# Innovations in teaching and learning microbiology – painting with pigment microorganisms

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***Innovation in teaching and learning of microbiology - painting with pigment microorganisms:*** *In the present work are presented innovations in teaching and training in microbiology. For the first time has been applied "microbio art" by painting with living pigmented microorganisms. The innovation makes the students active participants in the studying process. It is in a state to increase their involvement, collaboration and communication skills and activate their creative and critical thinking.*

*Besides, in the course of painting with microorganisms an innovative method for determination of the color of the microbial pigments has been applied. For the first time it has been measured instrumentally and presented numerically using L*a*b units in the CIELab system about which no data have been reported in the literature.*

***Key words:*** *innovations in teaching and learning painting with microorganisms, microbio art, microbial pigments, color, L*a*b*.*

## INTRODUCTION

The new age we live in requires a plastic and flexible style of teaching that matches the dynamics of reality. Teachers need new training methods, techniques and approaches.

In recent years, innovative teaching and learning is increasingly becoming a part of education, based mainly on information and communication technology, interactive learning.. These trends are in force in the teaching and learning of microbiology at universities. Following the interactive model to improve the learning process of students in medical microbiology and stimulate their interest, Struwig et al. [9] develop the game Med Micro Fun With Facts (MMFWF) in addition to the course of lectures.

Among the microbial community on a world scale is constantly discussed the content of curricula in microbiology undergraduate degree and incorporation in their innovative tasks to make the discipline more attractive, stimulate students to more in-depth knowledge and encourage them to think outside the boundaries of a given object [9, 11].

Conditionally, are formed two trends – one for teaching the basic principles first and then to be inserted applied aspects in the curriculum, and the other trend is to work for rousing initially the enthusiasm among the students on the topic in any possible way such as by focusing on applications of microbiology, and subsequently teaching basic principles [11]. Both approaches aim at acquiring skills and abilities, gaining practical experience, generation of creative talents, innovative solutions and creativity. They provide opportunities for innovative methods in the learning process, unusual interdisciplinary collaborations.

At first glance impossible, but very interesting and effective, the relationship with the art of microbiology turns out to be. Art enables the visualization and communication of science as well as a different kind of scientific creativity of students.

Surprisingly, the microorganisms themselves can provide both material and inspiration for art: many types of bacteria produce various pigments, including in light or dark shades and unusual colors like black, white, brown, gold, silver and fluorescence-green, yellow or blue. Pigmented microorganisms can be used for "painting" images on agar culture medium in Petri dishes [1, 8]. A group of scientists and artists around the world create beautiful color patterns by means of microorganisms known as "microbial art" (microbio art). This kind of art is something unusual and interesting. This is not an ordinary painting on a sheet of paper in water-color, oil or other paints. This is a drawing using live microorganisms synthesizing various pigments which are used as "live paint" for making various images. In 2015 Dr. Simon Park from the University of Surrey holder of Peter

Wildy Prize for his pioneering work that unites art and microbiology for making pictures with the aid of pigment live bacteria [3]. Microbial pigments are of importance in many aspects of science, technologies and society. With its good biodegradability and greater safety for the environment, they offer promising opportunities for various applications in food, pharmaceuticals, cosmetics and textiles [4, 5, 7]. They not only can lead to more sustainable and environmentally coloring the world around us in the future, but they have also the potential to facilitate the realization of a more productive relationship between science and art [1].

Microbial pigments are an untapped resource and teachers can include them in laboratory practice in clarifying a wide range of concepts and methods in the field of microbiology and biotechnology.

The color is one of the most important characteristics of the pigments. Colored substances absorb and convert the light beams of a specific wavelength in the visible part of the spectrum (Fig. 1), due to their atomic structure.

Violet: 400 – 420 nm
Indigo: 420 – 440 nm
Blue: 440 – 490 nm
Green: 490 – 570 nm
Yellow: 570 – 585 nm
Orange: 585 – 620 nm
Red: 620 – 780 nm

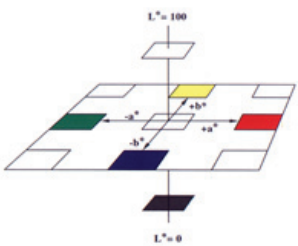
The color of most natural substances is related to the presence in their composition of d- or f- elements of the periodic table. For these elements are characteristic incomplete d- or f-electron orbitals. Under the action of light energy transitions of electrons are made from one orbital to another one which determines the color of the pigments.

