

# Study of the optimum growth conditions of *Bacillus subtilis* (strain WT 168), and *Pseudomonas fluorescens* (strain SBW25)

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**Objective:** determine what are the best temperature (between 30°C and 37°C) and pH (between 4 and 8) for the growth of these strains.

## 1. Determination of the combined influence of the pH and the temperature on the strains growth

An experience plan has been realized at different pHs, temperatures, and with a variable concentration of glucose in the medium culture. The **specific growth rate ( $\mu$ )** has been determined for each culture. The specific growth rate and the **doubling** time ( $T_d$ ) are linked by the following formula:

$$T_d = \frac{\ln 2}{\mu}$$

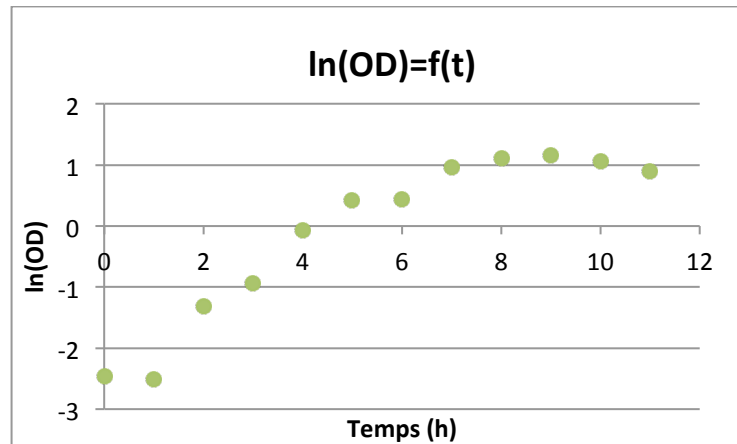
Three parameters are studied: the pH, the temperature, and the glucose concentration in the medium.

### a. Experimental protocole

#### Preparation of the LB medium

- Mix of the different products without adding the glucose
- Adjust the pH by adding HCl to acidify or NaOH to increase the pH with a pHmeter
- Put the medium in the autoclave
- Once liquids are cold, add glucose

The cultures were realized in LB medium. A sample of each culture was collected every hour during 10 hours to measure the OD. Then, the specific growth rate has been determined by plotting  $\ln(\text{OD})$  in function on the time, as shown on the figure below. The slope of the linear part of the graph gave the specific growth rate.



b. *Pseudomonas fluorescens*

According to a review, the concentration of glucose in the culture medium could influence the growth of *Pseudomonas* strains. Thus we tested cultures with 0% of glucose, or with 3%. We realized 8 cultures in different conditions.

	% Glucose	Temperature (°C)	pH	G.T	G.pH	T.pH	G.T.pH	Specific growth rate	Doubling time	Doubling time
	X1	X2	X3	X1X2	X1X3	X2X3	X1X2X3	$\mu$ (h <sup>-1</sup> )	T <sub>d</sub> (h)	T <sub>d</sub> (min)
1	0	33	4	1	1	1	-1	0	0	0
2	3	33	4	-1	-1	1	1	0	0	0
3	0	37	4	-1	1	-1	1	0	0	0
4	3	37	4	1	-1	-1	-1	0	0	0
5	0	33	8	-1	1	-1	-1	0,592	1,172	70
6	3	33	8	1	-1	-1	1	0,569	1,219	73
7	0	37	8	1	1	1	1	0,776	0,893	54
8	3	37	8	-1	-1	1	-1	0,748	0,926	56
Influence	0,0501	0,3639	2,6845	0,0047	0,0501	0,3639	0,0047			

First of all, the glucose does not impact the growth of *Pseudomonas fluorescens*, as the value of  $\mu$  does not change. Moreover, pHs that are too low are unviable for bacteria. Taking a look at the influence coefficient, we can note that it is quite low for the influence between the temperature and the pH (0,3639). As a consequence, the best temperature and the best pH can be studied independently as they do not have a combined effect on the cell growth.

