ALGALTOXKIT F

Test procedure

1

PREPARATION OF

ALGAL CULTURING MEDIUM

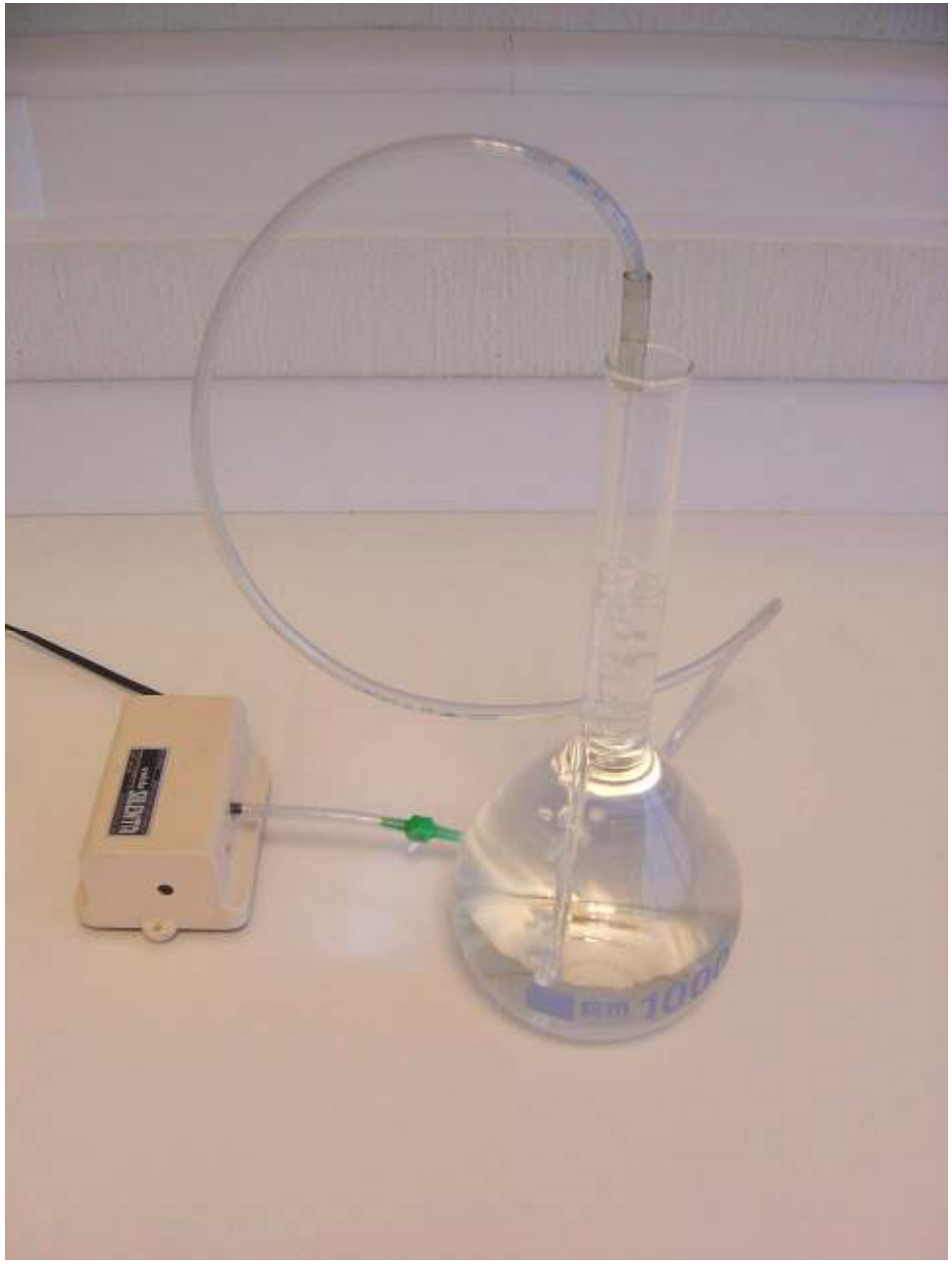
- VOLUMETRIC FLASK (1 liter)
- VIALS WITH NUTRIENT STOCK SOLUTIONS A (2 vials), B, C, D
- DISTILLED (or deionized) WATER

2

TRANSFER 10 ML FROM ONE OF
THE TWO “NUTRIENT STOCK A” VIALS
IN ± 800 ML DISTILLED WATER
IN THE 1 LITER VOLUMETRIC FLASK

3

TRANSFER 1 ML FROM THE NUTRIENT STOCK VIALS B, C AND D INTO THE 1 LITER VOLUMETRIC FLASK.

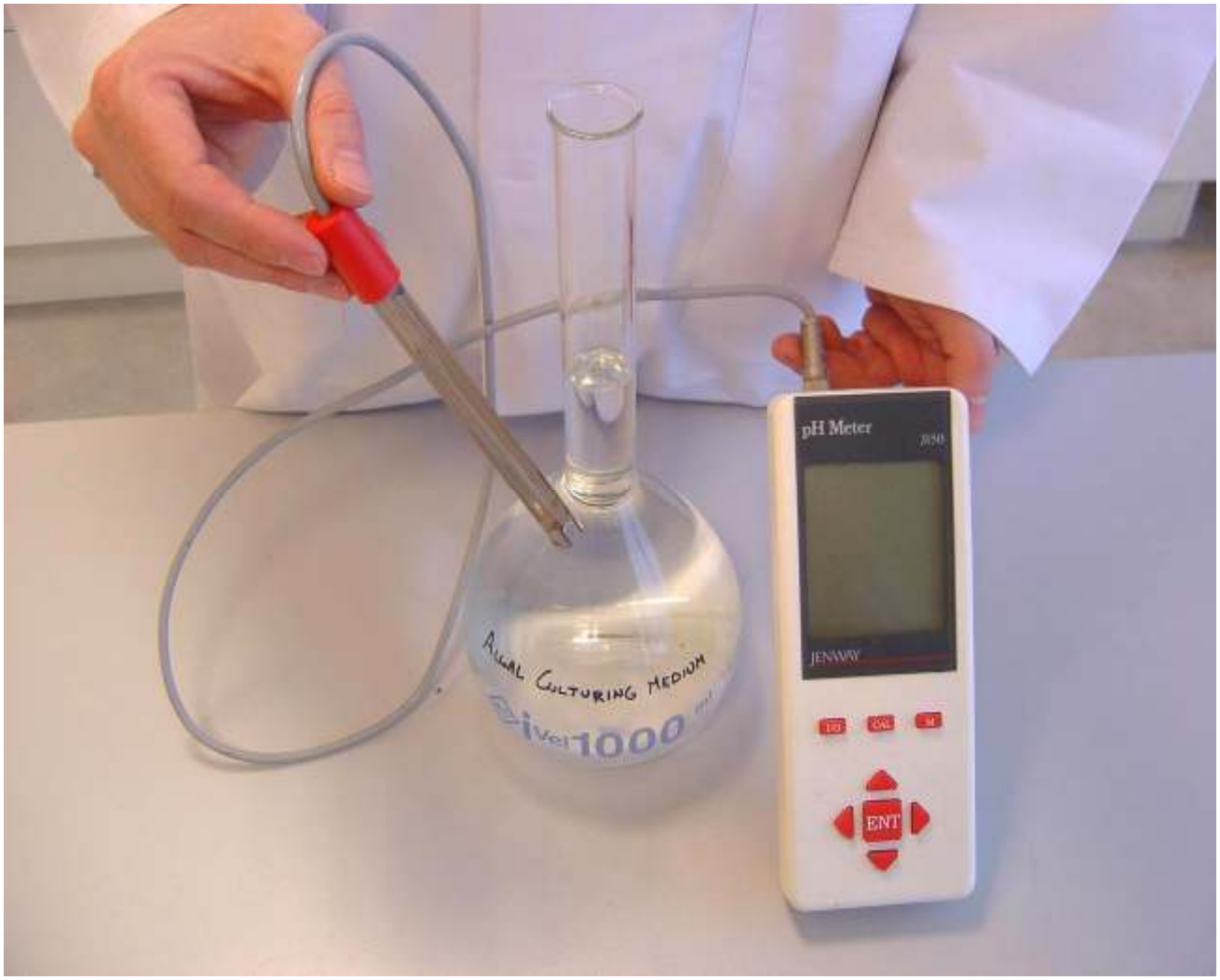


4

- FILL THE FLASK TO THE 1 LITER MARK WITH DEIONIZED WATER

- STOPPER THE FLASK AND SHAKE THOROUGHLY TO HOMOGENIZE THE CONTENTS

- AERATE THE ALGAL CULTURING MEDIUM FOR AT LEAST 30 MINUTES



5

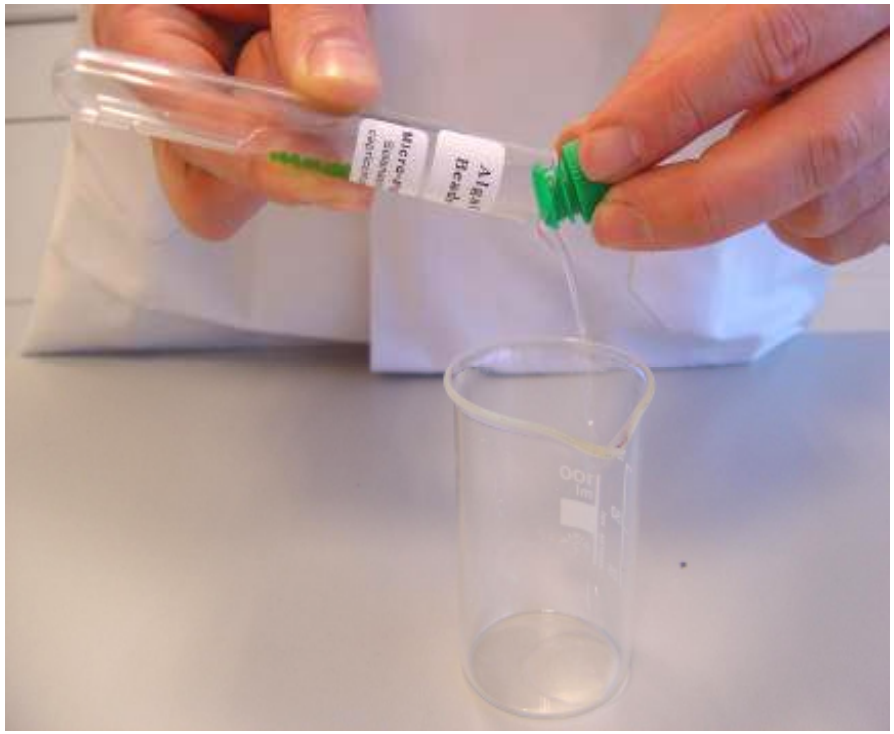
ADJUST THE pH

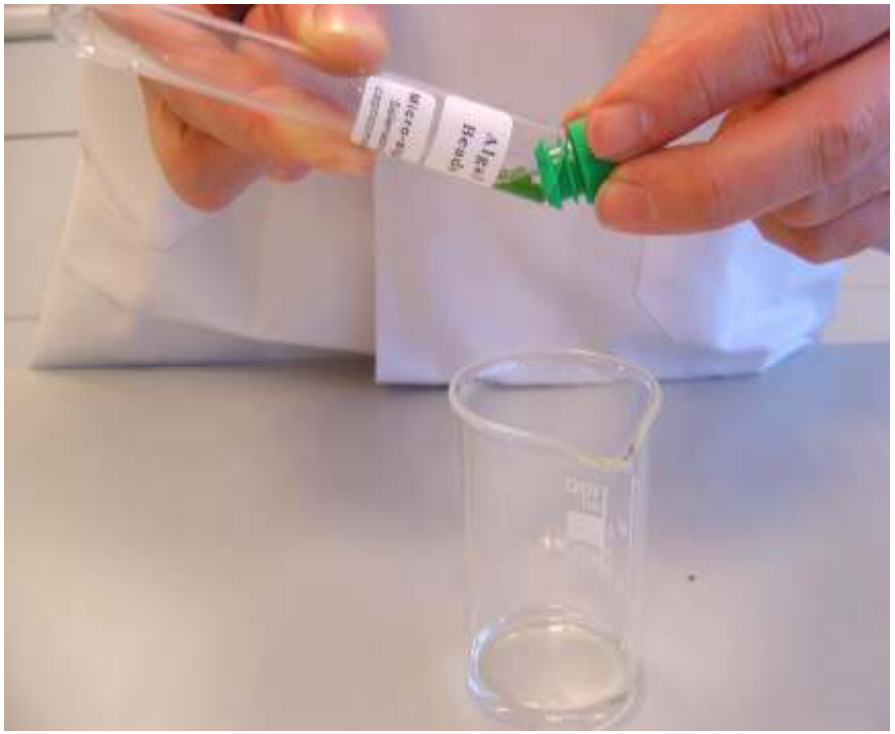
(if necessary)

