



## ROLE OF ALKALIS IN FLOCCULATION AND VIABILITY OF MICROALGAE TETRASELMIS SUECICA

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### ABSTRACT

This study is to evaluate the amount of alkalis required to flocculate microalgae and the percentage of viability in different types of dyes. The main objective of this study is to separate the micro algae. The study attained it using the flocculation method and the flocculant used were Sodium Hydroxide, Calcium Hydroxide and Potassium Hydroxide. These chemicals were used as flocculant to determine the range of flocculation and the exact amount of chemicals used to attain clear flocculation. This study of flocculation was done on micro algae, *Tetraselmis suecica*. Each chemical had the flocculation occurred at different range and the least being NaOH which showed the result from the range of 0.02 -0.09 g of the flocculate with pH of 11.1. The cell viability using different stains like trypan blue, Neutral red and Rodamine B were studied. This study concluded that the NaOH has more cell viability and flocculation to separate the micro algae by using least of Na(OH) as the flocculant.

**Key words:** Flocculation, Alkalis, Cell viability.

## INTRODUCTION

It is a well-known fact that the microalgae are the most efficient solar energy converters and the fastest in the biomass production. In addition, they are one of the sources for great variety of metabolites. This study mainly deals with *Tetraselmis suecica*, a unicellular flagellated green alga. It serves as feed for larvae and post-larvae of shellfish, penaeid shrimp larvae, abalone larvae, brine shrimp and rotifers (Becker, 2013). It is most extensively used in aquaculture and is considered to be an optimal source of long-chain PUFAs, and especially of eicosapentaenoic acid (EPA) (Fa'bregas *et al.*, 2001). Other main consumptions of these algae include the extraction of vitamins, amino acids, antibiotics and refining of industrial wastes as well (Sym and Pienaar, 1993). Most existing commercial systems use centrifugation for harvesting microalgae, but this is an energy-intensive process (Heasman *et al.*, 2000). For harvesting microalgae, the method used in this study is flocculation, which is cheaper and simple. This method gives us volume independent method for deriving concentrated algae cells when compared to the existing methods in use. Algal removal operations as function of treatment technique include micro straining, centrifugation, flocculation in combination with sedimentation or flotation, rapid/slow sand filtration, oxidation and direct filtration (Petrusevski, 1996). The procedure of algal removal by coagulation and sedimentation could be the most economical options for the mitigation of filter clogging problems in conventional water treatment plants that will require very least of the capital. Further, agglomeration of the charge-neutralized solids could be processed by Flocculation. Alkaline flocculants neutralize the repelling surface charge of algal cells, allowing them to floc. It was discovered long ago that  $Mg(OH)_2$  and  $Ca(OH)_2$  flocculate algal suspensions (Folkman and Wachs, 1973).

For viability of microalgae Neutral Red ((3-amino-7-dimethylamino- 2-methylphenazine hydrochloride) is widely used as the vital stain. Neutral Red (3-amino-7-dimethylamino- 2-methylphenazine hydrochloride) is a water-soluble, weakly cationic dye that passes through the intact plasma membrane and becomes concentrated in lysosomes of viable cells (Triglia *et al.*, 1991). In animal cells, the stain is stored in the lysosomes, while in plant cells Neutral Red accumulates in the cytoplasm and/or vacuoles (Crippen and Perrier, 1974). The exact definition of cell death is not clear-cut for phytoplankton and less readily determined than for zooplankton where motility provides an ultimate criterion (Garvey *et al.*, 2007). The disabling of the trans-membrane transport and the loss of physical integrity of the plasma membrane are major features distinguishing dead from live cells (Darzynkiewicz *et al.*, 1994). The stains Trypan blue, propidium iodide and the SYTOXw nucleic acid stains (Green, Blue and Orange stains from Invitrogen Ltd) are viability stains based on such membrane integrity.

## MATERIALS AND METHODS

Pure cultures of *Tetraselmis suecica* was obtained from CMFRI, Tutucorin. The microalgae were grown in one litre Borosil culture flasks and cultured in F/2 medium (Guillard,1965) at CAS in Marine Biology Algal Culture Lab. Cultures were illuminated by lamps to stimulate photosynthesis. This light intensity (2500 lux) was used when conduct the experiment under the temperature of 26 ° C at 29 ppt. For each experiment, 25 ml of microalgae were taken near their maximal cell densities ( $6 \times 10^4$ ) at exponential phase from sub culture. The pH of the algal suspension was 7.8. One-Way ANOVA performed for flocculation rate in SPSS 10.0 software.

