DEFINITION OF A NEW RANGE OF PORCELAIN ENAMELS WITH ANTIBACTERIAL CHARACTERISTICS AND THE METHOD OF THE ANTIBACTERIAL POWER CONTROL

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Definition of a New Range of Porcelain Enamels with Antibacterial Characteristics and the Method of the Antibacterial Power Control

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1. Introduction

The porcelain enamel is the covering of a glassy nature, historically used for its aesthetical and protective functionality in respect to the action of aggressive factors. In particular, the porcelain enamelling of metals that in the past was used as the decorative work for jewels and moniles and only in the XVIII. century began its use to protect metals from the corrosion. Nowadays this material is of common use in many areas of engineering and especially it's appreciated as a protective covering of the metal surfaces, so as to protect them against the corrosion of numerous chemical and atmospheric factors.

The purpose of this work is to extend functional capacity of such covering in terms of resistance to the biological factors, in particular to the bacteria. These, beyond to be able to attack the materials, among which the metals, through the corrosive production of biofilm[1,2,3,4], represent serious and often undervalued risk for the human, animal and phytological health.[5]

Unfortunately, the porcelain enamel for the metals appears to be, to this day, not much studied and few works with this argument don't treat its antibacterial characteristics, for less than one study by *Marzullo*[6] that recognises innate and superior antibacterial characteristic of this material in respect to the others. Last years this problem has attracted attention of some research group[7], but without production of the full works both of methodological functional point of view and industrial application. This group of the research already active in other studies about mechanical tribological characteristics of porcelain enamel for metals[7,8,9,10,11], has given an idea to improve considerably reply of porcelain enamels conveniently formulated in respect to bacteria, both in terms of velocity of antibacterial action, and of its duration in time, defining at the same time strong method to evaluate antibacterial power. To this day the analyse method the most qualified for evaluation of the antibacterial power of the surface is described in the norm JIZ Z 2801:2000. This method, at least provisionally in this application case, isn't immune to the problems and in the next paragraphs is also suggested alternative and optimised method based on what is given in *Pfaller et Al.*[7].

Leaving from the formulations in oxides of standard porcelain enamels that have already for itself aesthetic- functional specific characteristics, it was made a careful analysis of the literature regarding the capacity to interfere with bacterial proliferation by the side of metallic oxides of the common or extraordinary use in the ceramic area. In particular, there was found a number of studies which deal with the antibacterial activity of some metallic cations in solution or of some ceramic powders moved along in different matrix or in massive form[7]. The best known antibacterial inorganic factor is certainly Silver oxide (Ag_2O), of which properties are defined as oligodynamic. This term indicates that silver is active even if it's present in very low concentrations. It is used with success as addition