Fig. 1 Relationship between the wavelengths of the visible light with its color (the data from Charkoudian et al. [1])

Color as a subjective sensation characterized by the following three parameters: color tone or hue, color saturation and brightness of color. It is generally accepted that each color is considered as a combination of the three primary colors – red, green and blue denoted respectively as R, G and B. If instead of R (red), G (green), B (blue) are accepted the approved by the International Commission on Illumination (CIE) symbols x, y, z, then we talk about coordinates of color and a numerical representation of the visual sensation of the color can be given. Most accurately the color can be determined by use of measuring instruments.

Currently are used color spaces and numerical values for creation, presentation and visualization of colors in two- and three-dimensional space [6].

The CIELab system, Commission Internationale d'Eclairage (CIE), has been an international standard since 1976 in which the color coordinates are respectively:




- L* - brightness, L* = 0 – black color, L* = 100 – white color;
- a* - green color ( - ) / red color ( + );
- b* - blue color ( - ) / yellow color ( + ).

The parameters a* and b* are two chromatic components ranging from -120 to 120 color space (fig. 2). CIELab system is universal. It is applied for measurement of numerical values in the color of fruit, vegetables, wine, ceramic pigments, etc. [10].

Fig. 2 Color space of the CIELab system

The present work aims at presenting innovations in teaching and learning in microbiology of students of Specialty "Biotechnologies" at the Razgrad Branch of the

University of Rousse as drawing with live microorganisms and instrumental color measurement of microbial pigments by numerical expression in the CIELab system.

## PRESENTATION

### Materials and methods

*Pigment microorganisms*

When painting the images we worked mostly with *Saccharomyces cerevisiae* and *Rhodotorula rubra* yeast for coloration in white and red, and the yellow hues are achieved with the *Sarcina sp.* и *Xanthomonas campestris* bacteria. The microorganisms are from the Department of Biotechnologies and Food Technologies at the Razgrad Branch of the University of Rousse "A. Kanchev". Yeasts are maintained on Sabouraud slant agar (OXOID), and bacteria – on meat-peptone agar (NA, Difco) by periodic re-culturing. They are stored at 0÷4 ºC.

*Microbial pigments*

Among the microbial pigments can be met representatives of different classes of substances: porphyrin, carotenoids, phenazine pigments, pyrroles, azaquinones, azaphilones, anthraquinones, anthocyanins, etc. The microorganisms, selected in the experiment, contain mostly carotenoids and xanthomonadin.

Carotenoids are fat-soluble yellow to orange-red pigments. There are two groups of carotenoids: carotenes, composed only of carbon and hydrogen, and xanthophylls which are their oxidized analogs. In the latter oxygen may be part of a hydroxyl group (e.g. zeaxanthin), of oxy groups (e.g. in canthaxanthin) or a combination of both of (e.g. astaxanthin). Red yeast of the genus *Rhodotorula* can produce carotenoids such as β-carotene and astaxanthin. Sarcinaxanthin is the main carotenoid of the yellow species in the genus Sarcina. Xanthomonadin is the water insoluble yellow pigment of bacteria of the genus Xanthomonas.

*Nutrient media*

When creating unique color drawings by means of pigmented microorganisms we have used solid agar nutrient media cooled down in Petri dishes. Yeast were grown on Sabouraud medium (OXOID), Grape agar (grape juice was adjusted to pH 5.5 to 6.0 and is added 3% agar) medium Hansen (in g $l^{-1}$: glucose 50, peptone 10, potassium phosphate 3, magnesium sulphate 2.5, agar 25; pH 5.5 to 6.0). The bacteria were cultured on Meat-peptone agar, a together with yeast – on a Potato-dextrose agar (KDA, HiMedia).

*Painting with live pigmented microorganisms*

The technique that we have used in our work is painting by means of a sterile inoculating loop. Using it material is taken from selected young, 24-hour, microbial cultures in test tubes and seeded on the surface of the nutrient medium along the contours of the previously outlined on the bottom of the Petri dish model or on a placed underneath template. Sterile conditions of work are observed.

