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Aeration Effects on Metabolic Events during Sporulation of *Bacillus thuringiensis*

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The metabolism of *Bacillus thuringiensis* during its sporulation process was investigated under different concentrations of oxygen. At the beginning of sporulation, the aeration conditions were regulated to obtain different oxygen transfer rates (OTR) in four separate fermentations, representing interrupted, limited, non-limited, and saturated oxygenation, respectively. A higher OTR resulted in a higher pH, up to about 9 in the case of saturated oxygenation, while the interrupted oxygenation resulted in a significantly acidic culture. In contrast, the absence of oxygen resulted in rapid sporangia lysis and caused acidification of the medium, indicating a distinctly different sporangia composition and different metabolism. The bacterium also showed different CO_2 production rates during sporulation, although a maximum point was observed in every case. With a higher OTR, the maximal value was observed after a longer time and at a lower value (40, 26, and 13 mmol/L/h for limited, non-limited, and saturated cases, respectively). Despite the exhaustion of glucose prior to the sporulation phase, the interrupted oxygenation resulted in acetate, lactate, and citrate in the medium with a maximum concentration of 4.8, 1.3, and 5.0 g/L, respectively. Notwithstanding, while the metabolic events differed visibly in the absence of oxygen, once sporulation was triggered, it was completed, even in the case of an interrupted oxygen supply.

Keywords: *Bacillus thuringiensis* H14, fermentation, sporulation, bioinsecticide, aeration, metabolism

Introduction

Spore-forming bacteria are sources of various industrially important biological products, including enzymes, antibiotics, organic acids, and bioinsecticides (Arbige *et al.*, 1993). Typically, the maximal synthesis of these products occurs at the beginning of and/or during sporulation (Liu *et al.*, 1994). Although the synthesis of these products is not required for sporulation, their production is controlled by mechanisms common to those responsible for the initiation of sporulation (Sonenshein, 1989). It is already well recognized that sporulation is a normal metabolic phenomenon among sporogenic bacteria and the processes involved are quite different from those associated with vegetative growth (Nakata and Halvorson, 1960). Sporulation is triggered by stressful environmental conditions under which vegetative growth cannot be sustained (Stragier and Losick, 1996). However, despite quite extensive literature related to this phenomenon, our understanding regarding the effect of oxygen on the process of sporulation is still quite limited. With proper control of the extracellular environment, such as the dissolved oxygen concentration, the fermentation can be directed toward a desirable product and higher yield. Oxygen shows diverse effects on product formation in aerobic fermentation processes by influencing metabolic pathways and changing metabolic fluxes.

According to the cell growth conditions and based on metabolic pathway analyses, some bioprocesses require high oxygen transfer rate (OTR) conditions, while others require controlled oxygen transfer rates (Calik *et al.*, 1998). Thus, maintaining the appropriate concentration of dissolved oxygen has already been pointed out as an important factor in the fermentation of *Bacillus* species (Arbige *et al.*, 1993; Calik *et al.*, 1998). Oxygen balance data has also been used for estimating the biomass concentration in a culture (Silveira and Molina, 2005). However, the interactions between *Bacillus* species and their environment are highly complex, where one reaction often has a sequential effect on several other metabolic reactions (López-y-López and De la Torre, 2005). Carbon catabolite repression is considered one of the main regulation mechanisms in the synthesis of certain products from *Bacillus* species (Arbige *et al.*, 1993). The involvement of dissolved oxygen in such regulation has also been demonstrated by several authors (Zouari *et al.*, 2002; Ghribi *et al.*, 2007) who clearly demonstrated the importance of this parameter in fermentation control. *Bacillus thuringiensis* is a spore-forming bacterium that produces a potent insecticidal crystalline protein (ICP), making it a successful biopesticide (Buchanan and Gibbons, 1974; Roh *et al.*, 2007; Sarrafzadeh, 2012). ICPs are also known as Cry proteins and contain δ-endotoxins, which kill insects belonging to different orders, namely Diptera, Coleoptera, and Lepidoptera (Schnepf *et al.*, 1998; Sarrafzadeh *et al.*, 2007; Park *et al.*, 2013).