of metals used for the construction of the water tanks in public field and in past its salt was used to disinfect injures[7,8,9,10,11]. The other oxide, where are recognized antibacterial characteristics is rameic oxide (CuO), that for such characteristic is now of normal engagement in diverse fields. The most recent began the study of action mechanism of this element in contrast with the bacterial proliferation. It seems that in certain aquatic fields is active under form of Cu^{2+} already in concentration of 10 μg l^{-1}. However, in order to study the composition of battericides supports, must be bear in mind more recent works that have demonstrated how the total quantity of Cu(II) isn't directly connected to the bacterial activity, but like the previous depends solely on the presence of Cu^{2+} free in the solution of decontamination absorbed by *E. coli* e *S. aureus*, damaging irreparably its structure[7]. This quantity is measured through pCu[7,8,9] More recently only, thanks intensive study in fields of catalise it was discovered antibacterial power of TiO_2. The titanium oxide is considered antibacterial in so far factor that activated fotocatalitical reactions, through radiation with UV, that damage organic compounds, even those contained in cells. The number of studies is available about fotocatalitical effects on different microorganisms of TiO_2 on powder or nano-dust, moved-along in solutions or like covering for the fotocatalitical surfaces[7,8,9,10,11,12]. There are evidences[7,8,9,10] of the fact that fotocatalitical activity causes lipids peroxidation of the cellular membranes and subsequently of the intracellulare material, in way to kill the bacteria. Even if other coverings, different from that proposed here, have already demonstrated excellent foto-biocide capacity[7] they present the necessity of being activated through radiation UV. In addition according to *Sawai et Al.*[7,8,9,10,11] even ZnO would have some type of interaction with the bacteria, even if this would be considered bacteriostatico rather than bactericida. This reacts with major efficacy on Gram-positive bacteria and its action would attribute the interactions on surface of liquid-solid interface, in fact zinc is scarcely soluble and moreover it was noticed how increasing the fineness of the particles, increases also the bacteriostatical activity. Superficial moisturizing, thanks which are formed on the surface the groups of -OH, plays an important role in antibacterial activity: is sensible to the heat-treatments of high temperatures (1023K), that cause non hydrate structure stabilization (63%) in bond Zn-O. From other works of the same group emerge that the action mechanism of ZnO could be correlated to damage of ribosoms in cell of *E. coli*. What could derive from fact that ZnO creates H_2O_2. In regard is mention also the new certificate (WO 2006/088468 A2) of a new porcelain enamel for ceramic and metals zinc oxide containing and zinc borate having antimicrobical characteristics.

It's related finally as much as affirmed in one interesting comparative study between quartz, zinc oxide and titanic oxide[7] in nanometrical form. Here the authors attribute even though not so high but important antimicrobical power to nanometrical crystal silice. This peculiarity of the silice could be correlated on raw antimicrobical power of the porcelain enamel introduced at the beginning.
How it's hitherto affirmed, it can be said that the capacity of the inorganic oxide to act like antibactecal could depend on the following phenomena:

1. transfer of antibacterical factors, under form of cations, to the solution containing bacteria;
2. oxidation by fotocatalitical wayof the organic compounds from which bacteria are constituted;
3. production by catalitical way of oxidant factors like H_2O_2.

This work consecutively described, has an objective to study antibacterial efficacy of these and other substances when they enter to be part of the constitutional elements of the covering of the porcelain enamel for metals.

2. Experimatal details

2.1 *Preparation of samples*

Through the specific techniques of porcelain enamel field, were made and applied three different coverings: AMB1 (white porcelain enamel), AMB2 (porcelain enamel for water heaters), AMB3 (antiacid porcelain enamel).

When barbottines (*slip*) was obtained, coverings were applicated on substrated ultra low carbon steel DC04ED which form recovers the capsule Petri. One example of this capsule follows in Fig. 1.

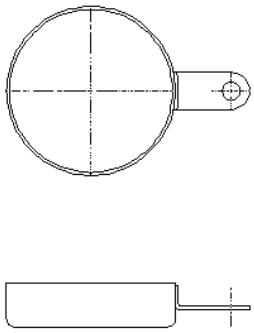


Fig. 1 Drawing of the pseudo-capsule Petri used for the microbiological test

The samples were then be fired for 6'30'' at 850°C. Pre-emptively, in order to evaluate the analytical method, one set of pseudo-capsule Petri was covered with standard porcelain enamel that contained no antibacterial factor and denominated NEUTRAL.