### Flocculants:

Four different alkalis Ca(OH), NaOH, KOH ,Mg(OH) were used as a flocculants for this experiment. The onset of flocculation was visually determined by a "grainy" appearance of the algal cells. The pH was continuously measured during flocculation with a pH electrode (Eco testr pH2 : Accuracy  $\pm$  0.1 pH). Flocculation rate was estimated by the observation of the displacement of the upper interface of the cell suspension with time (in min.) through the naked eyes. Once flocs were visually observed, pH and quantity of base add were recorded. Flocs were allowed to settle and supernatants were decanted after 15 min. The flocculation experiment was determined using the optical density data measured at 750 nm wavelength by Spectrophotometer. The flocculation efficiency (%) was calculated by using the following formula.

$$\text{Flocculation efficiency (\%)} = \frac{C_i - C_f}{C_i} \times 100$$

Where  $C_i$  is the concentration of microalgae before treatment and  $C_f$  is the final concentration of cells.

### Cell viability:

The stains used in this evaluation for viability of cells are very few commonly used non-fluorescent stains. Trypan blue, neutral red and rodamine b. For staining procedure, 10 $\mu$ l of samples were treated with 0.01  $\mu$ l of each dyes. The samples were allowed to stand at room temperature for at least 30 min and cells were observed microscopically under fluorescent microscope (Leica Dm-2500). Organisms should continue to stain until cellular activity has ceased. Dead intact individuals, which do not yet show any visible signs of disintegration or decomposition will remain unstained and therefore reduce the number of false positives.

Eva- Mariazetschei and Filip J.R.Meysmani (2012). The cell numbers were counted using haemocytometer and the percentage of viable cells was calculated using following equation.

$$\text{Cell viability (\%)} = \frac{\text{Viable cells}}{\text{Total cells}} \times 100$$

## RESULT AND DISCUSSION

The flocculation experiment helps to determine the range for the flocculation and the results are shown in Fig,1 & 2 with different alkali and pH. Taking 25 ml of microalgae (*Tetraselmis suecica*) as sample and Calcium Hydroxide (Ca OH) as flocculating agent, flocculation occurs at the range of (0.05 -0.1g) maintaining pH at 11.1. Clear flocculation is attained at 0.09g of alkali during this experiment. The flocculation efficiency using calcium hydroxide was found to be 93%. There are many theories and studies as above and the ones to follow depict that calcium and magnesium at very high level pH will result to flocculate algae. The same result was proved to initiate the flocculation with pH level of 11.5-12 ( Yahi *et al.*, 1994) and at pH of 10- 10.5 (Ayoub *et al.*, 1986). The present experiments also showed that similar results with flocculation at pH level of 11.3 for calcium hydroxide and KOH at 10.7 thus proving it.

Using Potassium Hydroxide (KOH) as alkali, flocculation is observed at the range of (0.01 – 0.04 g) where the pH was 10.7. The clear flocculation in KOH is attained at 0.04 g of alkali. When KOH used as a flocculant 95% of flocculation efficiency was obtained. While Sodium Hydroxide, (NaOH) used as alkali the flocculation ranged from 0.02 – 0.09g at 11.3 and the clear flocculation attained at 0.02g. The flocculation efficiency for sodium hydroxide is 90%. As the last, Magnesium hydroxide (Mg OH) used as alkali and no flocculation observed. To support the situation of Mg(OH) the same result was derived by Ami Schlesinger *et.al.*, 2012.

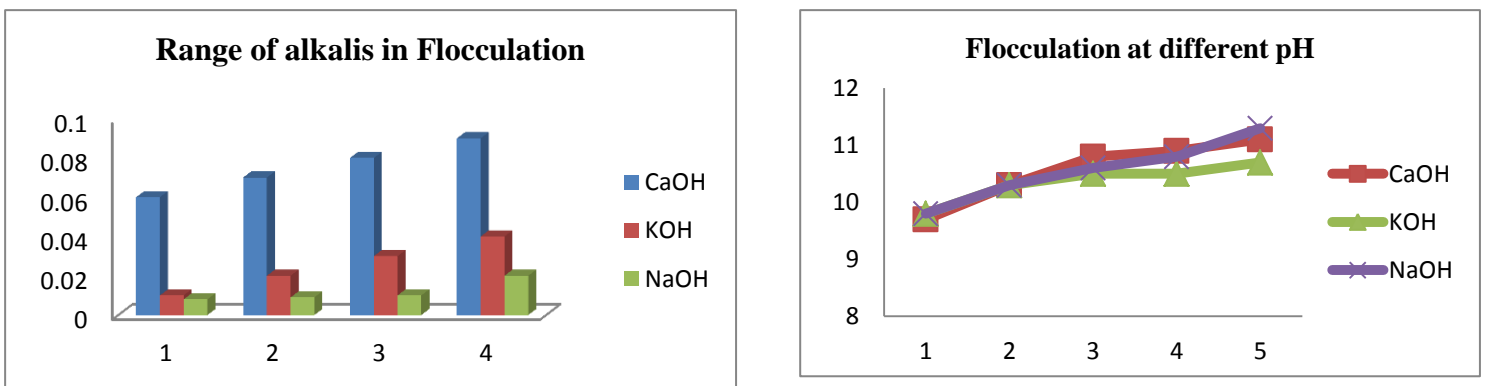


Figure 1 & 2: Range of alkalis and pH in flocculation

Using different alkali it was observed that the flocculation occurs at different ranges. For the same amount of sample, more amount of alkali is required when calcium hydroxide (CaOH ) is used and the least while using sodium hydroxide. It was revealed that pH plays an important role in the flocculation process. Floc particles begin to form well above pH 10 and flocculation is completed at pH 11. At the initial stage of this flocculation process, pH of the medium was high; which makes the small particles aggregate and settle down slowly due to gravity. The large loosely formed cells aggregate as dense packed and settles under gravitational force. After attaining the equilibrium, adding further flocculant might result to form larger