While it is well known that oxygen transfer is essential dur-

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ing the fermentation of *B. thuringiensis* (Avignone-Rossa and Mignone, 1995), the level of this requirement during the different culture phases needs more clarification. The environmental requirements, such as the temperature, pH, and dissolved oxygen concentration, generally differ for the growth phase and sporulation phase. Various reports have already identified some potentially interesting findings on the effects of dissolved oxygen. Yet, the analyses of the sporulation phase include observation discrepancies that were recently reviewed and discussed in the study by Boniolo *et al.* (2012). Inconsistencies in the definition of the sporulation phase are one of the main reasons for such different observations. Certain studies characterize the cell cycle of *B. thuringiensis* using three phases: vegetative, transition, and sporulation (Rowe *et al.*, 2003; Sarrafzadeh and Navarro, 2006), some use two phases: growth and sporulation (Ghribi *et al.*, 2007), and others use four phases: vegetative growth, transition to sporulation, sporulation, and cell lysis (Berbert-Molina *et al.,* 2008; Boniolo *et al.*, 2012). Sometimes, cells in the course of sporulation that contain refractile spores (sporangia) are counted as vegetative cells (Kraemer-Schafhalter and Moser, 1996), while other times they are counted as spores (Yang and Wang, 2000; Sarrafzadeh and Navarro, 2006).

Meanwhile, unavoidable differences in the fermentation conditions of *B. thuringiensis* and disturbances from the external environment can also be sources of discrepancy between reports on the effect of oxygen on sporulation and ICP synthesis. Therefore, for a better understanding of this bioprocess, it is important to relate the sporulation and toxicity results with more common fundamental aspects of this bacterium, such as metabolic events, and try to explain them in a causal basis. However, this is not an easy task, as the metabolic processes of microorganisms are very complex and generally not well understood (Wiechert, 2002). The metabolic pattern of *B. thuringiensis* is closely related to that of *B. cereus*. Glucose is degraded through the Embden-Meyerhof-Parnas (EMP) pathway to yield pyruvate and acetate as the main products and some other organic acids. Acetic acid is a key metabolite produced by the vegetative cells and stocked within the cells as poly-β-hydroxybutyrate (PHB), which is then consumed during sporulation (Mignone and Avignone-Rossa, 1996; Yang and Wang, 2000). Once the glucose is exhausted, the acids are oxidized via the tricarboxylic acid (TCA) cycle (Lüthy *et al.*, 1982). This process has also been reported to be closely associated with the onset of sporulation (Benoit *et al.*, 1990). Plus, Mignone and Avignone-Rossa (1996) reported that the oxygen demand decreases just prior to the initiation of sporulation, followed by a sudden increase at the beginning of sporulation. Meanwhile, acetic acid and some other organic acids concomitantly disappear from the medium with the second change in oxygen demand (Benoit *et al.*, 1990).

Accordingly, this study investigated the occurrence of these metabolic events during the sporulation of *B. thuringiensis* under different conditions of oxygenation. The same experimental conditions as used by Sarrafzadeh and Navarro (2006) were applied to allow a correlation of certain simple metabolic aspects with previous results and provide new insights on the general metabolic behavior of this bacterium during the sporulation phase that may help to explain the reported discrepancies on the effect of dissolved oxygen.

Materials and Methods

Microorganism and media

Bacillus thuringiensis serotype H14 (Ecautec S.A., Tahiti, French Polynesia) was used (Sarrafzadeh *et al.*, 2005b). The strain was maintained in a sporulated form on nutrient agar slants at 4°C. The inoculum was then prepared by transferring cells from the slants into 5 ml of a nutrient broth medium, following by incubation overnight at 30°C. Thereafter, the cells were inoculated into a 5 L preculture flask containing 500 ml of the culture medium, incubated at 30°C on an orbital shaker for 9 h at 150 rpm, and then used as the seeds for the fermentor. The composition of the culture medium was (g/L): glucose, 5; hydrolysed casein, 4.5; yeast extract, 0.5; ammonium sulphate, 6; K_2HPO_4, 1.4; KH_2PO_4, 1.4; $MgSO_4{\cdot}7H_2O$, 0.61; $CaCl_2$, 0.332 ; $MnSO_4{\cdot}H_2O$, 0.006. A concentrated medium containing (g/L): glucose, 240; hydrolysed casein, 155; yeast extract, 25, was then added during the fed-batch phase. The pH of the media was adjusted to 6.8 using 3 M NaOH.

