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Decontamination of Solid and Powder Foodstuffs using DIC Technology

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1. Introduction

1.1 Generalities

Micro-organisms correspond to a multitude of living organisms. Indeed, may be considered as micro-organisms: bacteria, yeast, fungi, protozoa, micro-algae, prions. As for viruses, belonging to micro-organisms is still debated, since some scientists consider them more as "objects" than living organisms. Pathogenic microorganisms are harmful to humans. Their destruction is completely required to adapt the processes to inhibit their proliferation.

It is important to note that many microorganisms has two forms: a "vegetative" form in which the microorganisms is present when the environmental conditions are favorable for its development and a "spore" form which appears when these conditions become unfavorable. The microorganisms have hence the particularity to "wrap" themselves with a sort of protective "shell". The "spore" forms are thus much less sensitive to potential unfavorable environmental situation. The destruction of these germs is generally carried out in two stages: the first aims to trigger the spores' germination and the second takes place destroying the germinated spores. If germination is incomplete germs inactivation will then be incomplete and will lead to final product partially treated but still infected. These microorganisms are present in our environment and can be found in our diet.

Decontamination techniques must also take into account the very strong ability of microorganisms to adapt themselves rapidly to their environment. Thus, the microorganisms that suffered sub-lethal stress factor can develop new resistance mechanisms (Hill et al., 2002; Lou & Yousef, 1997; Rajkovic et al., 2009; Rowan, 1999). This evolution is usually not abrupt but more gradual. (Rajkovic et al., 2009).

Safety standards become a shared concern increasingly limiting food market access. Facing this constraint, the food industry has only two solutions:

1. limiting the contamination of raw materials and probably during the manufacturing process,
2. applying final appropriate technologies to reduce microbial load.

Indeed, the precautionary measures settled in various manufacturing processes to acquire healthy foods are in no way sufficient to fully guard against various sources of microbiological contamination. On the one hand, the possibility of clean production processes (microbiologically speaking) is never perfect and the need to include a specific decontamination step is a vital reality, even in the most developed countries. On the other hand, the food industry must also provide products with high nutritional, gustative, etc. quality while remaining “natural”. On various industrial and traditional handwork levels, these operations have two seemingly contradictory objectives:

1. a relevant microbial destruction and
2. the best biochemical, nutritional, sensory... preservation.

The need for a specific optimization for each case is hence highly needed. There are so many constraints related to the need of a good decontamination operation in terms of technical performances (microorganism's destruction efficiency, energy consumption...) and great preservation of product attributes and quality.

1.2 Issues of some decontamination techniques

Early techniques of microorganisms destruction were established on the use of temperature, with different levels of temperature and different treatment time: pasteurization (temperature between 62 and 88°C) sterilization (temperature above 100°C) and canning (temperature around 121°C).

Pasteurization destroys a significant fraction of the microbial load. However, products that undergo this process require special storage conditions. In fact, these products still contain germs capable of multiplying. If they are placed under favourable environmental conditions to their multiplication, the products will then quickly become unfit for human consumption. Sterilization has been used for a long time in order to inactivate microorganisms (Hope, 1901; Kim et al., 2007). Unfortunately these heat treatments have very negative effects on nutritional, gustative, etc. qualities, (Jb et al., 2003).

Furthermore, the use of thermal shock (positive or negative) poorly controlled may lead to increase resistance to subsequent decontamination techniques. (Nevarez et al., 2010) have studied the case of *Penicillium glabrum*; (Chang et al., 2009), *Cronobacter sakazakii*; (Lin & Chou, 2004) and *Listeria monocytogenes*. (Broadbent & Lin, 1999) have studied application to increase the germs preservation (*Lactococcus lactis*) used as processing assessment.

Although relatively effective, conventional steam treatments often require a long period of rising and fall edges in temperature and a great temperature gradient, in other words a lack of homogeneity, which naturally affect the qualities of the end product.

The use of specific gases such as ethylene oxide or propylene oxide has long been practiced at room temperature. Nevertheless, standards are more and more stringent mainly regarding residual molecules introduced into the treated product. They hence tend to be completely banned such as ethylene oxide that has been prohibited in France since 1990.

The various processing operations applied to liquid or pumpable products are, somehow, more easily achievable.

An obvious statement can now be established in the decontamination field: after a great and long activity of studying, investing and optimizing the broadest possible range of technologies, several new types of treatment have been realized successfully. However, the heat treatment remains the major industrial operation adopted to destroy microorganisms in liquids mainly as UHT (e.g.: ohmic heating) (Lewis & Heppell, 2002).

Microorganism destruction in solid faces several types of difficulties. The heat transfer or radiation penetration phenomena are more difficult to achieve. Heat or radiation treatments

are almost impossible to be uniform. This is even more difficult since solids must be often processed in bulk. Dried food products as solid (spices, herbs, onions, garlic ...) or powder (flour ...) are known for their high microbial load sometimes coupled to a presence of insects, often because of their traditional way of production including harvesting, drying, grinding, storage, etc. Very few remediation technologies have been suggested to adapt to this type of products.

Although relatively effective, conventional steam treatments often require a long period of rising and fall edges in temperature, a great temperature gradient and in other words a lack of homogeneity, which naturally is capable to harm the qualities of the end product. The use of specific gases such as ethylene oxide or propylene oxide has long been practiced at room temperature. Nevertheless, standards are more and more stringent mainly regarding residual chemical molecules introduced into the treated product. They hence tend to be completely banned such as ethylene oxide that has been prohibited in France since 1990.

The ionization by nuclear gamma irradiation has for a very long time and for many solid and/ or powders foodstuffs emerged as the best decontamination treatment (Fan et al., 2003; Molins, 2001; WHO, 1988; R.A. Molins et al., 2001). This treatment has proven to be highly effective and very convenient since the product can be treated in its airtight packaging away from any recontamination. γ irradiation has been employed for the decontamination and/ or sterilization of dehydrated vegetables, fruits, seasonings and animal foods, and to prolong the storage period (Chwla et al., 2003; Fu et al., 2000; Mahrouf et al., 2003). The dose to deliver to food depends on the desired effect: "low" dose irradiations (50-150 Gy) don't decontaminate and are used to only inhibit sprouting of potatoes and onions. Food sterilization (e.g.: pre-cooked meals) requires much higher doses (10-50 kGy). (Thomas et al., 2008) irradiation dose of 7 kGy can be effective to control microbial growth in black tea and in extending their shelf life without any significant deterioration of quality constituents. This technology enables food processors to deliver larger amounts of high quality tea with extended shelf life.

Nevertheless some negative impacts of such treatment are possible. Because of the radiation energy, ionization can remove electrons from atoms and break molecular bonds leading then to the formation of highly reactive free radicals. New molecules could thus appear in the food as a result of chemical recombination. Irradiation of lipids causes the formation of cyclobutanones whose toxicity is well known. Although works are needed to identify these chemicals toxicity, studies done in 1950/ 1960 have revealed very disturbing effects including chromosomal damage.

Radiation can also induce the loss or the degradation of amino acids and vitamins (including A, B1, B6, B12, C, E, K, PP and folic acid) depending on the dose and the radiosensitivity of molecules. High dose irradiation kills bacteria but does not affect the toxins previously produced. However, these toxins are often responsible for many foodborne illnesses. Alternatively, such irradiation eliminates all micro-organisms in food, including those with useful features. Finally, some authors highlight the risk of a particular mutation induced by irradiation in insects and bacteria. In addition to these scientific and technical aspects and despite a big marketing effort developed by the concerned industry to attest the safety of irradiation (proved only for doses up to 10 kGy), ionizing radiation suffers from a very bad public opinion due to the confusion between radiation and radioactivity. Consumer rejection has been strengthened especially since the mandatory labelling in 2001 of all products processed by ionizing radiation. This set of elements and the relatively high equipment cost explain its very low approval.

The World Health Organization (WHO) expert committee on the wholesomeness of food irradiation agreed with the food and drug administration (CAC, 2003) that foods subjected to low dosages (10 kGy) of γ irradiation are safe and do not require toxicological testing (WHO, 1981; FDA, 2005; WHO, 1988). γ irradiation can extend the shelf life of treated foods without inducing the formation of any radionuclide in food products. (Lacroix & Quattara, 2000).

