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OPTIMISATION OF THE *FIBROBACTER* UNIT: PROCESS PARAMETERS

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1 INTRODUCTION

Fibrobacter succinogenes originally isolated from the bovine rumen, have culture conditions defined by Bryant and Doestch (1954) i.e. a temperature of 39°C and a pH of 6. Gaudet and al. (1992) have defined that the culture media must contain 0.3% of a mixture of VFA. In this report we test these culture conditions by performing experiments in order to define the range of temperature, pH and VFA concentration allowing *Fibrobacter succinogenes* growth.

Fibrobacter succinogenes is one of the major cellulolytic bacteria found in the bovine rumen (Stewart and Flint, 1989). Except for the lignin, all the parietal polymers of the vegetable cells are degraded by *Fibrobacter succinogenes* thanks to a complex cellulolytic system. The efficiency of this strain to degrade fibres is explained by the presence of various enzymes. We have tested in the previous TN 2.6 for the presence of endoglucanases and determined the activities of related fibrolytic enzymes produced when *Fibrobacter succinogenes* grown on three different carbon sources. Some of these enzymes were identified and seem to be endo and exoglucanases. To confirm the presence of endoglucanases and the activities of related fibrolytic enzymes produced by *Fibrobacter succinogenes* other methods can be perform like a viscosimetric method. So we decided to examine this method in our culture conditions. After the development of an existing technique (Béra, 1998), a correlation with the proportioning technique described by Miller (1971) was made in cells grown on vegetables in the bioreactor.

Finally, according to the last meeting, degradation of substrate from partner 1 was performed. In the previous TN 2.6 substrate from metanogenic unit was degraded by *Fibrobacter succinogenes* with a rate of 28% and with VFA production. But we were confronted a difficulty. It was to define a feeding procedure in terms of quantity (200g once) and of frequency (33g once a week). So for this loop we have added the totality of the substrate at the beginning of the fermentation to try to obtain a better rate of degradation.

2 VARIATIONS OF CULTURE CONDITIONS

2.1 Experimental set up

Fibrobacter succinogenes was grown anaerobically in serum bottle in a basal medium defined previously (Gaudet et al., 1992). 100mL of culture media was introduced in serum bottles and inoculated with 10 mL of a preculture grown on cellobiose (8 g/L). Glucose or vegetables (wheat straw, green cabbage and soya, 1/3 of each) were the carbon and energy source.

In order to evaluate the optimal culture conditions, the serum bottles were incubated 24h or 72h at several temperatures, pH and with different initial VFA concentrations. Samples were performed regularly and analyzed by HPLC to establish VFA production. The temperatures tested were 46°C, 39°C (standard), 30°C and 4°C. pH was modified by adding H_2SO_4 in the medium (pH value lower than 6) or by adding Na_2CO_3 (pH value higher than 6). The VFA concentrations tested were 0.15%, 0.3% (standard), 0.6%, 0.9% and 1.2%.

2.2 Results and discussion

2.2.1 Temperature influence

2.2.1.1 On glucose

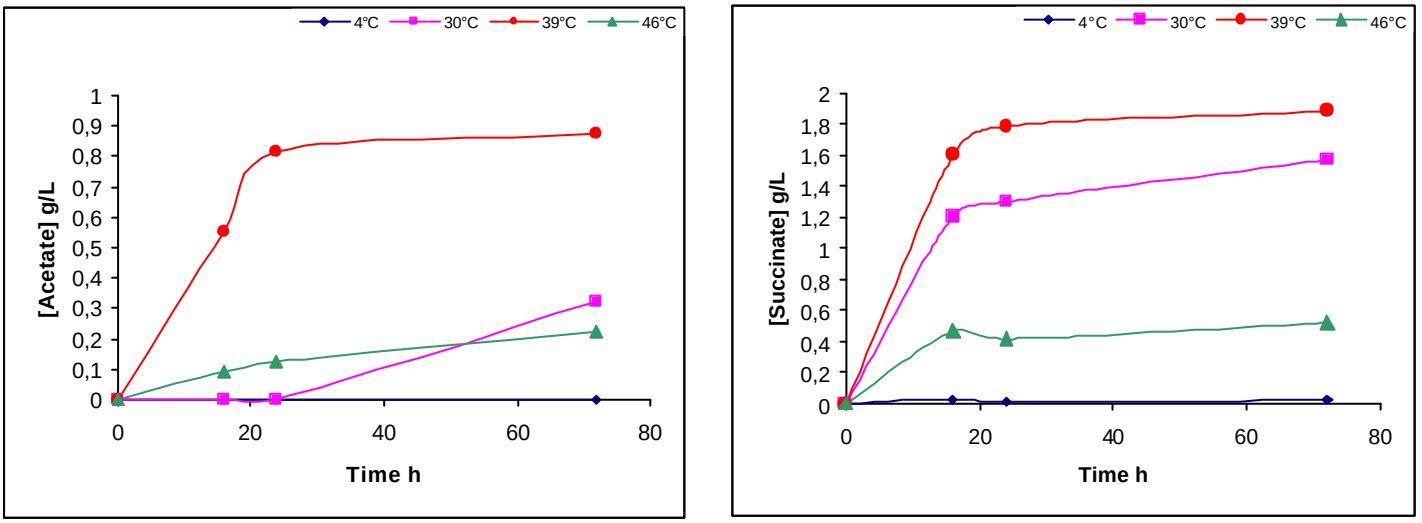


Figure 1: Acetate and succinate production on glucose with the time of culture

Figure 1 shows the production of acetate and succinate by *Fibrobacter succinogenes* growth on glucose in different conditions of temperature. At 39°C, the production of acetate and succinate is more important than with the others. According to these curves, we consider that the maximal productivity is achieved during the 22 first hours of the culture before the

substrates became limiting. The maximal production of acetate and succinate is for a temperature of 39°C with 0.87 g/L of acetate and 1.88 of succinate (Table 1).

	Acetate g/L	Succinate g/L
4°C	0	0.028
30°C	0.32	1.57
39°C	0.87	1.88
46°C	0.22	0.52

Table 1: Maximal production of acetate and succinate with the temperature

Figure 2 represents the maximal concentrations of acetate and succinate achieved on glucose for different temperatures of culture. So the optimal temperature of growth seems to be 39°C. Moreover a production of succinate and acetate is observed at 30°C and at 46°C. So a range of temperature can be used to have an acceptable production of succinate and acetate between 30 and 46°C