Fermentation procedure

A 20-L fermentor (Biolafitte, France) with a maximum working volume of 15 L was used, and 9 L of the culture medium was sterilized *in situ.* Four fermentations were initiated under identical conditions in a batch mode at 30°C, followed by a feeding period from the 4^{th} h to the 24^{th} h of fermentation at a constant feed rate of 150 ml/h. The dissolved oxygen (DO) was monitored continuously and maintained above 20% saturation during the growth period. During the sporulation phase a different oxygenation level was maintained. During the growth phase, the pH was maintained at 6.8 using 3 M NaOH, however, its increase during the sporulation phase was not controlled. The conductivity and permittivity data were collected using a Biomass System (Fogale Nanotech, France). The CO_2 concentration at the off-gas was measured using an infrared gas analyzer (Abiss CM12AT, France).

Detection of sporulation

Since this study was focused on the sporulation phase, determining the real boundaries between the culture phases was of particular importance. Recent advances in biomass monitoring systems provide new methods for real-time and on-line quantification and qualification of the biomass (Madrid and Felice, 2005). Therefore, this study detected the sporulation phase using on-line measurements of the permittivity and optical density, as described previously (Sarrafzadeh *et al.*, 2005a). A phase contrast microscope (Olympus BX60, Olympus Optical Co., Japan) and Thoma haemocytometer were also used to monitor the proportion of vegetative cells, sporangia, and mature spores. These diverse cell states were distinguished based on their morphological differences and the refractile nature of the endospores.

Table 1. Conditions of oxygenation during sporulation phase

Condition qualification	Agitation speed (rpm)	Aeration rate (vvm)	K_La (h^{-1})	OTRmax (mmol/L/h)	DO (%)
Interrupted	550	0	-	0	0
Limited	650	0.1	80	20	0–40
Non-limited	700	0.5	400	100	50±10
Saturated	700	0.25 (O_2)	200	250	100

Oxygenation conditions during sporulation

The aeration conditions were regulated to achieve oxygen transfer rates of 0, 20, 100, or 250 mmol/L/h, representing interrupted, limited, non-limited, and saturated, respectively. The saturated oxygen transfer rate was carried out using pure oxygen instead of air at the beginning of the sporulation phase (around 24^{th} h of culture). The oxygen transfer coefficient was determined according to (Nikolaev *et al.*, 1976; Dang *et al.*, 1977). Table 1 shows the details of the aeration conditions used during the fermentations.

Glucose and organic acid analysis

The concentrations of glucose and certain organic acids, such as pyruvate, acetate, citrate, and lactate, were determined using HPLC or enzymatic assays. The HPLC assays were performed using a Shimadzu LC-6A HPLC system (Japan) equipped with an Aminex HPX-87H BIORAD column maintained at 40°C, and two UV (210 nm) and refractive index serial detectors and two Shimadzu LC-A3 and LC-A5 integrators. A mobile phase of 6 mM H_2SO_4 with a flow rate of 0.8 ml/min was used. The mobile phase was filtered and degassed through a 0.2 µm cellulose nitrate membrane. The samples and standards were also filtered before injection into the HPLC. When the HPLC detection resolution was insufficient, the measurements were carried out using various enzymatic kits (Diffchamp Group, Sweden) according to the protocols defined by the manufacturer for each organic acid and glucose.

Results and Discussion

During the sporulation phase of the *B. thuringiensis* cultures, the oxygen transfer rate was regulated based on the four conditions mentioned above, which resulted in different levels of dissolved oxygen in each fermentation (Table 1). The effects of such conditions on the sporulation, δ-endotoxin synthesis, and toxicity of *B. thuringiensis* have already been reported (Sarrafzadeh and Navarro, 2006), and a summary of such sporulation results has been reviewed here for correlation with the metabolic events studied in this work and comparison with more recent publications. The final percent of spores obtained with the four oxygen levels (interrupted, limited, non-limited, and saturated) was 100, 93, 84, and 48%, respectively. Thus, a large percent of cells failed to sporulate with the saturated oxygenation. Boniolo *et al.* (2012) obtained very similar results under saturated oxygen conditions.