TO 8,1 ± 0,2

(with either 1 M HCl

or 1 M NaOH)





6

DE-IMMOBILIZATION OF THE ALGAE

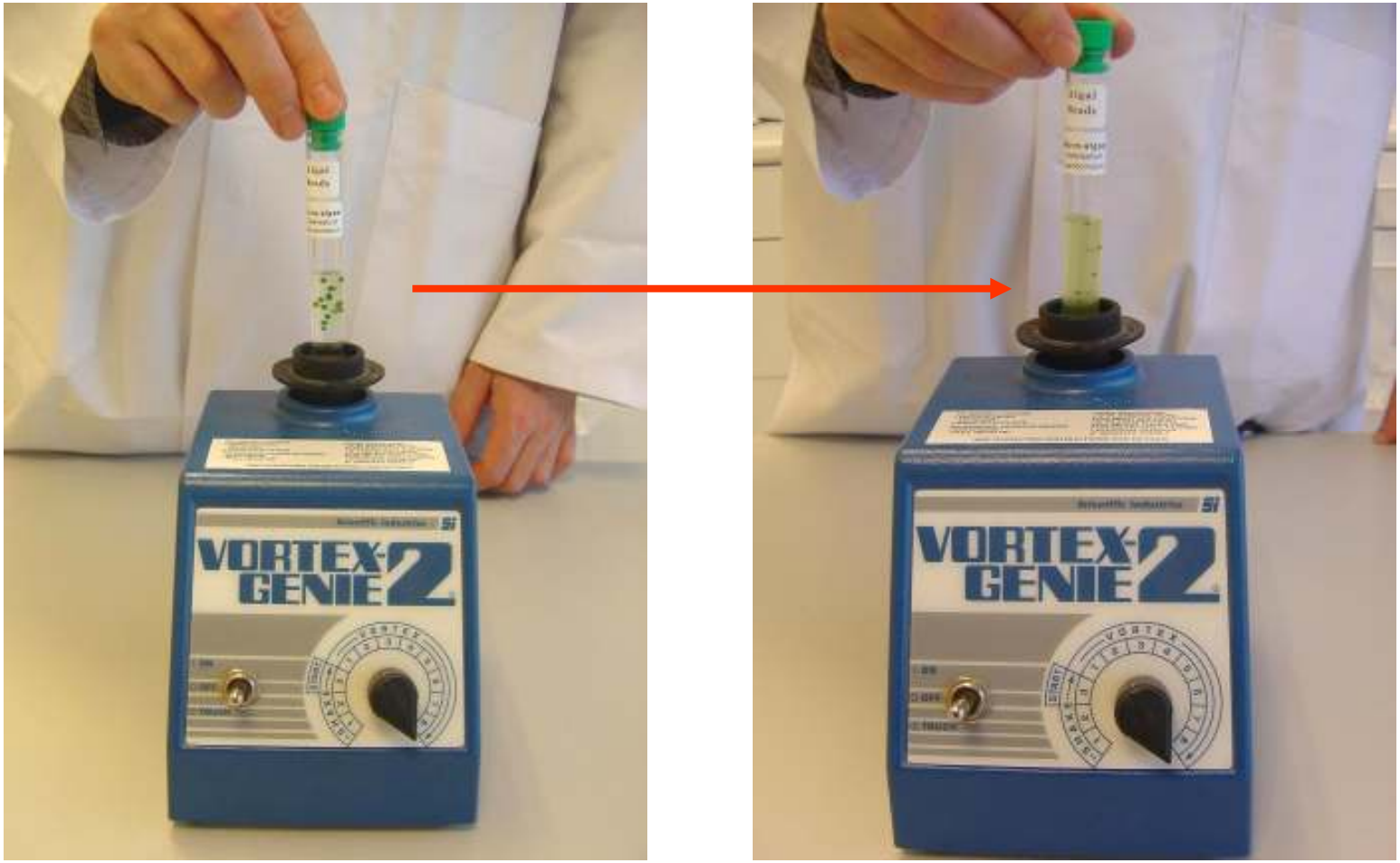
TAKE ONE TUBE CONTAINING ALGAL BEADS AND POUR OUT THE LIQUID

TAKE CARE NOT TO ELIMINATE ANY OF THE ALGAL BEADS DURING THE PROCESS !!

7

OPEN THE VIAL “MATRIX DISSOLVING MEDIUM” AND TRANSFER 5 ML

TO THE TUBE WITH ALGAL BEADS

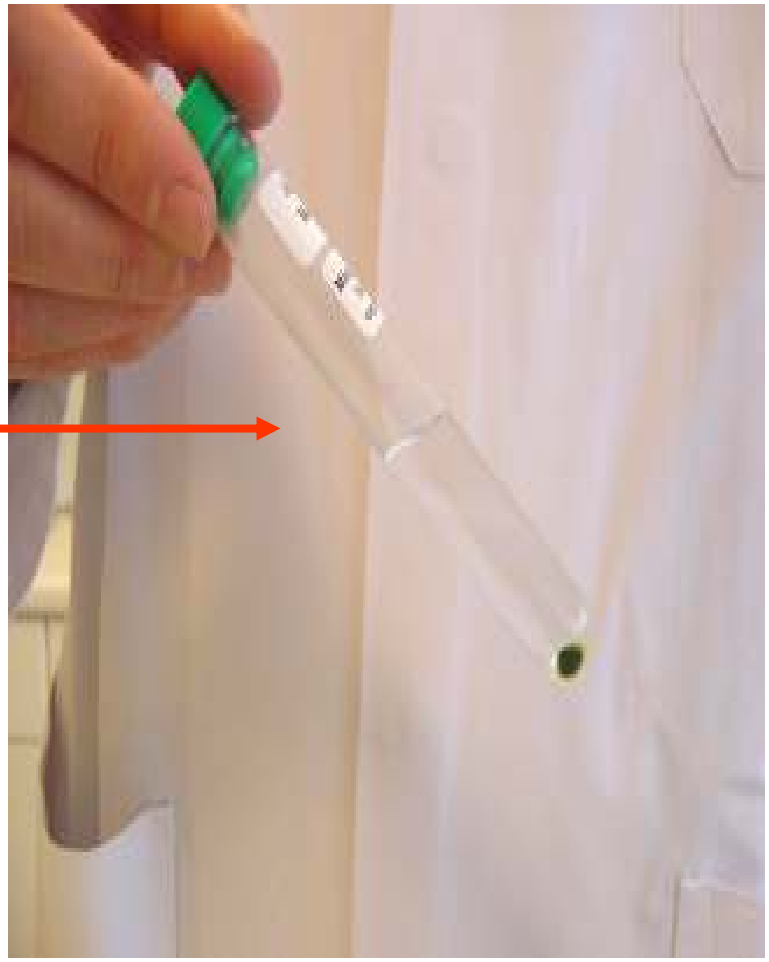


8

CAP THE TUBE AND SHAKE VIGOROUSLY TO DISSOLVE THE ALGINATE MATRIX OF THE ALGAL BEADS, PREFERABLY WITH THE AID OF A VORTEX SHAKER