### c. *Bacillus subtilis*

Four experiments were realized.

	pH	Temperature (°C)	pH.T	Specific growth rate	Doubling time	Doubling time
	X1	X2	X1X2	$\mu$ (h <sup>-1</sup> )	T <sub>d</sub> (h)	T <sub>d</sub> (min)
9	4	33	1	0	0	0
10	8	37	-1	0,9671	0,717	43
11	4	33	-1	0	0	0
12	8	37	1	1,2594	0,550	33
Influence	2,2265	0,2923	0,2923			

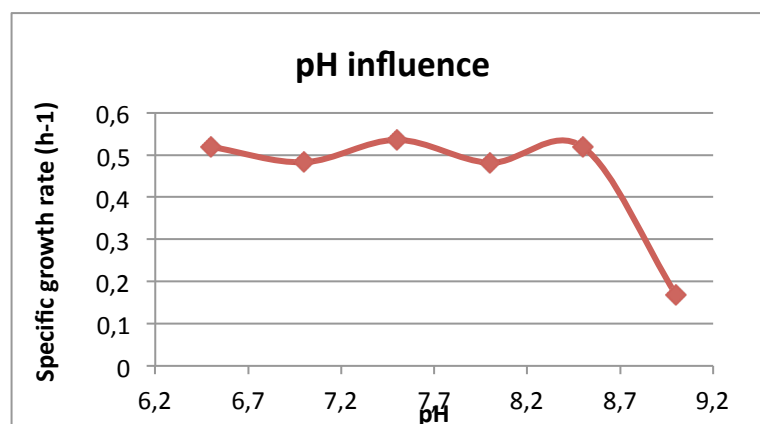
As we demonstrated for *Pseudomonas fluorescens*, the best temperature and the best pH can be studied independently as they do not have a combined effect on the cell growth.

## 2. Determination of the optimum temperature and pH

### a. *Pseudomonas fluorescens* – optimum pH

The pH has been studied at 6 different values, and at a constant temperature (30°C). A pH inferior to 6 has not been considered, as we have demonstrated that it is unviable for cells.

	pH variation					
	1	2	3	4	5	6
Temperature	30					
pH	6,5	7	7,5	8	8,5	9
$\mu$ (h <sup>-1</sup> )	0,4697	0,4828	0,5356	0,4815	0,4985	0,169
Doubling time (min)	89	86	78	86	83	246

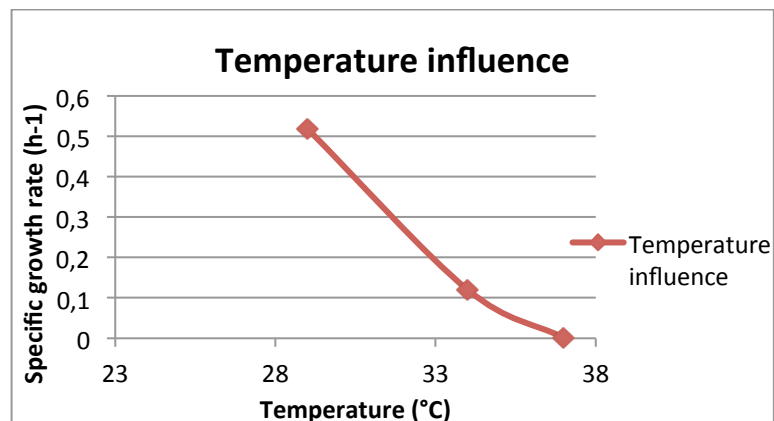


According to our results, the optimum pH for the growth of *Pseudomonas fluorescens* strain SBW25) is 7,5.

### b. *Pseudomonas fluorescens* – optimum temperature

The temperature has been studied at 3 different values, and at a constant pH (7). According to reviews, the optimum temperature growth of *Pseudomonas fluorescens* is between 25°C and 32°C. However, as the material at our disposal could not work at a temperature inferior to 29°C, we could not try this range of temperatures.

