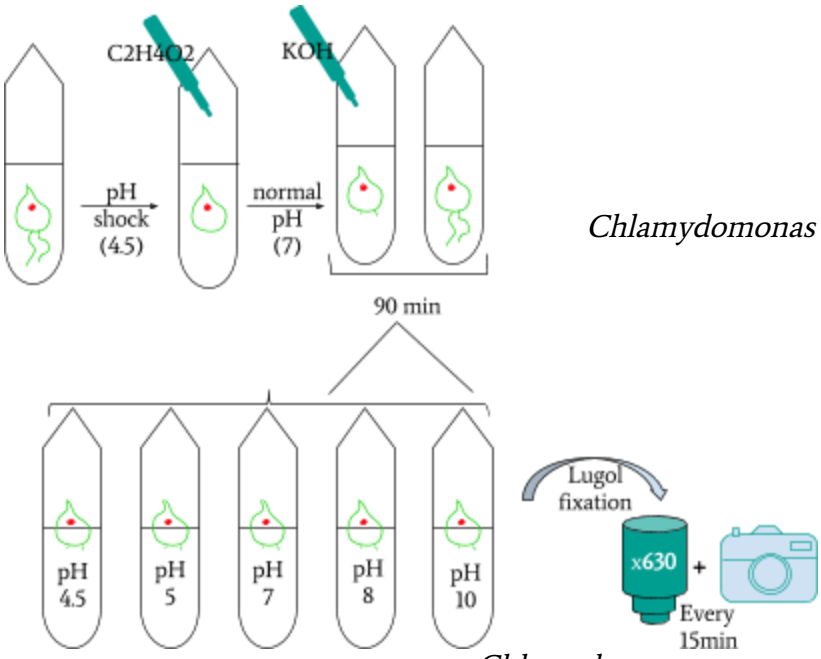
Lab Notebook

Aim of the first project :

Chlamydomonas reinhardtii are green algae, with two flagella. *Chlamydomonas* don't need these flagella to survive. They are used to lose their flagella - before dividing and mating. In a stressful environment, *Chlamydomonas* also detach their flagella to reduce their exposition to this stressful environment. For example, they are used to live in a medium of pH 7. When they are put in a very acid environment, a pH shock occurs, and a deflagellation appends. After this deflagellation, they regenerate their flagella.
We would like to observe the regeneration time and quantify if the pH environment after the shock has an influence on the regeneration.

Experiment 1 :



To induce the deflagellation, we occur a pH shock by using acetic acid and potassium hydroxide. In a solution of *Chlamydomonas*, add acetic acid (a solution at 0,5N) to decrease the pH and after add potassium hydroxide (solution 0,5N) to go back to a normal pH. In this step, we will add different quantity of potassium hydroxide in order to test different pH environments to test the ability of *Chlamydomonas* to regenerate their flagella.

Sample size : 5 different solutions
Repetitions : 8 measure per sample (one before the pH shock and then one every 15 minutes)
Replicates : 3 microscope slides per measure (2 or 3 *Chlamydomonas* on differents per slide)

6th February 2017 - Day #1

Observation of the under the microscope (liquid culture and solid culture)

The liquid culture of (send by Carolina™) contains a biofilm in the tube. During the observations under the microscope of 5µL of this solution, we observed three types of elements :

- "Pieces" of the biofilm (non-living)
- without flagella.
- with flagella.

The problem is that the concentration of flagellated is very low. We are afraid that we will not be able to observe flagellated in each step of our protocol.

Preparation of the acetic acid solution and the potassium hydroxide solution (25 mL at 0.5M)

- 0.7mL of acetic acid from "purum" solution,+ 24.4 mL of distilled water
- 0.74g of solid potassium hydroxide + 25 mL of distilled water.

Incubation of cultures

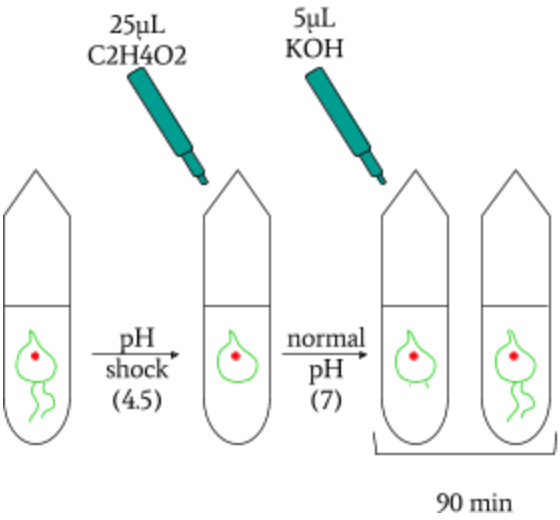
1. Since we had very few in liquid culture, we decide to transfer some of from the liquid culture of Carolina™to a TAP (Tris-Acetate-Phosphate) agar medium plate. We stored the plate at room temperature, in a "greenhouse" with constant ambient light. They are conserved at 25°C
2. But considering the repartition of phenotypes in the Carolina™ solution, we decided to start a new liquid culture of .

For that, we took another strain from Carolina™(on a solid medium) and put it in a liquid medium of oxygenated aquarium water. We made this because we had not the needed materials to make TAP liquid media. Knowing that their generation time is about ~5 to 8 hours, we were hoping to have sufficiently enough to run our experiment.

We incubated them during the night (~12 hours) at 25°C in light.

7th February 2017 - Day #2

Tests to determine the volume of acetic acid and potassium hydroxide needed to obtain the expected pH.