High Pressure Processing HPP treatment is generally considered to:

1. affect bacterial cell membranes and impair their permeability and ion exchange, but also
2. but also to inactivate some of the enzymes vital for survival and reproduction of bacterial cells (Cheftel, 1995; Hoover et al., 1989; Considine et al., 2008; Yaldagard et al., 2008).

Through HPP treatment, microorganisms undergo different range of resistance, with some strains being more resistant than others (Alpas et al., 1998; Chung & Yousef, 2008).

The inactivation of spores by HPP compared to efficiency in vegetative cells, is less efficient and requires higher pressures and higher temperatures (Heinz & Knorr, 2005 and references therein).

Bacterial spores were set up to survive up to 1200 MPa at room temperature (Zhang & Mittal, 2008 and references therein). Besides they compiled a review with data details showing that there can be significant variations in the requirements of high pressure and temperature among different bacterial spore species and also among strains of the same species. For a successful inactivation of spores, the optimization of the HPP conditions or the combination with other treatments and agents may be needed.

The use of a specific heat treatment similar to UHT in the case of solid or powder foods may be a necessary and indispensable solution. The needs of a very rapid heating and an instant cooling have been noticed through the use of an instant controlled treatment: the DIC.

Indeed, during over twenty-two years (since 1988), the LMTAI (Laboratory Mastering of Technologies for AgroIndustry) has managed to develop a specific research activity about the impact of the instantaneous pressure drop on cells structure and biological structures. This work, in addition to its apparent scientific interest, had an obvious technological impact. Saturated or superheated steam injection, at controlled pressure reaching a level of seven or eight bars of absolute pressure applied to products put initially under vacuum, implies a very rapid heating done mainly by condensation on the inner surface of the product. The definition of a limiting thickness can achieve this step in less than three seconds. The heating time is completely controlled by introducing a new stage in vacuum (around 5 kPa of absolute pressure), abruptly established (in less than 1/ 10th second i.e. a decompression rate of more than 50 MPa s⁻¹).

Thanks to the autovaporization the product temperature drops immediately to reach very low levels, necessarily lower than the equilibrium temperature of the water (which is in our case 33°C).

Other versions of this process consist in achieving proper heating by hot air, contact with hot plates, microwaves... possibly coupled with vibration or mechanical mixing for a more uniform treatment. The application of the instant pressure drop towards vacuum generates an instant cooling by autovaporizing a part of the product water.

Such treatment reflects perfectly the heat treatment required by the UHT process usually applied to liquids. However, studies conducted by the LMTAI (Debs-Louka et al., 1999) have proven efficiency twice as high. It was then possible to prove that after applying the DIC, the action of the instant pressure drop was not confined to the only impact of abrupt

cooling. A thermo-mechanical effect that may lead to the microorganism cells explosion (spores or vegetative forms) also occurs.

The higher the amount of "steam" generated within the cell and the smaller the pressure drop time, the more efficient the mechanical effect. Besides water, further molecules could be considered. The choice of the molecule is closely related to the importance of its possible mechanical action. This must be due to the extent of the difference between the initial and final pressures and the rate of pressure drop itself.

The use of carbon dioxide has been studied because of its high relative dissolution capacity during a high pressure stage. For each fluid used the most important point was its capacity to generate a force capable of breaking and even cracking the cell walls.

Therefore various industrial applications have been obviously established. The most immediate consisted in the definition of thermo-mechanical destruction of microorganisms while maintaining as well as possible, the various contents on the agro-food quality (texture, flavor, vitamins, protein activities, etc.). At the initial LMTAI (Allaf et al. 1998; Debs-Louka et al., 1999; Debs-Louka 2000) research lies in the definition of the various impacts of operations and determination of their main phases of intervention. The couple "temperature-time" will establish the level of decontamination. Quantifying the impact on various quality parameters can lead to the establishment of a multidimensional optimization generally very relevant.

Other researches, carried out subsequently by the company ABCAR-DIC process, have identified areas of treatment different from those initially planned. Application sectors are dramatically widening. Various studies have been made on many solids and powders, such as fruits, vegetables, meat and seafood, algae and microalgae, spices, ginger, etc.

In the case of this operation, the impact of the number of pressure drops towards vacuum has been quantified. The effect of decontamination by DIC has therefore been optimized according to various constraints related to: the product and its requirements in terms of quality, the microorganisms that we seek to eliminate and those that we want to preserve.

DIC specificity is related to the ability to define the degree of decontamination across the triumvirate "temperature-time-number of pressure drops" instead of the conventional torque "temperature-time". The impact of such possibility is immediate in terms of quality control of the finished product.

This work results from the identification and analysis of a new thermo-mechanical process of destruction of microorganisms, mainly valid for solid or powder products. DIC can advantageously substitute conventional processes in this field where many new treatments, such as radiation (γ , ultraviolet, acoustic...) or mechanical (UHP, ultra-sound), have had, when achievable, only very restrictive applications.

The efficiency of the instant controlled pressure drop DIC developed as microbial inactivation system was studied, analyzed, and optimized. This chapter aims to illustrate the main impact of saturated steam instant controlled pressure drop STEAM-DIC per se. Its double impact as heating and explosion effects allowed it to be very relevant in terms of industrial operation capable to intervene in both decontamination and preservation of functional, nutritional, and sensorial quality, in a large domain of very heat fragile foodstuffs. The study of the second version of this operation had, as objective, to reduce even more the thermal impact by inserting numerous pressure-drops maintaining the same temperature level and the total treatment time range. As the main functional and sensorial properties as well as the nutritional contents closely depend only on the processing temperature and the treatment time, such pressure drops normally intervened to improve the decontamination effect, while maintaining quality. It was even possible to improve some

functional quality by increasing the specific surface area. By inserting numerous pressure drops, the Multi-Cycle DIC treatment was studied for expanding the granule, creating internal pores, preserving the quality while implying more decontaminating effect.

1.3 The DIC and its application fields

The DIC Détente Instantanée Contôlée, French for Instant Controlled Pressure-Drop, is based on the principles of thermodynamics of instantaneity. It started in 1988 by the fundamental study on the expansion through alveolation and has targeted several industrial applications in response to issues of control and quality improvement, coupled with reduced energy costs. They are various operations studied such as steaming, extraction, drying, and sterilization. The approach has always involved the integration of phenomena of instantaneity to intensify the elementary processes of transfer. Several industrial projects have been developed, the first one in 1993. Several patents have been filed since 1993, (Allaf et al., 1993) and more than twenty Phd thesis have treated the subject from different angles.

1.3.1 Waterlogged wood

This application has been patented (Allaf et al., 1997). Archaeological investigations often renew pieces of wood having spent long periods in water (mostly seawater). Once emerged, and as they gradually lose water (dehydration), they deteriorate very quickly. The DIC treatment can stop these degradations.

1.3.2 Rice steaming

The results of this application have been prepared following several thesis and research works (Duong Thai, 2003). The DIC treatment is presented as an operation of pre-drying just after harvesting or otherwise as part of a treatment for rice steaming. In both cases, the DIC-rice has many advantages compared to the conventional method:

1. a time of particularly low heat treatment (30 seconds instead of 40 to 60 minutes with conventional drying),
2. a 2-hour drying instead of 8 hours without any tempering period and a higher quality end product,
3. a better performance (a percentage of broken rice generally less than 3% instead of 15% to 35% for conventional methods).

1.3.3 Sterilization

Three patents protect this application (Allaf et al., 1994; Allaf et al., 1998; Allaf et al., 1999). DIC treatment eliminates micro-organisms (even spore forms) through two main mechanisms: a particularly well controlled heat treatment and mechanical stress on the micro-organisms caused by the instant pressure-drop that lead to their explosion.

1.3.4 Drying and texturing

Both applications are very often associated, although we can approach one without the other. In the case of coupling the two processes, applications studied have been numerous on vegetables, fruits, fish, meat products... DIC treatment causes an expansion in response to the mechanical stress due to the autovaporisation by instant pressure drop, leading to a good textured, porous once the operation is performed near the glass transition. The final drying step is generally carried out by conventional hot air or TPG (Total Pressure Gradient).

1.3.5 Drying by TPG (Total Pressure Gradient)

This is another version of the DIC-drying. The autovaporization is used as a step of removing a certain amount of water, so a succession of pressure cycles followed by a pressure-drop towards vacuum will intensify dramatically the drying since it brings the solution to the paradox of (Al Haddad et al., 2008).

