalindimethanol (dioxidine) obtained from PJSC "Biosintez.".

Preparation of aqueous solutions of C_{60}

Fullerene C_{60} was added to solutions of urfactin 312.5 mg/l and TWEEN 80 2 % in deionized water and sonicated with an ultrasonic dismembrator Sonics Vibra -Cell VCX 130 at a power of 30 W pulses 5/5 s for 10 min with cooling in a refrigerant to prevent boiling of solubilizer solutions and to prevent a decrease in the solubility of fullerene in them due to heating solutions above 320 K (28). The concentration of dissolved C60 fullerenes was determined by the optical density of the solution measured using a BECKMAN COULTER DU ® 800 UV/ Vis spectrophotometer relative to solutions of the corresponding solvents.

Bioluminescence test

The study of the biological properties of fullerene C_{60} was carried out on a model biological object - *Escherichia coli*.

Bioluminescent tests were performed to study antioxidant and DNA protective activities. Bioluminescent strains *of E. coli* MG 1655 carry plasmids with reporter genes *lux-CDABE*, placed under the control of promoters of oxidative and genotoxic stress and the ampicillin resistance gene *bla*. To study the biological effect of fullerene C_{60} under conditions of genotoxic stress , strains of *E. coli* MG 1655 with SOS -inducible promoters PrecA and PcolD, which detect the penetration of DNA-damaging agents into the cell, for studying oxidative stress - with promoters PkatG and PsoxS , induced by peroxides and superoxide anion radical, respectively (29). The ColD promoter has a higher induction coefficient compared to pRecA due to the lower openness of the SOS promoter *Pcda* in the absence of stress. In addition to SOS regulation by the lexA repressor, expression of the Colicin D operon is controlled by a repressor (cdr), cotranscribed with cda, but terminated earlier and preventing overproduction of colicin D in the absence of stress (30).

Dioxidine was used as an inducer of genotoxic stress at a concentration of 2.25×10^{-5} M, an antibacterial drug with a DNA-damaging mechanism of action due to the generation of reactive oxygen species, including superoxide (31) and, possibly, hydrogen peroxide (32); inducers of oxidative stress - hydrogen peroxide 10^{-3} M and paraquat 10^{-3} M, under aerobic conditions causing the formation of superoxide radical O^{2-} (33).

Strains were grown in liquid Luria- Bertani (LB) medium supplemented with 100 μg/ml ampicillin (34). The overnight culture, depending on the strain, was diluted with fresh LB with ampicillin to a density of 0.01-0.1 McFarland units, the culture density was measured using a DEN-1B densitometer (Biosan), then grown in a thermostat at 37 ºC 1.5 h, after which 80 μl aliquots of this culture were transferred into sterile microplate cells. The control cells were cells with only solubilizers added to the culture - surfactin or TWEEN 80 (2%) (solvent control). After adding surfactin, TWEEN 80 and fullerene solutions were further incubated for 30 min in a 96-well microplate in a thermostat at a temperature of 37 ± 0.2 °C. Then inductors were added to the cells of the tablet: hydrogen peroxide and paraquat at a concentration of 10^{-3} M; dioxidine 2.25×10^{-5} M.

The microplate was placed in the FLUOstar multimodal reader Omega and incubated at 37 ºC. The bioluminescence intensity was measured every 10 min.

SOS response induction factor (I) was calculated using the formula:

$$I = \frac{L_e}{L_c} - 1 \qquad (2.1),$$

where: L_e – luminescence intensity of the sample with the inductor (in arbitrary units); L_c – luminescence intensity of the control sample (in arbitrary units).

Protective activity was calculated as

$$P = \left(1 - \frac{I_f}{I_c}\right) \times 100\% \qquad (2.2),$$

where I_f and I_c are induction factors of the surfactine solution with and without added fullerene, respectively.

Each experiment was conducted at least three times with six replicates, and statistical analysis was conducted using Student's t-test. Confidence intervals were calculated using MICROSOFT EXCEL software (Microsoft Corporation, Redmond, WA) with a significance level of P = 0.05.

Measuring the turbidity of bacterial cultures using a spectrophotometer

To determine the effect of a range of surfactin concentrations on the growth curves of *E. coli,* overnight cultures of bioluminescent strains were grown as for the bioluminescent test, then aliquoted in 80 µl quantities into sterile microplate wells. Solutions of surfactin and dioxidine were added in a volume of 10 µl immediately before the experiment. Measurement of the optical density of the culture at a wavelength of 600 nm was carried out using a FLUOstar device Omega every 10 min at a temperature of 37 ± 0.2 °C.

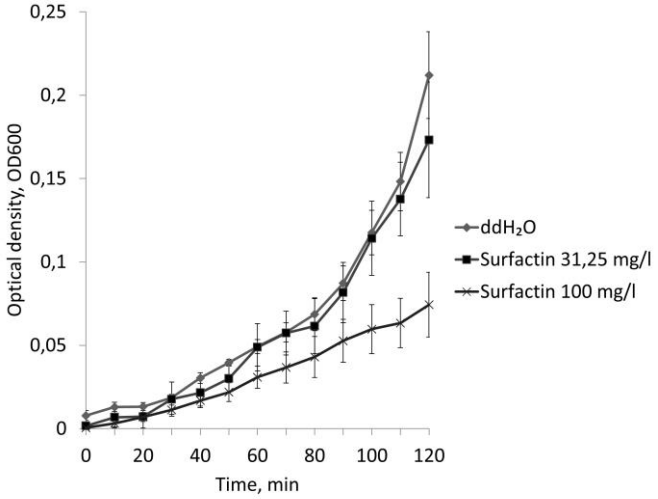
RESULTS

Selection of the optimal biocompatible concentration of surfactin for use as a surfactant in bioluminescent tests

To determine the biocompatible concentration of surfactin, which does not affect the growth rate of bacterial biosensors, as well as the influence of the range of surfactin concentrations in experiments with genotoxic substances, the optical density at a wavelength of 600 nm of the bioluminescent strain *E. coli* MG 1655 pRecA was measured. All surfactin concentrations used in this experiment are above the critical micelle concentration (0.017 g/L) and below the minimum inhibitory concentration, 3.125 g/L (20). In the absence of dioxidin, surfactin concentrations of 31.25 mg/l and 50 mg/l (data not shown) have no effect on the growth rate, while 100 mg/l statistically significantly reduces the growth rate by 1.5–2 times (figure 1, A). In the presence of dioxidin $2.25 \cdot 10^{-5}$ M, surfactin concentrations of 31.25 mg/l and 50 mg/l had no effect on the growth rate; a 100 mg/L surfactin concentration

supplemented with dioxidine reduced growth rates, possibly due to a greater increase in membrane and cell wall permeability to dioxidine (Figure 1B).

A



B

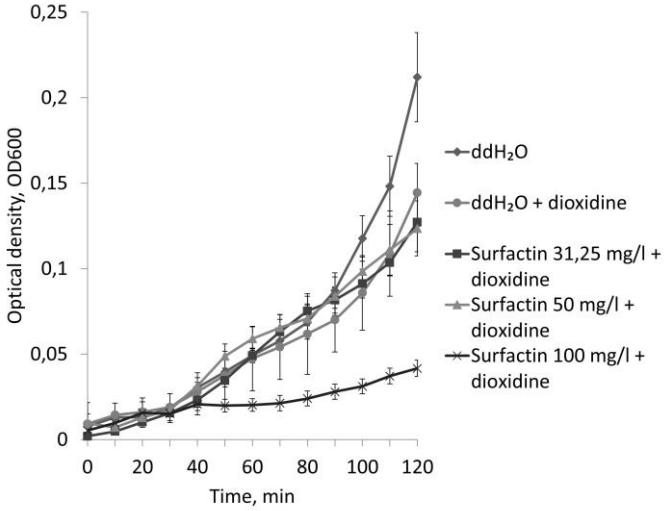


Figure 1 – Effect of surfactin on the growth of the biosensor strain *E. coli* MG 1655 pRecA in the absence (A) and presence (B) of dioxidine.

Surfactin concentrations of 50 mg/l increased the induction of bioluminescence relative to dioxidine. Thus, increasing the concentration of surfactin within the biocompatible range does not have a protective effect, but enhances the genotoxic effect of dioxidine (Figure 2).

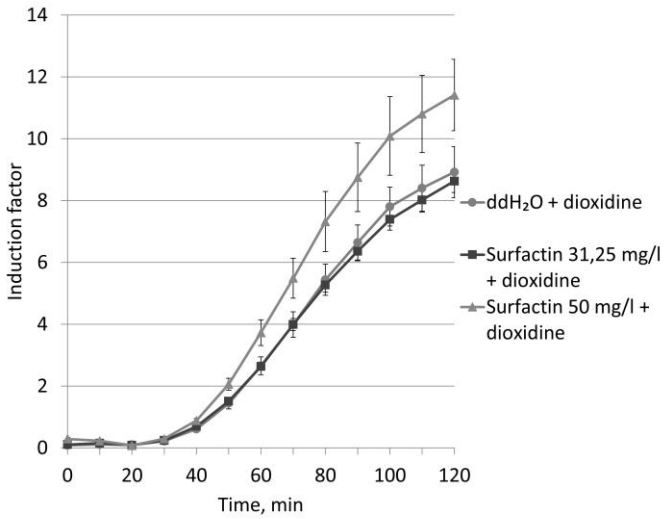


Figure 2. Effect of surfactin on the induction of bioluminescence by dioxidine of the biosensor *strain E. coli* MG 1655 pRecA - lux .

Based on the data obtained, for use as a solubilizer in experiments with toxic substances, it is sufficient to use a surfactin concentration of 31.25 mg/l, for the greatest increase in membrane permeability in the absence of toxicants for nanoparticles and drugs without reducing the growth rate - 50 mg/l. Surfactin reaches the micellization plateau at a concentration of 0.28 mM (290.2 mg/L) (36).

Biological activity of solutions C_{60}

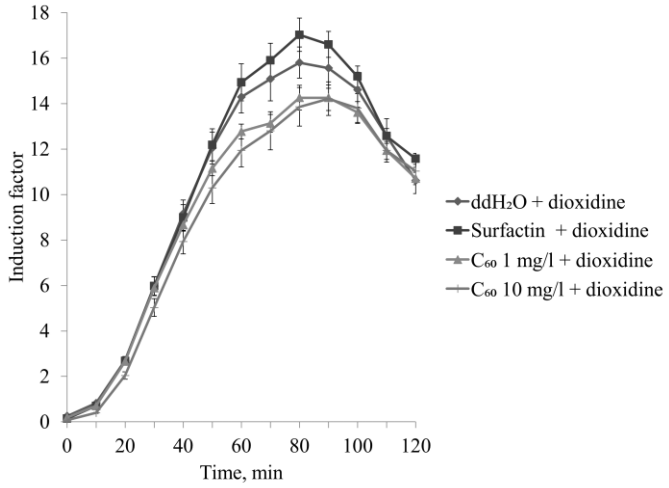
For preliminary screening, C_{60} concentrations of 0.01 – 10 mg/l in TWEEN 80 (2%) and surfactin 31.25 mg/l were tested on the *E. coli* MG1655 pRecA biosensor. All tested concentrations of C_{60} in TWEEN 80 (2%) and concentrations 0.01 and 10 mg/L in surfactin had no effect on the induction factor of bioluminescence relative to the solvent control. In addition, it turned out that TWEEN 80 reduced induction factor relative to the positive control (maximum induction factor with dioxidine 20.4, while the addition of TWEEN 80 or fullerene solutions reduced the maximum induction to 12.09-13.15) (data not shown).