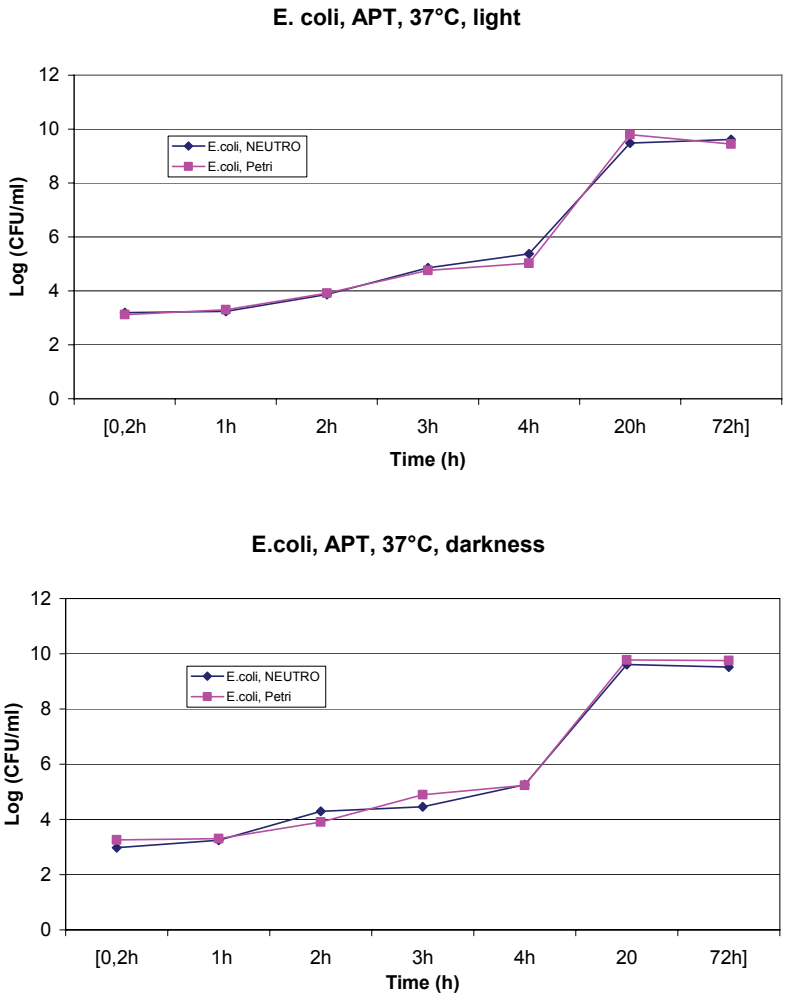
2.2 *Method of antibacterial activity analysis*

Data obtained from this work of research are based on the microbiological test expressly created so as to minimize the experimental mistake and to speed up analysis. Grounding on what described in the technical norm JIS Z 2801: 2000 and in recommendation of *National Committee for Clinical Laboratory Standards* (NCCLS) in matter of *Time-Kill Method* as mentioned above, was established a method of action antibacterial analysis of surface in function of time, that confirmed a strong and fast application. It's fundamental step to produce pseudo-capsule Petri, of which internal surface in contact with broth containing the bacterial colony is made and covered by material of which will be tested antibacterial capacity. The test were conducted using bacterial suspensions in APT and in physiological, di *Escherichia coli* e *Staphylococcus aureus*, prepared according to a normal microbiological standard having concetration of 10^8 CFU/ml. At the beginning of each test the known quantity of two bacterial suspensions, usually 10 ml or the sufficient volume to cover all the capsule surface, it was inoculated in covered pseudo-capsule Petri and in standard capsule Petri, that rappresent white test and were placed to incubate both in presence of light and darkness in 37°C. Leaving from zero (moment of inocule) and subsequently to set intervals of 6, 20 e 72 hours, were drawn rates of 100 µL of bacterial suspension directly from capsule submitted to a test and CFU residual were counted by technique of *agar plate count*. The variant of described method consists in using bacterial suspension of 10^3 CFL/ml in APT with which test the sample NEUTRAL, in order to desperse each doubt in goodness of the proposed technique, once verified equivalence of multiplicative capacity of bacteria in this samples and capsule Petri. For this version samplings were made in 0.2, 1, 2, 3, 4, 20 e 72 hours .