1.3.6 Volatile molecules extraction

Through the autovaporization of volatile compounds, the DIC technology allows removing a big part of essential oils present in the plant (aromatic herbs, fruits, flowers...). By assuring a Multi- DIC-Cycles, the complete extraction is generally carried out in some minutes (2-4 min), with low energy and low added water consumption.

1.3.7 Non-Volatile molecules extraction (solvent extraction)

The expansion of the solid matrix through the DIC treatment can act on the solvent extraction. Indeed, such a treatment implies increasing the porosity and the specific surface area of the treated plant. Therefore it subsequently allows the solvent to easily enter the matter and hence extract the requested material. DIC texturing is considered as a solvent extraction pretreatment, which generates a dramatic decrease of extraction time of non-volatile compounds.

2. Material and methods

2.1 Raw materials: Powders

Trials were carried out on various varieties of seaweeds, skim milk "low heat" powder manufactured at the INRA of Rennes, France (Research Laboratory of Dairy Technology), which was artificially contaminated by ASR spores and vegetative forms. Table 1 shows the initial chemical composition. Before treating by DIC, powder humidity is controlled from 4% to 22% dry basis depending on the spray-drying conditions (air temperature, speed of flow and humidity content).

Some seaweed industrial amounts from SETALG Co and microalgae powders such as spirulina were STEAM-DIC treated just after a first stage of drying (hot air drying, spray-drying, freeze-drying).

Sample N°	Powder				Spray-Drying Temperature	
	Casein	Whey protein	H ₂ O	a _w	T _{inlet} (°C)	T _{outlet} (°C)
	concentration (g.kg ⁻¹)		(%)			
H ₁	250.1	66.8	6.0	0.34	140	64
H ₂	246.2	65.9	7.5	0.41	110	47

Table 1. Physical and chemical characterization of classical spray-dried skim milk powder

2.2 Treatment equipment

Fig. 1 shows the operational protocol used in different trials. The experimental set-up has been largely described (Allaf et al., 1989, 1992, 1993a, 1993b). It comprises three main parts (Fig. 2):

The processing vessel (1), where steam or gas pressure may be established up to 1 MPa.

The vacuum system, which is mainly a vacuum tank (2) with a volume 100/ 150 times greater than the processing reactor, and an adequate vacuum pump capable of reaching and keeping the vacuum level constant at 5 ± 0.1 kPa in all our experiments just before dropping the pressure.

An abrupt pneumatic valve (3) that assures the connection/ separation between the vacuum tank and the processing vessel. It can be opened in less than 0.2 second, which ensures the “instant” pressure drop within the reactor.

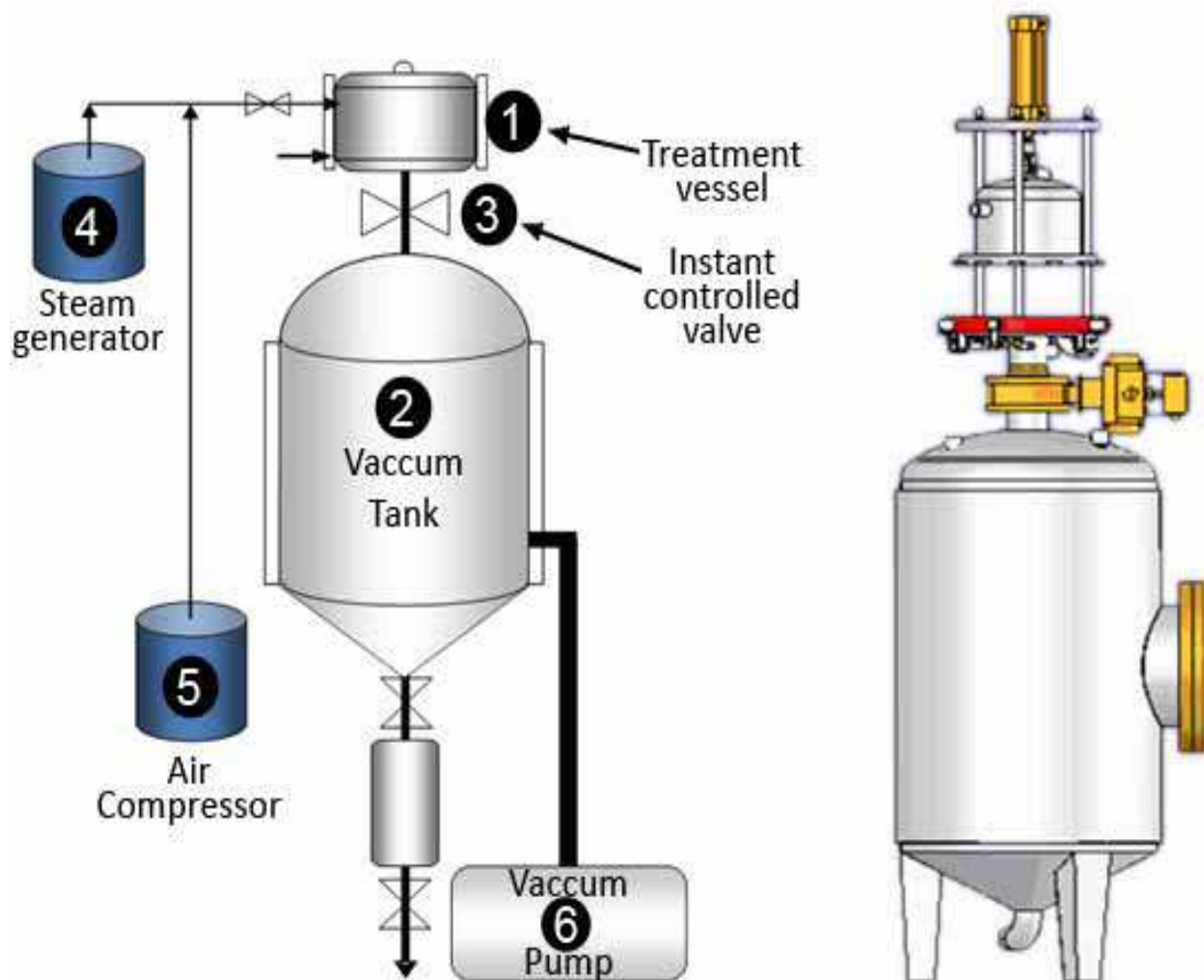


Fig. 1. Schematic presentation of DIC reactor: 1- treatment vessel; 2- vacuum tank with double Jacket as condensers; 3- controlled instant pressure drop valve; 4- steam generator; 5- air compressor; 6- water ring vacuum pump

Samples of approximately 60 g of powders are first placed in the DIC treatment vessel. The treatment consists in setting up a first vacuum stage, then injecting gas at a predefined pressure and maintaining it for a predefined time; the gas we use is compressed air in the case of Multi-Cycle DIC and saturated steam in the case of STEAM-DIC. By abruptly dropping the pressure ($\Delta P / \Delta t > 0.5$ MPa.s⁻¹) towards vacuum, instant autovaporization occurs, inducing texturing and “instant” cooling of the treated material (Figure 2). Similar

system was used at industrial scale. It is a 150 L processing treatment and 25 m³ as vacuum tank. Scaling up studies allowed us to adopt the same DIC processing parameter values to reach the same results.