The Petri dishes are thermostatically regulated at a suitable temperature – 28 ºC for the growth of the yeast and at 37 ºC for the bacteria. In our pictures the microorganisms are live and after their growth for 24-48 h they synthesize pigments in the characteristic for them white, yellow and orange-red color and making this art work visible. Thus our beautiful color images literally come alive. If necessary, for the addition of another color, in the pattern are plated white yeast *Saccharomyces cerevisiae* which after germination are colored with aqueous alcoholic solutions of aniline dyes which are used in preparation of stained microscopic preparations. When the pictures reach the desired pigmented condition the Petri dishes are stored in a refrigerator. Although the agar in the dishes continues drying more slowly at low temperatures the rate of drying is reduced by wrapping the edges of the Petri dishes with sellotape. Thus the life of our artworks gets extended. As an ephemeral form of art, however, they are safely destroyed and persist only as photographs and videos.

The students have the opportunity to work individually or in small groups (of two or three) together with a teacher, on a certain element in order to illustrate the relationship between art and microbiology. The awareness of the various interests of the students is important for provide an appropriate stimulating educational environment especially where the groups may be greater and their entry qualifications and abilities are quite diverse. There is no upper limit regarding the number of students who can take advantage of this opportunity to express their creativity.

*Color measurement of microbial pigments in L*a*b* units*

The color of the microorganisms is determined by sight or by color scales, but it is too subjective. We applied an innovative method for measuring the color of the microbial pigments spectrophotometrically using a tintometer RT 100 of the company Lovibond and it was presented numerically in L*a*b* units in CIELab system, about which no data have been found in the literature.

*Determination of the influence of the composition and the nutrient medium pH on the color of the microorganisms*

For determination of the influence of the composition of the nutrient medium on the pigment formation the yeast *Saccharomyces cerevisiae* and *Rhodotorula rubra* were seeded on grape agar, Sabouraud medium and on medium of Hansen where as a source carbon were used sucrose, glucose, and maltose. All the media were with pH 5.5 – 6.0.

For determination of the pH influence of the medium yeast are plated on medium of Hanzen with pH 5.5 – 6.0 and 4.5 – 5.0. All crops are made using a yeast suspension with a sufficiently high titer.

**RESULTS AND DISCUSSION**

The results of drawing with microorganisms are diverse, frequently creative and inspiring, and several works are used to illustrate this article (fig. 3). We are the first and so far the only in Bulgaria who in 2015 have applied “microbial art” in teaching microbiology to students from Specialty Biotechnology at the Razgrad Branch of the Rousse university. With imagination and artistry the students create their paintings which by sprouting of microorganisms on solid agar medium come to life in incredible aesthetic shapes and colors. This is a wonderful opportunity to show the beauty of these tinycreatures, of this invisible and unusual world that remains hidden for the naked human eye.


Fig. 3 Color images made using live pigment microorganisms

The work is incredibly funny and simultaneously educational. The students apply the acquired knowledge and skills, methods and manipulation techniques for microbiological work. Drawing with microorganisms allows us to teach and learn the same amount of material in the curriculum but more efficiently and with better results. The students retain the information more complete and for a longer time. The innovation makes the students active participants in the learning process. It is in a condition to increase the commitment, cooperation and communication skills, activate their creative and critical thinking and encourages them to appreciate the wonders and flexibility of microbiology. The

introduction of applied aspects in learning encourages them to more in-depth knowledge and charges them with even greater creative enthusiasm and desire for expression.

The students are very excited and proud to present their work. The works were shown at the 17th exhibition of equipment and technologies in the University of Rousse in 2015, the feast of the Razgrad Branch, were presented at the student scientific session in 2015 and caused universal admiration. For the acceleration of the innovation's diffusion their photographs may be used as decoration of corridors and lobbies in educational institutions, for making advertising materials, calendars and so on.

In the course of drawing with microorganisms we have applied an innovative method for determining the colors of the used yeast and bacteria. They were measured by spectrophotometry and expressed numerically with L* a* b* units in CIELab. This system is used to determine the color of plants [6, 10], but in the literature no data have been found for its usage in determining the color of microorganisms. Generally, on the biosynthesis of microbial pigments as well as on biologically active substances influence the composition and pH of the culture medium, carbon and nitrogen source, light, temperature and other environmental factors [4, 8]. Upon visual determination of the color, however, the extent of influence of these factors cannot be accurately established.

In our study we determined by sight the color of *Saccharomyces cerevisiae* as white, of *Rhodotorula rubra* - orange-red, and of *Sarcina sp.* and *Xanthomonas campestris* - yellow. When measuring the colors in the CIELab system were clearly revealed differences in brightness and the amount of red and yellow in the both types of yeast under the influence of the composition and pH and the type of the carbon source.