The surfactin concentration of 31.25 mg/l used for fullerene solubilization had no effect on the induction of bioluminescence of the biosensor strain *E. coli* MG1655 (pRecA),

p <0.05 with dioxidine relative to deionized water (ddH_2O), and subsequently the effect of C_{60} was assessed relative to this concentration (solvent control).

Solutions of C_{60} at concentrations of 1 – 10 mg/l in surfactin caused a statistically significant decrease in induction factor relative to the solvent control (Figure 3A) (induction graphs with C60 concentrations of 2.5 – 7.5·mg/l are not shown), thus demonstrating a DNA protective effect. It was found that the DNA protective effect in the concentration range from 1 to 10 mg/L in surfactin has no dose dependence (Figure 3B).

A



B

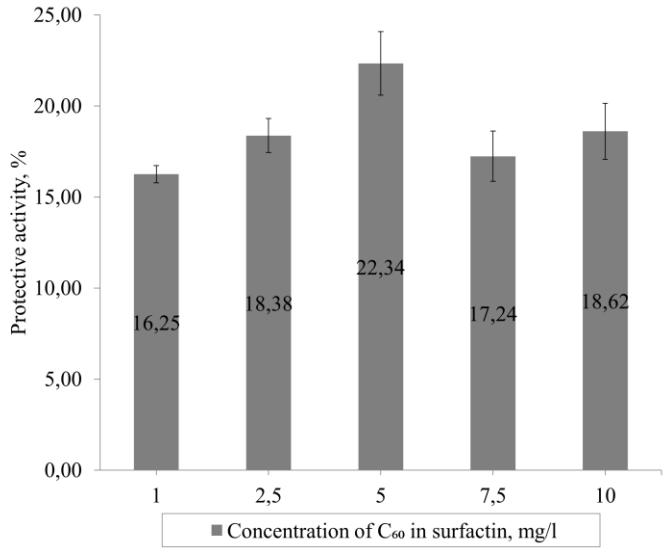
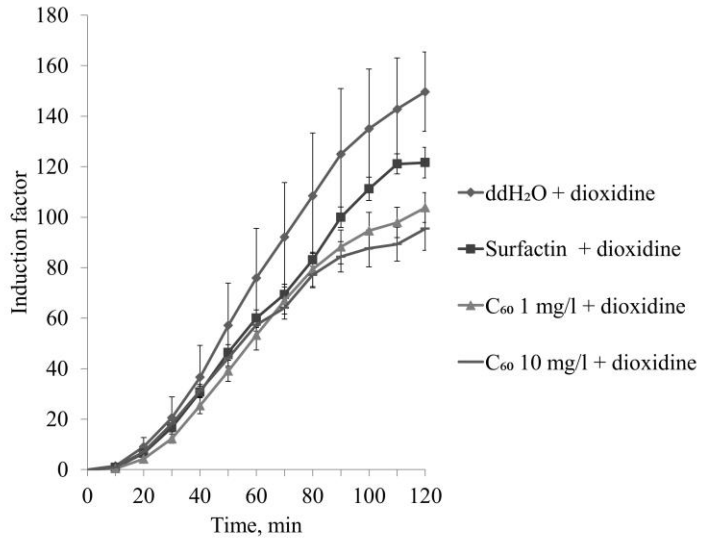


Figure 3. (A) Reduced induction factor caused by dioxidine 2.25×10^{-5} M ($p < 0.05$) of the biosensor strain *E. coli* MG1655 (pRecA) relative to the solvent control (surfactin) fullerene C_{60} at concentrations of 1 and 10 mg/l, solubilized in surfactin; (B) DNA protective effect of surfactin C60 concentrations during maximal induction

Thus, the biological activity of fullerene appears in surfactin solubilizer solution, but not in TWEEN 80 solution.

The results of the bioluminescent test using the *E. coli strain* MG1655 (pColD) are presented in Figure 4. Fullerene C_{60} concentrations of 0.01 and 0.1 m g/l did exhibit a statistically significant decrease in induction factor compared to the solvent control (surfactin) in the presence dioxidine, as in experiments using the biosensor *E. coli* MG1655 (pRecA) (data not shown). For the range of C_{60} concentrations 1 – 10 mg/l, a statistically significant decrease in induction factor was observed relative to the solubilizer and control (ddH_2O) (Figure 4A) (induction factor graphs with C_{60} concentrations of 2.5·– 7.5·mg/l not shown). Concentrations of C_{60} 10^{-3} – 10^{-2} g/l in surfactin on the biosensor *E. coli* MG1655 (pColD) had a DNA protective effect close in value to that obtained on the biosensor *E. coli* MG1655 (pRecA) (Figure 4B). which also does not have a direct dose dependence. Thus, when surfactin is used as a solubilizer, C_{60} has a DNA protective effect on both biosensor strains that detect DNA damage.

A



B

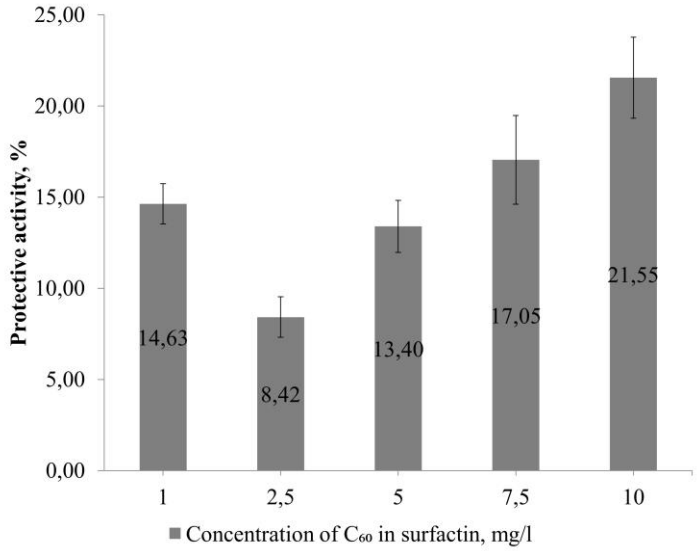
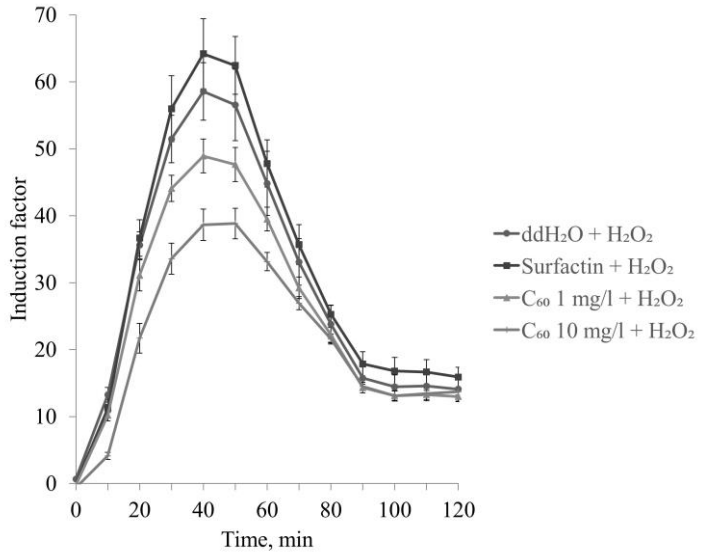


Figure 4. (A) Reduced induction of biosensor strain *E. coli* MG1655 (pColD) caused by dioxidin 2.25×10^{-5} M relative to the solvent control (surfactin) fullerene C_{60} at concentrations of 1 and 10 mg/l, solubilized in surfactin ($p<0.05$);. (B) DNA protective effect of C_{60} in surfactin during maximal induction by dioxidine of biosensor *E. coli* strain MG1655 (pColD), $p < 0.05$.

To test the hypothesis about the antioxidant mechanism of the DNA protective effect of fullerene C_{60}, a bioluminescent test was performed using *E. coli* strains MG1655 (pKatG) and *E. coli* MG1655 (pSoxS), reacting to peroxides and superoxide anion radical, respectively. As can be seen from Figure 5A and B, concentrations of C_{60} in surfactin, which

exhibit a DNA protective effect, exhibit an antioxidant effect against hydrogen peroxide 10^{-3} M. Correlation coefficient between the values of fullerene concentrations and the protective effect they provide against hydrogen peroxide during maximum induction and fullerene concentration was 0.884, which means a strong direct connection, i.e. the antioxidant effect of C_{60} is dose-dependent.

A



B

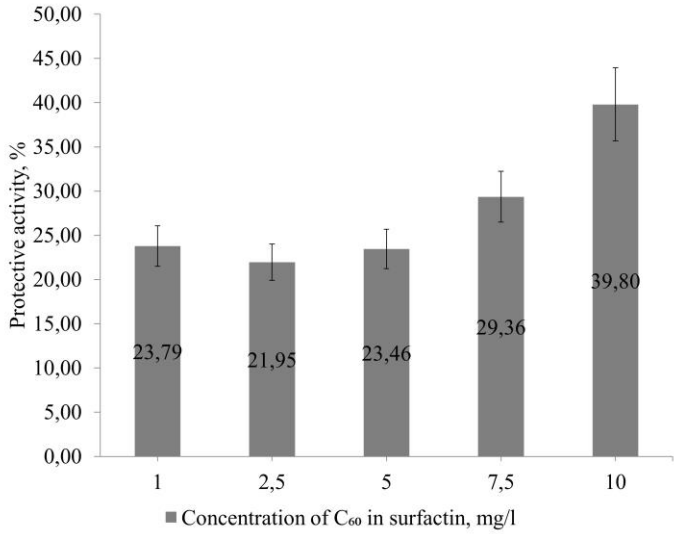


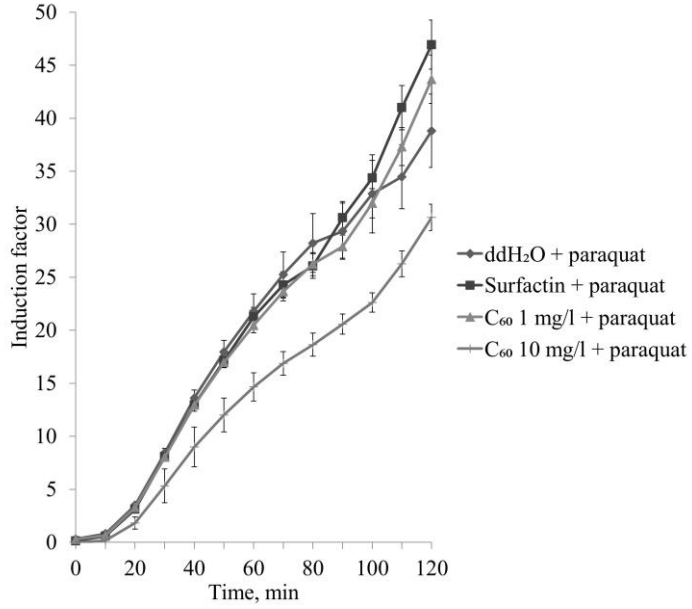
Figure 5. (A) Reduction of hydrogen peroxide-induced 10^{-3} M induction of the biosensor strain *E. coli* MG1655 (pKatG) relative to the control solvent (surfactin) with $_{C60}$ fullerene at concentrations of 1 and 10 mg/l,

solubilized in surfactin, $p<0.05$; (B) Protective (antioxidant) effect of C_{60} concentrations in surfactin during maximum induction, $p <0.05$.