	Temperature variation		
	1	2	3
Temperature	29	34	37
pH	7		
$\mu$ (h <sup>-1</sup> )	0,5185	0,1202	0,0015
Doubling time (min)	80	346	27726

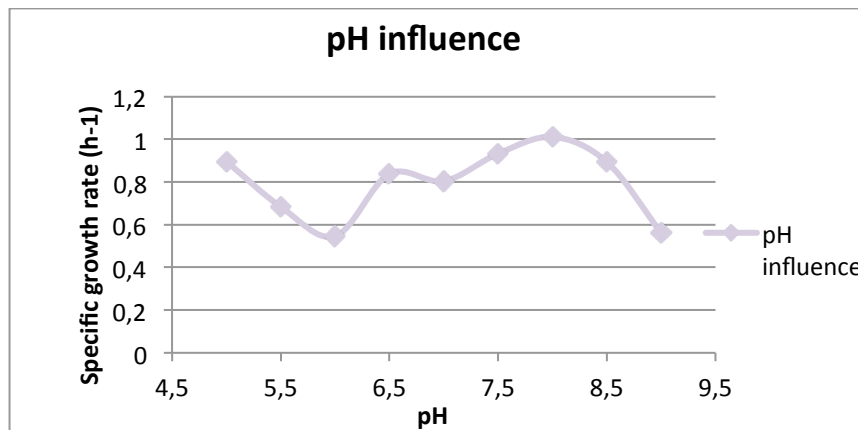


The optimum temperature for the growth of *Pseudomonas fluorescens* (strain SBW25) is obviously inferior to 29°C.

### c. *Bacillus subtilis* – optimum pH

The pH has been studied at 6 different values, and at a constant temperature (30°C). A pH inferior to 6 has not been considered, as we have demonstrated that it is unviable for cells.

	pH variation								
	1	2	3	4	5	6	7	9	10
Temperature (°C)	37								
pH	5	5,5	6	6,5	7	7,5	8	8,5	9
$\mu$ (h <sup>-1</sup> )	0,8945	0,6839	0,5459	0,8377	0,8035	0,9323	1,0104	0,8937	0,5617
Doubling time (min)	46	61	76	50	52	45	41	47	74

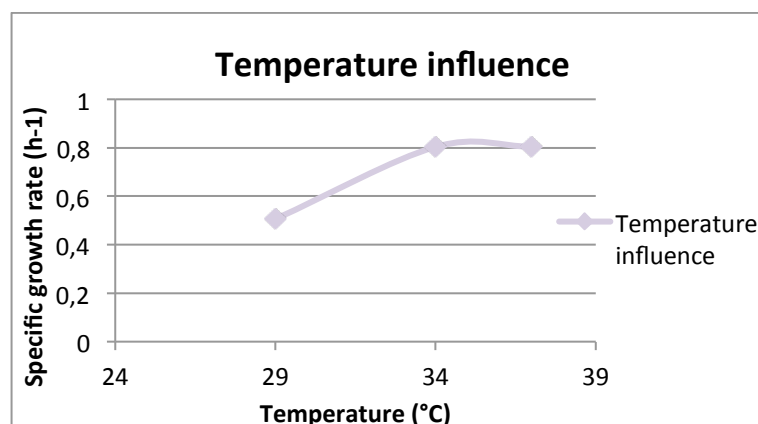


According to our results, the optimum pH for the growth of *Bacillus subtilis* (strain WT 168) is 8.

#### d. *Bacillus subtilis* – optimum temperature

The temperature has been studied at 3 different values, and at a constant pH (7). According to reviews, the optimum temperature growth of *Bacillus subtilis* is 37°C. However, as the material at our disposal does not allow studying more than three temperatures simultaneously, we could not try studying other temperatures.

	Temperature variation		
	1	2	3
Temperature (°C)	37	34	29
pH	7		
$\mu$ (h-1)	0,8035	0,8033	0,5059
Doubling time (min)	52	52	82



The optimum temperature for the growth of *Bacillus subtilis* (strain WT 168) is around 34°C and 37°C.

## Conclusion

	Optimum temperature (°C)	Optimum pH	Doubling time (minutes)
<i>Bacillus subtilis</i> (WT 168)	34-37	8	41
<i>Pseudomonas fluorescens</i> (SBW25)	<29	7,5	<80

Optimum temperatures and pH and the doubling times are summarized in the table for our two strains.