However, this contradicts other studies, where a higher spore count was reported with a higher dissolved oxygen concentration for *B. thuringiensis* var. *kurstaki* (Ghribi *et al.*, 2007). Meanwhile, Dingman and Stahly (1984) found that a high concentration of oxygen was toxic and prevented the sporulation of a *Bacillus* species. They related this effect of oxygen to the accumulation of H_2O_2 in the medium and the relative deficiency of catalase (and NADH peroxidase) in this strain. It has also been reported that an oxygen partial pressure higher than about 1 bar can show inhibitory effects in aerobic cultures (Amicarelli *et al.*, 2010). Such a high partial pressure of oxygen can only be achieved when pure oxygen is used instead of air, as in the case of the saturated oxygen fermentation. For *B. thuringiensis*, while intracellular accumulation of H_2O_2 could explain the effect of the high oxygen concentration, according to the current results, the absence of oxygen did not prevent sporulation in this bacterium, which puts into question the real role of oxygen in the sporulation process. However, there is no agreement between previous studies on the observations after interrupting the aeration. For example, Boniolo *et al.* (2012) reported severe restriction of sporulation in their 0% dissolved oxygen assay, which is totally different from the current results. Meanwhile, according to Avignone-Rossa *et al.* (1992), once sporulation has been triggered, it will be completed even if the oxygen supply is interrupted. Therefore, this explains the importance of selecting the appropriate time for interrupting the aeration, which should be just prior to the initiation of sporulation when the culture transfers from vegetative growth to the sporulation phase. Since this time point has been selected in different ways in different studies, this could be the origin of the discrepancies.

However, an even better explanation for the discrepancies can be provided by comparing such previous studies from a metabolic point of view. The initiation of sporulation coincides with huge changes in the cellular metabolism. As such, the sporulation process includes a large number of complex and highly interactive biochemical reactions, which need to be understood in order to control the optimal aeration conditions for improving process yields and reducing production costs. While the biochemical reactions in a fermen-

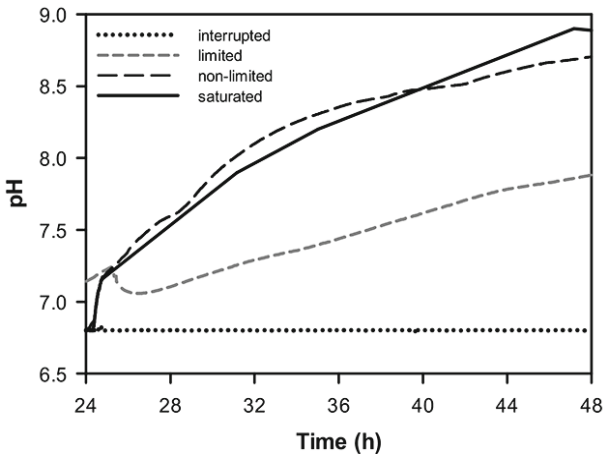

Fig. 1. pH profiles obtained with different concentrations of dissolved oxygen during sporulation phase of *B. thuringiensis* H14.

tation process are complicated and difficult to follow, they affect the culture environment and can provide simple indicators of what is happening. For example, the pH is one such indicator. In *B. thuringiensis* fermentations, as a member of the *B. cereus* group, the pH is normally maintained above a fixed value by the addition of a base in order to control the nature and amount of intermediate changes (Nakata, 1963), yet with the initiation of sporulation, this behavior changes and the pH starts to increase. According to the present results, in the absence oxygen, as opposed to the other oxygen conditions, the pH did not increase during sporulation and even NaOH had to be added to prevent the pH from falling (Fig. 1). Therefore, this may indicate that the sporulation in the absence of oxygen passed through a different group of metabolic reactions. It is possible that the lower pH during sporulation without oxygen actually increased the activity of a lytic system and accelerated the spore liberation. This hypothesis is based on previous observations of the current authors in the absence of oxygen (interrupted case), where a fully sporulated culture was obtained several hours prior to other cultures with oxygen. Reaching the highest percent of sporulation in a shorter time period could be related to the activity of lytic enzymes that can be affected by the concentration of dissolved oxygen. While the intervention of a lytic system during spore liberation has already been reported, the mechanisms are still unknown (Smith and Foster, 1995). Two different types of lytic enzyme, named enzyme V and system S, have been observed in the sporangia of *B. cereus*, where enzyme V is involved in spore liberation, while system S plays a role in germination (Vinter, 1969).