Bioluminescence test with biosensor reacting to superoxide anion radical, *E. coli* MG 1655 (pSoxS) showed no effect of surfactin on induction factor with a standard inducer (paraquat (methyl viologen) 10^{-3} M) up to 110 min (Figure 6A). C_{60} at a concentration of 1 mg/l in surfactin did not have a statistically significant effect on the induction factor of bioluminescence relative to the solvent control and the protective effect. C_{60} at concentrations of 2.5·–10 mg/l had a decrease in induction factor relative to the control solvent (data for concentrations of $2.5\cdot10^{-3}$ – $7.5\cdot10^{-3}$ mg/l are not shown) and a dose-dependent protective effect (Figure 6B). C_{60} at a concentration of 10 mg/l also had a statistically significant decrease in induction relative to the control (deionized water with paraquat). The correlation coefficient between the values of fullerene concentrations and the protective effect they provide against paraquat during maximum induction and the fullerene concentration was 0.884 (as well as between the values of fullerene concentrations and the antioxidant effect they provide against hydrogen peroxide), which means a strong direct relationship, those. The antioxidant effect of C_{60} on the superoxide anion radical is dose-dependent.

Thus, it can be noted that concentrations that have a DNA protective effect also demonstrate an antioxidant effect.

A



B

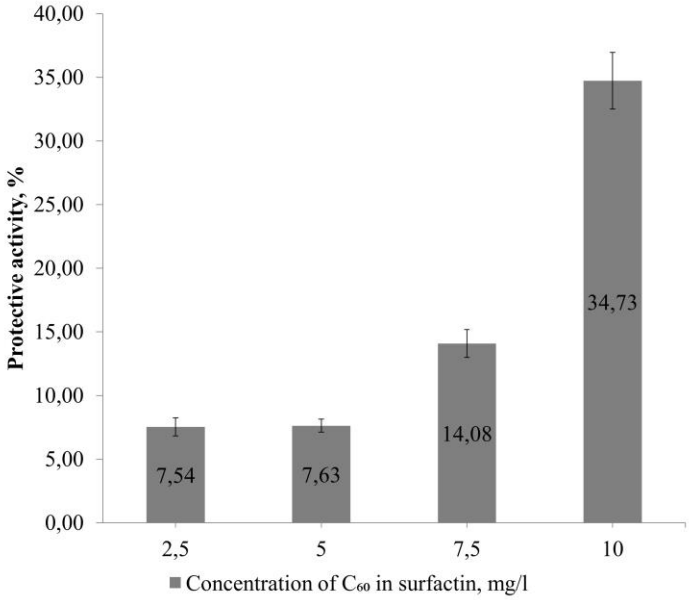


Figure 6. (A) Reduction of paraquat-induced induction factor of the biosensor strain E. *coli MG1655* (pSoxS) relative to the control - deionized water (ddH_2O) and the solvent control (surfactin) with fullerene C_{60} at a concentration of 10 mg/l, solubilized in surfactin, ($p<0.05$); (B) Protective (antioxidant) effect of C_{60} concentrations in surfactin during maximum induction factor, $p<0.05$.

DISCUSSION

From the presented data it can be seen that fullerene C_{60} has and DNA protective effect, one of the mechanisms of which is the antioxidant activity of fullerene. This is

confirmed by the dose dependence of the protective effect of C_{60} on one of the ROS – hydrogen peroxide. It is also impossible to exclude the chemical interaction of fullerene with dioxidine via the Diels–Alder reaction, since the dioxidine molecule is a derivative of the heterocycle of the benzene and pyrazine rings, quinoxaline , the derivatives of which are capable of forming adducts with C_{60} (37). Perhaps the chemical interaction or adsorption of dioxidin C_{60} is the reason for the deviation from the dose dependence of the biological DNA protective effect of C_{60}.

The mechanism of the protective effect against hydrogen peroxide may be the attachment of hydroxyl ion, a component of the dissociation of hydrogen peroxide (38,39); in relation to the superoxide radical , the product of partial reduction of superoxide, a hydroxyl radical, can be added to the double bonds of fullerene C_{60}. It has been shown that C_{60} is capable of attaching a large number of radicals per molecule (2), including the trichloromethyl-peroxyl radical $CCl_3OO\bullet$, protecting the liver of rats from the metabolic products of carbon tetrachloride (40). It is also known that fullerenes and their derivatives, fullerenols, are also capable of acting as a powerful radical scavenger (40) and reducing ROS levels in vivo under the influence of oxidative stress inducers, such as Juglone (5-hydroxy-1,4-naphthoquinone) (41).

According to (42), the size of C_{60} aggregates and the concentration of the solution can depend on each other. In a study (43), aggregate size was found to influence biological activity. This may explain deviations in the dose dependence of the protective effect of fullerene when solutions are diluted by an order of magnitude or higher.

In our experiments, C_{60} solubilized in surfactin has a DNA protective effect, but in TWEEN 80 it does not. Therefore, it can be assumed that the bioactivity of C_{60} in surfactin is associated not so much with solubilization, but with an increase in bioavailability due to increased membrane permeability. This hypothesis explains why in the experiment of Baati et

al. (14) in which fullerene C_{60} in olive oil prolonged the life of rats. Perhaps surfactin or other biosurfactants secreted by the intestinal microbiota led to an increase in membrane permeability and absorption of fullerene in the gastrointestinal tract. Surfactin-producing, in addition to potentially probiotic strains of the well-known producer *B. subtilis* (44,45) are probiotic bacteria *Bacillus clausii* (46) as well as representatives of the genus *Lactobacillus* (47) – components of the normal intestinal microflora of mammals (48) and rats in particular (49).

The lack of effect of C_{60} in olive oil on the lifespan and health of mice in the study (15) may be due to differences in the microbiota and lower levels of surfactin (or other biosurfactants) production compared to the intestinal microflora of rats in the experiment (14).

Selected for use as a solubilizer for C_{60} fullerene and to maximize the permeability of membranes for protectors, the concentration of surfactin (31.25 mg/l) is lower than the minimum concentration at which surfactin exhibits hemolytic activity (50 mg/l) (19), which opens prospects for studying suspensions of fullerene in surfactin in animal models .

Fullerene C_{60} can thus become the basis for a new type of medicine. Fullerenes can be used as antioxidants, geroprotectors, for drug delivery and in photodynamic therapy, but the above-described features of aqueous solutions, the influence of the solvent, the size of aggregates and intense lighting, especially the UV spectrum, should be taken into account.

The data obtained also indicate the potential for studying fullerene in combination with surfactin-producing bacillary probiotics in animal model organisms.

Funding

The research was financially supported by the Ministry of Science and Higher Education of the Russian Federation (№ FENW-2023-0008).

Conflict of interest statement: The authors declare no conflict of interest.

References

1. Schur D V, Zaginaichenko SY, Veziroglu TN. The hydrogenation process as a method of investigation of fullerene C60 molecule. Int J Hydrogen Energy. 2015;40(6):2742–62.

2. Krusic PJ, Wasserman E, Keizer PN, Morton JR, Preston KF, J. KP, et al. Radical reactions of C60. Science. 1991 Nov 22;254(5035):1183–5. Available from: https://doi.org/10.1126/science.254.5035.1183

3. Sayes CM, Gobin AM, Ausman KD, Mendez J, West JL, Colvin VL. Nano-C60 cytotoxicity is due to lipid peroxidation. Biomaterials. 2005;26(36):7587–95.

4. Quick KL, Ali SS, Arch R, Xiong C, Wozniak D, Dugan LL. A carboxyfullerene SOD mimetic improves cognition and extends the lifespan of mice. Neurobiol Aging [Internet]. 2008 Jan [cited 2022 Nov 1];29(1):117–28. Available from: https://pubmed.ncbi.nlm.nih.gov/17079053/

5. Nozdrenko D, Matvienko T, Vygovska O, Bogutska K, Motuziuk O, Nurishchenko N, et al. Protective effect of water-soluble C60 fullerene nanoparticles on the ischemia-reperfusion injury of the muscle soleus in rats. Int J Mol Sci. 2021;22(13):6812.

6. Lan J, Gou N, Gao C, He M, Gu AZ. Comparative and mechanistic genotoxicity assessment of nanomaterials via a quantitative toxicogenomics approach across multiple species. Environ Sci Technol [Internet]. 2014 Nov 4 [cited 2022 Oct 17];48(21):12937–45. Available from: https://pubs.acs.org/doi/full/10.1021/es503065q

7. Jacobsen NR, Pojana G, White P, MÖller P, Cohn CA, Smith Korsholm K, et al. Genotoxicity, cytotoxicity, and reactive oxygen species induced by single-walled carbon nanotubes and C60 fullerenes in the FE1-Muta^{TM} Mouse lung epithelial cells. Environ Mol Mutagen. 2008;49(6):476–87.

8. Mori T, Takada H, Ito S, Matsubayashi K, Miwa N, Sawaguchi T. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. Toxicology. 2006;225(1):48–54.

9. Sharoyko V V, Iamalova NR, Ageev S V, Meshcheriakov AA, Iurev GO, Petrov A V, et al. In Vitro and In Silico Investigation of Water-Soluble Fullerenol C60 (OH) 24: Bioactivity and Biocompatibility. J Phys Chem B. 2021;125(32):9197–212.

10. Gaponenko IN, Ageev S V, Iurev GO, Shemchuk OS, Meshcheriakov AA, Petrov A V, et al. Biological evaluation and molecular dynamics simulation of water-soluble fullerene derivative C60[C(COOH)2]3. Toxicol Vitr [Internet]. 2020;62:104683. Available from: https://www.sciencedirect.com/science/article/pii/S0887233319306083

11. Chistyakov VA, Smirnova YO, Prazdnova E V, Soldatov A V. Possible mechanisms of fullerene C60 antioxidant action. Biomed Res Int. 2013;2013.