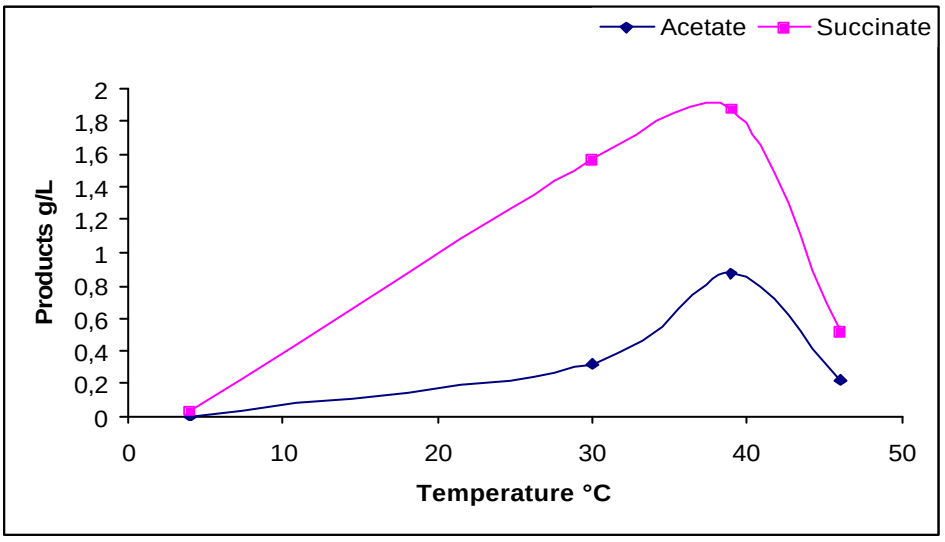


Figure 2: Acetate and succinate production on glucose with the temperature

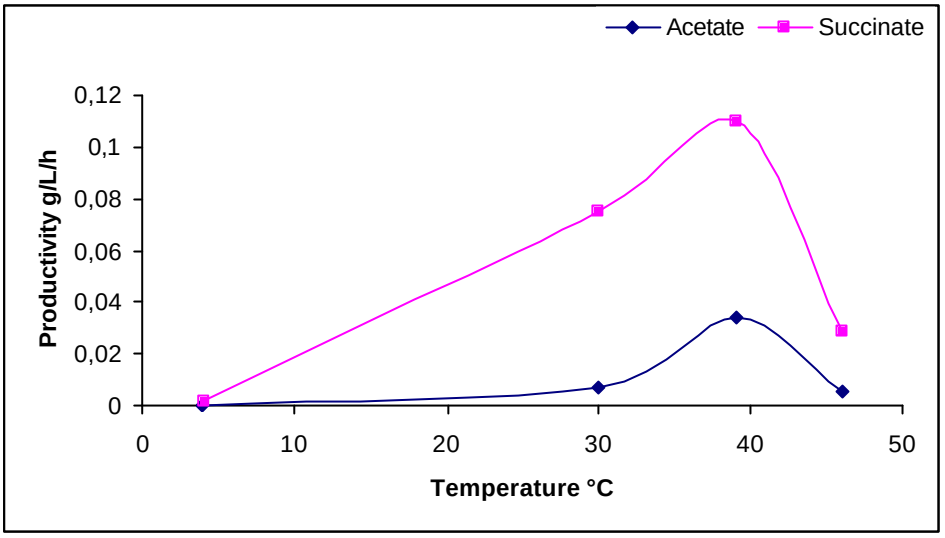


Figure 3: Productivities of acetate and succinate

Figure 3 represents the productivities for these temperatures. The optimal production of acetate is for a temperature of 39°C with about 0.034 g/L/h. Whereas at 46°C and 30°C the production is respectively $5.6x10^{-3}$ g/L/h and $6.7x10^{-3}$ g/L/h. In the same way, the production of succinate is maximal (0.11 g/L/h) for the same temperature (39°C) and at 4°C, 30°C and 46°C the production is respectively $1.9x10^{-3}$ g/L/h, 0.075 g/L/h and 0.029 g/L/h.
From the above, the best temperature seems to be 39°C. However production of acetate and succinate are also observed at 30°C and 46°C. In order to more precisely determine the optimal temperature, an experimental setup should be done in a narrower range of temperature, namely between 35°C and 43°C in order to correctly assess the optimal temperature of growth of *Fibrobacter succinogenes*. Neverthe less the general shape of the curve of productivity against temperature is classical of microbial growth, showing that the optimal temperature remains in the vicinity of 38-40°C.

2.2.1.2 On vegetables

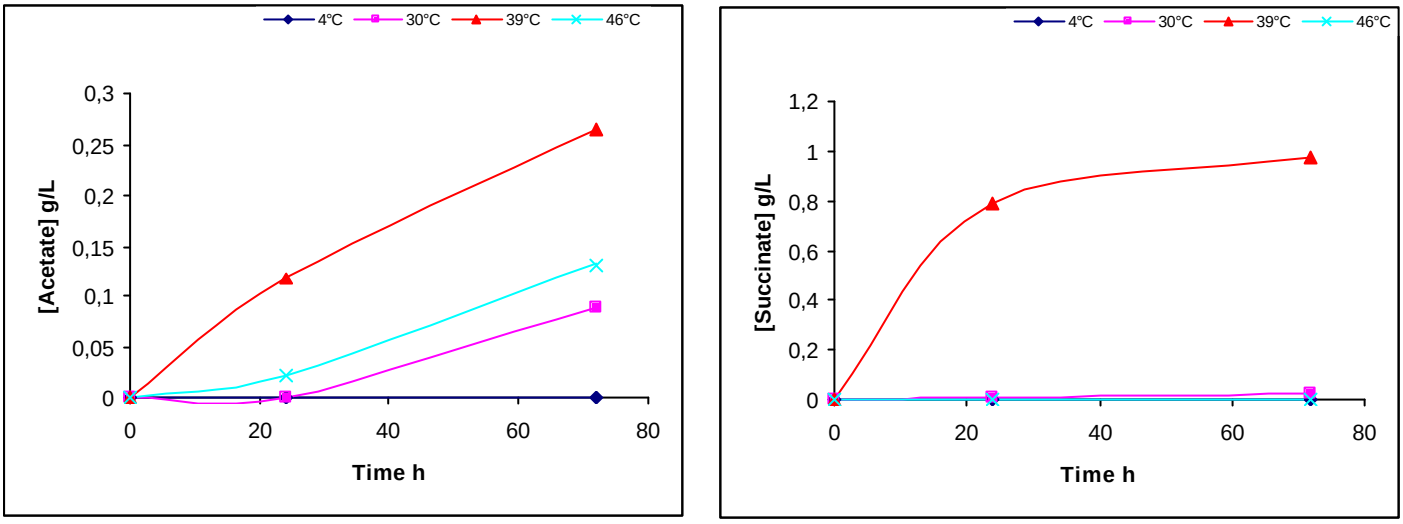


Figure 4: Acetate and succinate production on vegetables with the time of culture

At 39°C, the production of acetate and succinate is more important than with the others (figure 4). The maximal production of acetate and succinate is for a temperature of 39°C with about 0.26g/L and 0.97 g/L. We can also observe a production of acetate at 30°C and 46°C. All the maximal concentrations are gathered in table 2

	Acetate g/L	**Succinate g/L**
4°C	0	0
30°C	0.088	0.026
39°C	0.26	0.97
46°C	0.13	0

Table 2: Maximal concentration of acetate and succinate with the temperature

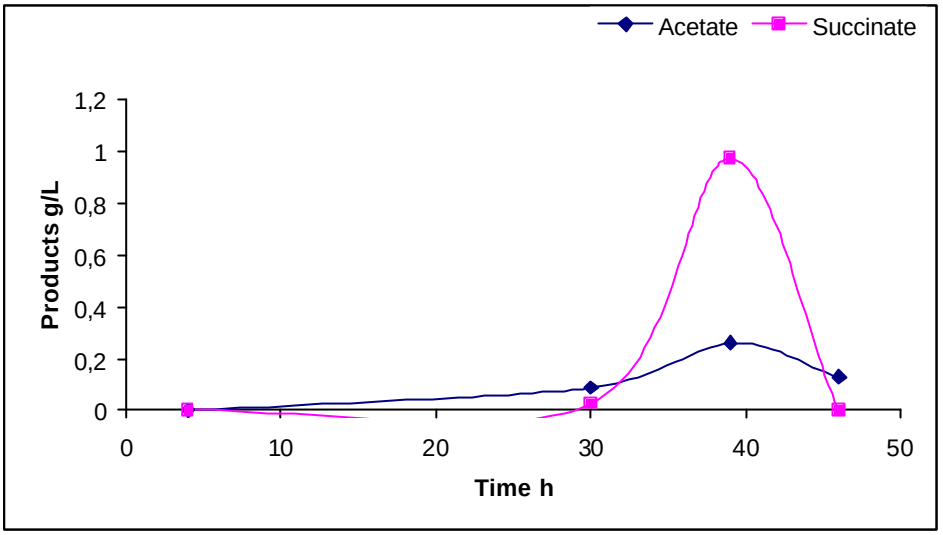


Figure 5: Acetate and succinate maximal production on vegetables with the temperature of incubation

In the same way as on glucose there is a range of temperature (30-46°C) for the production of acetate but only a temperature of 39°C allows a production of succinate (Figure 5). So the optimal temperature of growth seems to be 39°C on vegetable.

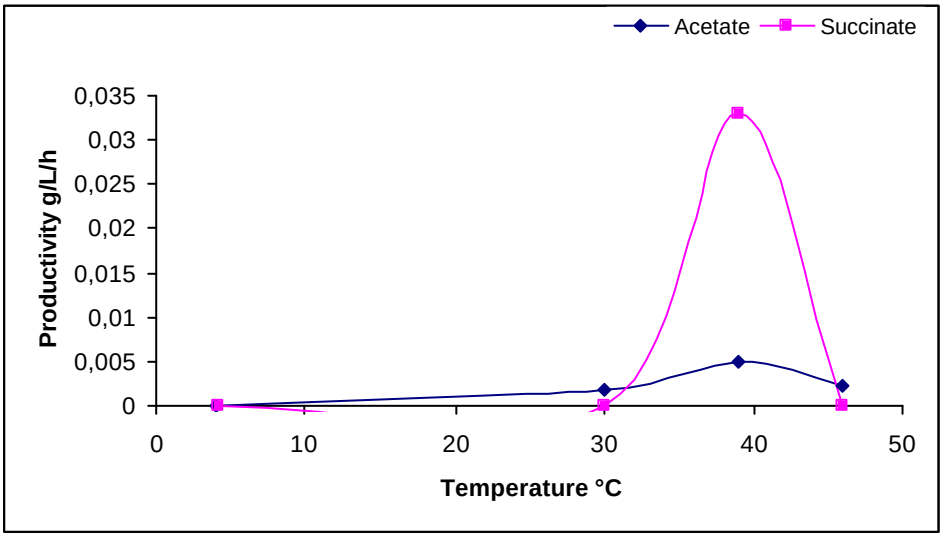


Figure 6: Productivities of acetate and succinate

Figure 6 represents the productivities for these temperatures. The optimal production of acetate is for a temperature of 39°C with about $5x10^{-3}$ g/L/h. At 30°C and 46°C the production is respectively $1.8x10^{-3}$ g/L/h and $2.25x10^{-3}$ g/L/h.
At 39°C *Fibrobacter succinogenes* on vegetables produces a relatively large quantity of succinate (0.033 g/L/h) whereas at the other temperatures: 4, 30 and 46°C, no production is observed after 72h of culture. One explanation is that the bacterium is stressed and reconsumes succinate preventing it to be observed before 24h of culture. This is observed in bioreactor when *Fibrobacter succinogenes* does not have enough easily degradable substrates.