The data from the studies of *Saccharomyces cerevisiae* are shown in Table 1, and of *Rhodotorula rubra* - in Table 2.

Table 1

Color coordinates of *Saccharomyces cerevisiae*

| Culture medium | L* | a* | b* |
|---|---|---|---|
| Sabouraud medium with pH 5.5 – 6.0 | 65.98 | -1.98 | 9.38 |
| Grape agar with pH 5.5 – 6.0 | 48.67 | -0.80 | 7.59 |
| Hanzen medium with sucrose with pH 4.5 – 5.0 | 60.95 | -2.79 | 5.10 |
| Hanzen medium with sucrose with pH 5.5 – 6.0 | 62.41 | -2.77 | 4.72 |
| Hanzen medium with glucose with pH 5.5 – 6.0 | 61.46 | -3.28 | 5.14 |
| Hanzen medium with maltose with pH 5.5 – 6.0 | 56.49 | -2.81 | 3.81 |

L* - brightness, L* = 0 – black color, L* = 100 – white color; a* - green color ( - ) / red color ( + ); b* - blue color ( - ) / yellow color ( + ).

Unlike the visual determination of the white color of *Saccharomyces cerevisiae* the measurement in the CIELab system acknowledges the presence of green - a*( - ) and yellow - b*( + ) color. The brightness of the yeast color is highest on Sabouraud medium wherein the amount of yellow is also highest, ranging from 9.38 to 3.81 units.The content of green color is comparatively low. The lowest is the brightness of color and amount of the green color on grape agar. On this medium, however, *Saccharomyces cerevisiae* form a significant amount of yellow in color pigment – 7.59 units.

Similarly to *Saccharomyces cerevisiae* and *Rhodotorula rubra* have the brightest color on Sabouraud medium wherein the amount of red and yellow pigment is also greatest. The visually determined orange-red color of the yeast is confirmed. The brightness and the amount of the red color are lowest on grape agar where the growth of the species is the weakest. In this medium, however, is formed a relatively large amount of yellow pigment.

Table 2 Color coordinates of *Rhodotorula rubra*

| Nuttrient medium | L* | a* | b* |
|---|---|---|---|
| Sabouraud medium with pH 5.5 – 6.0 | 51.55 | 29.30 | 18.34 |
| Grape agar with pH 5.5 – 6.0 | 36.14 | 8.15 | 17.89 |
| Hanzen medium with sucrose with pH 4.5 – 5.0 | 43.64 | 13.28 | 10.74 |
| Hanzen medium with sucrose with pH 5.5 – 6.0 | 46.93 | 15.84 | 9.63 |
| Hanzen medium with glucose with pH 5.5 – 6.0 | 49.27 | 14.39 | 9.43 |
| Hanzen medium with with maltose with pH 5.5 – 6.0 | 42.17 | 14.44 | 8.08 |

L* - brightness, L* = 0 – black color, L* = 100 – white color; a* - green color ( - ) / red color ( + ); b* - blue color ( - ) / yellow color ( + ).

The analyses of the results in tables 1 and 2 show that after acidification of the medium the brightness of the color is reduced, but the b* units of yellow color in both types of yeast increase. In *Rhodotorula rubra* is observed a considerable reduction of the red color – by 2.56 units.

The studied carbon sources exert different influence on the values and parameters of the color of the both types of yeast. A pattern is observed only in the influence of maltose. It reduces the color brightness and the amount of the yellow color in *Saccharomyces cerevisiae* and in *Rhodotorula rubra.* In white yeast sucrose exerts greatest impact on the color brightness, and in the red – the glucose. But in them the amount of red and yellow color increases with the presence of sucrose.

The measurement of *Xanthomonas campestris* color in numerical values shows brightness of color L* = 48.09, confirms the availability of yellow pigment in the bacteria (b* = 16.30 units) and proves the presence of green color (a* = - 1.56).

## CONCLUSION

The innovation applied in teaching and learning of microbiology by painting with live microorganisms on solid agar medium increases commitment, cooperation and communication among the students and teachers. Introducing applied aspects within the frames of compulsory curriculum and interdisciplinary relationship of microbiology with art stimulates students for more in-depth knowledge and activates their creative and critical thinking in a pleasant for learning environment.

For the first time an innovative approach was applied for measuring the color of microorganisms and its numerical expression in L* a* b* units in the CIELab system.

By measuring in the CIELab system was established the influence of composition, pH of the medium and the type of the carbon source on the values and the parameters of microbial pigments.

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