Another interesting observation regarding the pH in Fig. 1 is the higher pH value attained at the end of the culture with the higher dissolved oxygen concentrations. The consumption of accumulated organic acids during the previous growth phase increases the pH, which will be discussed later. However, this cannot be the only reason for the pH to increase up to almost 9 in some cases. The conversion of residual proteins or amino acids to ammonium through an ammonification process or the release of the ammonia or another alkaline substance during cell lysis can raise the pH. Kraemer-Schafhalter and Moser (1996) characterized the induction of sporulation with several concomitant events. While they reported an increase in the pH, they also mentioned the use of ammonia, which is confusing, since ammonia consumption during fermentation normally results in a decrease of the pH. However, despite frequent reports of this pH pattern during the sporulation of *B. thuringiensis*, its origin has not well been explored. Clifton and Sobek (1961) reported the release of considerable amounts of ammonia during the endogenous respiration of *B. cereus,* which probably also occurred here during the sporulation of *B. thuringiensis*, thereby increasing the culture pH.

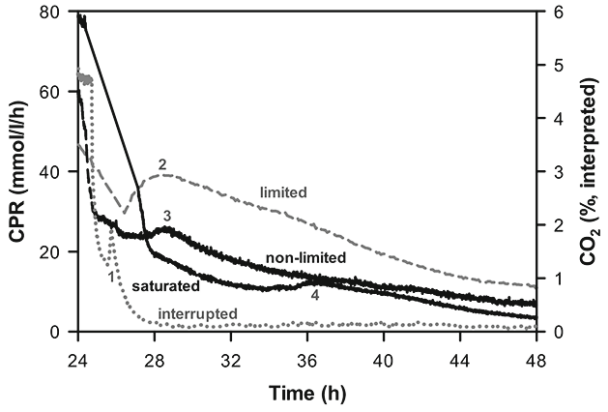

Fig. 2. Comparison of carbon dioxide production by *B. thuringiensis* H14 during sporulation phase of fermentation with different oxygen transfer rates. For interrupted case, CO_2 concentrations are presented instead of CPR. Numbers 1–4 in figure correspond to peaks observed in each case. The thin parts of the saturated and non-limited curves during the initial period correspond to the times when the on-line measurements were not possible due to technical problems.

The CO_2 production rate (CPR) is another indicator of metabolic activity during the fermentation process (Royce, 1992), thus its measurement during the course of *B. thuringiensis* sporulation was also conducted in this study. The profiles of this parameter are presented for all the cultures in Fig. 2. For the interrupted case, the CPR was replaced by the CO_2 concentration, as the CPR could no longer be defined when the aeration was arrested. The CO_2 concentration was also measured during first hours of sporulation when there was still enough pressure to evacuate the CO_2 from the fermentor. A decreasing CPR pattern was generally observed in all cases, which can be interpreted as the diminution of metabolic activity according to the development of the sporulation phase. As seen in Fig. 2, a lower concentration of dissolved oxygen resulted in a higher CPR, indicating that the metabolic reactions producing CO_2 were more active when oxygen was limited. A significant maximum for the CPR or CO_2 concentration was also observed for each case, identified as points 1 to 4 in Fig. 2. Such maximum points in the CPR profile during the sporulation process have also been observed by other authors (Kraemer-Schafhalter and Moser, 1996). The order of their appearance is also interesting; when the concentration of oxygen was higher, the maximal value was observed after a longer time, yet with a lower value (40, 26, and 13 mmol/L/h for limited, non-limited, and saturated cases, respectively). It should be noted that these maxima are different from the CPR peak that is normally seen during vegetative growth and has a much higher value.

Limited knowledge of the metabolic pathway used by *B. thuringiensis* during sporulation restricts any evaluation of the contribution of different possible origins of CO_2 production, such as residual growth or fermentative CO_2 releases. According to Nakata and Halvorson (1960), oxidative decarboxylation of pyruvic acid could be another origin of CO_2 liberation. In another study (Rowe *et al.*, 2003), the oxygen uptake rate (OUR) was measured during the fermentation of *B. thuringiensis* subsp. *kurstaki*, which that can be compared with the present CPR results, since both parameters are respiratory in nature. Rowe *et al.* (2003) presented their OUR values in a specific form and reported a decreasing pattern throughout the fermentation. Although not specifically mentioned, two significant peaks can be seen in one of their experiments (Run 1), one during vegetative growth