2.2.1.3 Discussion

The experiments performed on the variation of temperature show that the optimal temperature of growth for *Fibrobacter succinogenes* is in the vicinity of 39°C, which confirms the value classically recorded in the literature (Bryant and Doest, 1954). The quantity of succinate produced on glucose is higher than on vegetables. Indeed, on glucose the bacterium can directly assimilate the substrate and thus begin its growth whereas on vegetables, a lag time is necessary for the synthesis of the cellulolytic enzymes which enable it to assimilate cellulose in the vegetable and to produce succinate.
The production of succinate and acetate on glucose at 30°C and 46°C means that *Fibrobacter succinogenes* is able to grow at these temperatures. On the other hand, at these temperatures on vegetables there is a production of acetate but not of succinate, these temperatures thus don't allow an efficient growth.
The data obtained are in agreement with the observation of Bryant and Doest (1954) which observed that *Fibrobacter succinogenes* is able to grow at a temperature of 25°C to 45°C.

2.2.2 pH influence

2.2.2.1 On glucose

Figure 7 shows the production of acetate and succinate when varying the pH with glucose as carbon source. At 39°C, the production of acetate and succinate is more important than with the others.
The optimal production of acetate is obtained for pH 6 with about 0.034 g/L/h. Whereas at pH

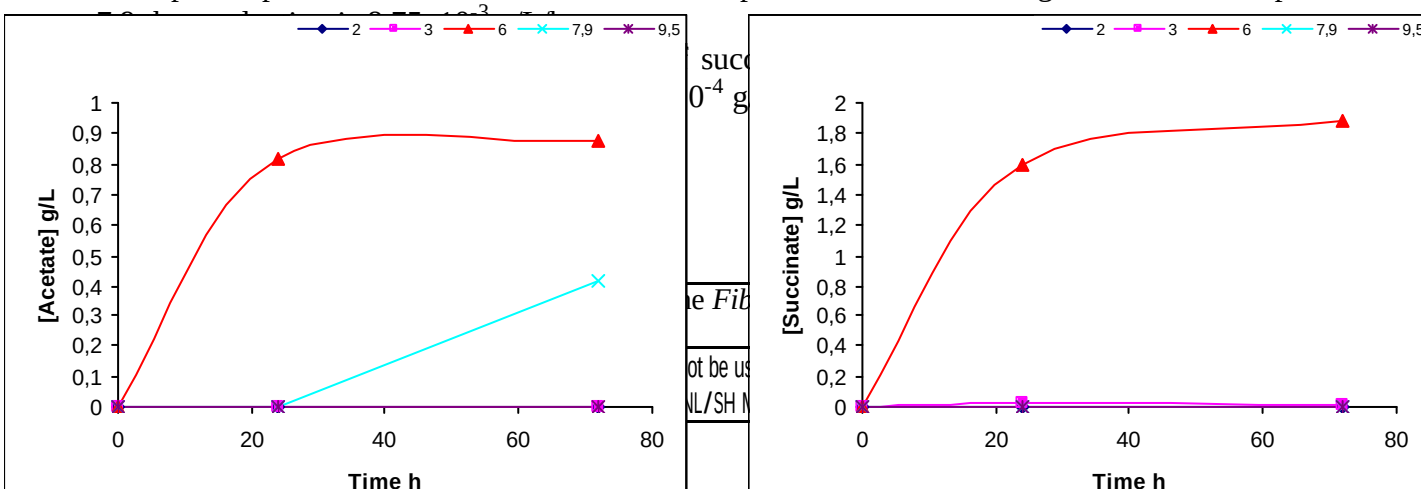


Figure 7: Acetate and succinate production on glucose with the time of culture

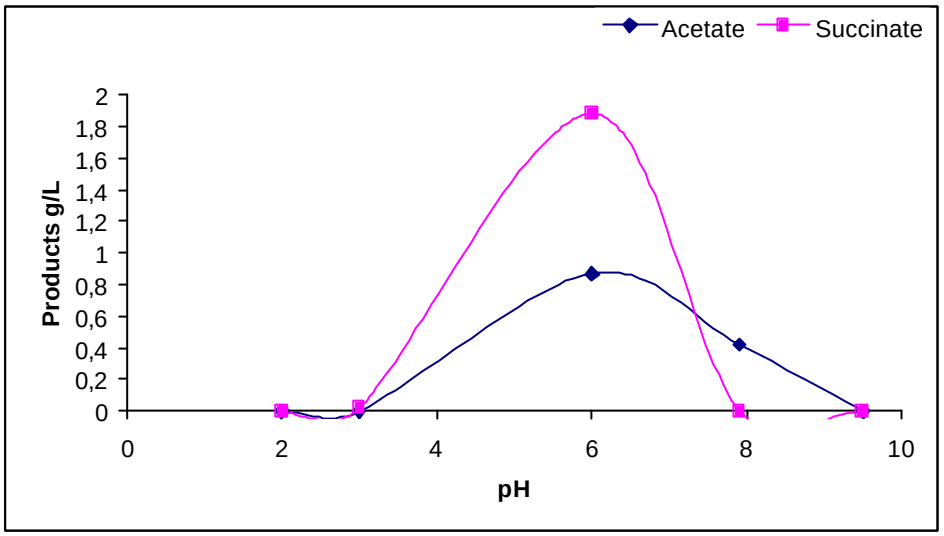
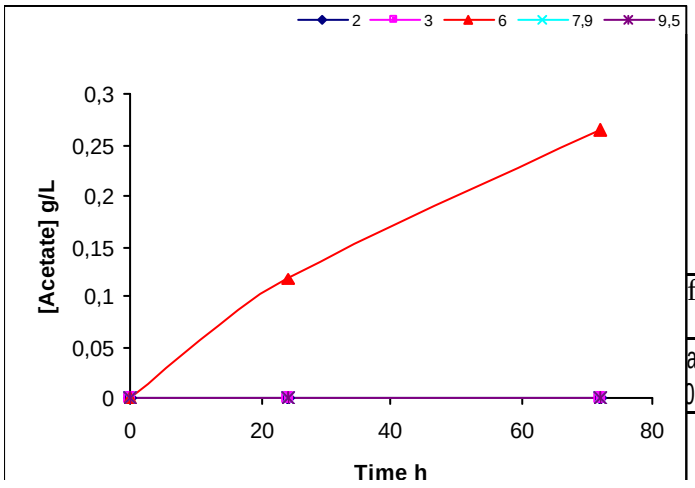


Figure 8: Acetate and succinate production on glucose with the pH of incubation

Acetate production is the highest at pH 6 (figure 8). None growth is observed when the pH is 2 or 9.5. A low production is observed when pH is 7.9. Succinate production is observed for a pH 3 this could be explained by the low pH conditions in the rumen but it is re consumed after 24h of culture.
A more narrow range of pH values, namely 5.2-7 should be necessary in order to precisely account of the effect of pH on growth, knowing that this range also contains the pK values of acetate, succinate and bicarbonate dissociation.