12. Korshunov SS, Skulachev VP, Starkov AA. High protonic potential actuates a

mechanism of production of reactive oxygen species in mitochondria. FEBS Lett [Internet]. 1997 Oct 13 [cited 2018 Nov 20];416(1):15–8. Available from: http://doi.wiley.com/10.1016/S0014-5793%2897%2901159-9

13. Chistyakov VA, Prazdnova E V, Soldatov A V, Smirnova YO, Alperovich I. Can C60 fullerene demonstrate properties of mitochondria-targeted antioxidant from the computational point of view. Int J Biol Biomed Eng. 2014;8:59–62.

14. Baati T, Bourasset F, Gharbi N, Njim L, Abderrabba M, Kerkeni A, et al. The prolongation of the lifespan of rats by repeated oral administration of [60]fullerene. Biomaterials [Internet]. 2012 Jun 1 [cited 2019 Apr 25];33(19):4936–46. Available from: https://www.sciencedirect.com/science/article/pii/S0142961212003237

15. Grohn KJ, Moyer BS, Wortel DC, Fisher CM, Lumen E, Bianchi AH, et al. C60 in olive oil causes light-dependent toxicity and does not extend lifespan in mice. GeroScience [Internet]. 2021;43(2):579–91. Available from: https://doi.org/10.1007/s11357-020-00292-z

16. Wei Y-H, Wang L-F, Chang J-S. Optimizing Iron Supplement Strategies for Enhanced Surfactin Production with Bacillus subtilis. Biotechnol Prog [Internet]. 2004 Jan 1;20(3):979–83. Available from: https://doi.org/10.1021/bp030051a

17. Abdel-Mawgoud AM, Aboulwafa MM, Hassouna NA-H. Optimization of Surfactin Production by Bacillus subtilis Isolate BS5. Appl Biochem Biotechnol [Internet]. 2008;150(3):305–25. Available from: https://doi.org/10.1007/s12010-008-8155-x

18. Prazdnova E, Kuznetsova A, Rudoy D, Olshevskaya A, Odabashyan M, Kulikova N, et al. Systemic and antimutagenic properties of probiotics. In: E3S Web of Conferences. EDP Sciences; 2022.

19. Santos VSV, Silveira E, Pereira BB. Toxicity and applications of surfactin for health and environmental biotechnology. J Toxicol Environ Heal Part B. 2018;21(6–8):382–99.

20. Abdelli F, Jardak M, Elloumi J, Stien D, Cherif S, Mnif S, et al. Antibacterial, anti-adherent and cytotoxic activities of surfactin (s) from a lipolytic strain Bacillus safensis F4. Biodegradation. 2019;30:287–300.

21. Ishigami Y, Osman M, Nakahara H, Sano Y, Ishiguro R, Matsumoto M. Significance of β-sheet formation for micellization and surface adsorption of surfactin. Colloids Surfaces B Biointerfaces [Internet]. 1995;4(6):341–8. Available from: https://www.sciencedirect.com/science/article/pii/0927776594011836

22. Yeh M-S, Wei Y-H, Chang J-S. Enhanced Production of Surfactin from Bacillussubtilis by Addition of Solid Carriers. Biotechnol Prog [Internet]. 2005 Jan 1;21(4):1329–34. Available from: https://doi.org/10.1021/bp050040c

23. Heerklotz H, Seelig J. Detergent-Like Action of the Antibiotic Peptide Surfactin on Lipid Membranes. Biophys J [Internet]. 2001;81(3):1547–54. Available from: https://www.sciencedirect.com/science/article/pii/S0006349501758080

24. Sen R, Swaminathan T. Characterization of concentration and purification parameters and operating conditions for the small-scale recovery of surfactin. Process Biochem [Internet]. 2005;40(9):2953–8. Available from:

https://www.sciencedirect.com/science/article/pii/S1359511305001194

25. Reddy AS, Chen C-Y, Baker SC, Chen C-C, Jean J-S, Fan C-W, et al. Synthesis of silver nanoparticles using surfactin: A biosurfactant as stabilizing agent. Mater Lett [Internet]. 2009;63(15):1227–30. Available from: https://www.sciencedirect.com/science/article/pii/S0167577X09001372

26. Joshi H, Shirude PS, Bansal V, Ganesh KN, Sastry M. Isothermal Titration Calorimetry Studies on the Binding of Amino Acids to Gold Nanoparticles. J Phys Chem B [Internet]. 2004 Aug 5;108(31):11535–40. Available from: https://doi.org/10.1021/jp048766z

27. Zhang L, Xing X, Ding J, Zhao X, Qi G. Surfactin variants for intra-intestinal delivery of insulin. Eur J Pharm Biopharm. 2017;115:218–28.

28. Ruoff RS, Malhotra R, Huestis DL, Tse DS, Lorents DC. Anomalous solubility behaviour of C60. Nature [Internet]. 1993;362(6416):140–1. Available from: https://doi.org/10.1038/362140a0

29. Bazhenov S V, Novoyatlova US, Scheglova ES, Prazdnova E V, Mazanko MS, Kessenikh AG, et al. Bacterial lux-biosensors: Constructing, applications, and prospects. Biosens Bioelectron X [Internet]. 2023;13:100323. Available from: https://www.sciencedirect.com/science/article/pii/S2590137023000201

30. Ghersa P, Lurz R, Dobrinski B, Deshusses J, Belet M, Frey J. Genetic evidence for the existence of a repressor that modulates colicin D expression on plasmid ColD-CA23. Mol Gen Genet MGG [Internet]. 1988;213(1):36–41. Available from: https://doi.org/10.1007/BF00333395

31. Chistyakov VA, Semenyuk YP, Morozov PG, Prazdnova E V, Chmykhalo VK, Kharchenko EY, et al. Synthesis and biological properties of nitrobenzoxadiazole derivatives as potential nitrogen (ii) oxide donors: SOX induction, toxicity, genotoxicity, and DNA protective activity in experiments using Escherichia coli-based lux biosensors. Russ Chem Bull. 2015;64(6):1369–77.

32. Mazanko MS, Chistyakov VA, Prazdnova E V, Pokudina IO, Churilov MN, Chmyhalo VK, et al. Dioxidine induces bacterial resistance to antibiotics. Mol Genet Microbiol Virol. 2016;31:227–32.

33. Hassan HM, Fridovich I. Paraquat and Escherichia coli. Mechanism of production of extracellular superoxide radical. J Biol Chem [Internet]. 1979 [cited 2022 Mar 28];254(21):10846–52. Available from: https://pubmed.ncbi.nlm.nih.gov/227855/

34. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Vol. 545. Cold spring harbor laboratory Cold Spring Harbor, NY; 1982.

35. Rosche WA, Foster PL. Determining mutation rates in bacterial populations. Methods. 2000;20(1):4–17.

36. Han Y, Huang X, Cao M, Wang Y. Micellization of Surfactin and Its Effect on the Aggregate Conformation of Amyloid β(1-40). J Phys Chem B [Internet]. 2008 Nov 27;112(47):15195–201. Available from: https://doi.org/10.1021/jp805966x

37. Sander M, Jarrosson T, Chuang S-C, Khan SI, Rubin Y. Approaches to Open

Fullerenes: Synthesis and Thermal Stability of cis-1 Bis(isobenzofuran) Diels−Alder Adducts of C60. J Org Chem [Internet]. 2007 Apr 1;72(8):2724–31. Available from: https://doi.org/10.1021/jo061987b

38. Afreen S, Kokubo K, Muthoosamy K, Manickam S. Hydration or hydroxylation: direct synthesis of fullerenol from pristine fullerene [C 60] via acoustic cavitation in the presence of hydrogen peroxide. RSC Adv [Internet]. 2017 Jun 21 [cited 2022 Mar 9];7(51):31930–9. Available from: https://pubs.rsc.org/en/content/articlehtml/2017/ra/c7ra03799f

39. Emelyantsev S, Prazdnova E, Chistyakov V, Alperovich I. Biological Effects of C60 Fullerene Revealed with Bacterial Biosensor—Toxic or Rather Antioxidant? Vol. 9, Biosensors . 2019.

40. Gharbi N, Pressac M, Hadchouel M, Szwarc H, Wilson SR, Moussa F. [60] Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. Nano Lett. 2005;5(12):2578–85.

41. Cong W, Wang P, Qu Y, Tang J, Bai R, Zhao Y, et al. Evaluation of the influence of fullerenol on aging and stress resistance using Caenorhabditis elegans. Biomaterials. 2015;42:78–86.

42. Arndt M, Nairz O, Vos-Andreae J, Keller C, van der Zouw G, Zeilinger A. Wave–particle duality of C60 molecules. Nature [Internet]. 1999 Oct 14 [cited 2018 Nov 20];401(6754):680–2. Available from: http://www.nature.com/doifinder/10.1038/44348

43. Lyon DY, Adams LK, Falkner JC, Alvarez PJJ. Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. Environ Sci Technol. 2006;40(14):4360–6.

44. Lee JH, Nam SH, Seo WT, Yun HD, Hong SY, Kim MK, et al. The production of surfactin during the fermentation of cheonggukjang by potential probiotic Bacillus subtilis CSY191 and the resultant growth suppression of MCF-7 human breast cancer cells. Food Chem. 2012;131(4):1347–54.

45. Mohammadipour M, Mousivand M, Salehi Jouzani G, Abbasalizadeh S. Molecular and biochemical characterization of Iranian surfactin-producing Bacillus subtilis isolates and evaluation of their biocontrol potential against Aspergillus flavus and Colletotrichum gloeosporioides. Can J Microbiol. 2009;55(4):395–404.