3. Results and discussion

3.1 *Stoutness of the method*

To control the efficacy of analyse method of the capacity of bacterial charge fall, proposed test was conducted also in its variant with bacterial concentration of 10^3 CFU/ml, in parallel with NEUTRAL enamel, which composition is limited to the minimum of constituting oxides, and on the set of Petri capsules. The test were conducted with 5 repetitions every single one. On the Graphs 1 is clearly shown how process of growing curves of *E. coli* in APT with the initial concentration of 10^3 CFU/ml, varying conditions of lightness and darkness, is completely equivalent for two types of capsules. Processes completely similar were obtained for *S. aureus*. Limited case, is given by experimental evidence that, as in the Petri capsule, as in that covered with NEUTRAL, bacterial suspensions in APT having initial concentrations of 10^8 CFU/ml, bring already after few hours, to the formation of extended bacterial coat and non accounting.



Graph 1 Growing curve of *E. coli* in APT in NEUTRAL and Petri capsule

3.2 *Evaluation of the antibacterial capacity*

Experimental data obtained for three types of covering, submitted to an analyse with bacterial suspensions in physiological solutions and in APT, are respectively related in Schedule 2 e in Schedule 3. Moreover, Graphs 2, 3 describe process of the growing curves for *E. coli* e *S. aureus* in physiological solution and APT for enamel AMB3, which gave the best results.

Enamel	Conditions	Bacteria strain	15 min (Log CFU/ml)	6 hours (Log CFU/ml)	20 hours (Log CFU/ml)	72 hours (Log CFU/ml)
AMB1	Light	E.coli	8.34	8.03	5.98	5.82
		Petri	8.16	8.09	7.75	7.64
	Darkness	E.coli	8.36	8.12	6.07	6.01
		Petri	8.42	8.22	7.83	7.51
	Light	S.aureus	8.11	7.26	6.21	5.36
		Petri	8.17	7.81	7.65	7.23
	Darkness	S.aureus	8.01	7.04	5.94	5.01
		Petri	8.30	8.21	8.08	7.49
AMB2	Light	E.coli	8.22	5.08	1.36	0.45
		Petri	8.14	8.06	7.29	7.25
	Darkness	E.coli	8.24	5.11	1.45	0.85
		Petri	8.34	8.16	7.49	7.33
	Light	S.aureus	8.09	1.97	1.40	-0.02
		Petri	8.32	7.11	7.02	6.75
	Darkness	S.aureus	8.12	2.65	1.75	0.98
		Petri	8.34	7.56	7.35	7.21
AMB3	Light	E.coli	8.34	4.78	0.95	-0.05
		Petri	8.26	8.18	7.78	7.74
	Darkness	E.coli	8.36	4.91	0.95	-0.05
		Petri	8.45	8.26	7.79	7.56
	Light	S.aureus	8.11	0.95	1.30	-0.05
		Petri	8.30	8.15	8.12	7.98
	Darkness	S.aureus	8.01	0.95	0.95	0.78
		Petri	8.30	8.21	8.01	7.85