2.2.2.2 On vegetable wastes



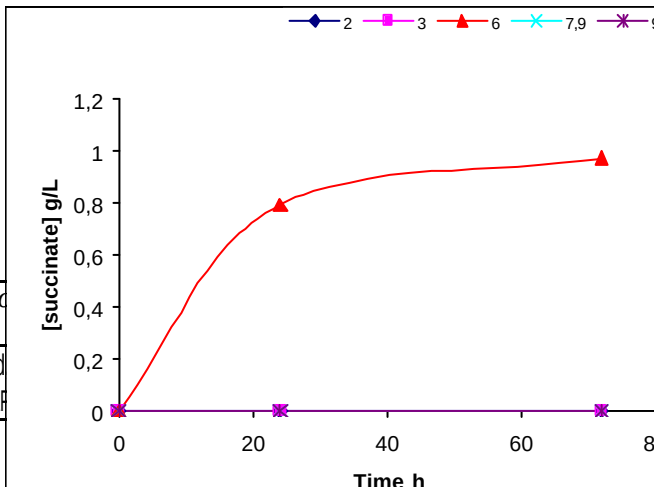


Figure 9: Acetate and succinate production on vegetables with the time of culture

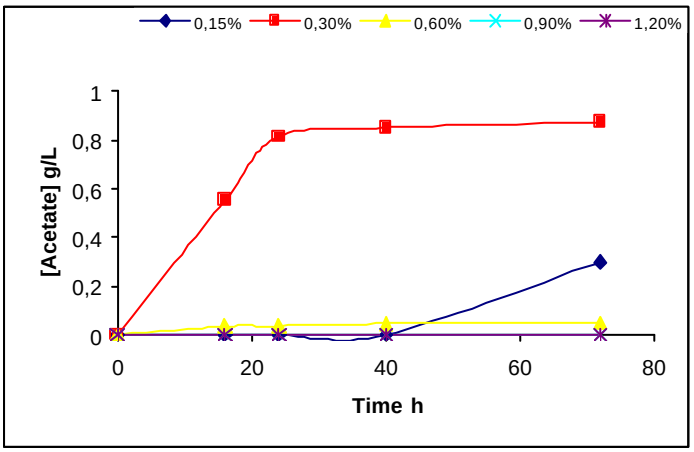
Figure 9 shows the production of acetate and succinate by *Fibrobacter succinogenes* growth on vegetables in different conditions of pH.
The only production of acetate and succinate is observed for pH 6 with respectively 5x10-3 g/L/h and 0.033 g/L/h. No production is observed when pH was 2, 3.5, 7, 9 and 9.5.

2.2.2.3 Discussion

The best pH for *Fibrobacter succinogenes* growth is 6 (standard). At pH 7.9 the bacterium has a lower production of acetate compared to that obtained with the control (6). With a very acid pH (3) there is no production of acetate.
This value of pH is thus a limiting factor for the growth of the bacterium like it was already noticed by Séon and al. (TN 2.3). It is for this reason that the bioreactor comprises today a regulation of pH which preserves a pH of 6 per automatic carbonate addition when the medium becomes acid.
Actually, the experiment performed show that *Fibrobacter succinogenes* has a tolerance of a range of pH between 6 to 7, whereas Bryant and Doest (1954) showed that *Fibrobacter succinogenes* tolerate a range of pH: 5.5 to 7.7.

2.2.3 Influence of the initial VFA concentration

The experiments were only performed on glucose as carbon and energy source.



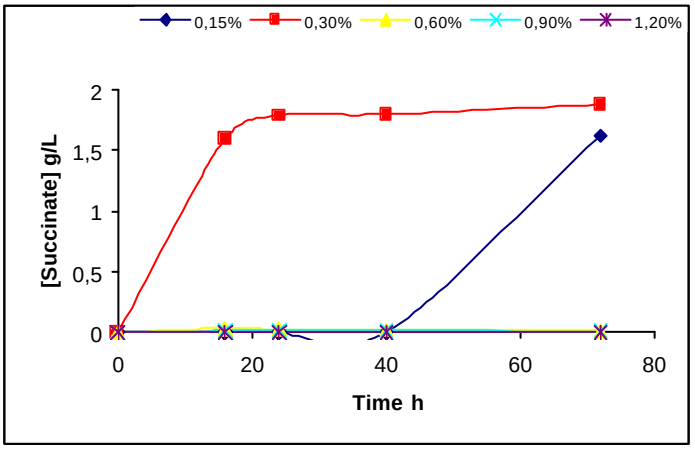


Figure 10: Acetate and succinate production on glucose with several initial concentration of VFA

Figure 10 shows the production of acetate and succinate by *Fibrobacter succinogenes* growth on glucose in different initial concentration of VFA. The media usually contains 0.3 % of a mixture of VFA. Indeed with this concentration, acetate production is important (0.34g/L/h) but the curves reveal that there is a production of acetate for a concentration of 0.15 % with about $9.3x10^{-3}$ g/L/h and for a concentration of 0.6 % with about $2.13x10^{-3}$ g/L/h.
Succinate production is the highest for 0.3 % of VFA with about 0.11 g/L/h but there also an important production for a concentration of 0.15 % of VFA with 0.05 g/L/h. Indeed weak productions are observed with 0.6 and 0.9 % of VFA with respectively $1.9x10^{-3}$ g/L/h and $6.9x10^{-4}$ g/L/h.
For concentrations higher than 0.9% of VFA, the VFA act like a limiting factor until completely inhibiting the growth. These experiments thus show that the growth of *Fibrobacter succinogenes* also depends on the concentration in VFA.
It will thus be necessary to find a solution to be able to control the quantity of VFA present in the medium in order to be able to optimize the growth of *Fibrobacter succinogenes*. However the addition of VFA in the culture media tends to decrease the pH value so it's possible that the inhibition was only due to the acidification and not to the presence of VFA. Indeed the addition of 0.9% of VFA decreases the pH value to 4.5 and the limiting value for *Fibrobacter succinogenes* growth is 5.5.

3 CELLULASE ACTIVITIES

3.1 Experimental set-up

3.1.1 Culture conditions

Fibrobacter succinogenes has been grown anaerobically under 100% CO_2 in a basal medium (Gaudet et al., 1992). 100 mL of this media was introduced in serum bottles and sterilized (20', 121°C). Cystein (0.5 g/l) and Na_2CO_3 (4 g/l) were introduced in the hot medium to decrease redox potential (–350 mV) and increase pH (6.0-6.5). Then, as *Fibrobacter succinogenes* is a strictly anaerobic organism, serum bottles were flushed with CO_2 during 3 hours in order to completely fill the atmosphere with this gas.
Three carbon sources have been used: glucose, microcristalline cellulose (Avicel PH 101) and vegetables constituted of green cabbage, soya and wheat straw (400 mg/L of each).

After these preparation steps, the serum bottles were inoculated with an overnight preculture (10 mL) grown on cellobiose (8g/l). The serum bottles were then incubated 24h, 48h or 72h at 39°C (figure 6).

3.1.2 Enzymes extraction

As it was explain in the previous TN (2.6) some problems were encounted with the cell associated proteins extraction so we have suppressed a centrifugation (figure 11)

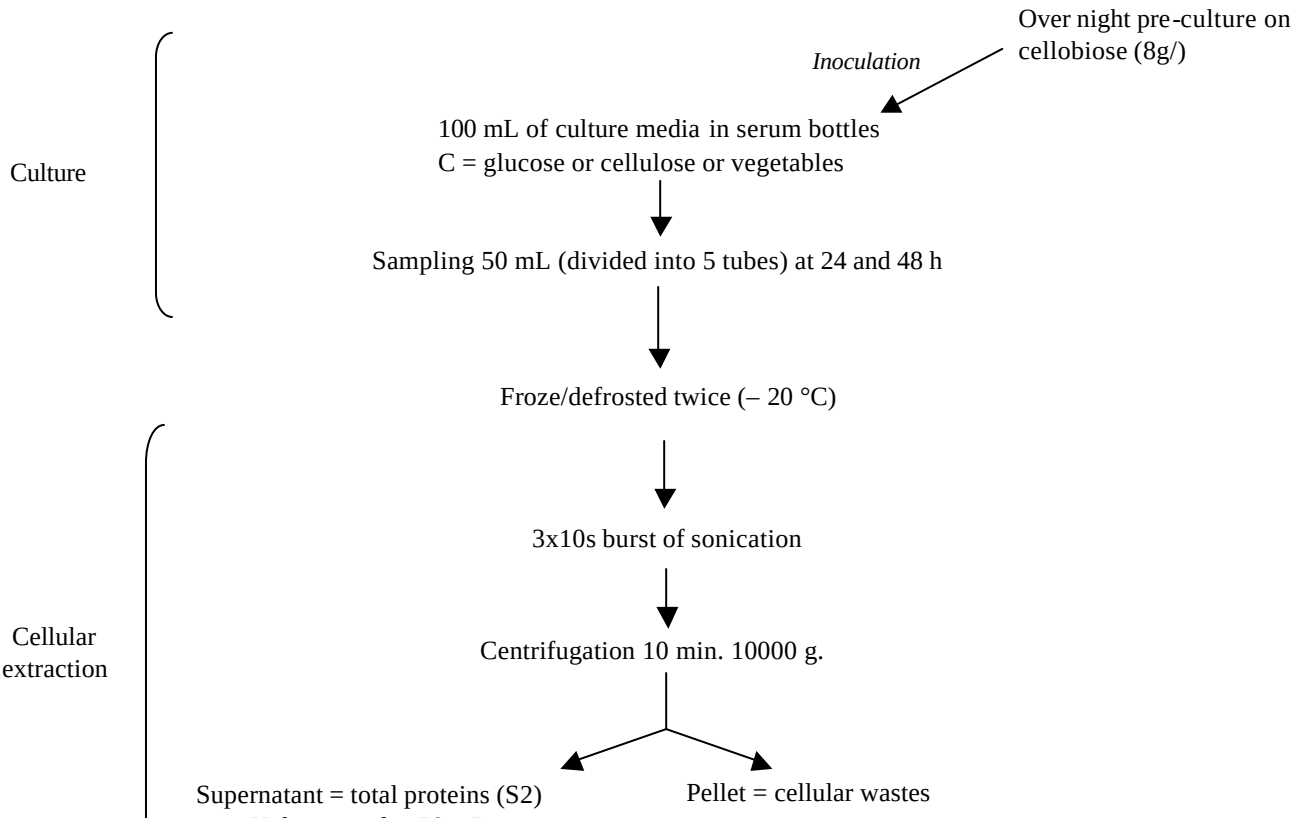


Figure 11: Cellular extraction

3.1.3 Viscosimetric method

The viscosimetric method is a quantitative method which measures essentially the cellulase activity. The viscosity of the mixture is measured by the flow time of this solution through a 1 ml pipette (T test). This time is proportional with the viscosity of the studied solution. The variations with time are expressed in % of decrease of viscosity compared to two tests (T1 and T2).
Figure 12 shows the protocol used to measure the time of flow (T test).