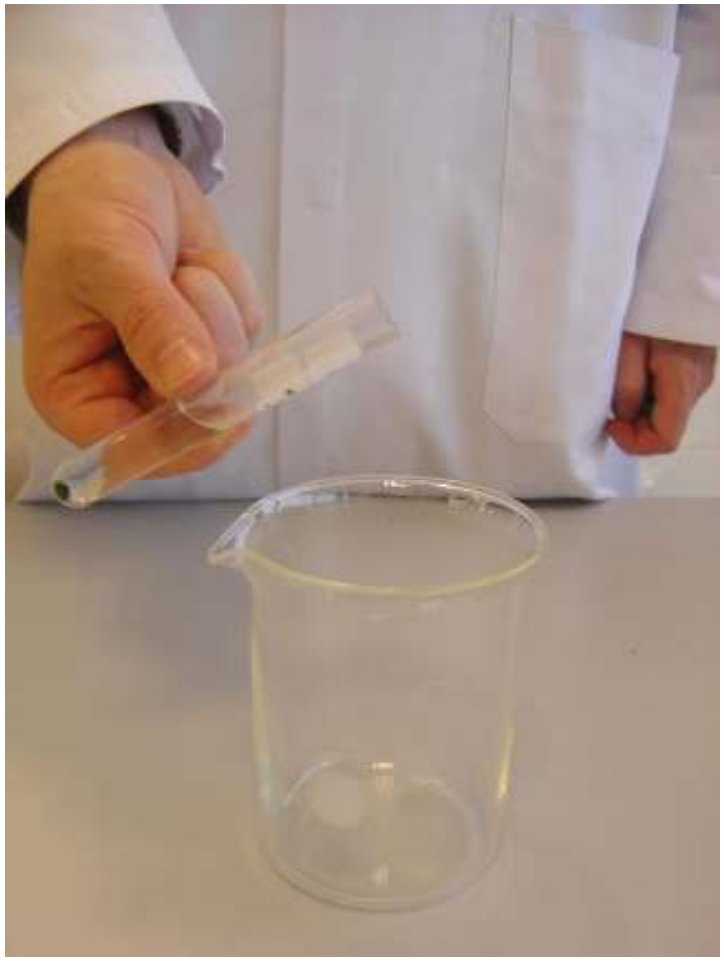
9

CONTINUE THE SHAKING UNTIL THE ALGAL BEADS ARE TOTALLY DISSOLVED



10

CENTRIFUGE THE TUBE FOR 10 MINUTES AT 3000 RPM IN A CONVENTIONAL LAB CENTRIFUGE

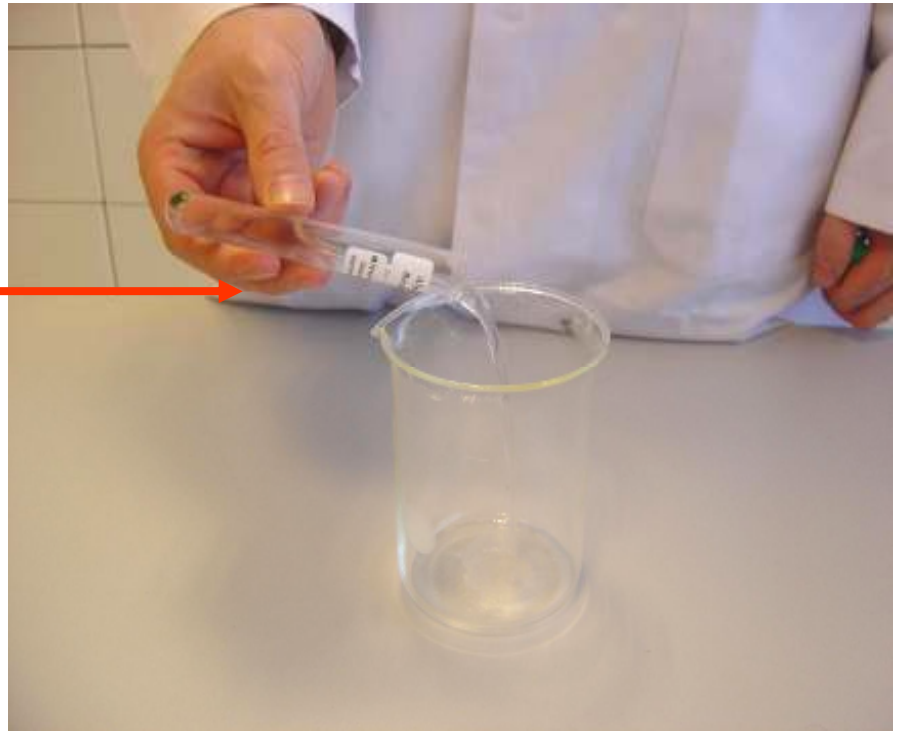


11

POUR OUT THE SUPERNATANT FROM THE TUBE

12

- ADD 10 ML DISTILLED WATER TO THE TUBE
- CAP AND SHAKE THE TUBE TO RESUSPEND THE ALGAE



13

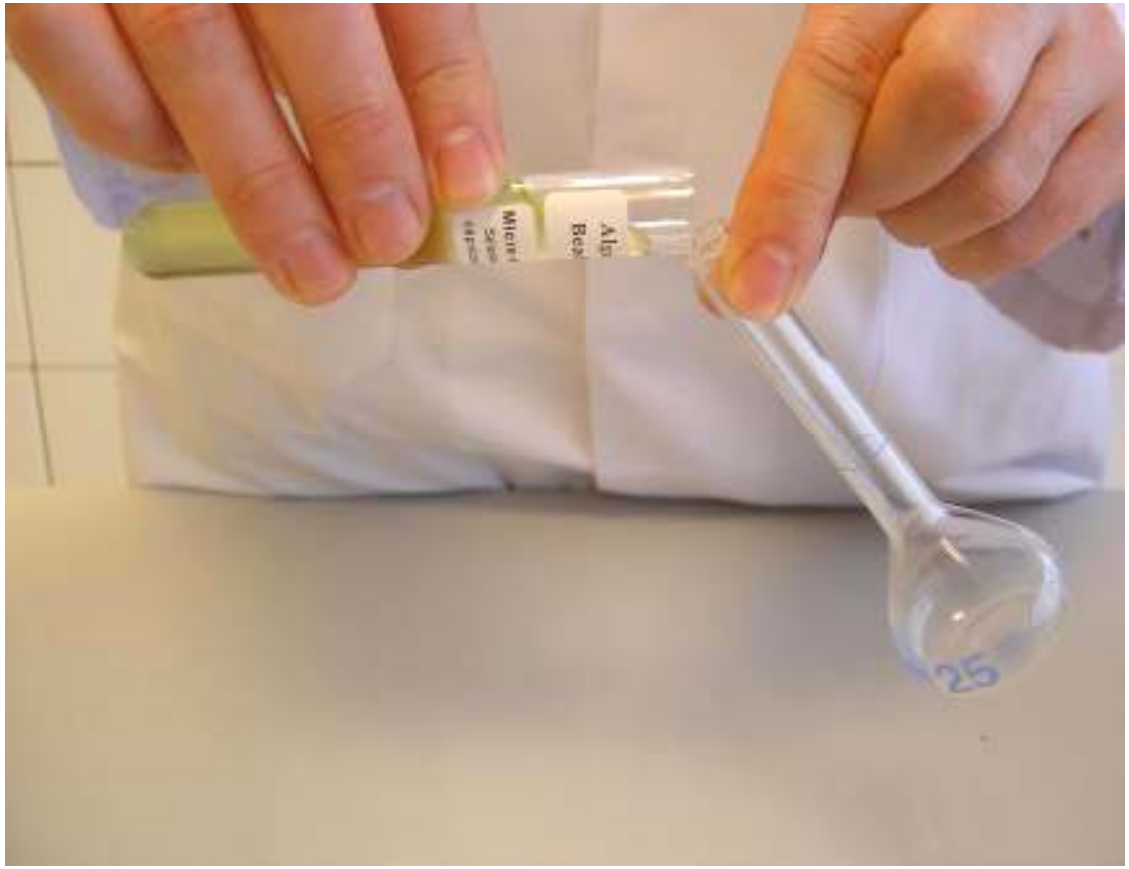
CENTRIFUGE THE TUBE AGAIN AT 3000 RPM FOR 10 MINUTES

AND THEN POUR OUT THE SUPERNATANT

14

- ADD 10 ML ALGAL CULTURING MEDIUM TO THE TUBE

- CAP THE TUBE AND SHAKE TO RESUSPEND THE ALGAE



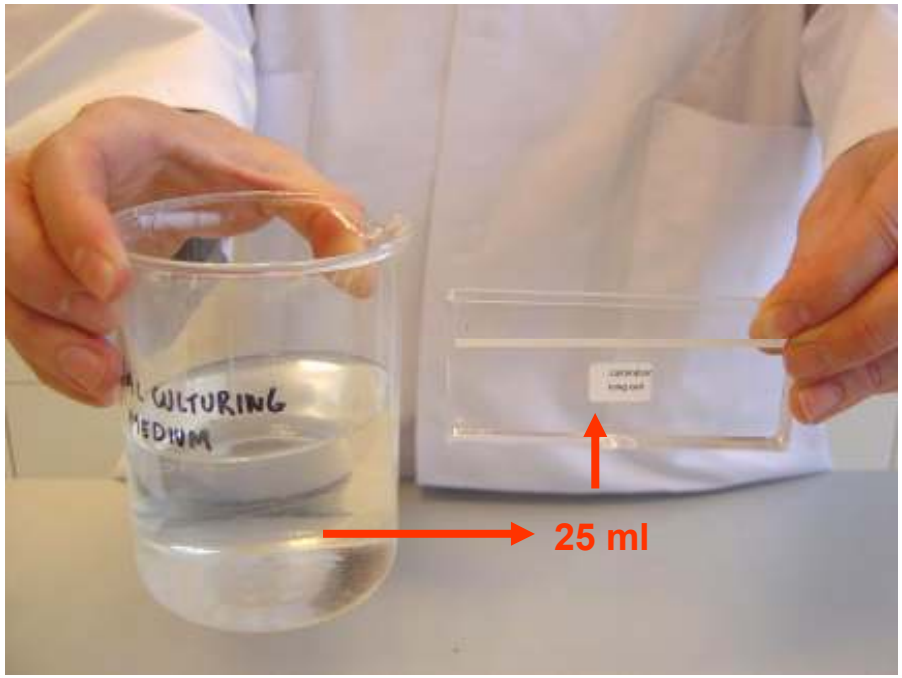
15

PREPARATION OF CONCENTRATED ALGAL INOCULUM

TRANSFER THE ALGAL SUSPENSION FROM THE TUBE INTO A 25 ML CALIBRATED FLASK

16

- ADD ALGAL CULTURING MEDIUM TO THE 25 ML MARK OF THE FLASK
- STOPPER THE FLASK AND SHAKE TO HOMOGENIZE THE ALGAL SUSPENSION