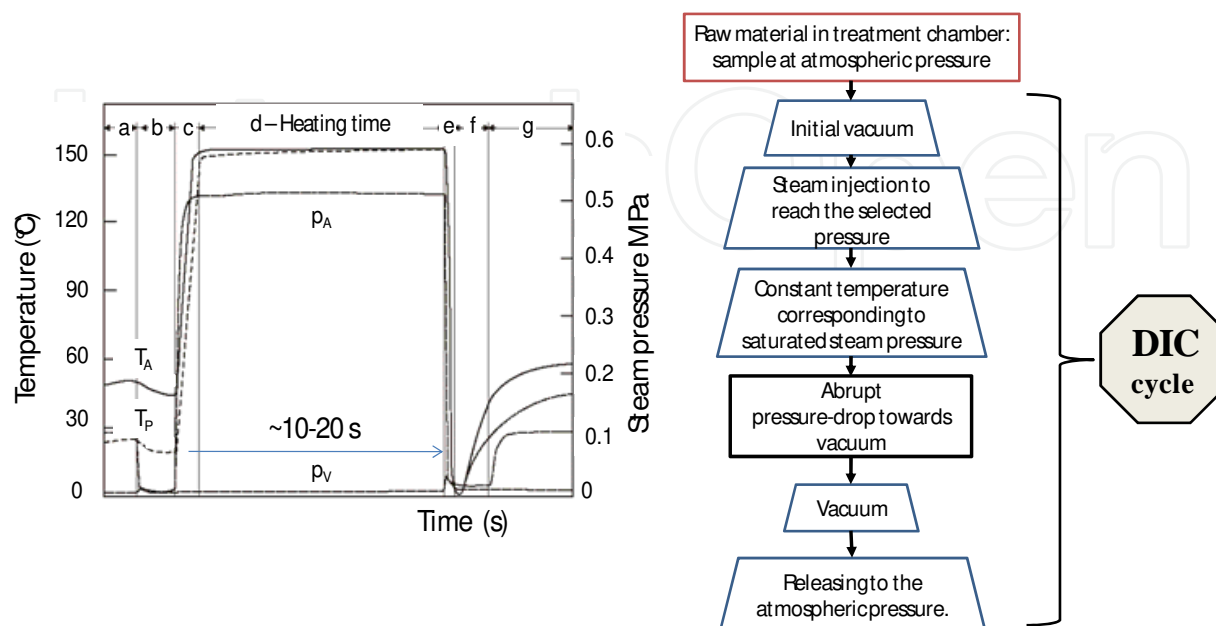


Fig. 2. Temperature and pressure history of a STEAM-DIC processing cycle. P_A is the steam pressure in autoclave, P_V pressure in vacuum tank, T_A temperature in autoclave, T_P temperature of product: (a) sample at atmospheric pressure; (b) initial vacuum; (c) saturated steam injection to reach the selected pressure; (d) constant temperature corresponding to saturated steam pressure; (e) abrupt pressure drop towards vacuum; (f) vacuum; (g) releasing to the atmospheric pressure.

In the Multi-Cycle DIC treatments, products were directly heated up to 155°C through a heating plate whose temperature was defined at various levels depending on an adequate experimental design. To intensify the cooling process of the powder surface, airflow was introduced and released towards vacuum, till reaching the high pressure level.

In some cases, a final drying stage had to intervene after DIC treatment. It usually was a convective drying carried out in an independent drier with a stream of dry air at 50°C in order not to modify the decontamination state; the dried samples were then recovered and ready for microorganism characterization.

In order to achieve a relevant experiment study on the impact of various operating parameters and optimize the DIC treatment, a five-level Central Composite Rotatable Experimental Design method was adopted. In the STEAM-DIC, two DIC operating parameters were studied: steam pressure (P), and processing time (t), while keeping the other operating parameters constant. Other three operating parameters were studied in the case of Multi-Cycle DIC: the heating plate temperature (T), the total processing time (t), and the number of cycles, (keeping the other operating parameters constant). In both cases, the experiments were run at random to minimize the effects of unexpected variability of responses due to unrelated factors. Experiments of DIC treatment were then carried out using the operating conditions described in Tables 2 and 3.

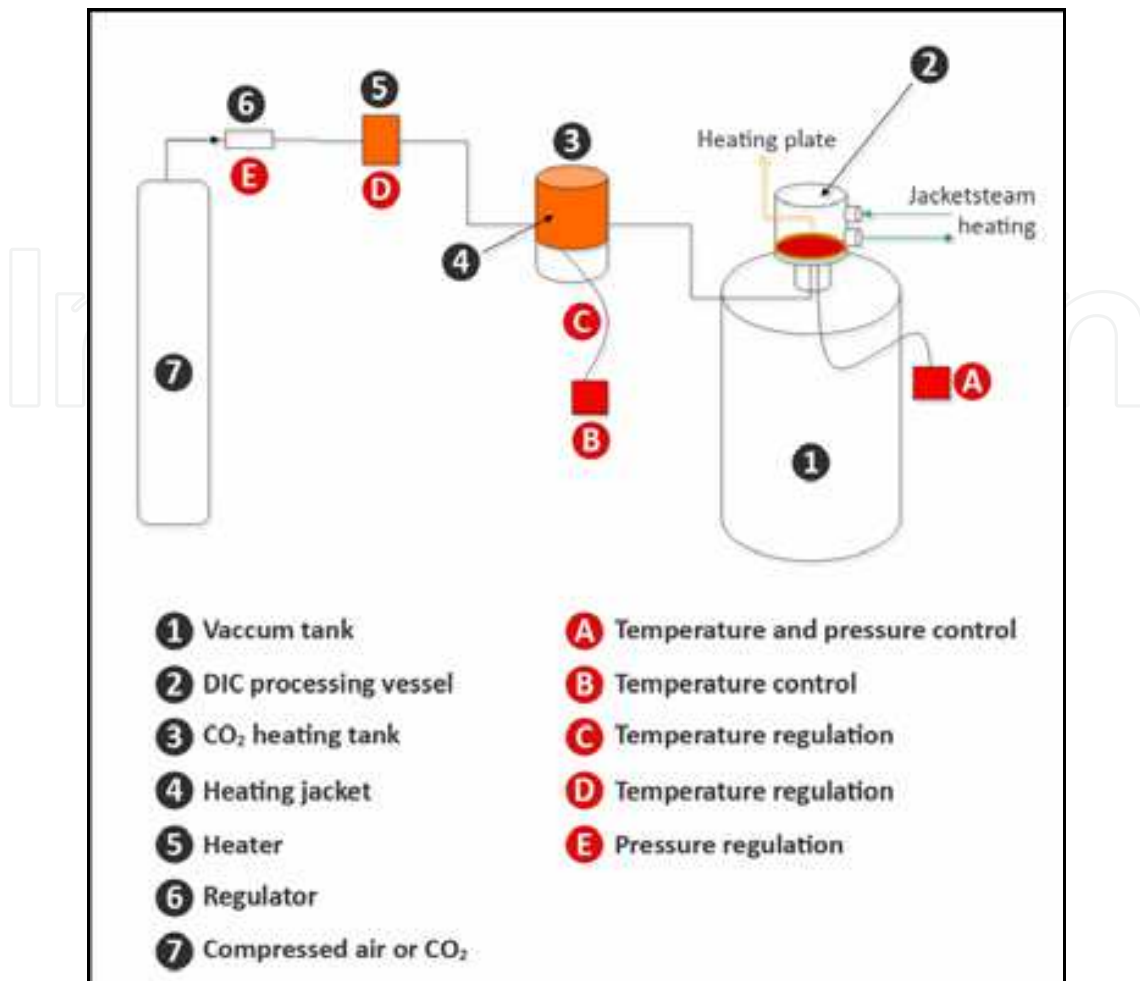


Fig. 3. Schematic presentation of Multi-Cycle DIC reactor

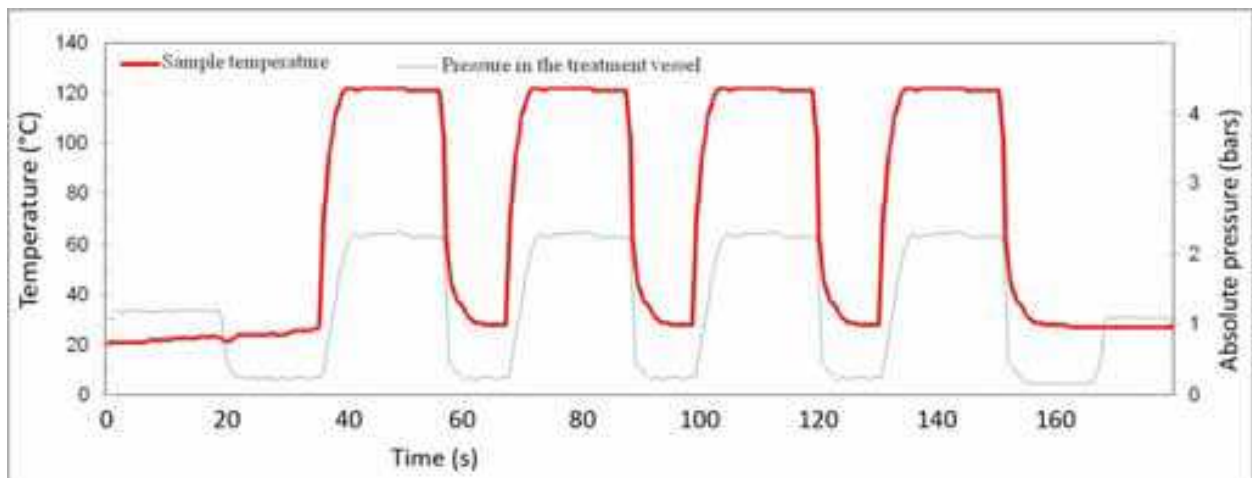


Fig. 4. Temperature and pressure history of a Multi-Cycle DIC. P_{atm} is the atmospheric pressure in the treatment vessel, P_{Vac} pressure vacuum level, P Processing pressure

The statistical treatment leading to Pareto Chart, main trends, surface responses, empirical model and R^2 were determined through the analysis design procedure of Statgraphic Plus software for Windows (1994-4.1 version- Levallois-Perret, France).