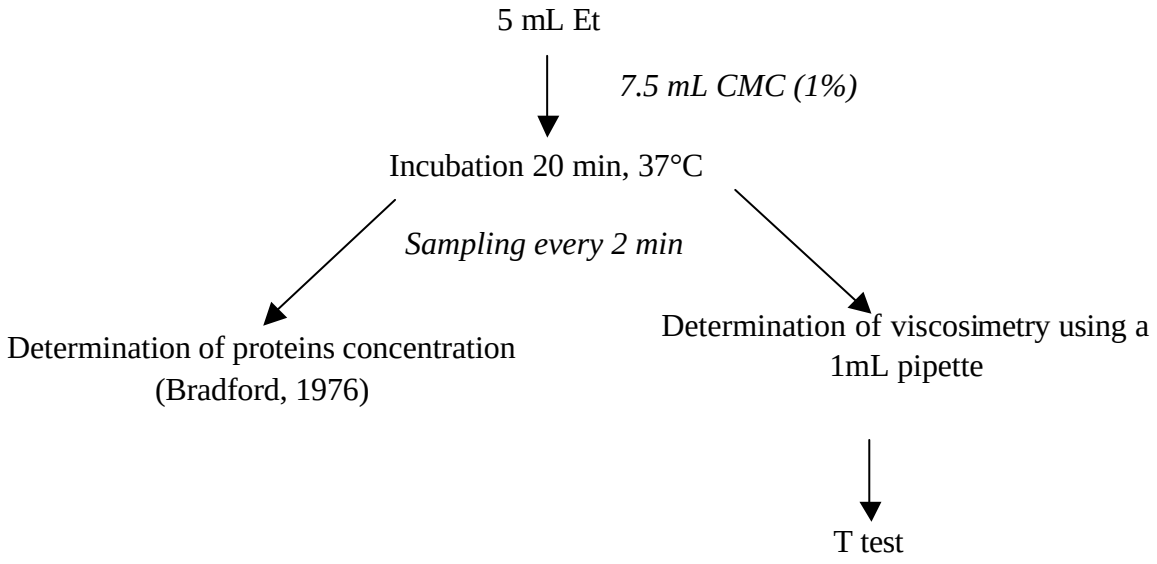


Figure 12: Determination of cellulase activity

From T1, T2 and T test we can calculate a percentage of viscosity reduction during the time (Béra, 1998).

T1 - T test

% of decrease of viscosity = $\frac{\text{T1 - T2}}{\text{T1 - T2}} \times 100$

T1 = speed flow of 1mL of CMC with inoculate media incubated 5 min. at 100°C.
T2 = speed flow of 1 mL of the buffer.
T1 and T2 are measured at the beginning and at the end of the proportioning.

3.2 Results and discussion

3.2.1 Preliminary experiments

At the beginning of the experiments several tests were carried out such as the use of various concentrations of CMC (0.5 and 1%), of various sizes of pipette (5 or 1 ml) and various time of incubation for the test in order to optimized the technique to our culture conditions.
First, we choose 1% for the CMC solution because the decrease of viscosity is visible and significant with this concentration whereas with 0.5% the 100% of decrease is quickly reached. Then, we used a 1 ml pipette because the measures are more exact, indeed the 5 ml pipette generates significant variations over flow time of the solutions.
Finally, time of incubation was determined by the fact that after 20 minutes the decrease of viscosity is very weak and slow.
Moreover, some experiments were carried out in order to validate the method of proportioning by viscosimetry. The medium (no inoculated) was incubated one night at 39°C, to check that no compound of the medium has influence on the viscosity of the CMC. Then, the medium just after the inoculation was test to check that the quantity of enzyme, brought by the inoculum, was not enough to make decrease of the viscosity of the CMC. These two tests were negative so the proportioning was possible.

3.2.2 Profile of the curve obtained

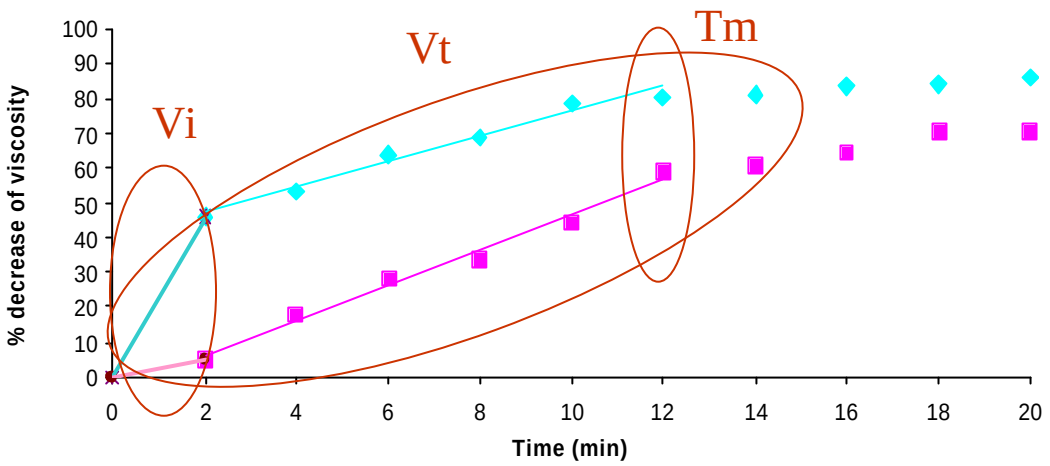


Figure 13: Profile of the curve obtained by the viscosimetric method

The proportion gives these profiles of curves (figure 13) in which three parameters are important:

- Vi = the initial speed,
- Tm = the time for the maximum decrease,
- Vt = the total speed.

3.2.3 Cellobiose and cellulose (Avicel PH 101) cultures

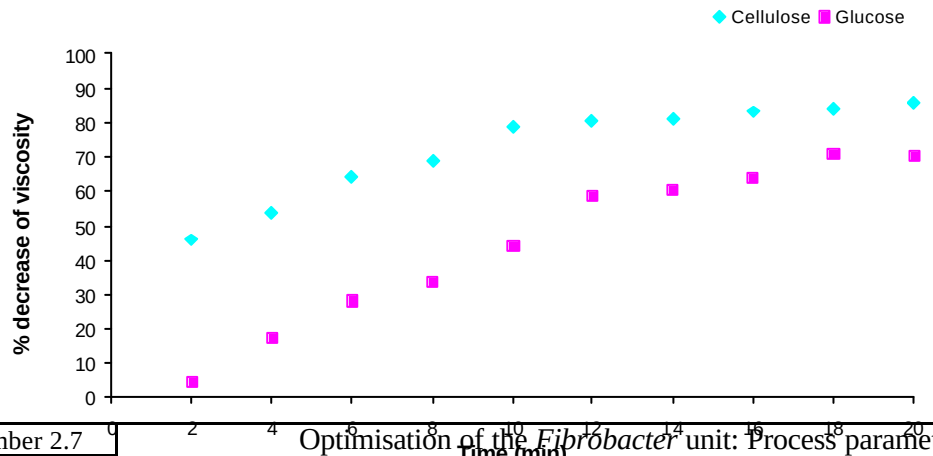


Figure 14a: % of decrease of viscosity with the time of the total protein extract after 24h of culture