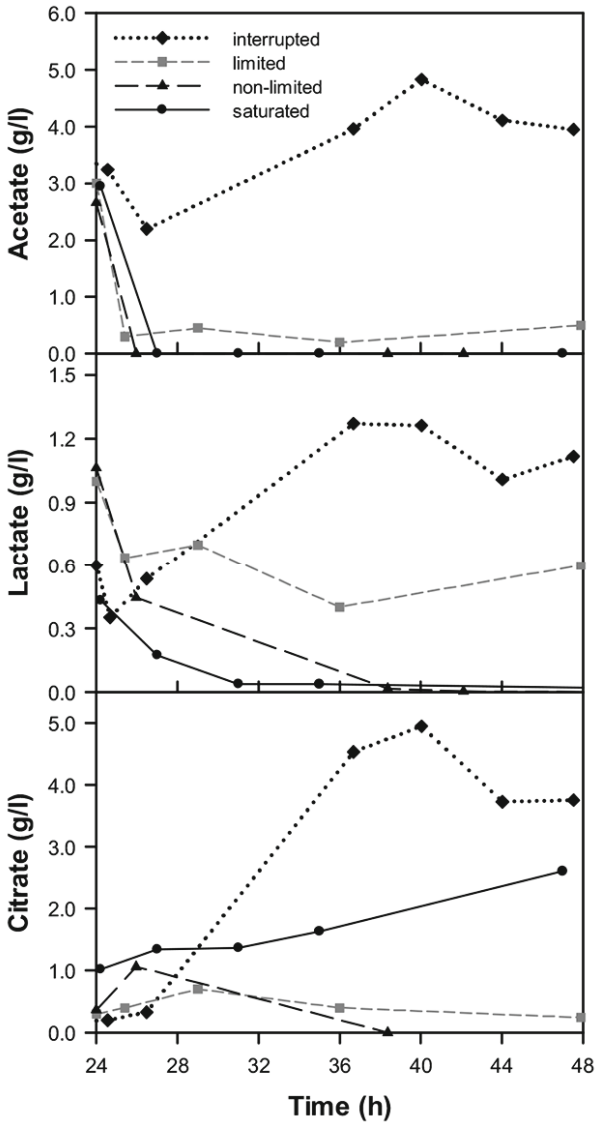

Fig. 3. Changes in acetate (top), lactate (middle), and citrate (bottom) concentrations during sporulation phase with different oxygen transfer rates. Interrupted, (◆); limited, (■); non-limited, (▲); saturated, (●).

and the other during the sporulation phase, where the latter can be correlated with the CPR peak in the present study. While the physiological cause of the appearance of the CPR peak the during sporulation phase remains to be determined, it may simply result from the growth of new cells on the released cytoplasm as a new substrate after sporangia lysis. In the report by Rowe *et al.* (2003), the OUR reached its minimum at the end of the culture, which is also similar to the present CPR results, as with the completion of sporulation, no further oxygen consumption or carbon dioxide production can normally be measured. The residual values of the OUR and CPR at the end of a culture depend on the remaining portion of active cells.

A quantitative study of supernatant samples removed at various intervals during the sporulation was conducted to determine whether the production and utilization of certain organic acids were affected by the oxygenation rate and whether these acids were responsible for the pH changes in the medium. In the absence of oxygen, the organic acids secreted into the medium during the growth phase remained during the sporulation phase, and their concentrations even increased, reaching about 4.8, 1.3, and 5.0 g/L for acetate, lactate, and citrate, respectively, as shown in Fig. 3. Kraemer-Schafhalter and Moser (1996) also reported considerable concentrations of acetate and lactate under limited oxygen (dissolved oxygen concentration under 5%). This agrees with the observed pH changes and may explain why the medium in the interrupted case was acidified and the addition of sodium hydroxide was required to keep the pH constant. Meanwhile, acetic acid was rapidly utilized in the case of saturated and non-limited aeration (Fig. 3), which supports previous reports of the disappearance of this acid intermediate derived from glucose at the beginning of sporulation (Lüthy *et al.* 1982; Benoit *et al.*, 1990; Mignone and Avignone-Rossa, 1996). The sudden and rapid utilization of acetate from the medium indicates that a new enzyme or enzyme system is induced during the transition from vegetative growth to sporulation. Yousten and Rogoff (1969) were successful in preventing the removal of acid by adding α-picolinate to inhibit aconitate hydratase, thereby inhibiting sporulation. Among the enzymes associated with acetate metabolism, the one most likely to be involved in the induction process is the one responsible for activating acetate into acetyl phosphate or acetyl coenzyme A (CoA). Yet, evidence concerning the fate of acetate taken up by sporulating cells is still lacking. Acetate is generally considered as the substrate for sporogenesis that provides the energy and precursors needed for the synthesis of spore materials (Yousten and Rogoff, 1969).