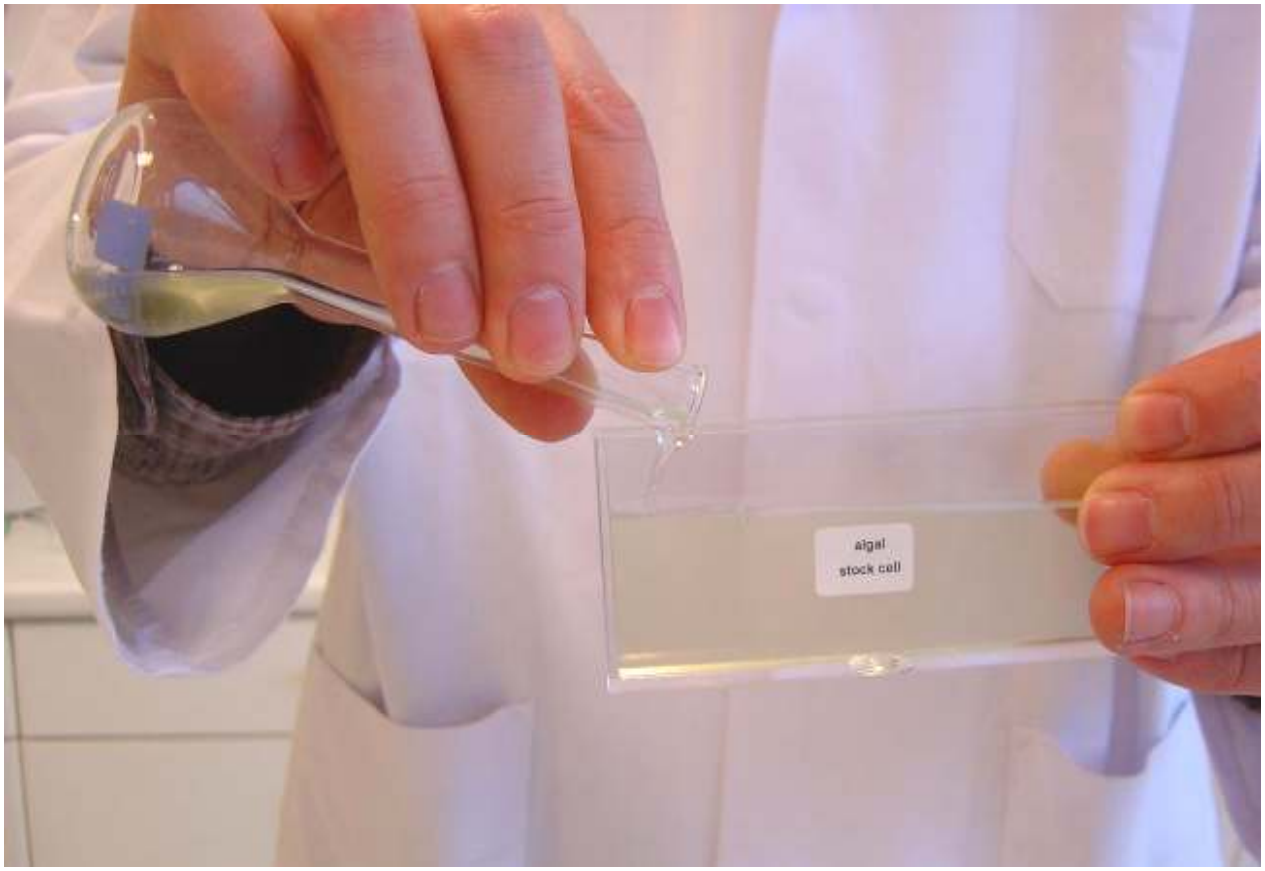
17

- PUT 25 ML ALGAL CULTURING MEDIUM IN THE CALIBRATION LONG CELL AND CLOSE THE CELL WITH THE LID

- PLACE THE CELL IN THE SPECTROPHOTOMETER

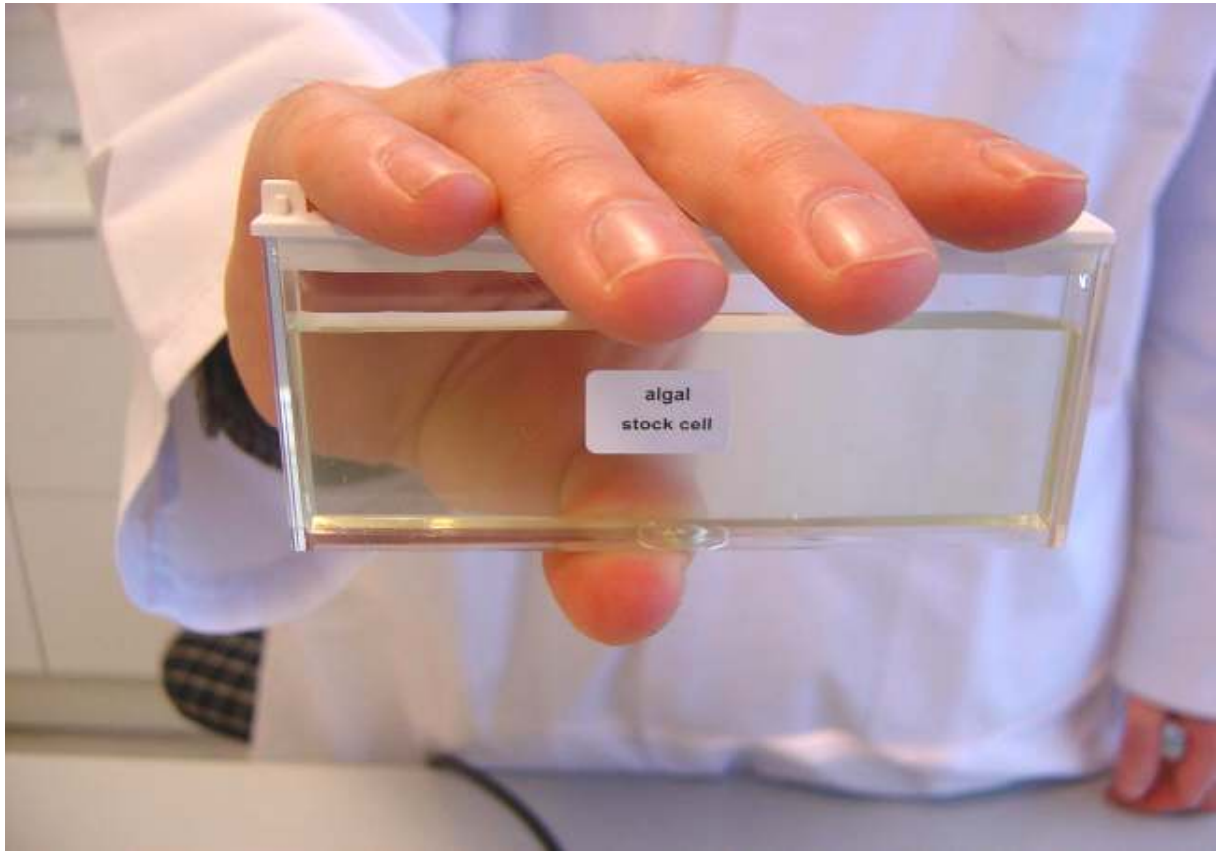
18

ZERO-CALIBRATE THE INSTRUMENT AT A WAVELENGTH OF 670 NM



19

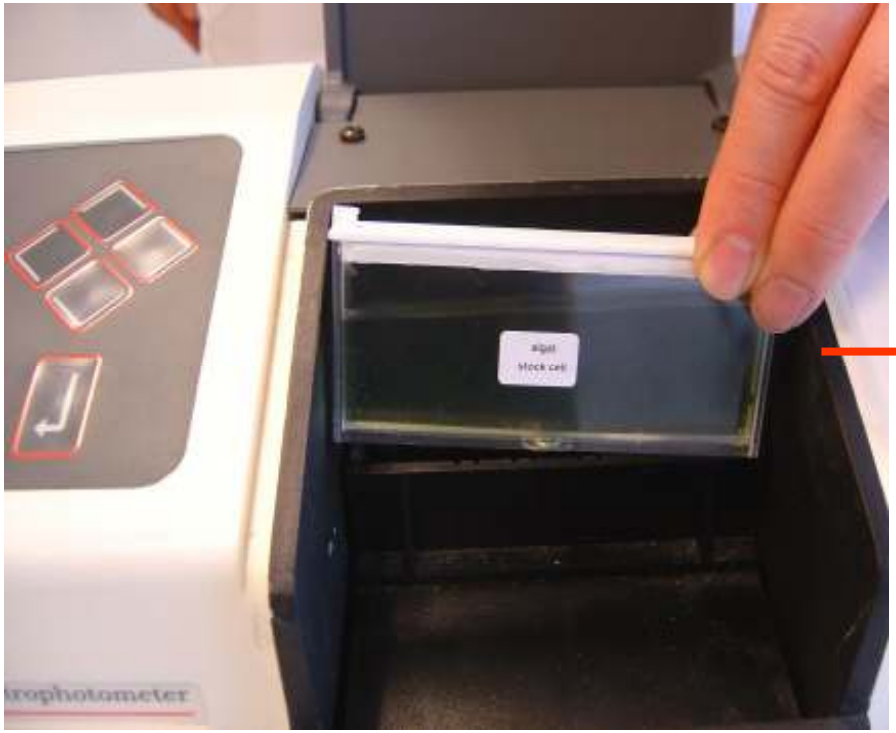
TRANSFER THE 25 ML ALGAL SUSPENSION INTO THE ALGAL STOCK CELL



20

CLOSE THE ALGAL STOCK CELL WITH THE LID

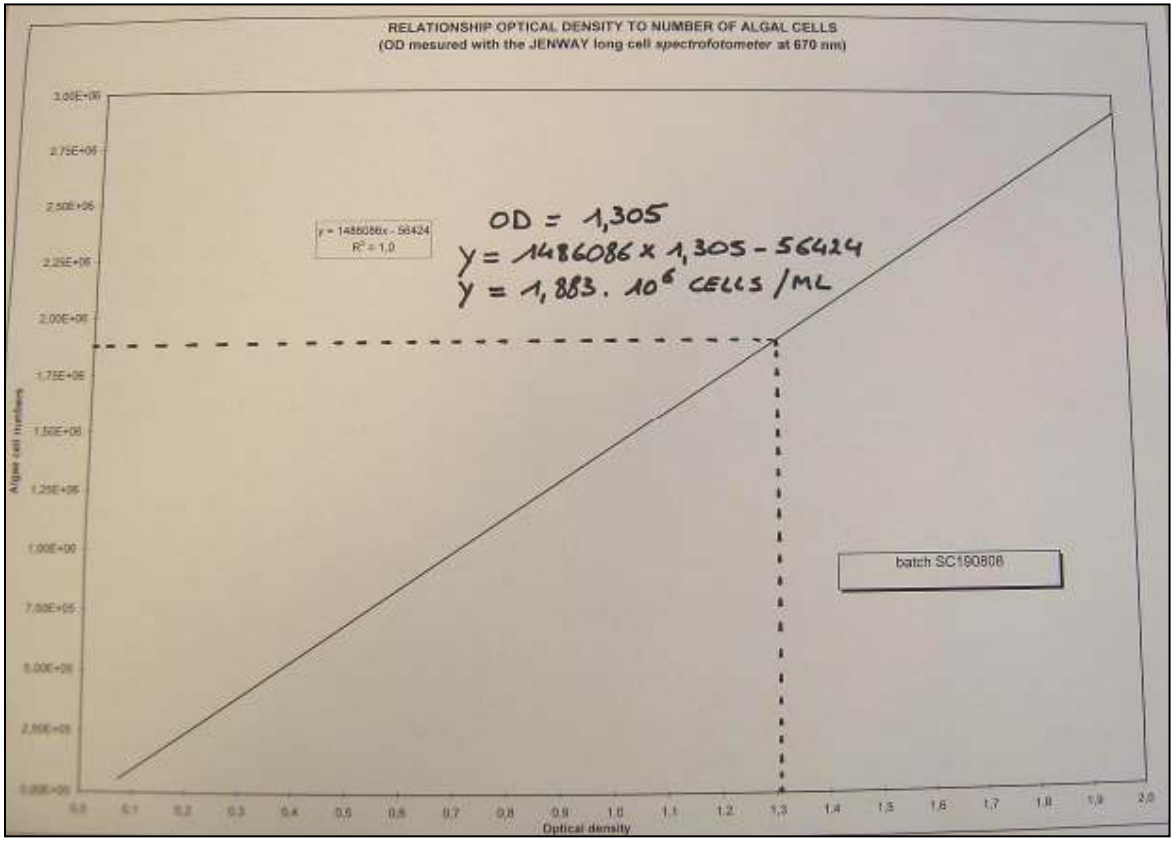
AND SHAKE TO DISTRIBUTE THE ALGAE EVENLY



21