Trials N°	Saturated steam Pressure (MPa)	Total treatment time (s)
1	0.44±0.02	40±2
2	0.3±0.02	12±2
3	0.2±0.02	60±2
4	0.3±0.02	40±2
5	0.4±0.02	60±2
6	0.3±0.02	68±2
7	0.3±0.02	40±2
8	0.16±0.02	40±2
9	0.3±0.02	40±2
10	0.2±0.02	20±2
11	0.4±0.02	20±2
12	0.3±0.02	40±2

Table 2. Experimental design of decontamination of spirulina powder by STEAM-DIC treatment.

Trial n°	Heating plate temperature T (°C)	Total heating time (min)	Number of cycles
1	125	5	6
2	125	5	6
3	116	6	4
4	140	5	6
5	125	7	6
6	110	5	6
7	134	4	4
8	125	5	10
9	116	6	8
10	116	4	8
11	125	5	6
12	125	5	6
13	134	6	4
14	125	5	6
15	125	5	6
16	125	3	6
17	134	4	8
18	116	4	4
19	134	6	8
20	125	5	6
21	125	5	6
22	125	5	2

Table 3. Experimental design of Multi-Cycle DIC treatment of a mixture of spray-dried skim milk powder.

2.3 Assessment protocol

2.3.1 General assessment

Water content, expressed as % dry matter (g water/ 100 g dry basis), was determined by the desiccation method in a Mettler Toledo LP-16 Infrared Dryer/ Moisture Analyzer with Mettler Toledo PE360 Balance – Bishop International Akron, OH, USA LP16 balance. The measurement of water content by calibration with a drying oven at 105°C during 24 hours was carried out two times: just before DIC treatment and after final drying to generally be expressed in g H₂O/ 100 g of dry matter.

2.3.2 Enumeration of living microorganisms.

The main enumeration of living microorganisms was achieved at the “Laboratoire d’Analyses Sèvres Atlantique (LASAT)” for measuring total flora, faecal coli, Salmonella, Clostridium P B., Cereus, Yeast/ Mold, Staph Aureus, ASR spores...

3. Results and discussion

3.1 Physical characterization

Whatever the type of treatment may be, the bulk density of skim milk powder is significantly influenced by either air or steam pressures depending on the two versions of DIC-decontamination. Color, expansion ratio, porosity, as well as functional properties were quantified, thus contributing to the optimization of the process. Furthermore, industries participating to the present study or those adopting the STEAM-DIC in their manufacturing process, were able to define the processing parameters to get the best preservation of total quality.

3.2 STEAM-DIC decontamination; case of spirulina

	Points	Efficiency of Decontamination Ratio
STEAM-DIC DECONTAMINATION	1	100%
	2	88%
	3	100%
	4	100%
	5	100%
	6	100%
	7	100%
	8	97%
	9	100%
	10	96%
	11	100%
	12	100%
Low-temperature (30°C) Multi-Cycle DIC		98%
Freeze-Drying		19%

Table 4. Decontamination ratio for different Steam-DIC samples (see Table 2.) in comparison with low-temperature (30°C) Multi-Cycles DIC and freeze-drying

Trials of raw *Spirulina* do not necessarily have the same microbial load. In order to compare the effect of each process on the quality of the final product depending on the initial microbial load of raw material, we calculated the decreasing ratio of this load using the following formula:

$$\text{Efficiency of decontamination ratio} = \frac{(\text{Initial microbiological charge} - \text{final microbiological charge})}{\text{Initial microbiological charge}} (\%)$$

Although the spray-drying air flow temperature is high, the final product microbiological charges are normally greater than that of non processed raw material. This is due to a product temperature during drying very close to the ideal temperature for the growth of microorganisms. Meanwhile, freeze-drying did not imply any detectable decontamination, while, the STEAM-DIC treatment did give very relevant results, with a decreasing ratio systematically higher than 87%; some STEAM-DIC treated spirulina could be considered as sterile, with 100% as a decreasing ratio. The statistical study of the experimental design provided the following results:

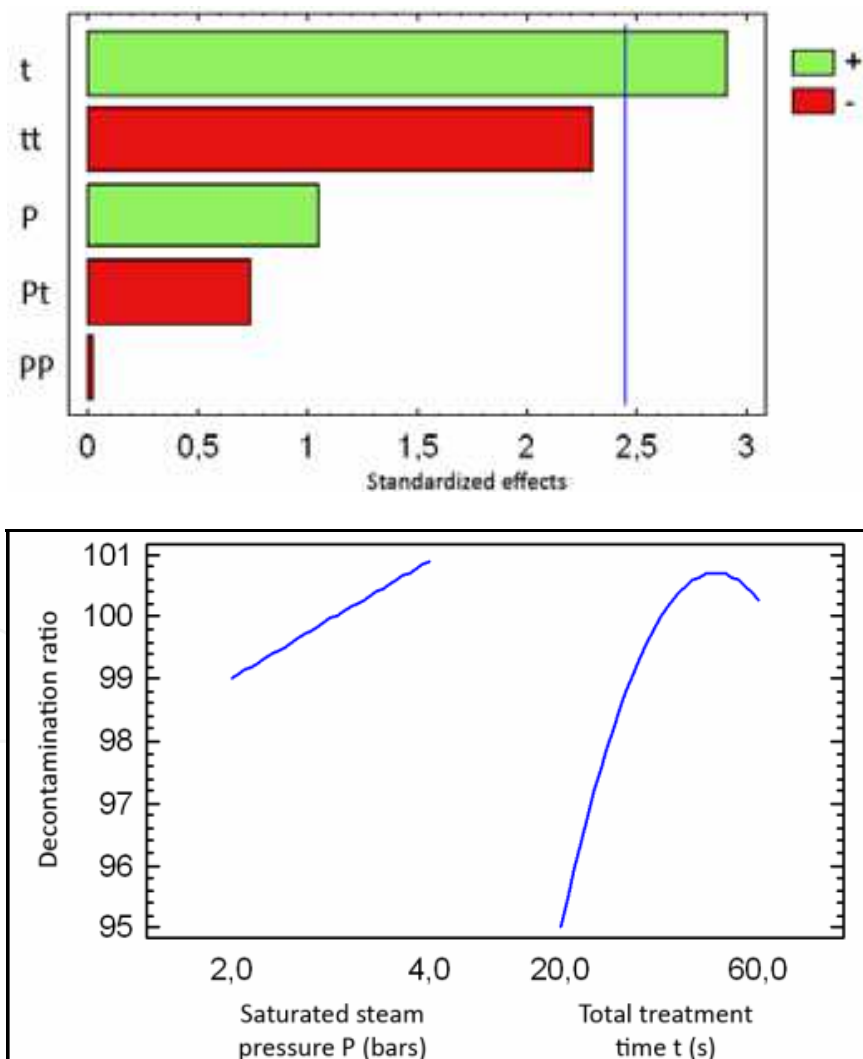


Fig. 4. Statistical treatment of experimental design of STEAM-DIC decontamination of spirulina.

Both operating parameters, total processing time and saturated steam pressure, have a positive influence on lowering the microbial load. The influence of processing time is the most significant because of its large range used during this study.

By observing the absolute level of the microbial load of mesophilic flora, one can note that different Steam-DIC products have less than 100 UFC/ g product.

Finally, the Multi-Cycle DIC process used as low temperature drying (30°C), allowed to notably reducing the microbiological load.