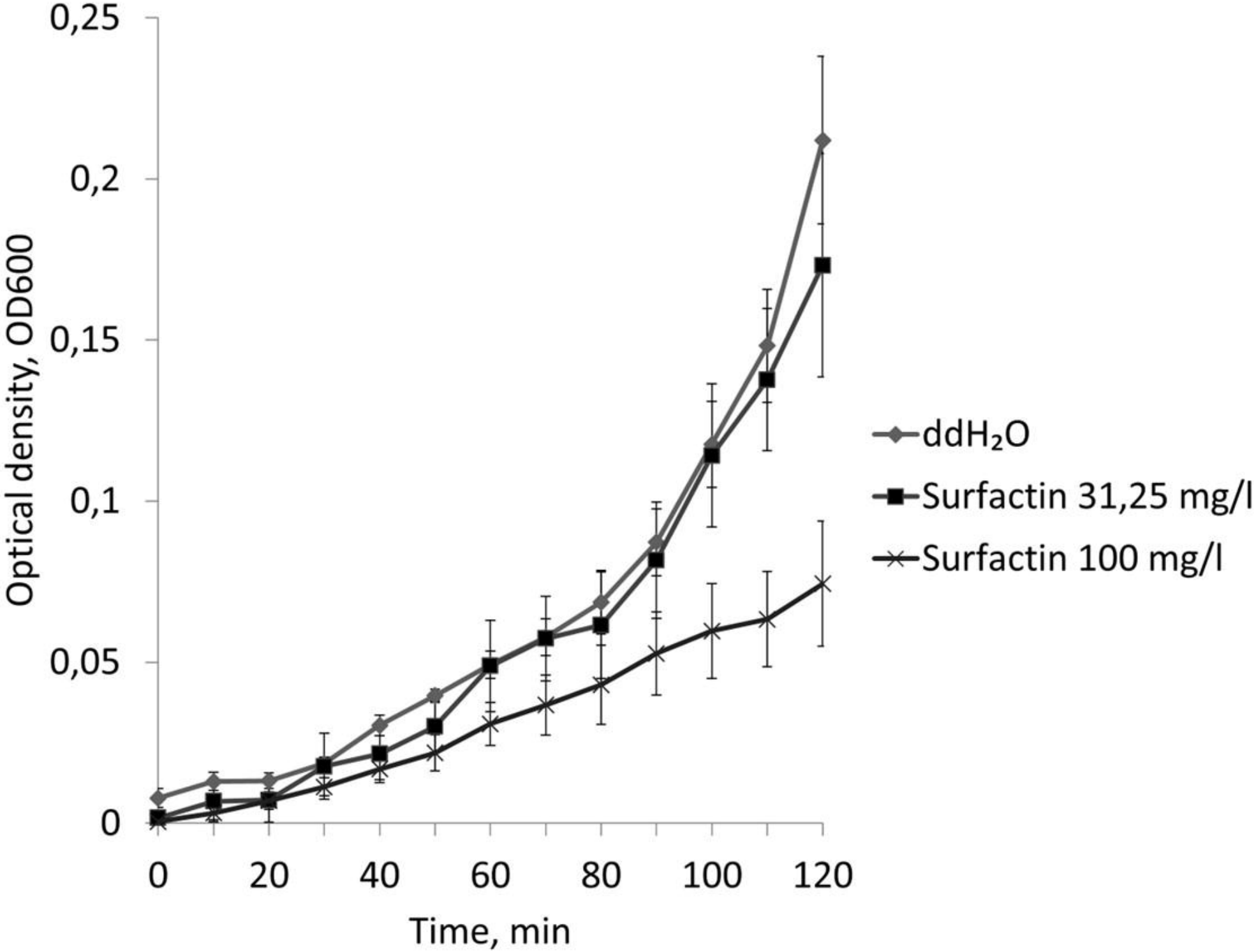
46. Liu CX, Xu QM, Yu SC, Cheng JS, Yuan YJ. Bio-removal of tetracycline antibiotics under the consortium with probiotics Bacillus clausii T and Bacillus amyloliquefaciens producing biosurfactants. Sci Total Environ [Internet]. 2020 Mar 25 [cited 2022 Nov 1];710. Available from: https://pubmed.ncbi.nlm.nih.gov/31918182/

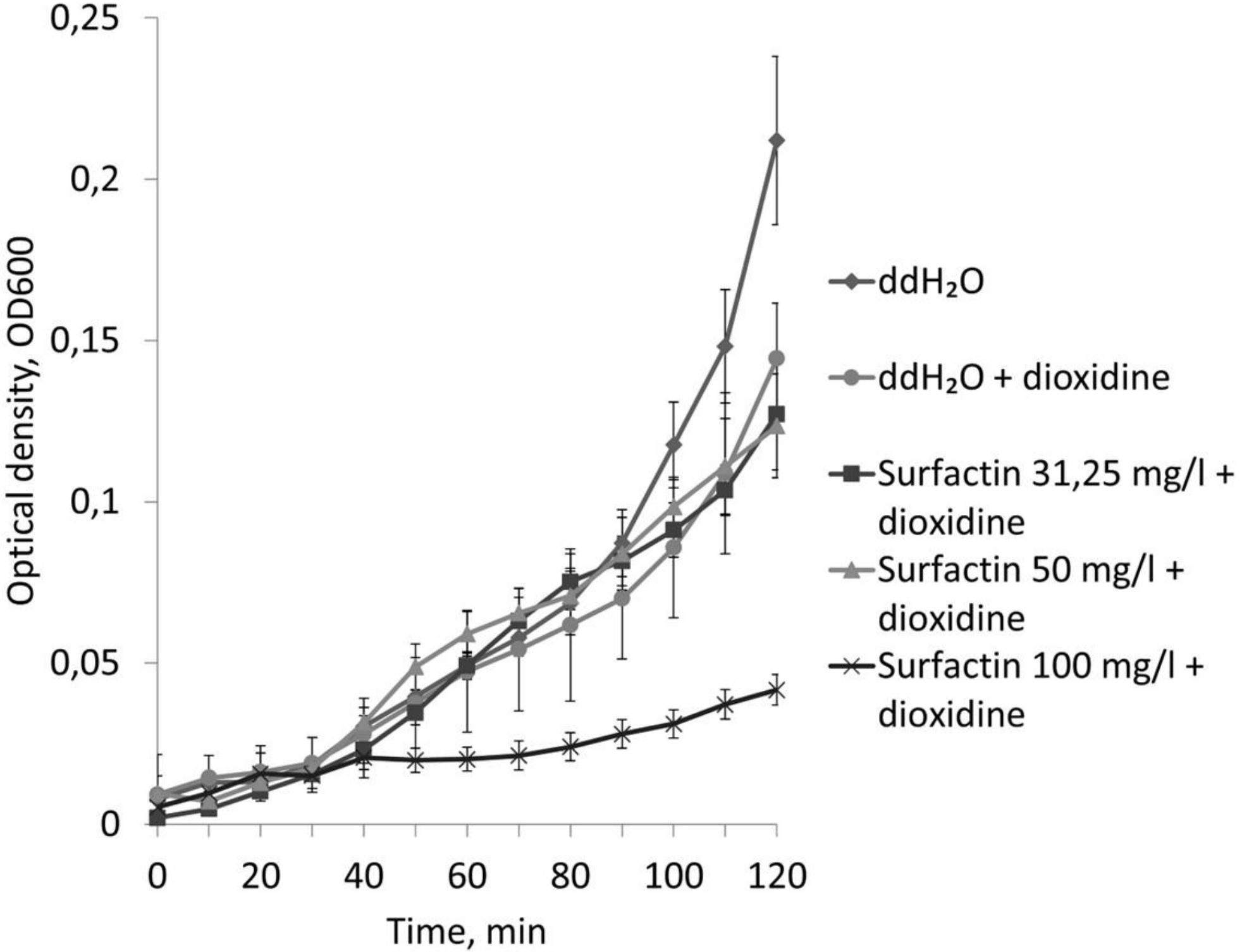
47. Nelson J, El-Gendy AO, Mansy MS, Ramadan MA, Aziz RK. The biosurfactants iturin, lichenysin and surfactin, from vaginally isolated lactobacilli, prevent biofilm formation by pathogenic Candida. FEMS Microbiol Lett [Internet]. 2020 Aug 1;367(15):fnaa126. Available from: https://doi.org/10.1093/femsle/fnaa126

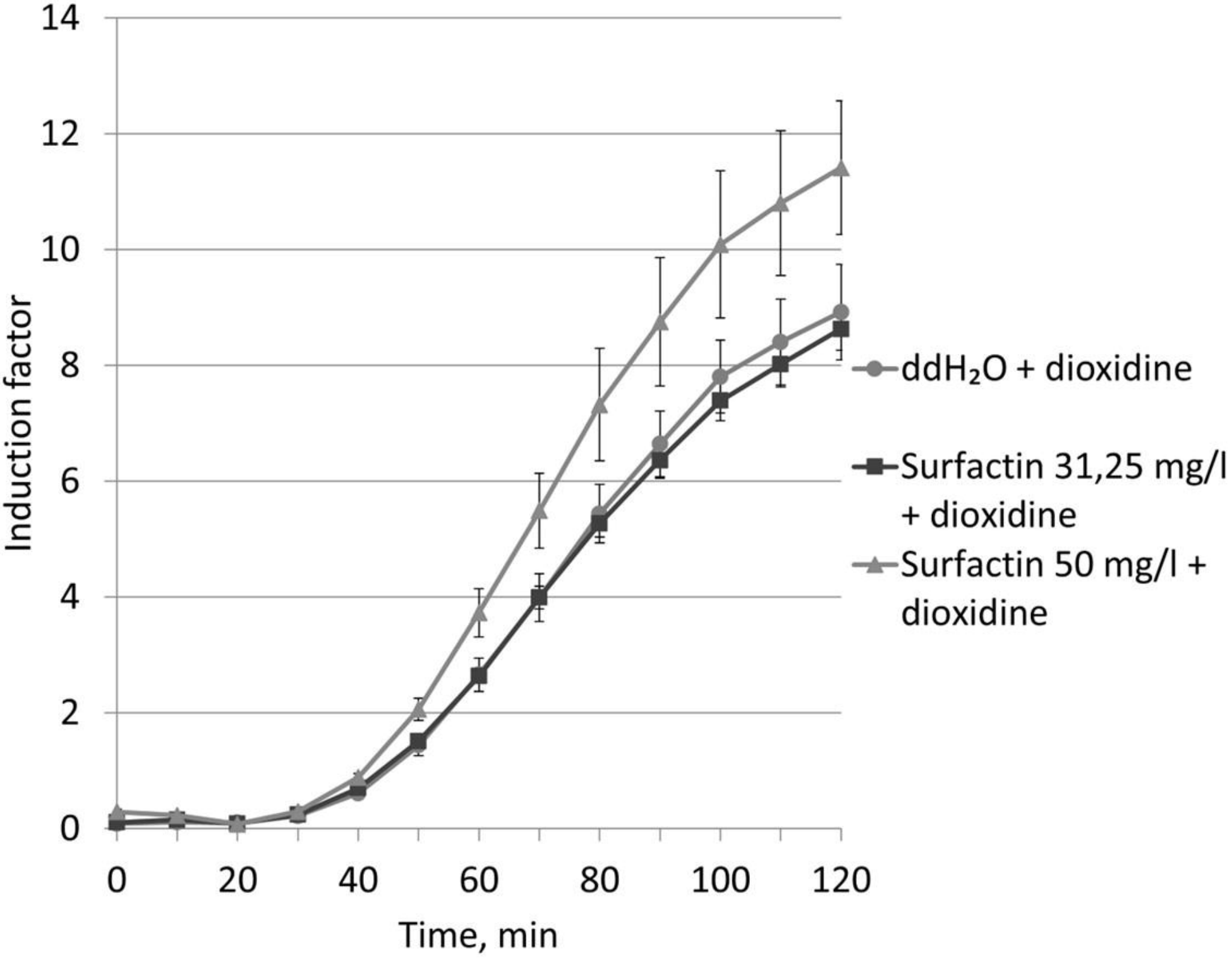
48. Urdaci MC, Bressollier P, Pinchuk I. Bacillus clausii probiotic strains: antimicrobial and immunomodulatory activities. J Clin Gastroenterol [Internet]. 2004 [cited 2022