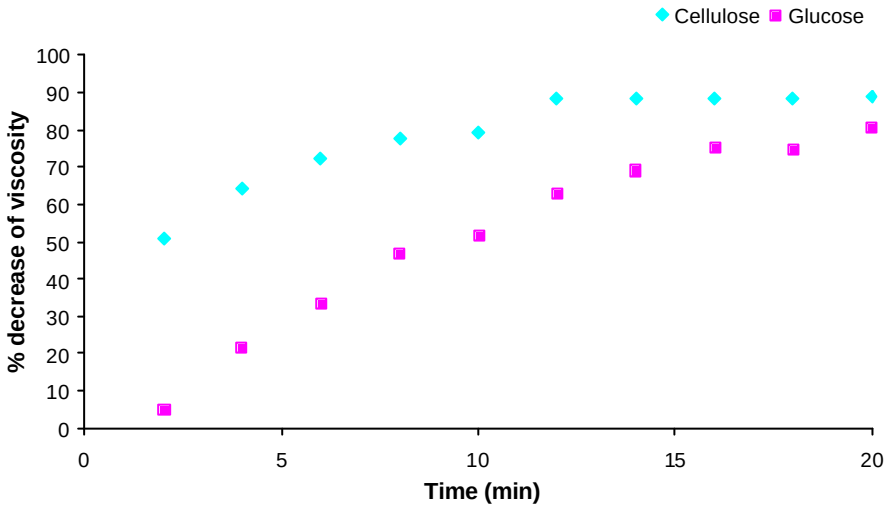


Figure 14b: % of decrease of viscosity with the time of the total protein extract after 72h of culture

Figures 14a and 14b represent the results of decrease of viscosity during the time of incubation of the extracts after 24 and 72h of cultures. These cultures were carried out on glucose and cellulose.
After 24 or 72h of culture there is a higher activity on cellulose than on glucose. The presence of an activity on glucose (simple sugar) shows that there is a basal synthesis of the enzymes. *Fibrobacter succinogenes*, when it develops on a medium whose only source of carbon is glucose, produces enzymes able to degrade the cellulose even if it does not use them. On cellulose, *Fibrobacter succinogenes* needs more specific enzymes such as avicelases but some of these enzymes don't have a CMCase activity (Béra, 1998) that why the 100% of decrease of viscosity is not reached here
The cellulolytic activity of the enzymes increases on simple sugars with time of culture, but in small proportion.

3.2.4 Cultures on vegetables

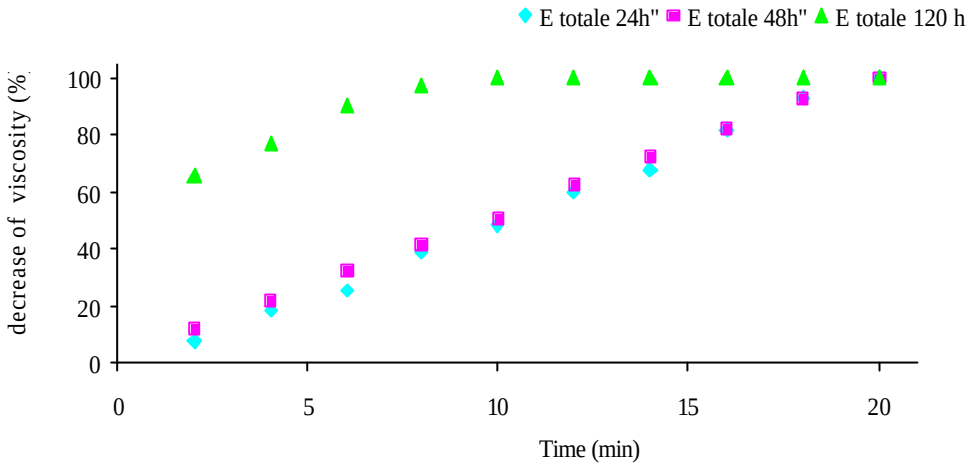


Figure 15: % of decrease of viscosity with the time of incubation for the total protein extract after 24, 48 and 120h of culture

Figure 15 represents the % of decrease of viscosity with the time of incubation for the total protein extract after 24, 48 and 120h of culture on vegetables in bioreactor. The same activity is observed after 24h and 48h of culture and there is a important increase after 120h of culture. On vegetable wastes *Fibrobacter succinogenes* hydrolyses first of all easily degradable substrates, such as cellulose, then recalcitrant substrates and don't synthesize lots of cellulases. After 120h of culture, there is not enough simple sugars so *Fibrobacter succinogenes* synthesizes cellulases.

3.3 Conclusion

	Glucose		Cellulose		Vegetables	
Time of culture (h)	24	48	24	48	24/48	120
Vi (% / min)	2.47	2.68	23.01	25.42	4.89	**33.15**
Tm (min)	18	16	14	12	20	**10**
Vt (% / min)	3.93	4.68	5.79	7.34	5	**10**

Table 3: The important parameters

Table 3 summarized the three important parameters explained in § 3.2.2. We can observed that Vi increase and Tm decreases with the time of culture. Indeed theses two parameters are linked, the more speed is the Vi the less is the Tm.
We can also notice that Vt and Vi are highest on vegetables. In the same way Tm is weakest on vegetable, which means that the activities of the extracts from vegetables cultures are higher than the activities of the extracts from glucose or cellulose culture.

Generally, an enzymatic activity is present in all the extracts and this activity increases with the age of the culture. Simple sugars are used by *Fibrobacter succinogenes* to provide energy necessary to synthesize proteins; the cellulases synthesis in this case is not necessary (Mc Dermid et al., 1990). This explains why the activities of the extracts from glucose culture are lower than that the activities of the extracts from cellulose or vegetables culture.
In the same way, the activity of the extracts from a vegetables culture is the same after 24h or 48h of culture but increases after 120h of culture. Mc Dermid et al. (1990) notes that all hydrolases, such as endoglucanases, identified in *Fibrobacter succinogenes* cells seem to be synthesized in the presence of cellulose, what suggests a mechanism of regulation. These enzymes have a basal level of expression in the cell, when the substrate is glucose or any other simple sugar and this level increase if the substrate is a more complex sugar such as cellulose or on vegetables, what suggest a mechanism of induction.

3.4 Correlation

The results obtained by the two methods show an increase of activities with the time of culture, the same action on vegetable after 24 or 48h of culture (*Fibrobacter succinogenes* uses first simple sugars to provide energy), a higher activity on cellulose than on glucose, a basal level on glucose and induction on cellulose and on vegetables (*Fibrobacter succinogenes* synthesizes on these substrates enzymes necessary to degrade more complex sugars). All these points, led us to ask if there is a possibility of a correlation between the two methods. So extract from a culture on cellulose, after 72h, was proportioned with the two methods (figure 16).

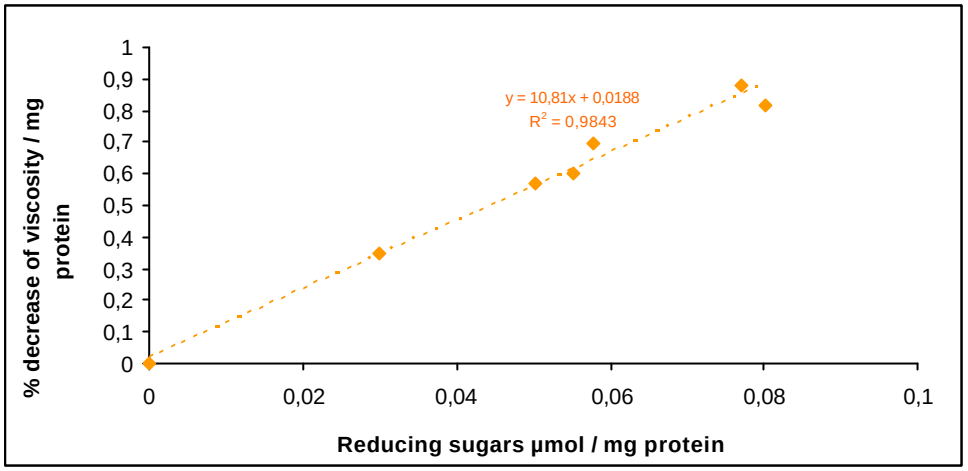


Figure 16: Correlation between the two methods of proportioning

The two methods are correlated (R^2= 0.9843) but don't give the same information. The DNS Miller (1971) proportioned the reducing sugars which represent the activity of the entire enzymatic complex (endoglucanases, exoglucanases, cellulases…), whereas the viscosimetric method gives the activity of the cellulases only. This correlation between the two methods confirms the efficiency of each method for the proportioning of enzymatic activities.