PUT THE ALGAL STOCK CELL IN THE SPECTROPHOTOMETER

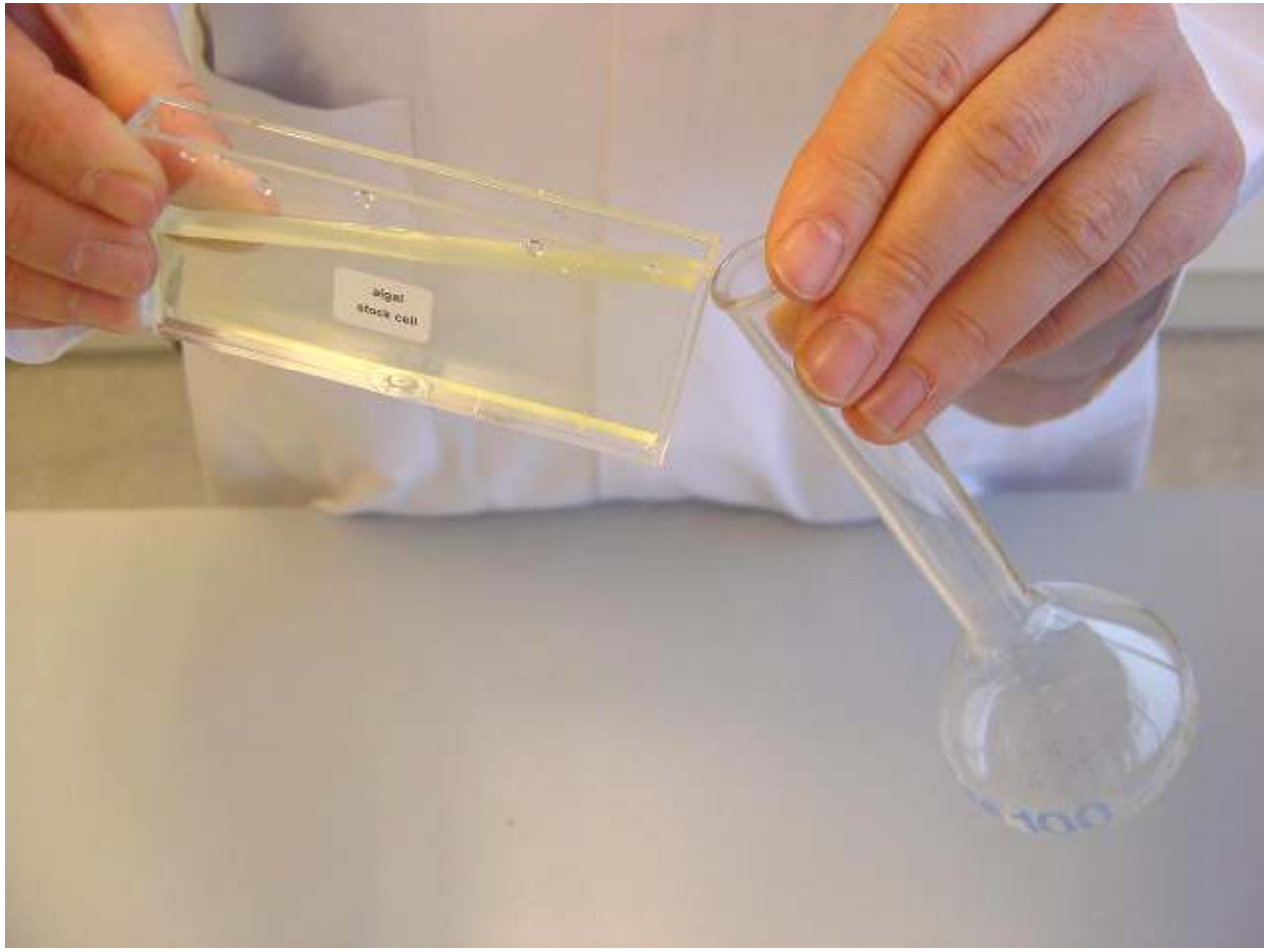
AND READ THE OPTICAL DENSITY (**OD1**) AFTER 10 SECONDS



- TAKE THE **OD/N** (optical density/algal number) SHEET

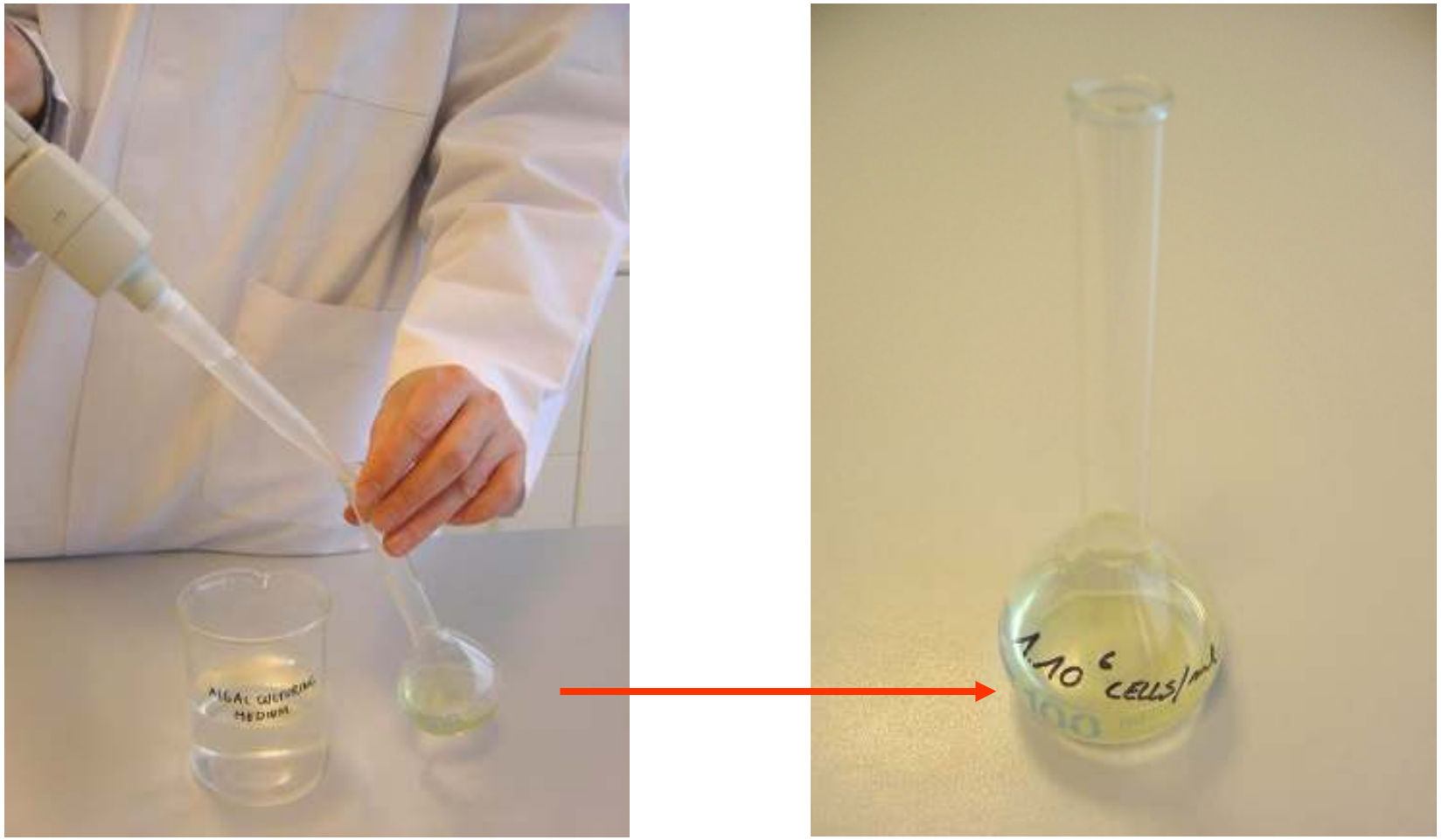
- WITH THE AID OF THE REGRESSION FORMULA CALCULATE THE NUMBER OF ALGAE **N1** CORRESPONDING TO THE MEASURED **OD1** IN THE ALGAL STOCK CELL

- WITH **N2** = 1.10^6 ALGAE/ML, CALCULATE FROM THE **N1/N2** RATIO THE DILUTION FACTOR NEEDED TO REACH **OD2** (corresponding to 1.10^6 algae/ml)