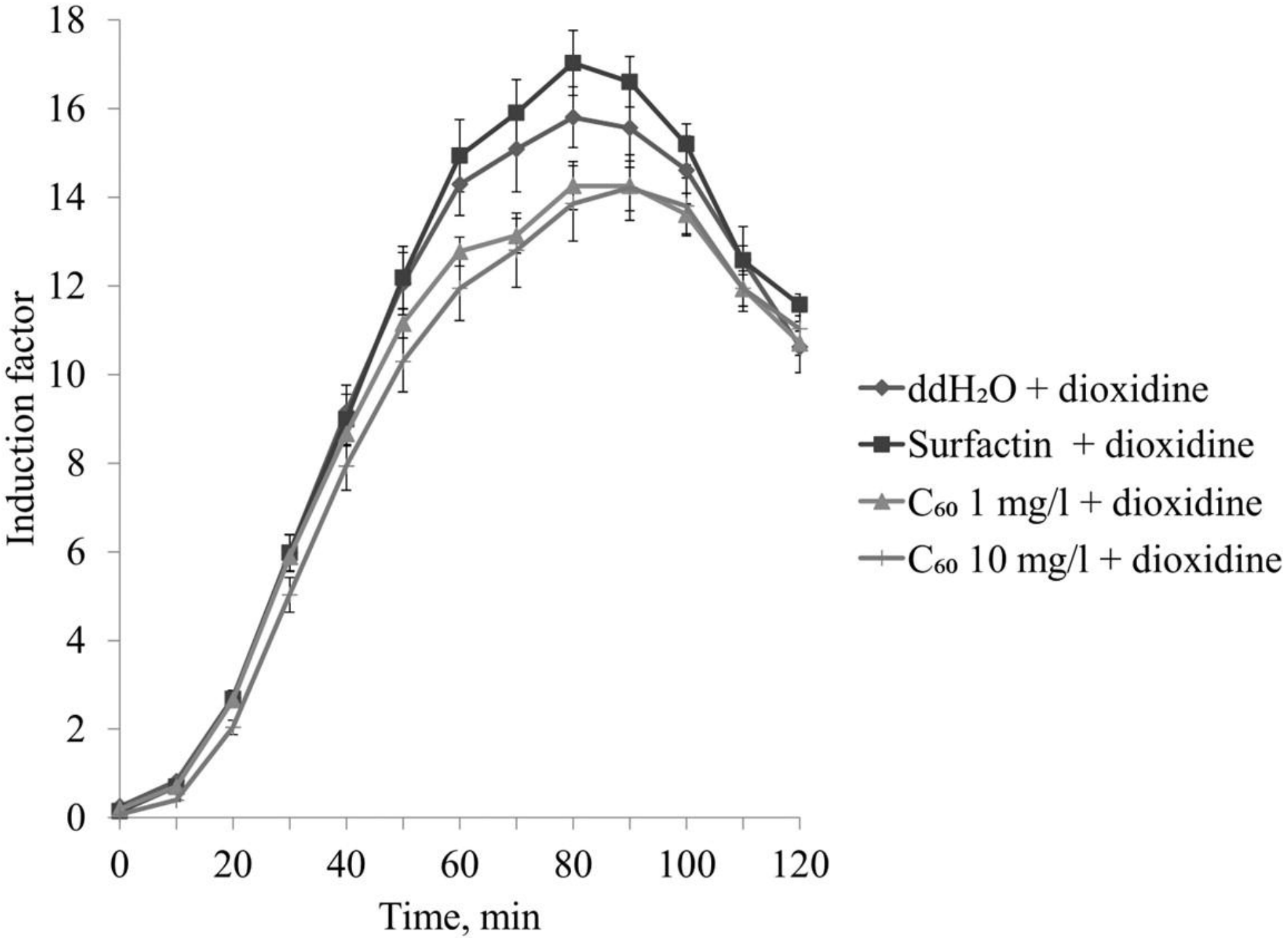
Nov 1];38(6 Suppl). Available from: https://pubmed.ncbi.nlm.nih.gov/15220667/

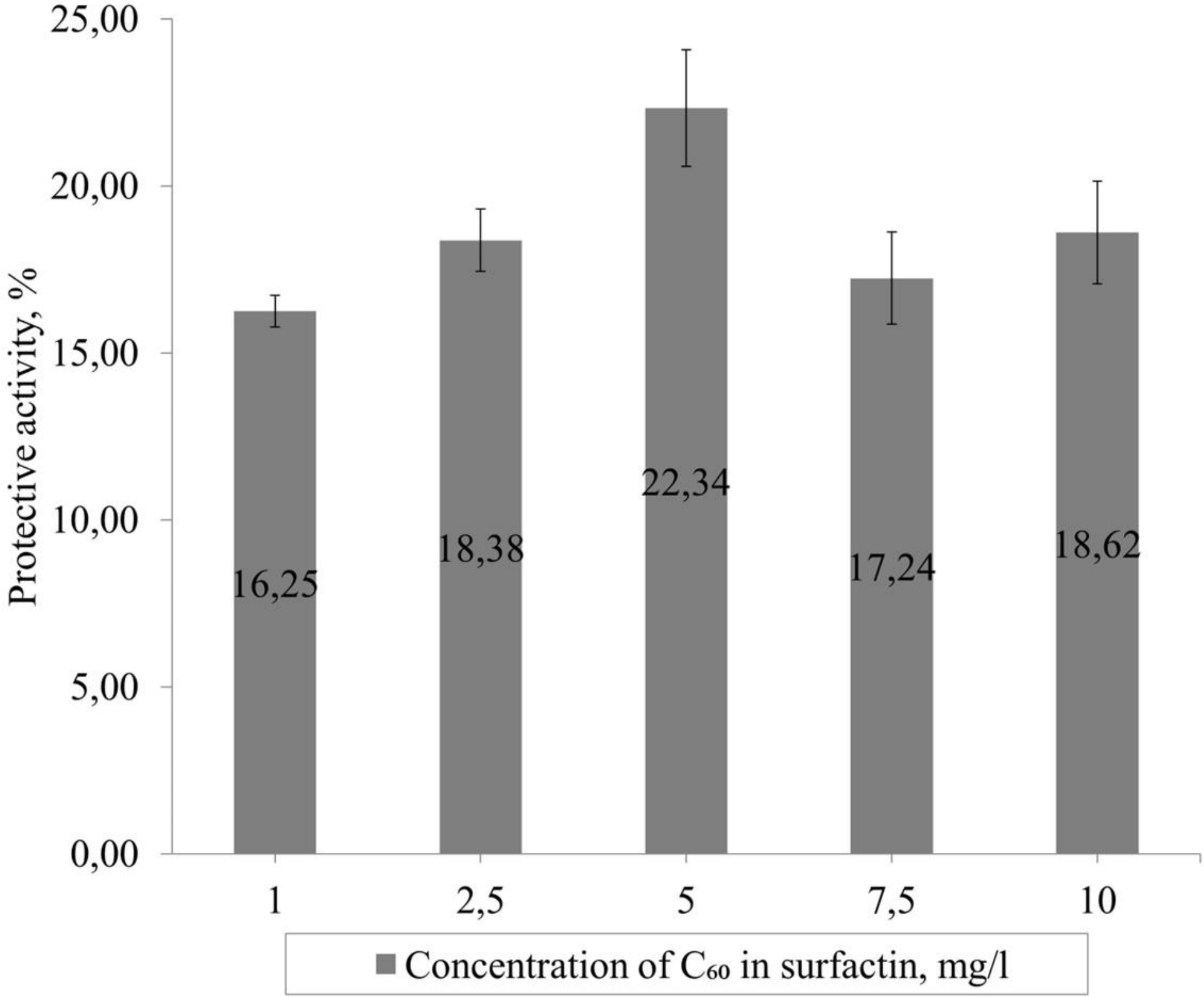
49. Klimina KM, Batotsyrenova EG, Yunes RA, Gilyaeva EH, Poluektova EU, Kostrova TA, et al. The effects of desynchronosis on the gut microbiota composition and physiological parameters of rats. BMC Microbiol [Internet]. 2019;19(1):160. Available from: https://doi.org/10.1186/s12866-019-1535-2

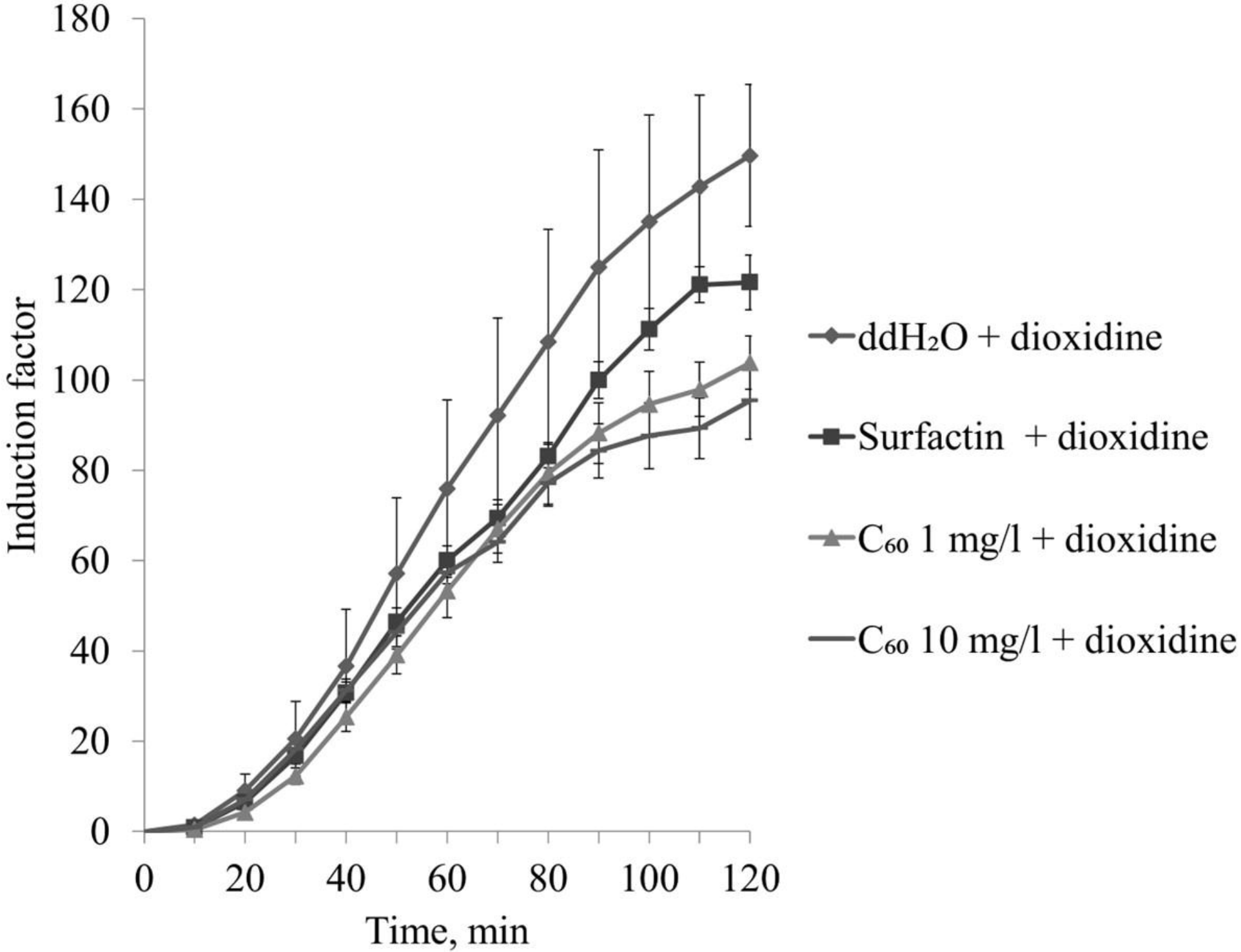

0,25
0,2
0,15
0,1
0,05
0
Optical density, OD600
0
20
40
60
80
100
120
Time, min
ddH₂O
Surfactin 31,25 mg/l
Surfactin 100 mg/l