4 LOOP

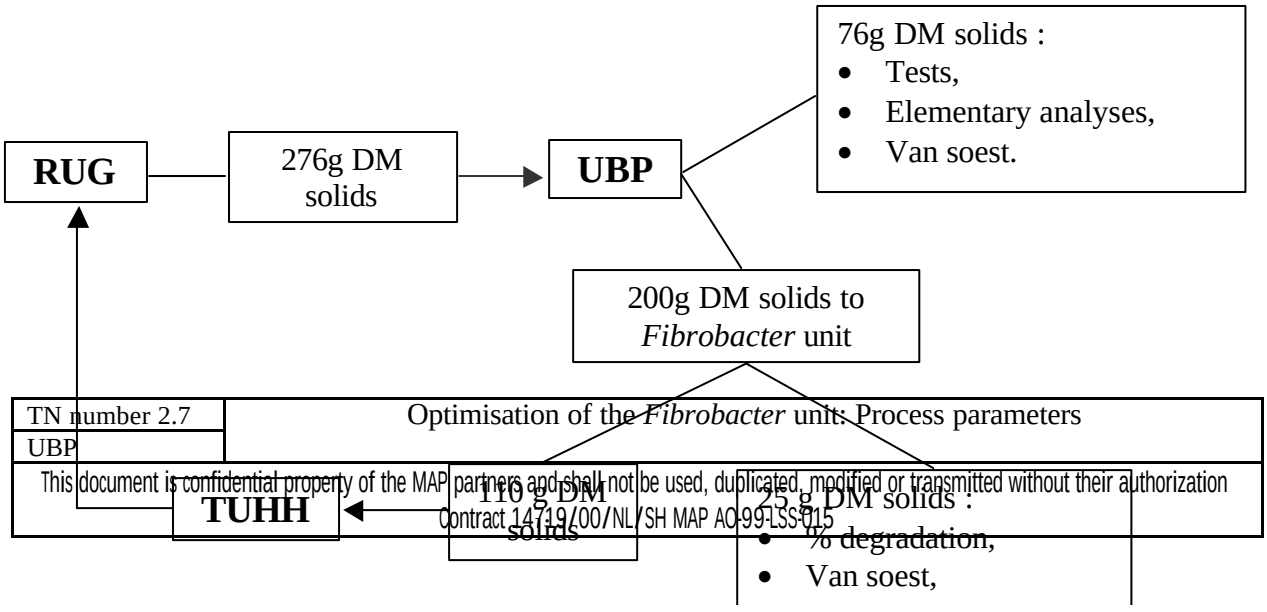


Figure 17: Organisation of the substrate exchanges between MAP partners

4.1 Preliminary experiments

4.1.1 Analysis of the liquid phase

The analysis classically performed for *Fibrobacter succinogenes* process characterisation were realised on the liquid effluent. The pH value was found to be 7.6 and the VFA concentrations were analysed by HPLC. The liquid effluent contained 3.25 g/L of acetate, 1.21 g/L of propionate and 0.86 g/L of butyrate. These concentrations are higher than those found in the TN 2.4 and TN 2.6 (table 4).

	Acetate (g/L)	Propionate (g/L)	Butyrate (g/L)	pH
TN 2.4	0.7	0.87	0.017	10.2
TN 2.6	1.7	0.9	1.1	7.2
TN 2.7	3.25	1.21	0.86	7.6

Table 4: Comparison of VFA concentrations and pH of LabMET substrate between TN 2.4, TN 2.6 and TN 2.7

4.1.2 Residence time

In order to determine the optimal residence time of LabMET substrate we used the RUSITEC. The general set up of the technique was the same as described in TN 2.6.
600 mL of basal medium is prepared in a flask with the nylon bag (porosity 100 µm) containing LabMET substrates (4.8g) and 4.8g of glucose to improve the growth. After the sterilisation (20', 121°C), Na_2CO_3 (4g/L) and cystein (0.5g/L) are added to induce the reduction of the medium under CO_2 atmosphere. Finally this medium with the nylon bag is introduced in the reactor under a laminar flow hood.
In the same time, 100 mL of medium are prepared in a flask with a double picking with cellobiose (8g/L) as carbon source. This double picking allows the connection to the gas flow to preserve anaerobic conditions and the inoculation. The medium is then inoculated with an overnight culture (5mL) and incubated one night at 39°C.
The reactor inoculation is done with the overnight preculture prepared previously under CO_2 atmosphere and the vertical agitation is launched and the pressure brought to 1.35 bars.

	Products (g/L)				**Degradation (% DM)**
Time of culture (h)	Acetate	Succinate	Propionate	Butyrate	
24	0	0,04	0	0,83	15,3
50	0,29	0,046	0	1,14	13,06
70	0,086	0	0,091	1,35	22,86
150	0,24	0	0,1	1,64	**35,7**

Table 5: Concentration of VFA and degradation rate with the time of culture.

Table 5 shows the production of VFA and the degradation rate with the time of culture. VFA classically produced with LabMET substrate are observed and the maximum of degradation is for 150 h of culture. It would be interesting to test longer residence times but we did not have enough substrate.

4.2 Degradation in bioreactor

4.2.1 Experiment set-up

Fibrobacter succinogenes was grown under 100% CO2 on a basal medium. The general set up of the culture was the same as described in TN 2.5. However during the previous loops we were confronted to a difficulty. It was to define a feeding procedure in term of quantity (200g once) and in term of frequency (33g once a week). So we decided to add all the substrate at the beginning of the culture to try to obtain a better rate of degradation. According to the previous loop results, we also decided to add with the substrate 8 g/L of glucose to improve the bacterial growth.

The process was maintained during 350h with three opening of the reactor after the stabilisation of CO_2 production to sample and to determine the metabolite productions.

Absolute pressure, redox potential, pH and temperature were on-line monitored with probes connected on the reactor and related to acquisition software. HPLC analyses were performed on liquid samples in order to follow the production of volatile fatty acids. N-NH_3 concentration was measured in the liquid phase by Patton and Crouch procedure. pH was automatically (peristaltic shear) maintained at 6.0 at least by addition of a concentrated solution of Na_2CO_3 (100 g/l) under CO_2 atmosphere.
Percentage of degradation was estimated by dry mass measurement of substrates and residue of fermentation (105° C, 48 h).
Percentage of carbon in the solid substrates, in the solid residue of fermentation and in biomass was determined by elementary analysis. Biomass concentration in the reactor was estimated by a correlation with succinate and acetate concentration.

4.2.2 Liquid phase

4.2.2.1 pH and redox

Figure 18 shows the evolution of redox potential and pH during the time course of the experiment. The redox value is always comprised between –300 and –450 mV (relative value) during all the experiments, which validates the strictly anaerobic conditions during the culture. The profile of the redox curve is due to a problem with the redox probe because of the density of the substrate.