However, acid accumulation can also be attributed to other reasons, such as oxygen limitation, blockage of the TCA cycle, saturation of this cycle, or subsequent respiratory chains (Yousten and Rogoff, 1969; Arbige *et al.*, 1993; Liu *et al.*, 1994). As seen in Fig. 3, in the interrupted case, a concentration of about 1 g/L of lactate still remained in the culture. While its origin is uncertain, residual activity un-

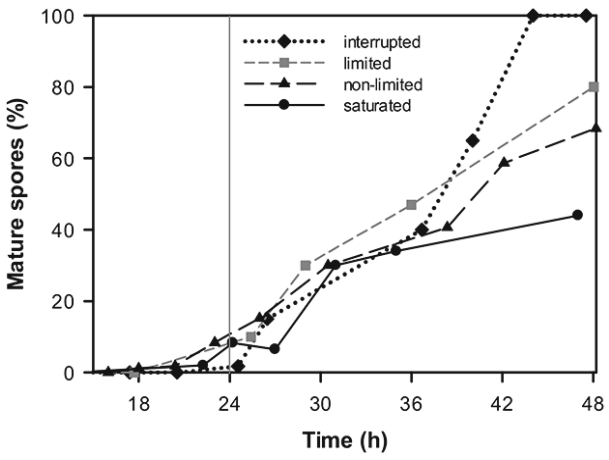

Fig. 4. Evolution of mature spores percent during culture of *B. thuringiensis* H14 in different conditions of oxygen transfer rate.

der limited oxygen or the liberation of intracellular acids during sporangia lysis could be the origins of this lactic acid. The data showing the time course of the appearance of mature spores during the sporulation phase (Fig. 4) can exclude the latter explanation, as the sporangia lysis was only accelerated at about the 36th h of the culture and at the end of the sporulation phase. However, the synthesis of citric acid (Fig. 3) shows that the TCA cycle was at least partially operational. Although *B. thuringiensis* lacks α-ketoglutarate dehydrogenase, this metabolic step is circumvented by the γ-aminobutyric acid pathway (Aronson *et al.*, 1975). Yet, since there is no report on the effect of oxygen on the activity of this pathway, a special study is needed to verify whether the malfunction of this pathway under anaerobic conditions could be considered as a reason for acid accumulation during sporulation.

The impact on the respiratory chains should also be taken into account in such a study. Another important aspect of *B. thuringiensis* fermentation is the final activity of the produced insecticide that is under special metabolic regulations, such as carbon catabolite repression (Ghribi *et al.*, 2007). Ghribi *et al.* (2007) suggested that the aeration level, affecting the carbon source assimilation, could affect the pathways of carbon catabolite repression and influence the delta-endotoxin production. Thus, an interesting study would be to correlate the metabolic events, such as those presented here, with the insecticidal activity (toxicity) and synthesis of different ICPs. Such a study would need to follow the time courses of different ICPs syntheses, taking particular account of the nitrogen metabolism. This would necessitate intensive researches toward better understandings of *B. thuringiensis* metabolism in relation to the ICPs production. However, it has already been reported that the final toxicity drastically decreases in the absence of oxygen during sporulation (Sarrafzadeh and Navarro, 2006; Boniolo *et al.*, 2012). Therefore, this implies that anaerobic conditions are unfavourable for *B. thuringiensis* sporulation, even with the advantage of a shorter sporulation time.

In conclusion, a high concentration of oxygen was found to prevent sporulation, whereas the absence of oxygen accelerated the lysis of sporangia and consequently decreased the sporulation period. The absence of oxygen strongly affected the metabolic reactions of sporulation, yet did not inhibit it. In this case, while the origin of the accumulated acids is unclear and their presence unusual, the cells used other unknown ways to continue the sporulation. Therefore, the current results suggest that adequate control of the oxygenation conditions during the sporulation phase of a spore-forming bacterium may be a valuable parameter for controlling the metabolic events during sporulation and thereby directing the culture towards the required product in a cost-effective way. While an intensive evaluation of metabolic events should consider the exact biochemical pathways of all nutrients, the present study was limited to some measurable yet important metabolic-related parameters. Therefore, the present work is of significance in clarifying certain aspects of the physiology of *B. thuringiensis* H14 as an important industrial bacterium, particularly the relationship between sporulation and metabolism under different aeration conditions.

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