23

POUR THE 25 ML ALGAL SUSPENSION FROM THE ALGAL STOCK CELL INTO A 100 ML FLASK



24

ADD THE CALCULATED VOLUME OF ALGAL CULTURING MEDIUM

TO THE FLASK, TO MAKE UP A SUSPENSION OF 1.10^6 ALGAL CELLS / ML

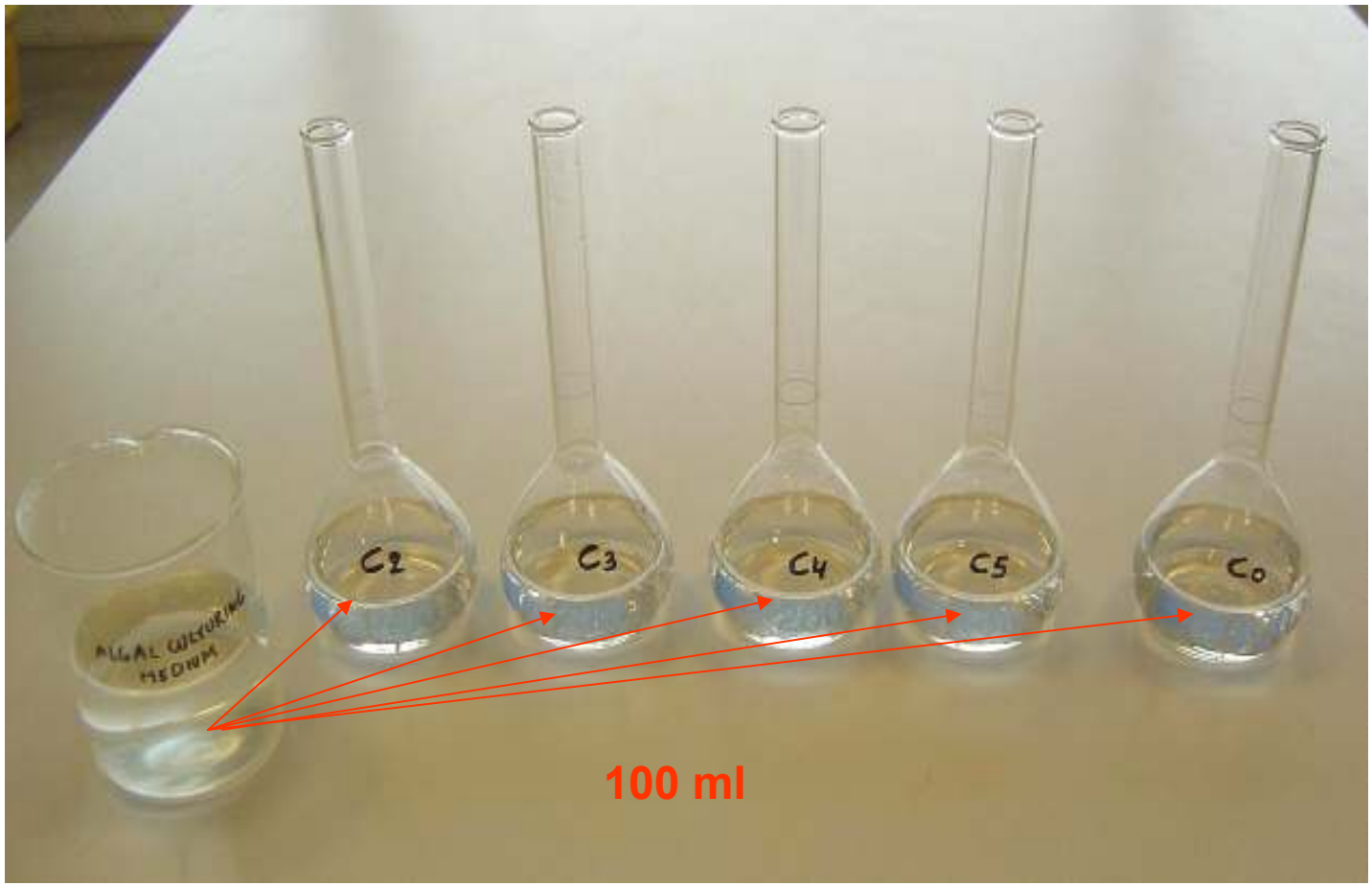
25

PREPARATION OF THE TOXICANT DILUTION SERIES

TAKE SIX 200 ML CAILBRATED FLASKS AND LABEL THEM FROM C0 TO C5

26

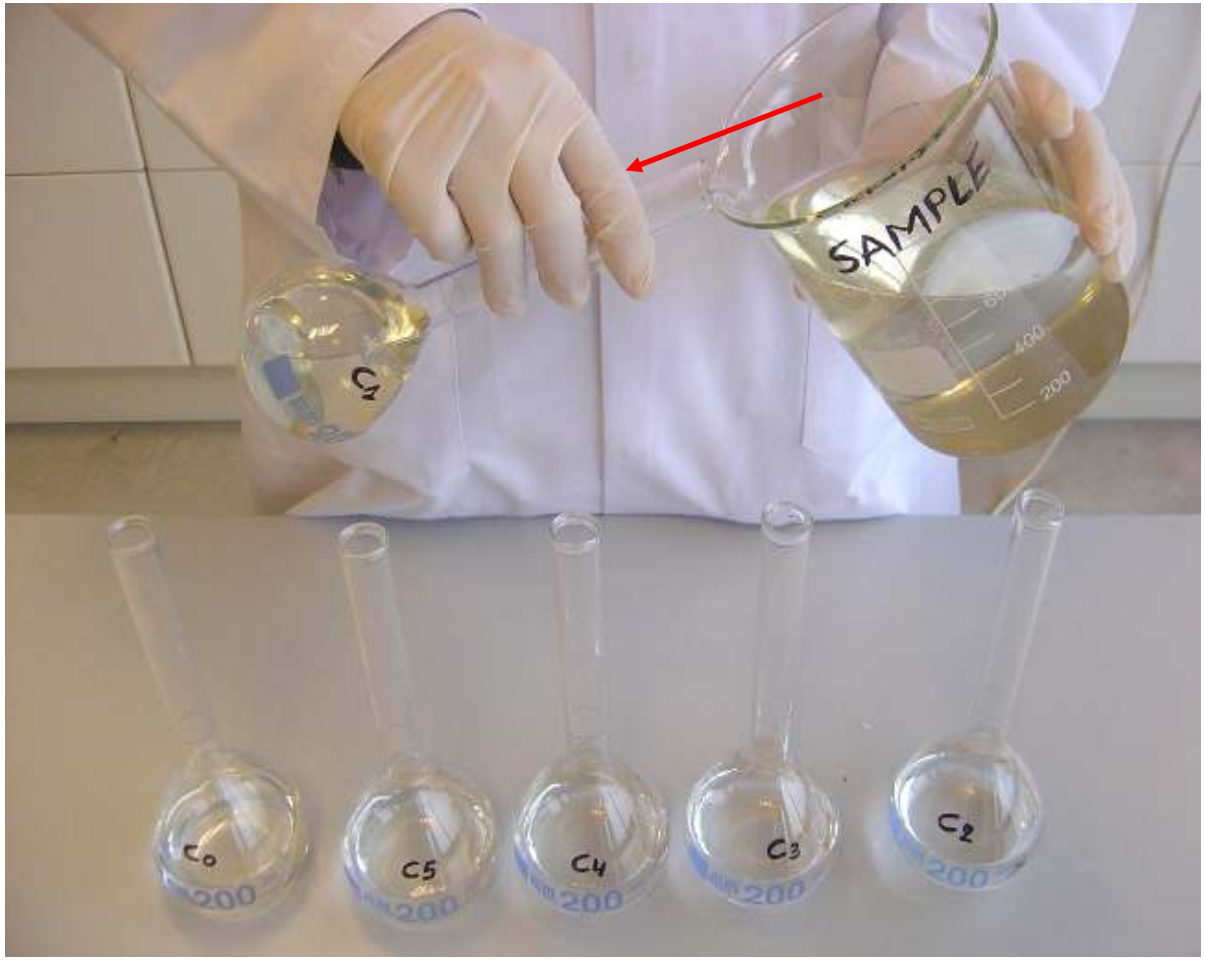
TO ELIMINATE TURBIDITY,
SAMPLES MUST BE
FILTERED BEFORE TESTING
(e.g. over a membrane filter
of 0.45 µm),



27

PUT 100 ML ALGAL CULTURING MEDIUM

IN THE 200 ML FLASKS C0, C2, C3, C4 AND C5

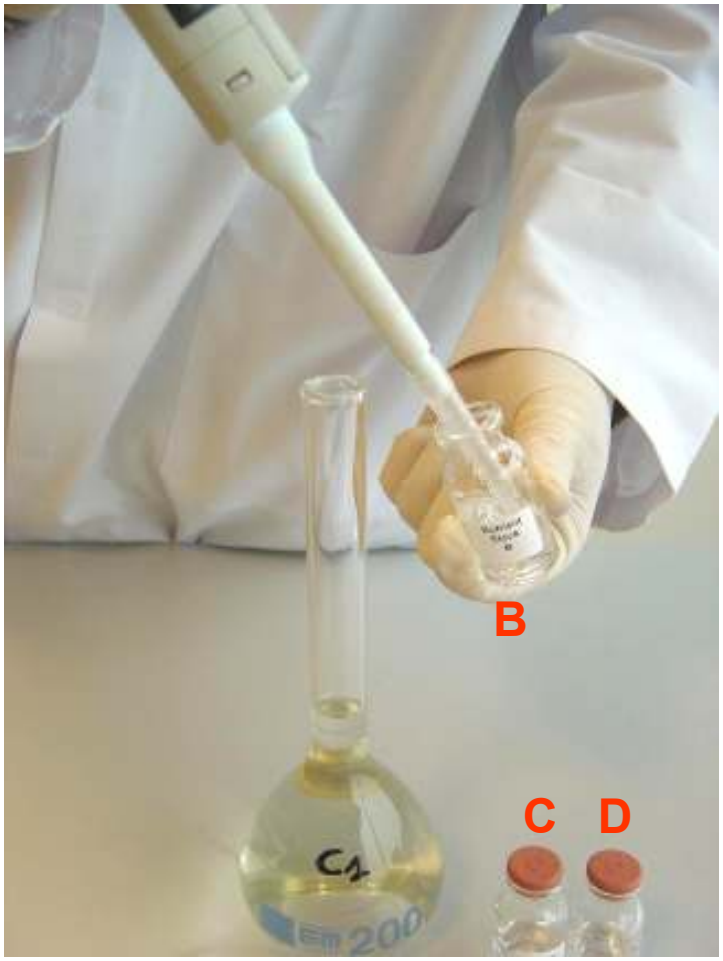


28

FILL FLASK C1

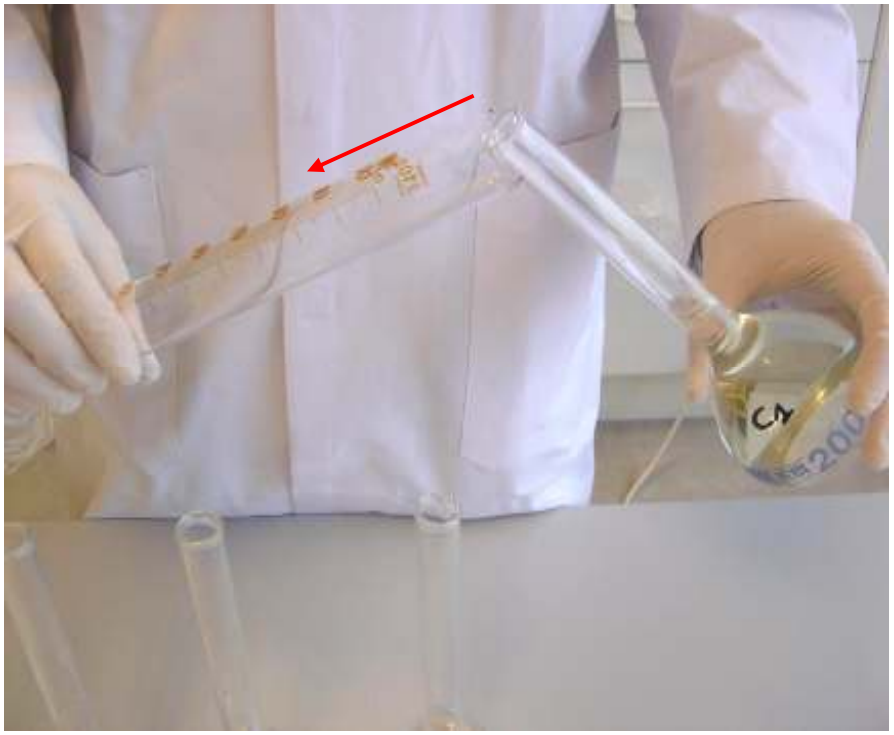
TO THE 200 ML MARK

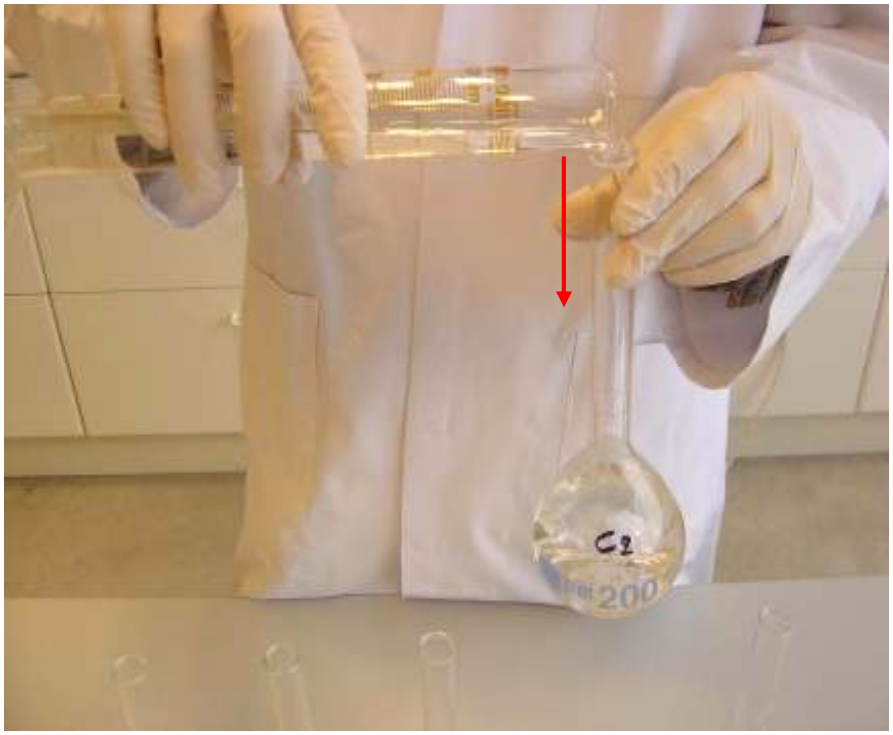
WITH THE FILTERED SAMPLE



29

- ADD 2 ML “NUTRIENT STOCK SOLUTION A” AND 0.2 ML OF NUTRIENT STOCK SOLUTIONS B, C AND D TO FLASK C1
- STOPPER THE FLASK AND SHAKE TO MIX THE CONTENTS