On a liquid solution of , we test variations of pH due to acetic acid and potassium hydroxide.
The initial solution has a pH 7.
We add 25µL of acetic acid, and the pH becomes 4.5 We took our measurements with a pH paper (it has a precision of 1.)
Then, add 5µL of KOH, and the pH increases to 7 (according to pH paper).
These tests help us to be aware of pH variations that occur in our solution during the deflagellation.

Microscopes test

We observed with each of the microscopes available in the lab and decided to do our experiment with the Fluorescence microscope with a x630 magnification.

Developing new strains

We observed that the strain we used for now was a multiflagellate strain of named " cc-2530 (vfl2 mt+)". Knowing that this specie can show a phenotype of 0 to 6 flagella, we decided that it could affect our results. One way of dealing with this bias is to change our population by one with less phenotype possible..
We looked for strains that were both wild type for their flagellum and with one mating type (they should not be able to reproduce and therefore enter a non-flagellate stage of their life cycle). We found 3 different strains on the Chlamydomonas Resource Center website :

- Chlamydomonas reinhardtii cc-602 (pf1 mt-)
- Chlamydomonas reinhardtii cc-620 (R3 NM mt+)
- Chlamydomonas reinhardtii cc-621 (NO mt-)

The idea would be to use them in order to avoid counting naturally non-flagellate

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But their growth was insufficient for the time available and we decided to keep our strain of multi flagellate . Our negative control will help us to determine which part of the population was already deflagellated.

8th February 2017 - Day #3

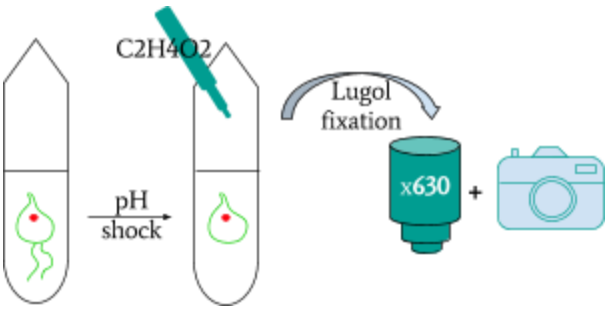
Second project :

We decided to pivot on the third day because our experiment was not showing conclusive results. It was really hard to determine if their flagella were still attached or not and if they were regenerating. Thus was part of our mistake because we forgot one step in the protocol taken from our bibliography. A centrifugation of 2000 rpm for 5 minutes was needed to separate the unfixed flagella from the soma of the Chlamydomonas.

On the other hand, 2 other groups decided to work with the fluorescence microscope and knowing that we have a limited amount of time in the laboratory. We thought of a new protocol taking less time to execute.:

Experiment 2 : Response of Chlamydomonas to pH-induced stress

To occur the deflagellation, we do the pH shock by using acetic acid : in a solution of , we add acetic acid (0,5N) to decrease the pH. In this step, we will add different quantity of acetic acid in order to test different pH environments to test the ability of to keep their flagella.



Sample size : 3 solutions
Repetitions : 3 slides for each sample
Replicates : 3
Details : For 20µL of solution, you need 1µL of Lugol to fix the solution.

Preparation :

1. With pH paper, control the pH of the stain and the acetic acid solution.
2. Prepare 3 solutions of 400µL and 3 Lugol solutions at 1µl.

For each solution :

1. Add to the solution :
    a. 0µl of acetic acid → Negative control
    b. 10µl of acetic acid
    c. 25µl of acetic acid → Positive control
2. Measure the pH of the solution with 10µL of it and a piece of pH paper.
3. After 1 minute of pH shock, fix 20µl of the solution in a lugol solution.
4. Take 5µl of the fixed solution and put it on a slide in order to observe it under the microscope (repeat this step for 3 different slides).
5. Count the number of with and without flagella

We made the first replicate of this experiment.

Volume of Acetic acid solution added (in µL)	pH shock value	Number of Chlamydomonas with a flagella			Number without flagella		
		Slide 1	Slide 2	Slide 3	Slide 1	Slide 2	Slide 3
0	7	15	19	15	0	31	9
10	5.5	15	12	6	15	16	20
25	4	14	16	12	53	32	25

9th February 2017 - Day #4

We made the second and third replicate of this experiment.

Volume of Acetic acid solution added (in µL)	pH shock value	Number of Chlamydomonas with a flagella			Number without flagella		
		Slide 1	Slide 2	Slide 3	Slide 1	Slide 2	Slide 3
Sample 2							
0	7	10	18	27	17	14	20
10	5.5	23	16	17	28	40	33
25	4	19	20	8	20	17	18
Sample 3							
0	7	31	25	28	13	33	17

10	5.5	15	13	20	31	41	41
25	4	13	24	4	53	52	37

We noticed that observing the presence of flagella was very complicated and that it could be interesting to measure statistically the bias induced by the difficulty to distinguish flagellated from deflagellated .

Our results :

Volume of Acetic acid solution added (in µL)	**pH shock value**	**% of flagellated Chlamydomonas**								
		Slide 1.1	**Slide 2.1**	**Slide 3.1**	**Slide 1.2**	**Slide 2.2**	**Slide 3.2**	**Slide 1.3**	**Slide 2.3**	**Slide 3.3**
0	**7**	100	38	62.5	37.04	56.25	57.45	70.45	43.1	62.22
10	**5.5**	50	42.86	23.08	45.1	28.57	34	32.61	24.1	32.77
25	**4**	48.72	54.05	30.77	20.9	33.33	32.43	19.7	31.6	9.76