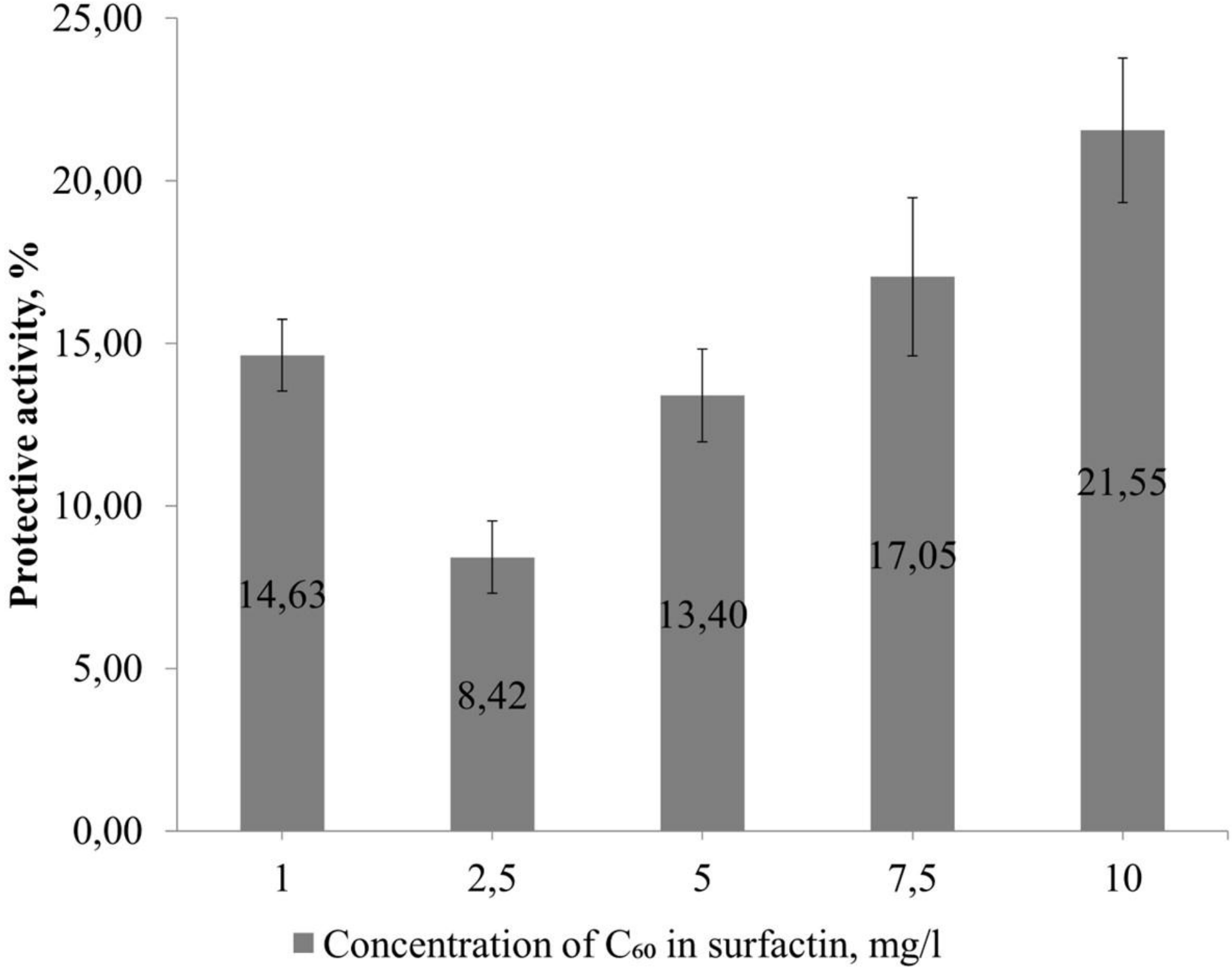

0,25
0,2
0,15
0,1
0,05
0
Optical density, OD600
0
20
40
60
80
100
120
Time, min
ddH₂O
ddH₂O + dioxidine
Surfactin 31,25 mg/l + dioxidine
Surfactin 50 mg/l + dioxidine
Surfactin 100 mg/l + dioxidine

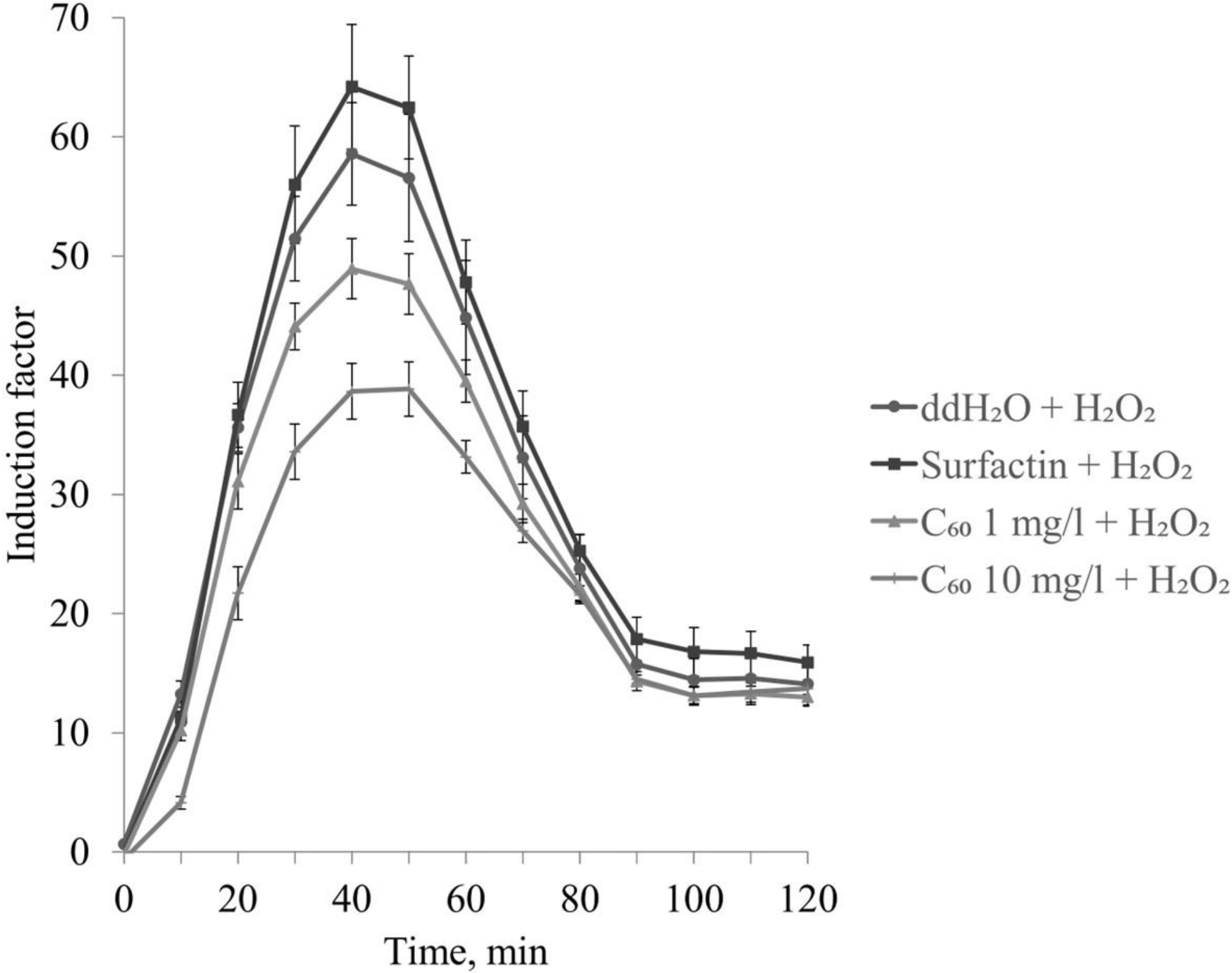

14
12
10
8
6
4
2
0
Induction factor
0
20
40
60
80
100
120
Time, min
ddH₂O + dioxidine
Surfactin 31,25 mg/l + dioxidine
Surfactin 50 mg/l + dioxidine