3.3 Analysis of Multi-Cycle DIC decontamination

Analysis of Multi-Cycle DIC decontamination was studied in the case of skim milk powder on the two aspects of vegetative and spore impacts. The results obtained with progressive decompression $\Delta P/ \Delta t = 50 \text{ kPa s}^{-1}$ (Debs-Louka, 1999) demonstrated that decontamination ratio could improve versus temperature and time, but also versus the number of cycles. This impact was observed with spores as well as with vegetative forms. Overall, Multi-Cycle DIC decontaminating powder has an impact through the pressure drops because a possible explosion of such microorganism cells. Debs-Louka et al. (1999) observed such an explosion in the case of steam-explosion. In this operation, the decompression rate $\Delta P/ \Delta t$ is presented as the principal factor since the system quickly evolves from an initial non-equilibrium thermodynamic state towards an equilibrium state very far from the starting situation.

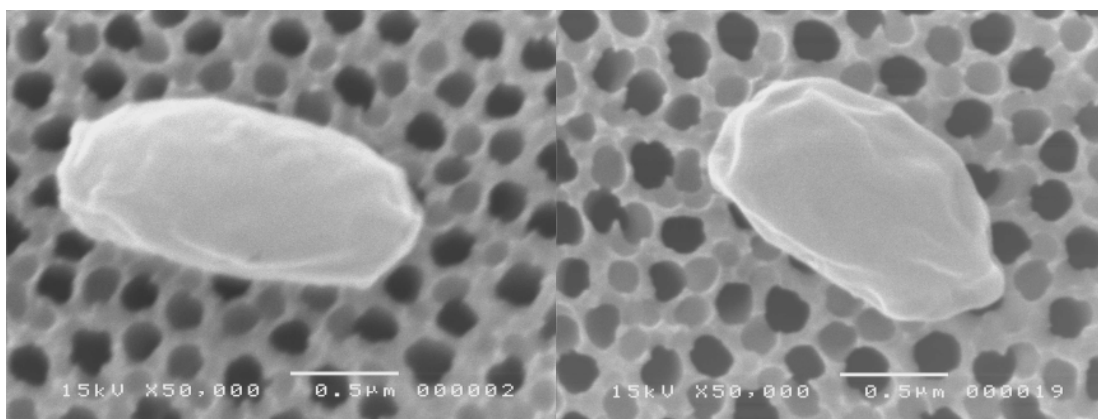


Fig. 5. Spores de *Bacillus stearothermophilus*: (a) untreated (b) treated at 130°C and 30 s

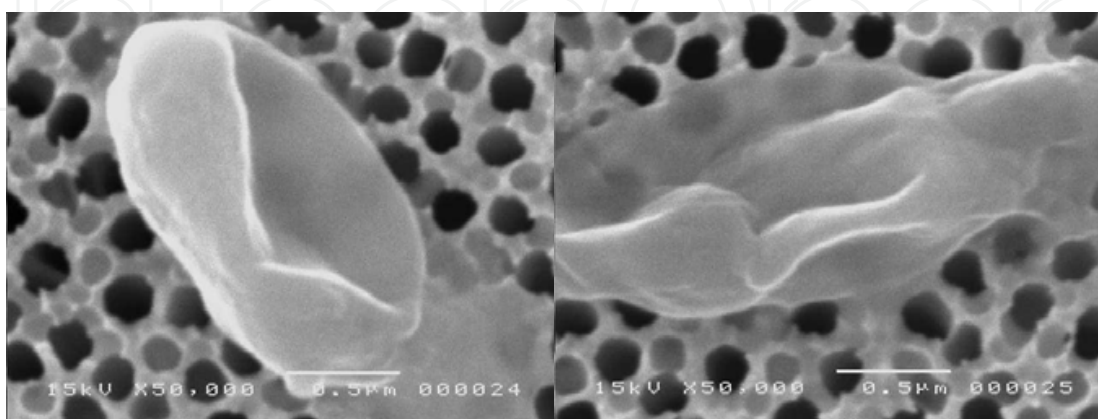
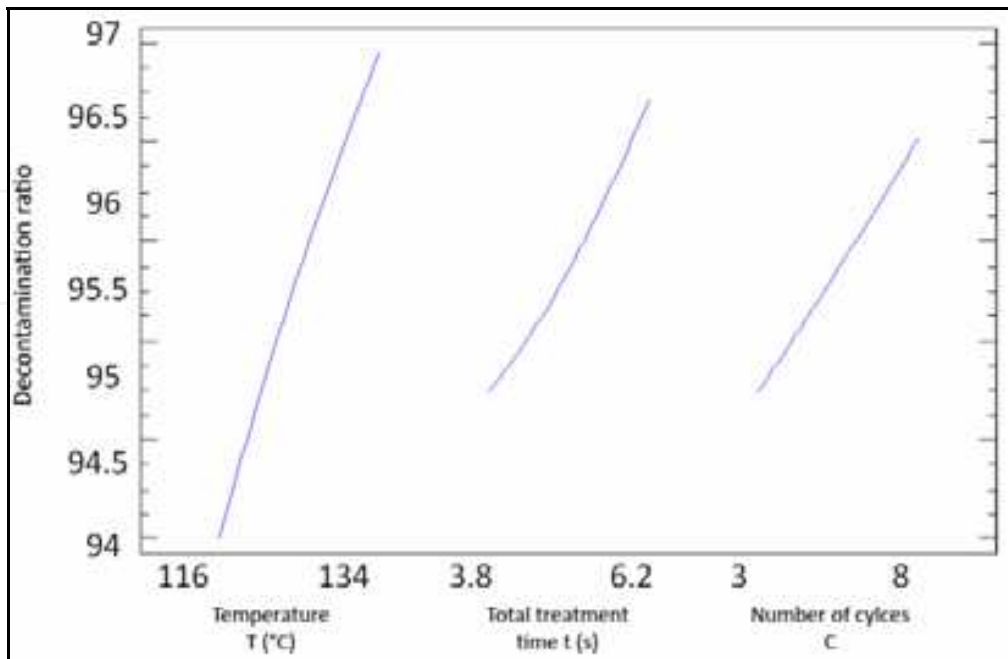
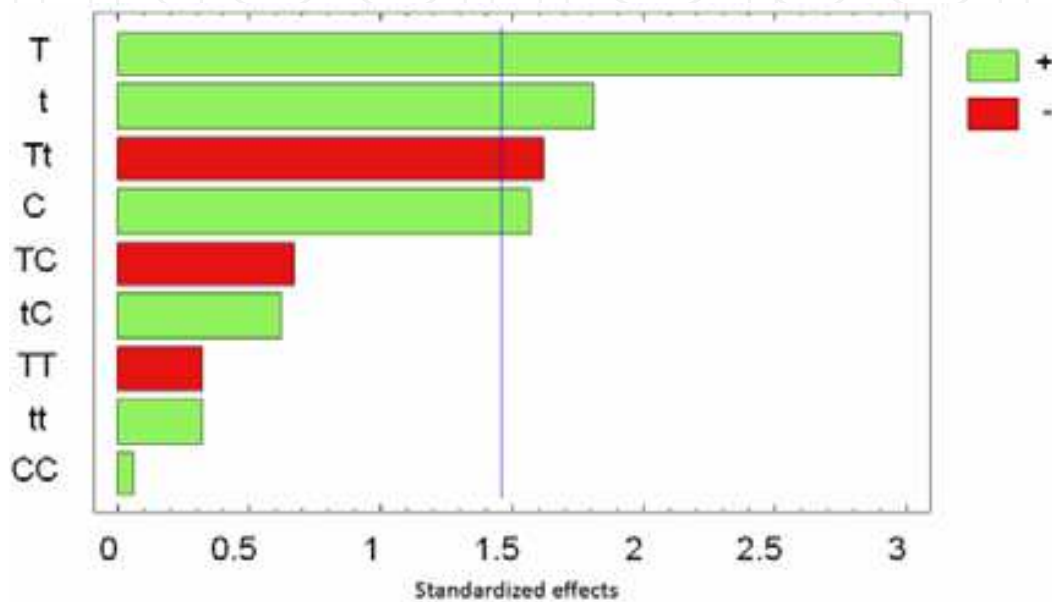


Fig. 6. Spores de *Bacillus stearothermophilus* treated by STEAM-DIC at 130°C and 30 s with instant pressure drop till vacuum at decompression $\Delta P/ \Delta t = 3 \text{ MPa s}^{-1}$ (Debs-Louka, 1999)

As experiments in this work were undertaken with an instant release of pressure ($\Delta P / \Delta t > 5 \text{ MPa s}^{-1}$), from high temperature towards the equilibrium temperature of water/ vapor at 5 kPa, the operation was carried out in the “explosion conditions” defined by Lin et al. (1991, 1992^a). In the cases we adopted, only 0.2 s were necessary to reach the complete vacuum stage from 0.6 MPa ($\Delta P / \Delta t = 3 \text{ MPa s}^{-1}$) in the processing vessel. No explosion effect was observed when we adopted the same treatment conditions but with 12 s as a decompression time ($\Delta P / \Delta t = 50 \text{ kPa s}^{-1}$).