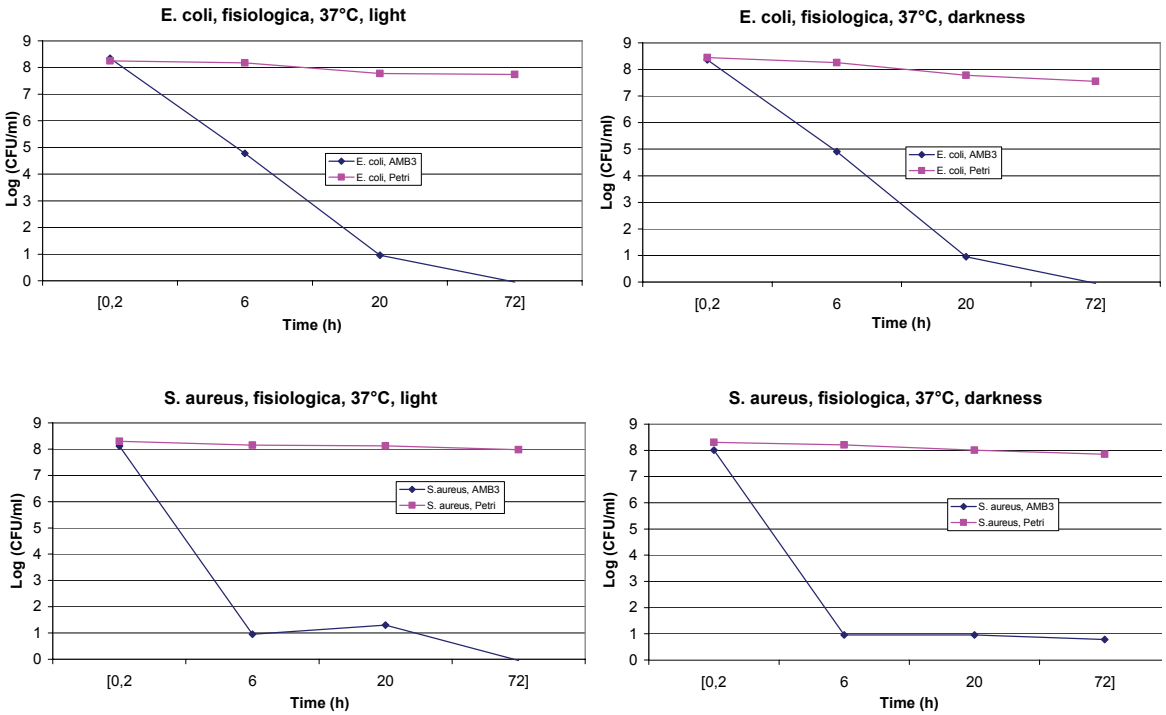
Schedule 2 Results of the fall test of *E. coli* ed *S. aureus* in phisiological solutions

Enamel	Conditions	Bacteria strain	15 min (Log CFU/ml)	6 hours (Log CFU/ml)	20 hours (Log CFU/ml)	72 hours (Log CFU/ml)
AMB1	Light	E.coli	8.16	n.c. patina	n.c. patina	n.c. patina
		Petri	8.24	n.c. patina	n.c. patina	n.c. patina
	Darkness	E.coli	8.62	n.c. patina	n.c. patina	n.c. patina
		Petri	8.41	n.c. patina	n.c. patina	n.c. patina
	Light	S.aureus	8.17	n.c. patina	n.c. patina	n.c. patina
		Petri	8.19	n.c. patina	n.c. patina	n.c. patina
	Darkness	S.aureus	8.34	n.c. patina	n.c. patina	n.c. patina
		Petri	8.30	n.c. patina	n.c. patina	n.c. patina
AMB2	Light	E.coli	8.12	7.44	5.25	5.20
		Petri	8.26	n.c. patina	n.c. patina	n.c. patina
	Darkness	E.coli	8.31	6.99	5.71	5.64
		Petri	8.27	n.c. patina	n.c. patina	n.c. patina
	Light	S.aureus	8.39	7.36	5.40	4.76
		Petri	8.25	n.c. patina	n.c. patina	n.c. patina
	Darkness	S.aureus	8.25	7.34	5.81	4.42
		Petri	8.02	n.c. patina	n.c. patina	n.c. patina
AMB3	Light	E.coli	8.31	7.18	5.05	5.00
		Petri	8.33	n.c. patina	n.c. patina	n.c. patina
	Darkness	E.coli	8.25	7.11	5.22	5.14
		Petri	8.41	n.c. patina	n.c. patina	n.c. patina
	Light	S.aureus	8.02	7.00	5.30	4.15
		Petri	8.33	n.c. patina	n.c. patina	n.c. patina
	Darkness	S.aureus	8.20	7.04	5.51	4.42
		Petri	8.27	n.c. patina	n.c. patina	n.c. patina