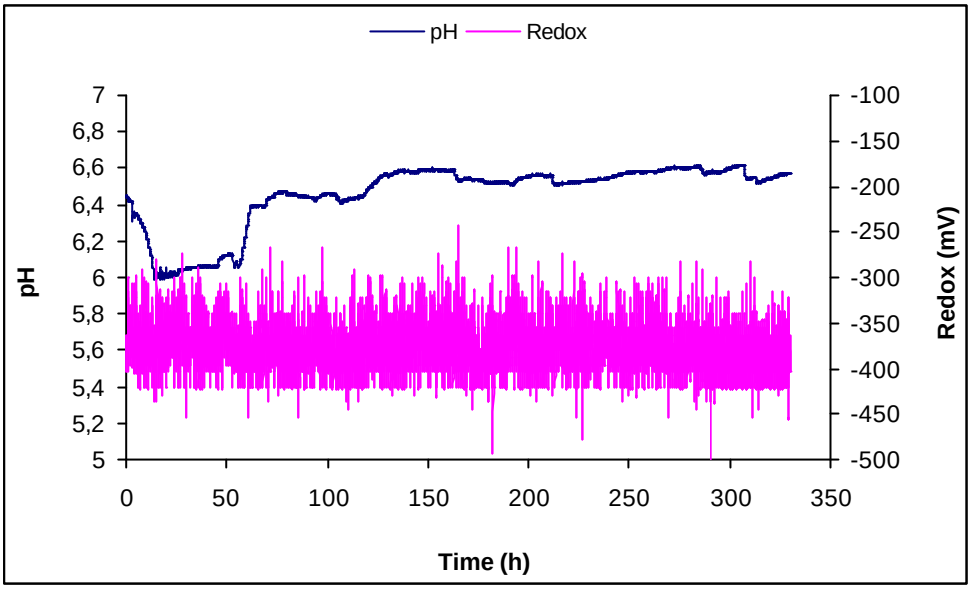


Figure 18: pH and redox evolution

4.2.2.2 VFA

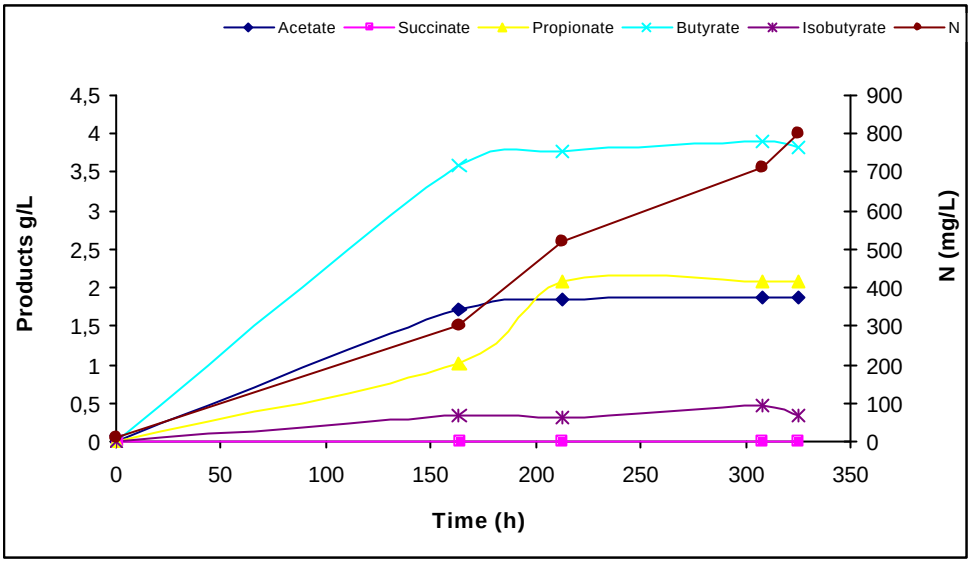


Figure 19: VFA and N concentrations during the experiment

Figure 19 represents the evolution of metabolites concentrations during the experiment. No sample was performed in the first hours of the experiment in order to preserve the pressure into the reactor. After 325 hours the concentration of butyrate, acetate and propionate was respectively 3.82g/L, 1.87g/L and 2.08g/L. The total VFA production during all the experiment was around 8.11g/L. The metabolic profile was similar to the previous loop and to degradation of vegetable wastes (soya, green cabbage and wheat straw) but with lower VFA production (20g/L at the end of the process on vegetable wastes) but about the same as during the previous loop (9g/L at the end of the degradation of LabMET substrate). So we can think that the first hours of culture are characteristic of *Fibrobacter succinogenes* metabolism with production of mainly succinate and acetate (Miller, 1978) and that as it was observed in the experiment on vegetable wastes and on LabMET substrate, all the succinate was reconsumed for the production of other VFA (butyrate and propionate).
The final N-NH_3 concentration is about 800 mg/l. N-NH_3 production is similar to what was observed during the previous culture in bioreactor. We observed an increase of the pH (ever observed in the previous loop) and at the end of the culture the ratio [VFA]/[N-NH_3] is lower than these observed previously on vegetable wastes. The difference between the two ratios could explain the profile of the pH curve.

4.2.3 Gas phase

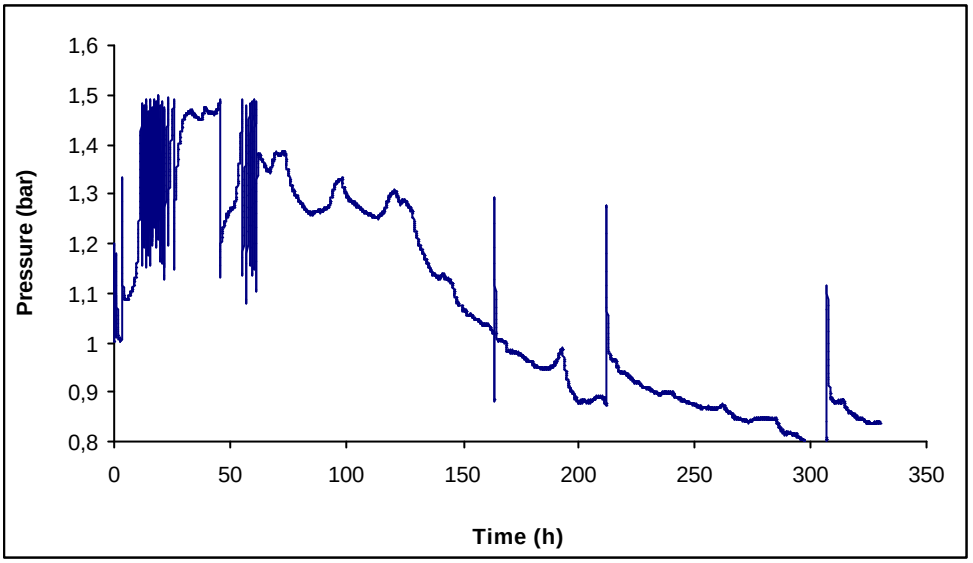


Figure 20: Monitoring of the CO_2 pressure and cumulative pressure

Figure 20 describes the evolution of gas pressure during the fermentation. Pressure, which is manually increased to 1.2 bars just after substrate addition, decreases during a few minutes because of chemical equilibrium between gaseous and dissolved CO_2. Pressure increases during the 75 first hours of the culture and the curve has a profile similar to degradation of vegetables. After this phase, CO_2 production became linear and has a low rate. So, two phases are observed: a high production and a low production. This may be explained by the degradation of two types of substrates: during a first step, the more easily degradable substrate (single sugars liberated by autoclave, cellulose with simple access and degradation of the glucose added at the beginning of the culture…) is degraded, which allow a very active metabolism of *Fibrobacter succinogenes*; then, the degradation of recalcitrant substrates. The kinetics of degradation of these substrates is clearly lower and results in a deceleration of *Fibrobacter succinogenes* metabolism.

4.2.4 Solid phase

4.2.4.1 Van soest

	Total fibers (% DM)	Hemicellulose (% DM)	Cellulose (% DM)	Lignin (% DM)
Substrate (200 g)	58.68	20.84	16.15	21.68
Residue (135 g)	37.99	11.13	12.37	30.95
Degradation (%)	56.3	63.95	48.3	3.65

Table 6: Analysis of cell wall degradation by Van Soest method

The percentage of degradation was determined by: $\% = \frac{[(\%s \times Ms)-(\%r \times Mr)]}{\%s \times Ms} \times 100$ (TN 2.6)

The cell wall components were determined by Van Soest (1967) method described in the TN 2.6. Table 6 shows the composition of the substrate from LabMET and the composition of the residues after *Fibrobacter succinogenes* unit.
The proportions of fibers are different before and after *Fibrobacter* unit. This confirms the efficiency of *Fibrobacter succinogenes* to degrade fibers.

4.2.4.2 Degradation

All the content of the reactor was centrifuged (15', 13 000g) and the liquid and solid phase were separated. Percentages of degradation were determined by the two methods described in previously TN (desiccation and filtration). The percentage of degradation estimated by desiccation was 32.19% and by filtration was 32.31%. This also confirms the efficiency of *Fibrobacter succinogenes* to degrade LabMET substrate.

4.2.5 Determination of C and N mass balance

	Input		Output			
Source	substrates	carbonate	solid residue	VFA	CO_2	Biomass
Total C (g)	87.09	6.98	63.45	12.95	7.87	4.25
Total C (g)	94.07		88.53			
Mass balance	**94.10 %**					

Table 7: Determination of C mass balance

The results for the determination of C mass balance are summed up in table 7. The C mass balance was 94.1% for the overall experiment, which confirms the efficiency of the control and regulation of *Fibrobacter succinogenes* process.

	Input		Output		
Source	substrates	$N\text{-}NH_3$	solid residue	$N\text{-}NH_3$	Biomass
Total N (g)	8.58	0.026	4.74	2.56	0.65
Total N (g)	8.61		7.95		
Mass balance	**92.33 %**				

Table 8: Determination of N mass balance

As well as for C mass balance, the results obtained for N mass balance (92.33 %, see table 8) also confirms the efficiency of the control of *Fibrobacter* process.
C and N mass balances indicates that the process is controlled for the two principal elements of the culture. These results obtained for carbon and nitrogen mass balances also show the efficiency of the control on *Fibrobacter succinogenes* process and confirm the methods and techniques used to determine the quantities consumed and produced.

5 CONCLUSION / OUTLOOKS

In this study we examined the influence of culture conditions on the growth of *Fibrobacter succinogenes*, on acetate and succinate production and on the rate of degradation of vegetables. It seems that the optimal culture conditions are pH 6, 39°C and 0.3% of VFA. However if we only examine the degradation rate we show that the optimal efficiency is for a range of pH (3.2-9) and a range of temperature (30-46°C).

Cellulases identified seem to be the more important enzymes in the efficiency of plant cell walls degradation. Moreover the correlation establish between the two methods confirms the validity of these methods. But it was known that *Fibrobacter succinogenes* have other enzymes like xylanases so we tested for the presence of these enzymes in our extracts.

Finally, substrate from metanogenic unit was degraded by *Fibrobacter succinogenes* with a rate of 32% and with VFA production.

R E F E R E N C E S

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