Statistical carried out on the results concerning the inhibition of vegetative form:



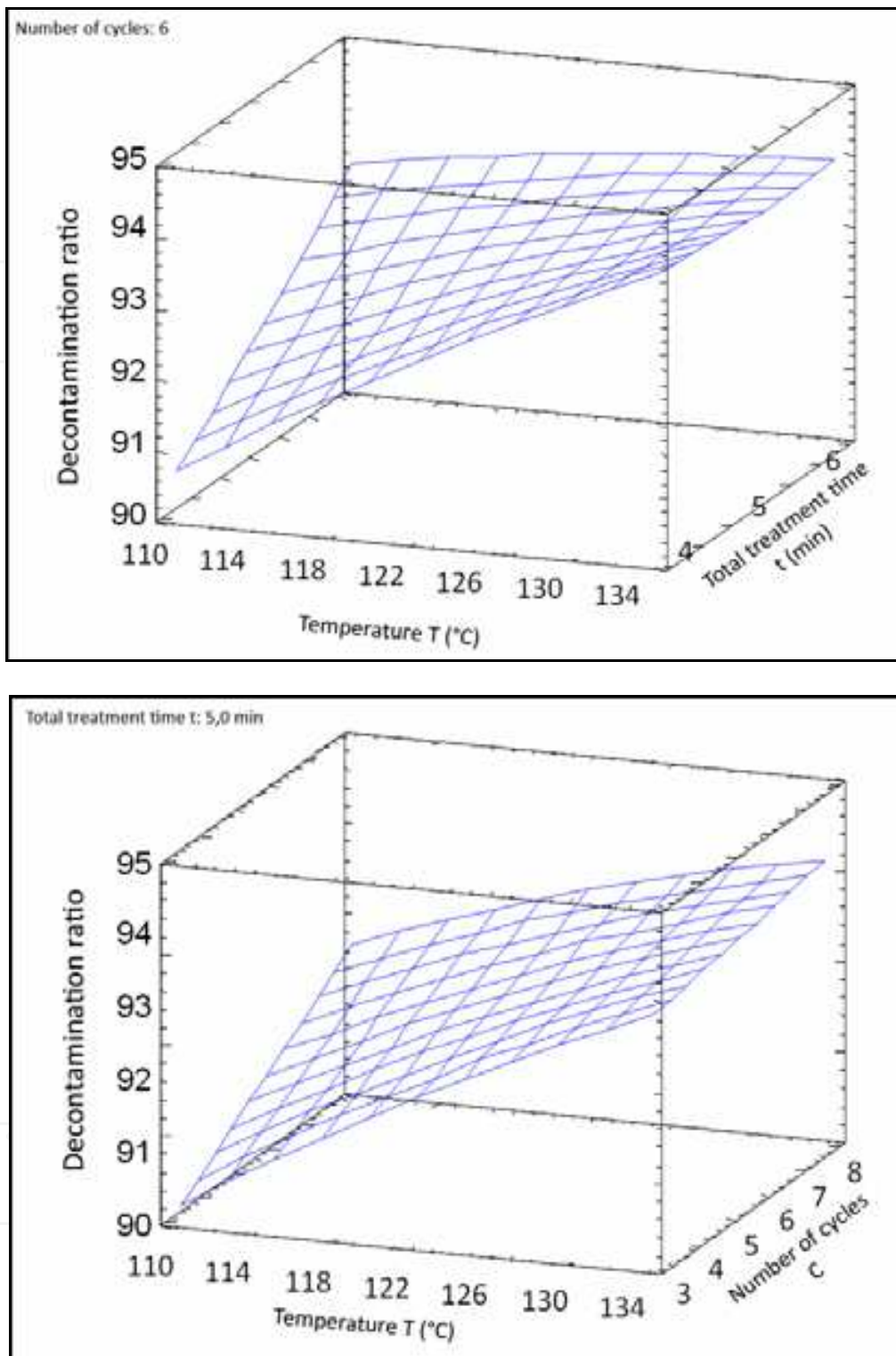


Fig. 7. Pareto Chart, trends of main effects and response surfaces of pressing temperature T ($^{\circ}\text{C}$), total thermal treatment time t (s), and number of cycles C as Multi-Cycle DIC operating parameters from five-level central composite rotatable RSM experimental design in the case of ASR vegetative forms

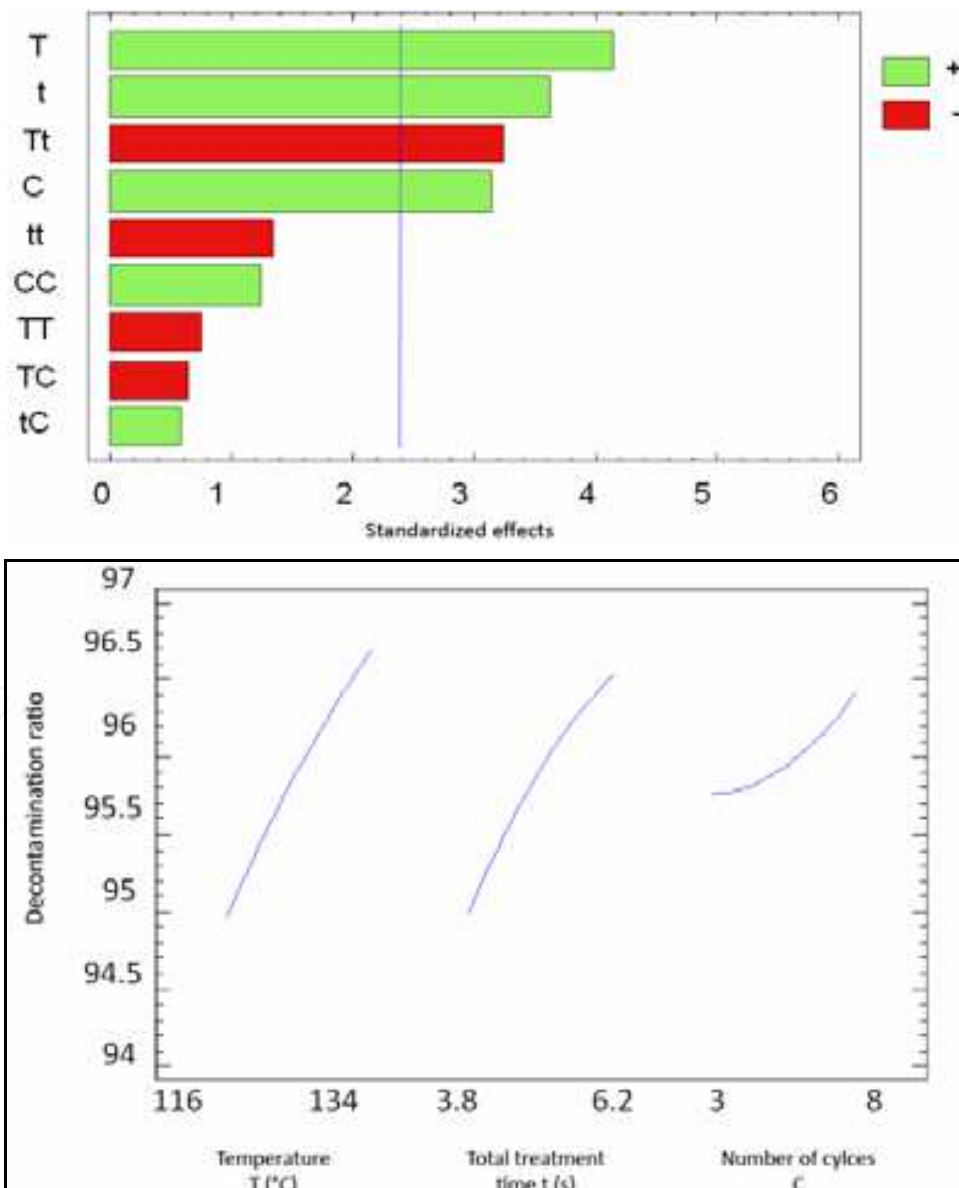
In the case of Multi-Cycle DIC, the effects of various processing parameters on decontamination showed that the processing temperature was the most relevant parameter,

whereas the total thermal treatment time (t) and the number of cycles (C) have non negligible impacts. It is worth noting that the higher T, t and C, the higher the direct Multi-Cycle DIC decontamination impact.