Schedule 3 Results of fall test of *E. coli* e *S. aureus* in APT

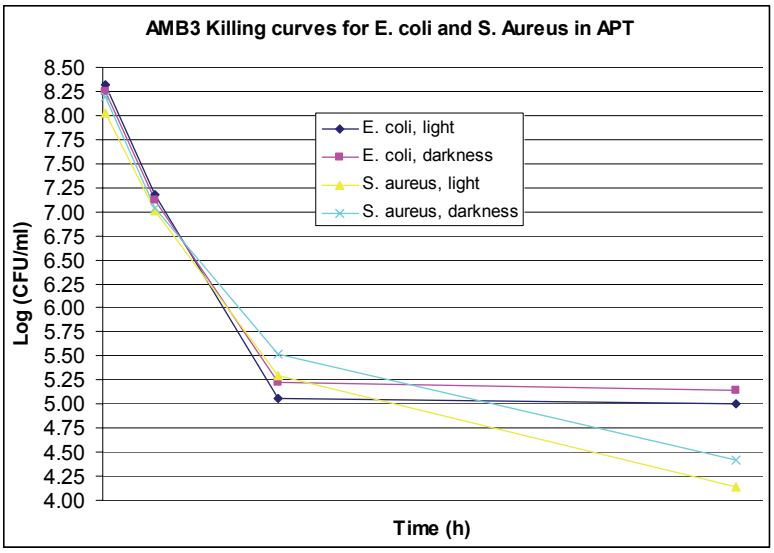
As it's noted, all whites of test don't give any interferences with bacterical activity, in line of what is coherent waiting in optimal conditions. On the contrary, the curves obtained from the count of bacteria in suspension in physiological solution, inoculated in psedo-capsule Petri covered with AMB3, incubated in light and darkness, demonstrate fall of initial bacterial charge of at least three orders of greatness. In particular the fall of *E. coli* in physiological solution, on the light and darkness,

is resulted to be of three orders of greatness already after six hours of incubation, to arrive up to the factor 10^7 after 20 hours.



Graph 2 Process of the growing curves for *E. coli* e *S. aureus* in physiologic in AMB3

For physiological *S. aureus* in, already after six hours of incubation, initial inocule was reduced to the factor 10^7.



Graph 3 Growing curves of *E. Coli* e *S. aureus* in APT in AMB3; points are referred to 0,2, 6, 20 e 72 hours of contact

In the case that two bacterial fettersesis APT instead, following their evolution in time, the contact with the coverig AMB3, in condition of lightness and darkness, giving a fall no more that one logarithm after 6 hours and 3 logarithms after 20 hours respect to initial inocule; in 72 hours from inocule the sampling confirms the number stability of CFU counted for *E. coli* from previous sampling, while register ulterior fall for *S. aureus*, that is attested on the total reduction of factor 10^4 with respect to initial inocule. Even if the porcelain enamel AMB2 gave remarkable results in terms of contrast to bacterial proliferation, even if slightly inferior of that obtained for AMB3. Finally, enamel AMB1 is demonstrated to be more limited than other two, even having permit total fall of *E. coli* in physiological solution, in lightness and darkness, of 2 logarithms after 20 hours that remain unchanged in 72 hours. Lightly better results were obtained in the same operative conditions for *S. aureus*, that was falled by the order of greatness after 72 hours. It must be register the missed efficacy of AMB1 towards 2 bacterial fetterses (Petri capsule), both in pseudo Petri covered with this porcelain enamel.

4. Conclusions

This work described in present document, not at all definitive, offers new possibilities of the research and of market for porcelain enamel for metals. In particular, shows in evident manner the possibility to manipulate formulation of this ceramic coverings for obtaining new functional properties, like the capacity of contrasting the bacterial proliferation. However, it's necessary to persevere in experimentation of the new antibacterial factors, as much as to conduct test on different bacterial fetterses. It's noted finally how, beyond defining the precise and fast analytical method and of effective realization of the porcelain covering already industrializable, our study made possible demonstration one more time of the effective capacity of some inorganic compounds contrasting bacterial proliferation, even after that these were heat treated in significant way and repeated and included in glassy amorphous matrix.

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