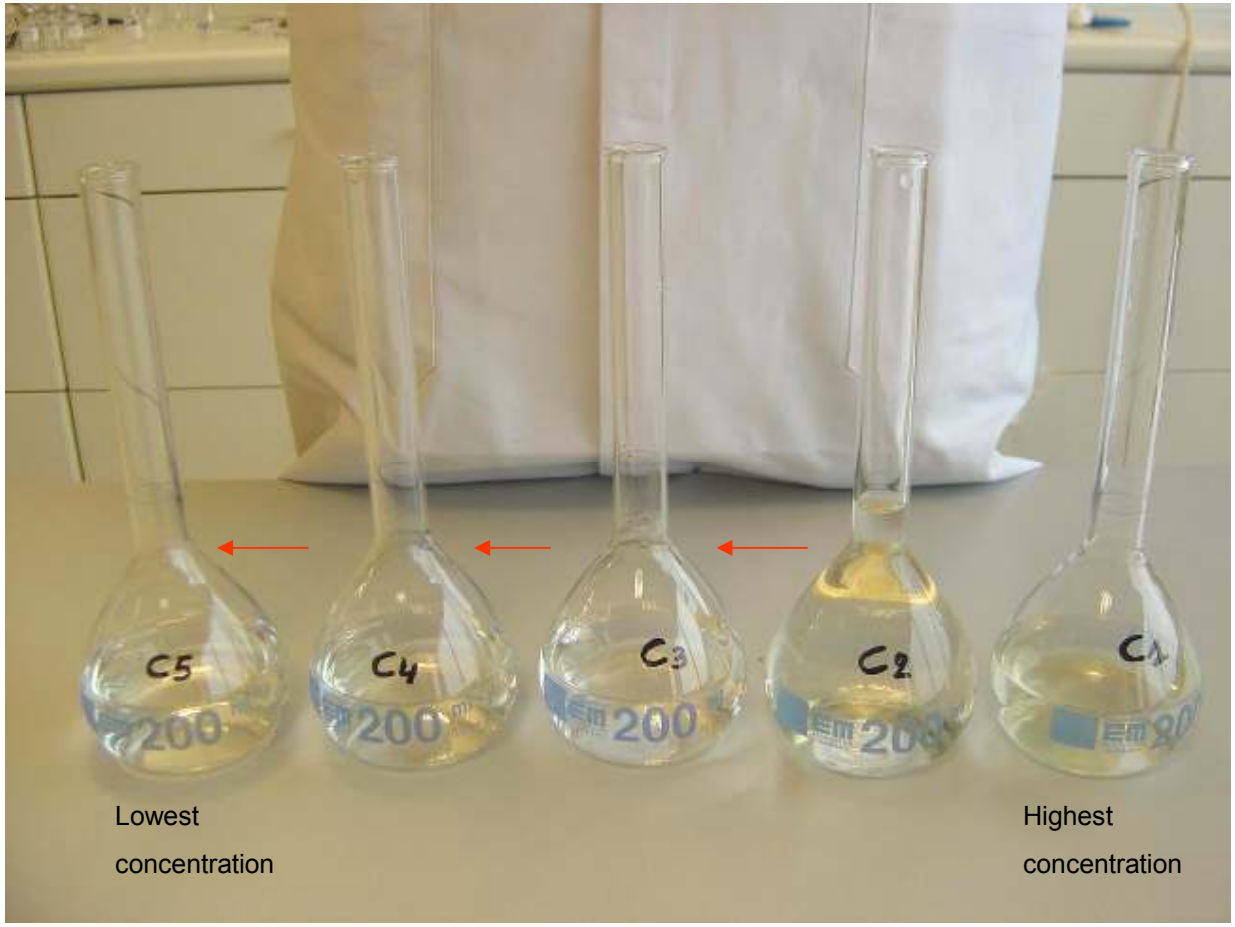




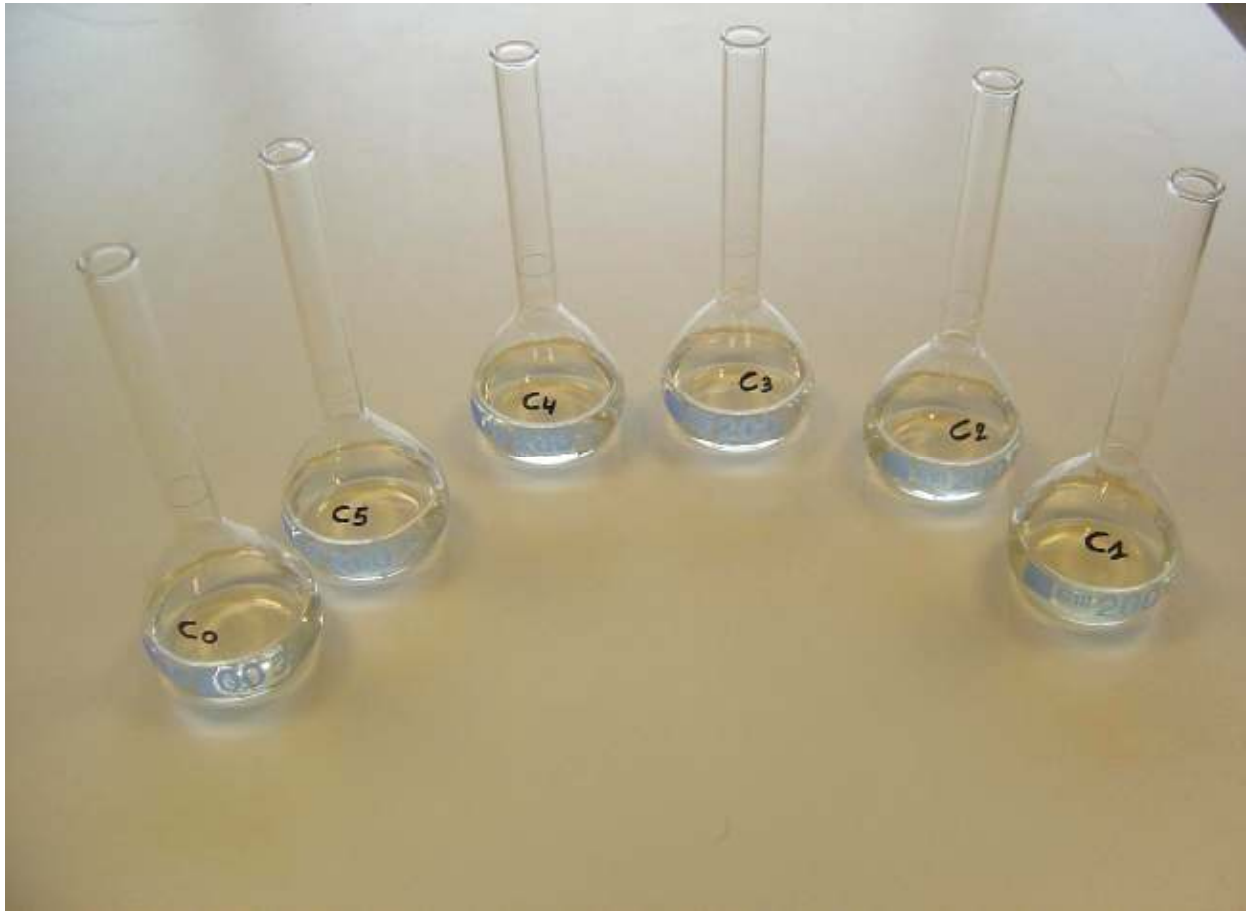
30

- POUR 100 ML SAMPLE FROM FLASK C1 INTO A GRADUATED CYLINDER
  AND TRANSFER THIS VOLUME INTO FLASK C2 TO MAKE THE FIRST 1:1 DILUTION

- STOPPER FLASK C2 AND SHAKE TO HOMOGENIZE THE CONTENTS



REPEAT THE FORMER
DILUTION PROCEDURE
FOR THE OTHER FLASKS
(i.e.. 100 ml from C2 to C3, etc.)



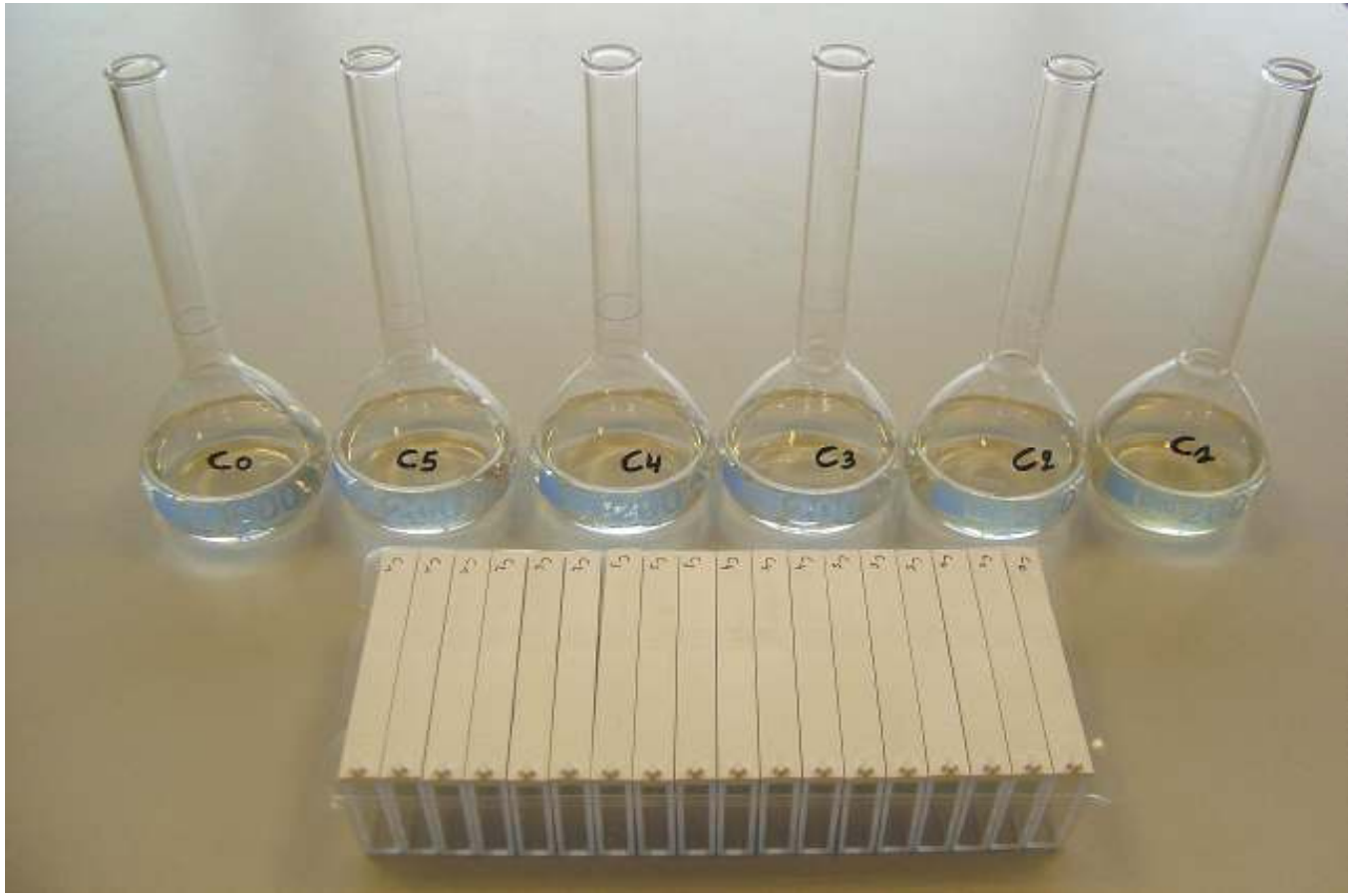
32

REMOVE AND DISCARD
100 ML SOLUTION FROM
FLASK C5 TO HAVE 100 ML
SOLUTIONS IN EACH FLASK

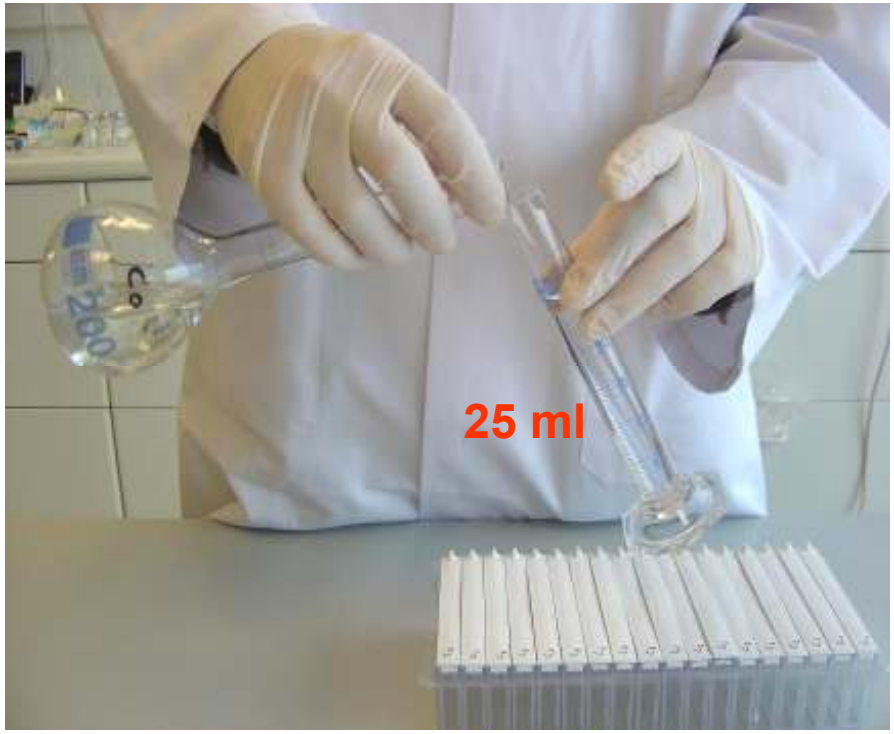
33

- TAKE THE FLASK CONTAINING THE 1.10^6/ML ALGAL SUSPENSION AND SHAKE IT GENTLY

- ADD 1 ML ALGAL SUSPENSION TO EACH OF THE 6 FLASKS C0 TO C5, IN ORDER TO OBTAIN AN INITIAL ALGAL CONCENTRATION OF 1.10^4 CELLS/ML IN EACH FLASK