It was then possible to establish an empirical model of the MC-DIC decontamination ratio of ASR vegetative versus the DIC processing parameters. The R^2 value ($R^2 = 60.2\%$) directly proved that such a study would have to be carried out with more precise experiment measurements.

$$\text{Decontamination ratio of vegetative ASR} = -806,293 + 8.07574T + 73.0524t + 13.716C - 0.0119666T^2 - 0,641782 * Tt - 0.131539TC + 0.666216t^2 + 0.907552tC + 0.0269125C^2$$

Each processing parameters has an effect, including the number of cycles C; this last coupled with temperature, and treatment time can define the highest decontamination rate. The specificity of C is due to its positive impact in terms of preservation of quality, whereas t and T normally imply an inevitable degradation.



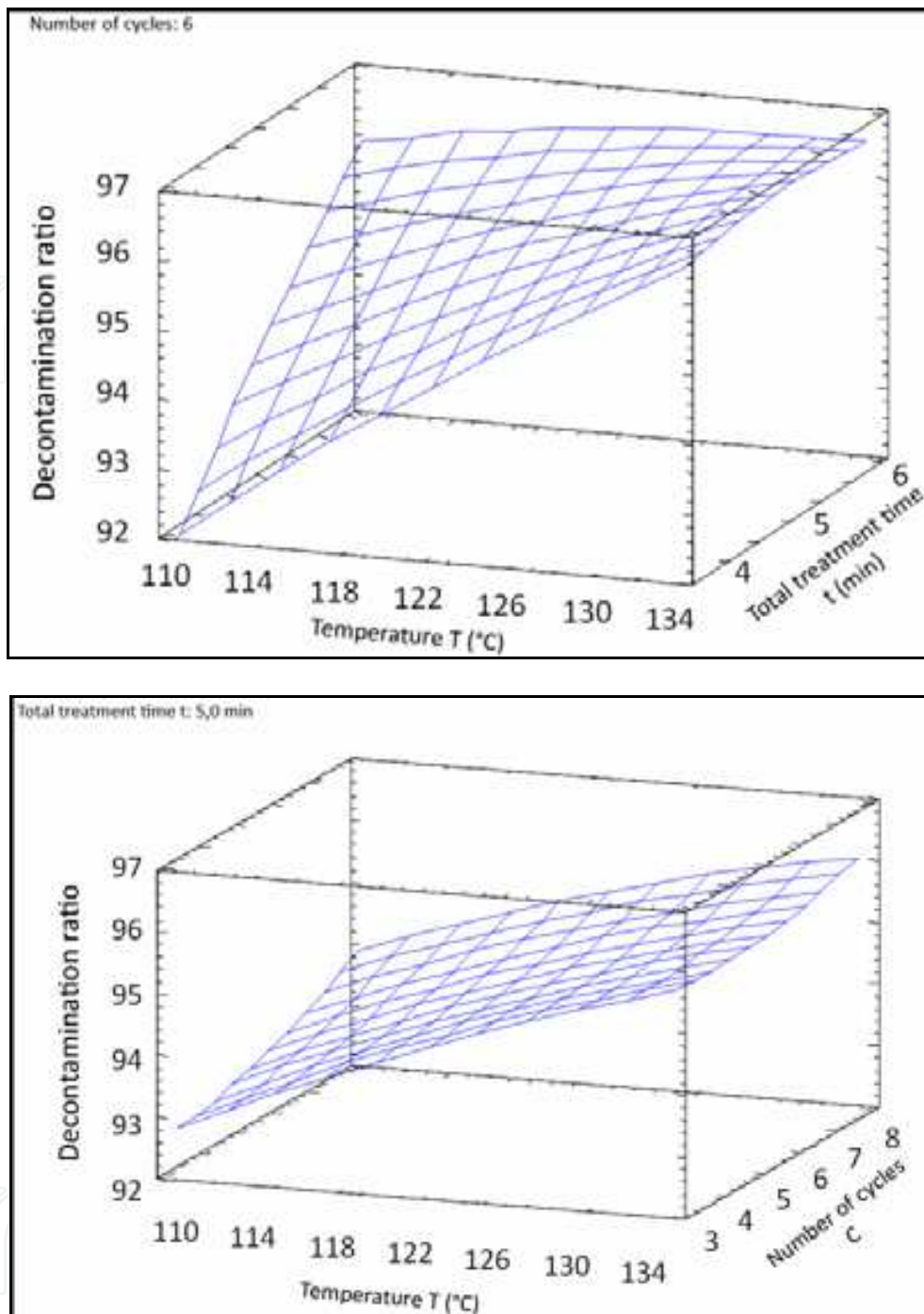


Fig. 8. Pareto Chart, trends of main effects and response surfaces of pressing temperature T ($^{\circ}\text{C}$), total thermal treatment time t (s), and number of cycles C as Multi-Cycle DIC operating parameters from five-level central composite rotatable RSM experimental design in the case of ASR spores

With the aim to inhibit ASR spores, the results issued from various Multi-Cycle DIC processing parameters showed that the processing temperature, the total thermal treatment time (t) and the number of cycles (C) were all with non negligible impacts. Here too, it is worth noting that the higher T , t and C , the higher the direct Multi-Cycle DIC decontamination impact.

It was then possible to establish decontamination ratio of ASR vegetative versus the DIC processing parameters. The R^2 value ($R^2 = 76.77\%$) of empirical model of the Multi-Cycle DIC directly proved the relatively good impact of such a model, however this study has to be carried out with more precise experiment measurements.

$$\text{Decontamination - ratio - of - spores - ASR} = 1365.23 + 13.4322T + 162.997t + 6,23863C - 0,023054T^2 - 1,09387Tt - 0,101794TC - 1.97197t^2 + 0,667969tC + 0,45166C^2$$

The impact of the number of cycles C as processing parameter has a specific impact because this parameter would normally have positive effect or no impact on preservation of quality, whereas t and T normally imply an inevitable degradation.

4. Conclusion

The aim of this work was to study and define more precisely the instant controlled pressure-drop DIC technology as a very relevant decontamination process which has been used since twenty years for inhibiting spores and vegetative forms more specifically in the cases of thermally sensitive dried solids and powders. The two versions of one-cycle saturated-steam (STEAM-DIC) and Multi-cycle air DIC used in this work were both relevant in the cases of thermal sensitive products (seaweed, microalgae, skim powder...). The coupled mechanical and thermal impacts allowed us to obtain high decontamination levels, differently and relevantly defined in order to perfectly master the final product quality. DIC technology as an innovative technique has been designed and developed at industrial scale by ABCAR-DIC Process more especially for decontamination of various products such as seaweeds, herbs, mushroom... and different industrial sectors. DIC reactors are currently operating at laboratory, semi-industrial and industrial fields. Thus, there are several models of infrastructure with features and abilities. (Besombes et al. 2010) could calculate the energy consumption to be 0.110 kWh per kg and per cycle.

5. References

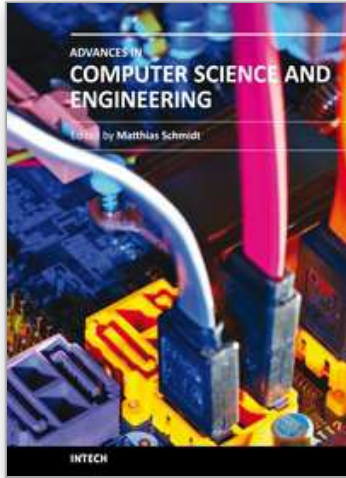
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