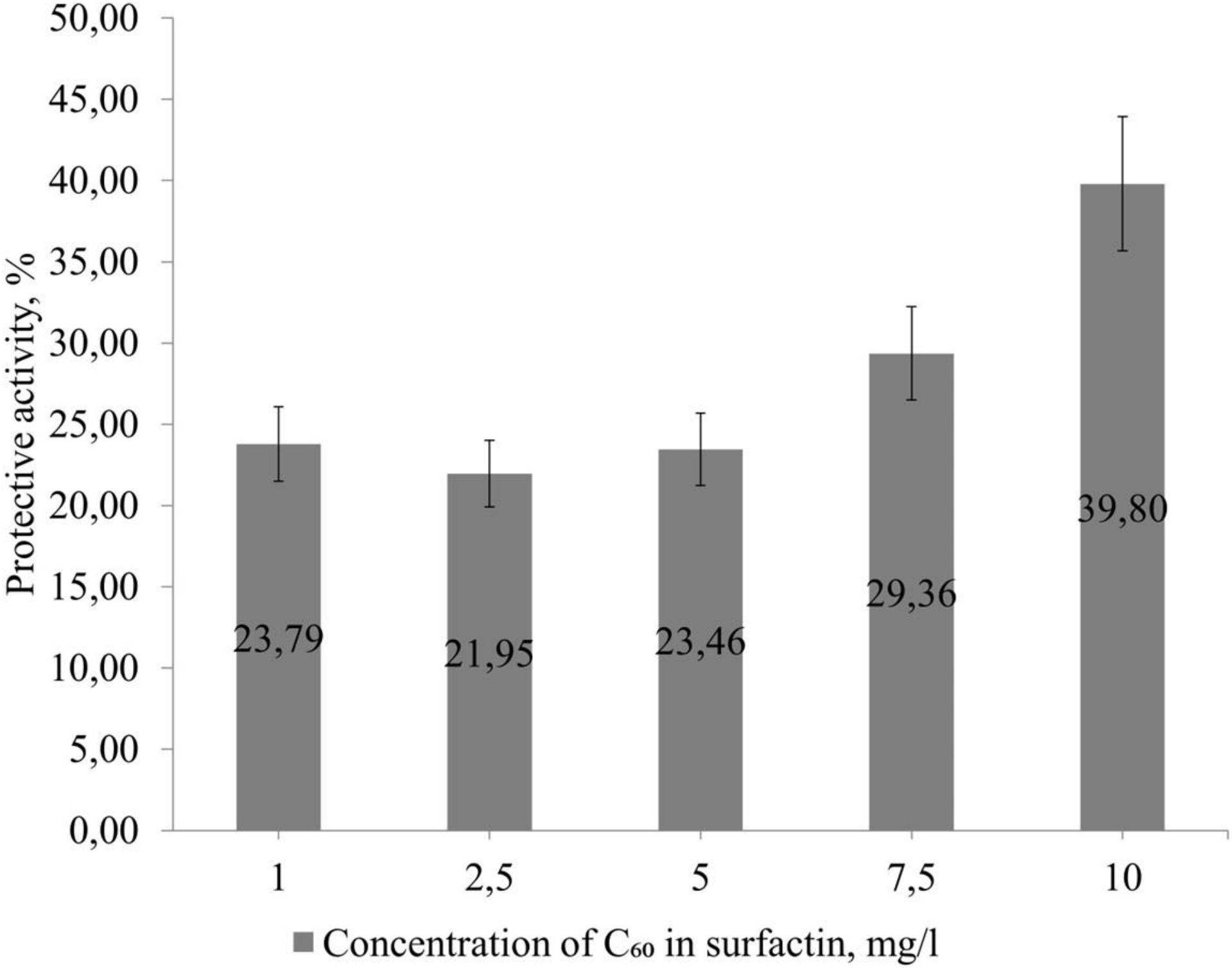

18
16
14
12
10
8
6
4
2
0
Induction factor
0
20
40
60
80
100
120
Time, min
ddH₂O + dioxidine
Surfactin + dioxidine
C₆₀ 1 mg/l + dioxidine
C₆₀ 10 mg/l + dioxidine

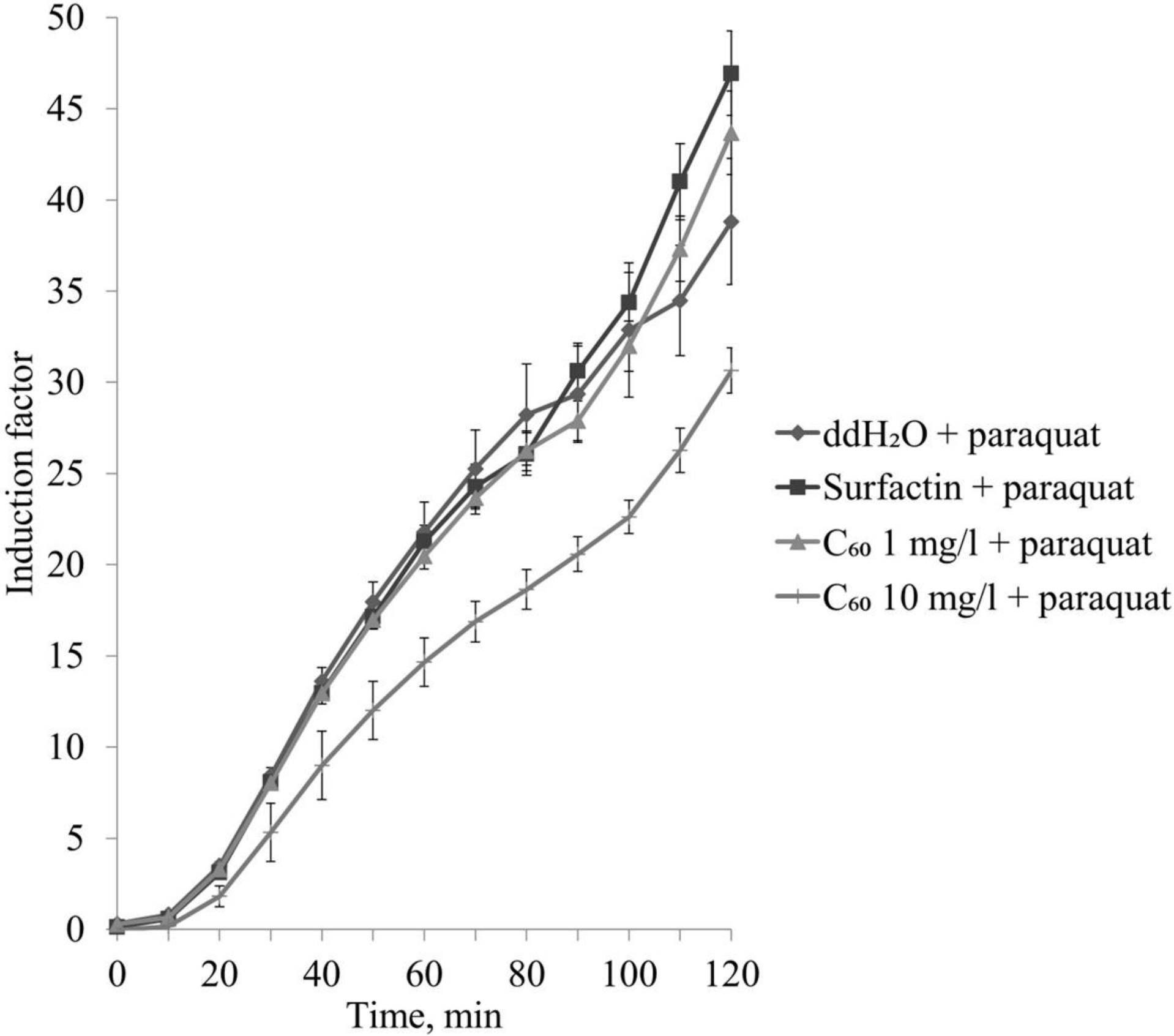

25,00
20,00
15,00
10,00
5,00
0,00
Protective activity, %
16,25
18,38
22,34
17,24
18,62
1
2,5
5
7,5
10
Concentration of C60 in surfactin, mg/l

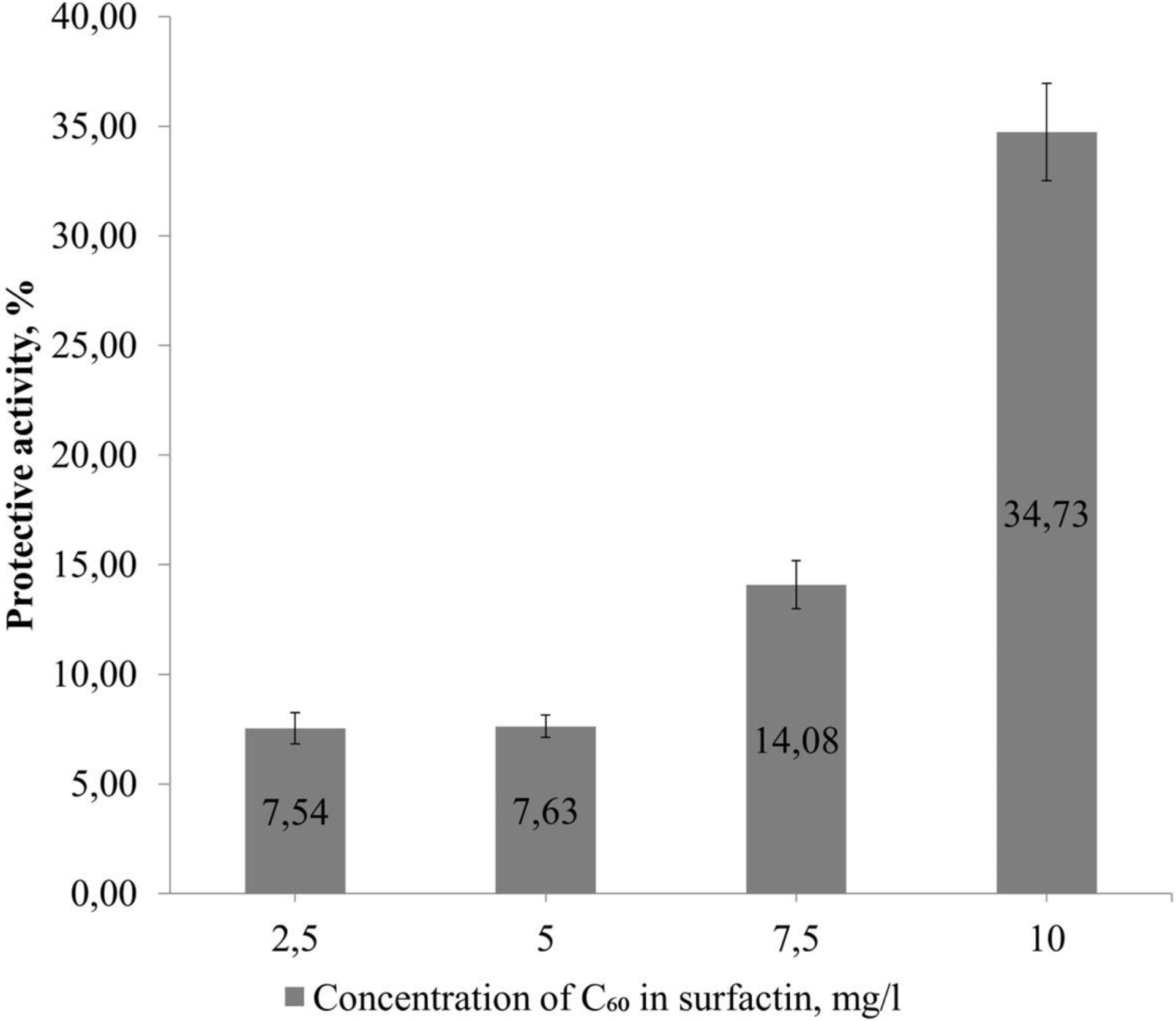

180
160
140
120
100
80
60
40
20
0
Induction factor
0
20
40
60
80
100
120
Time, min
ddH₂O + dioxidine
Surfactin + dioxidine
C₆₀ 1 mg/l + dioxidine
C₆₀ 10 mg/l + dioxidine


25,00
20,00
15,00
10,00
5,00
0,00
Protective activity, %
14,63
8,42
13,40
17,05
21,55
1
2,5
5
7,5
10
Concentration of C60 in surfactin, mg/l


70
60
50
40
30
20
10
0
Induction factor
0
20
40
60
80
100
120
Time, min
ddH₂O + H₂O₂
Surfactin + H₂O₂
C₆₀ 1 mg/l + H₂O₂
C₆₀ 10 mg/l + H₂O₂


50,00
45,00
40,00
35,00
30,00
25,00
20,00
15,00
10,00
5,00
0,00
Protective activity, %
23,79
21,95
23,46
29,36
39,80
1
2,5
5
7,5
10
Concentration of C60 in surfactin, mg/l


Induction factor
50
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
Time, min
ddH₂O + paraquat
Surfactin + paraquat
C₆₀ 1 mg/l + paraquat
C₆₀ 10 mg/l + paraquat


40,00
35,00
30,00
25,00
20,00
15,00
10,00
5,00
0,00
Protective activity, %
7,54
7,63
14,08
34,73
2,5
5
7,5
10
Concentration of C60 in surfactin, mg/l