TRANSFER OF THE ALGAE-TOXICANT DILUTIONS INTO THE TEST VIALS



35

- LABEL ALL THE LONG CELLS ON THEIR LID (3 replicates per dilution)

- AFTER THOROUGH SHAKING, TRANSFER 25 ML ALGAE-TOXICANT DILUTION FROM EACH FLASK INTO A GRADUATED CYLINDER, FOR FURTHER TRANSFER INTO THE CORRESPONDING LONG CELL (3 replicates per toxicant dilution)

36

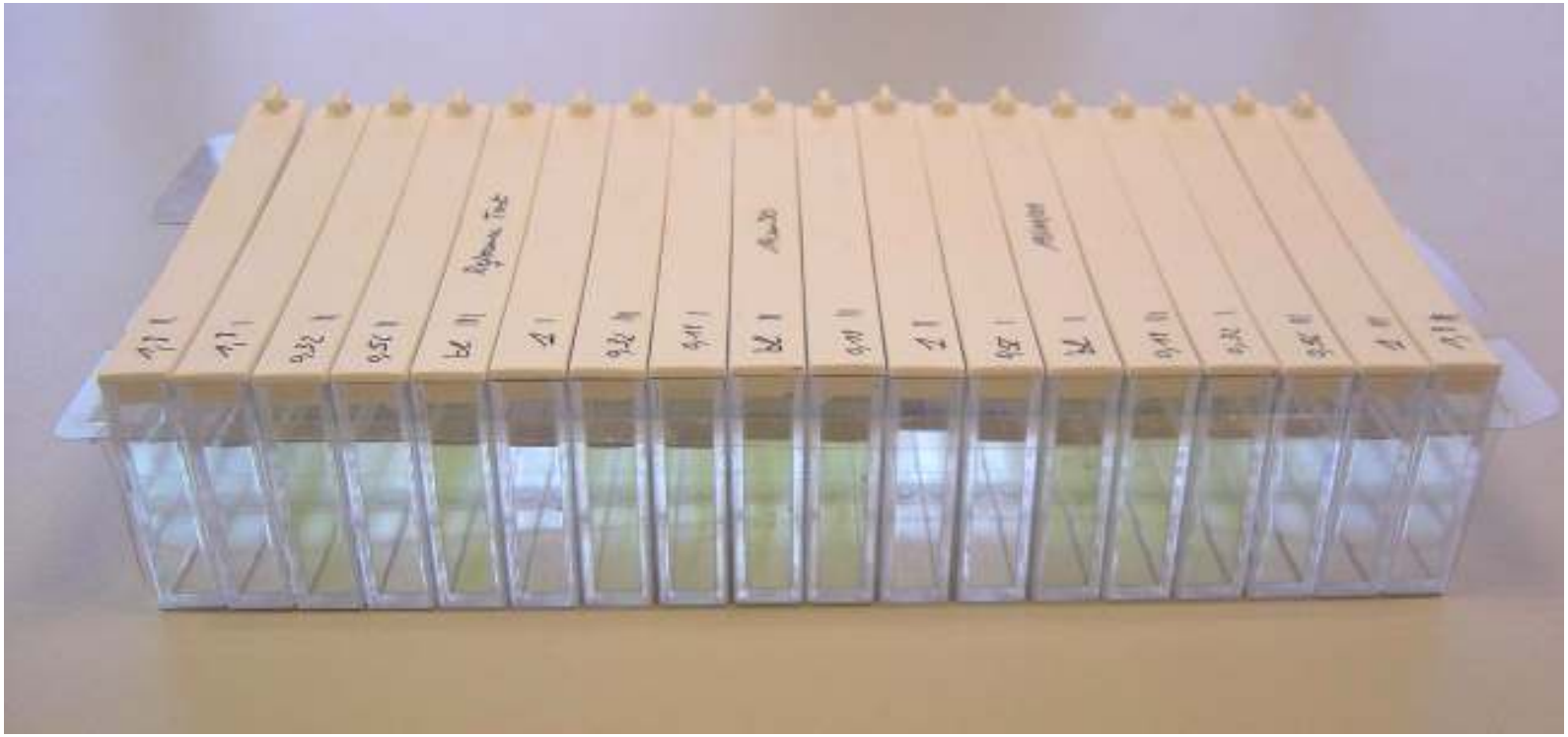
- REDISTRIBUTE THE LONG CELLS IN THE HOLDING TRAY IN A RANDOM WAY

- LIFT UP THE LIDS OF THE CELLS A LITTLE AT ONE SIDE, AND SLIDE THE PLASTIC STRIP OVER THE OPEN PART OF THE LONG CELLS TO KEEP THEM SLIGHTLY OPEN DURING THE INCUBATION PERIOD

37

INCUBATE THE HOLDING TRAY WITH THE LONG CELLS FOR 72h IN AN INCUBATOR AT 23°C ± 2 °C, WITH CONTINUOUS ILLUMINATION:

- SIDEWAY ILLUMINATION = 10000 LUX
- OR BOTTOM ILLUMINATION = 3000-4000 LUX

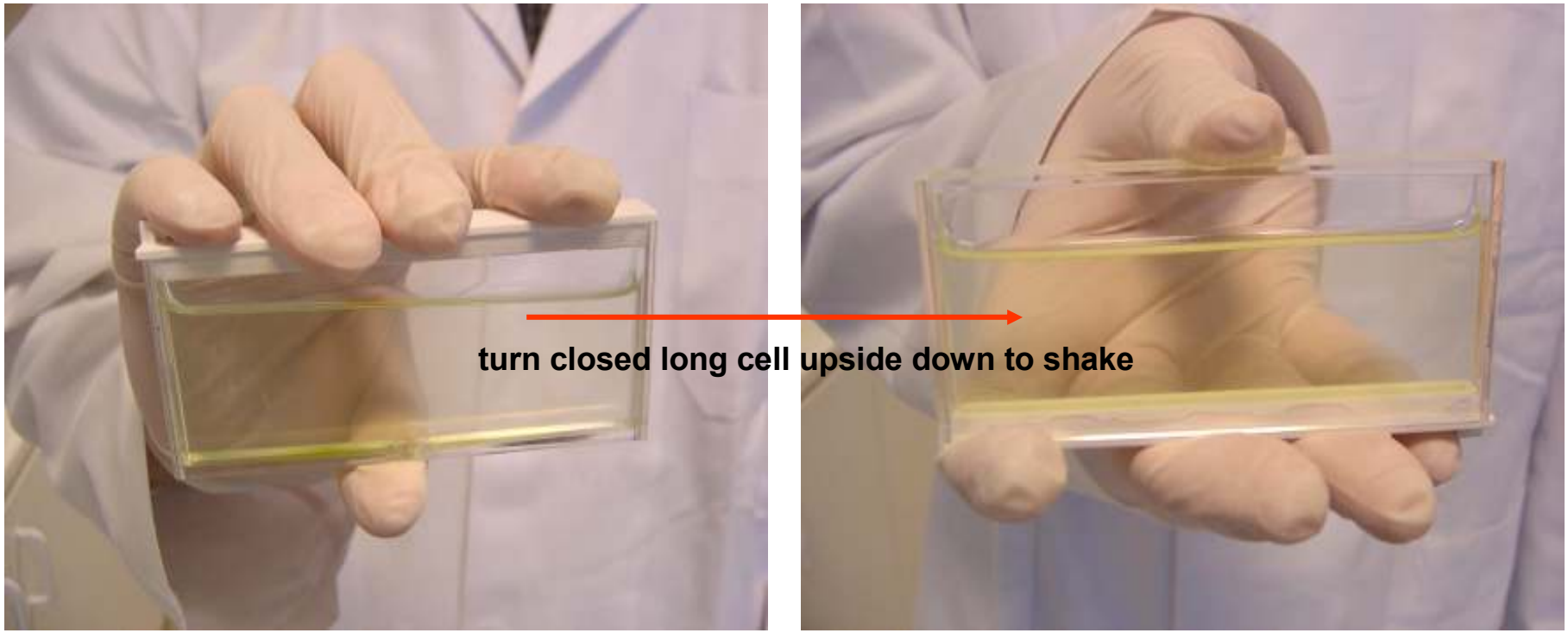


SCORING OF THE RESULTS

THE **OD** OF THE ALGAL SUSPENSIONS SHALL BE MEASURED EACH DAY DURING THE 3 DAYS OF THE TEST, I.E. AFTER 24h, 48h AND 72h EXPOSURE TO THE TOXICANT

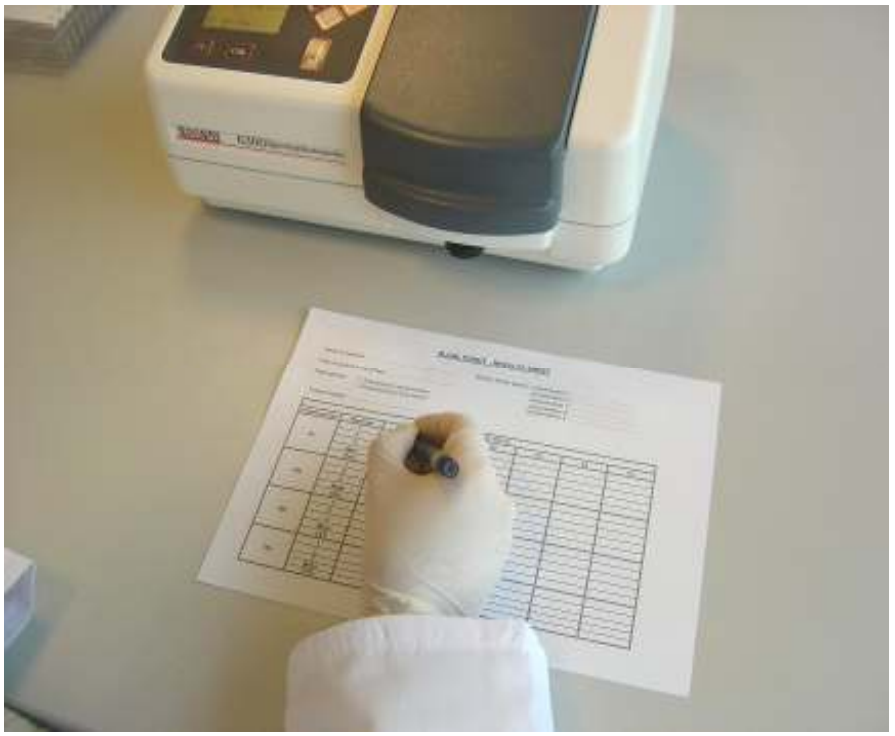
39

ZERO-CALIBRATE THE
SPECTROPHOTOMETER
PRIOR TO THE DAILY MEASUREMENT
OF THE **OD** IN THE LONG CELLS,



40

IMMEDIATELY BEFORE MEASURING THE **OD** IN A LONG CELL, CLOSE THE CELL, TURN IT UPSIDE DOWN AND SHAKE GENTLY TO RESUSPEND THE ALGAE EVENLY



41

- SCORE THE DAILY **OD** RESULT OF EACH LONG CELL ON THE “RESULTS SHEET”

- PERFORM THE DATA TREATMENT OF THE RESULTS WITH AN APPROPRIATE PROGRAMME