aggregates. This might cause higher settling rates with minimal addition of flocculants (Owen *et al.*, 2002). The cell viability was also determined using Ca(OH), KOH and Na(OH) as flocculants with three similar dyes such as Neutral red, Rodamine B, Trypan blue. (Fig: 3, 4&5). The results showed that Na(OH) has the maximum in cell viability followed by KOH and Ca(OH) respectively. ANOVA gave ( $p < 0.99$ ) indicating a significant difference between pH and flocculation given in Table 1.

### CONCLUSION

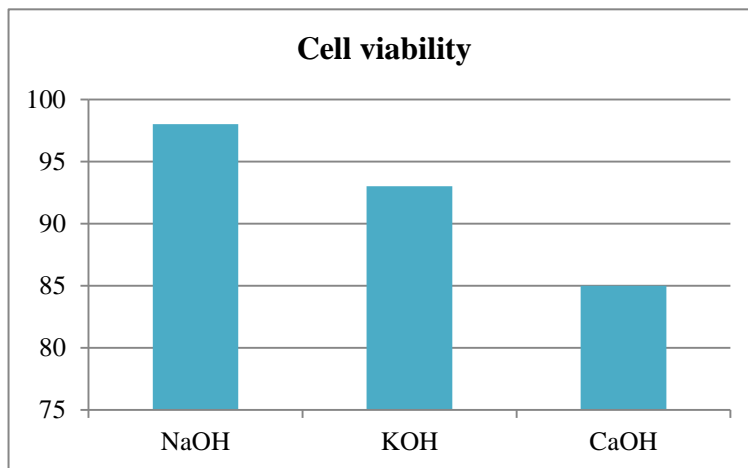
The objective of the study was to determine flocculation and cell viability in microalgae (*Tetraselmis suecica*) using different alkali. From this study, it is concluded that among the 3 alkali used in the experiment, the only flocculant to attain clear flocculation with least amount of alkali used (0.02 g) is NaOH. The algal removal efficiency was high in KOH (95%). The study determines the cell viability using these alkalis as flocculants in three different stains. The outcome showed 98% of cell viability in Na(OH) used as a flocculant.

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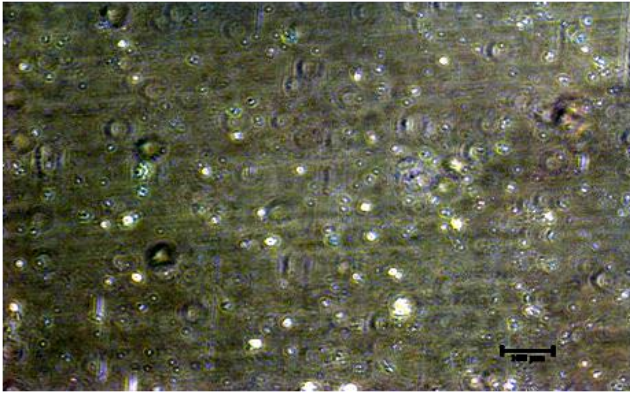
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.421738	3	0.140579	0.002222	0.99983	6.591382
Within Groups	253.0331	4	63.25826			
Total	253.4548	7				

**Table 1:** ANOVA on data of flocculation Efficiency (20-25 ml algae) at different pH in *Tetraselmis suecica*

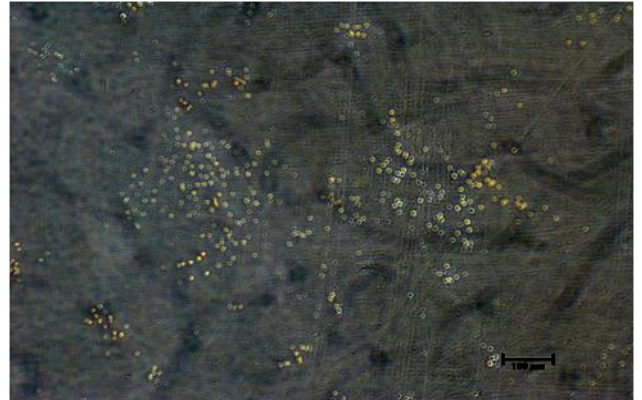




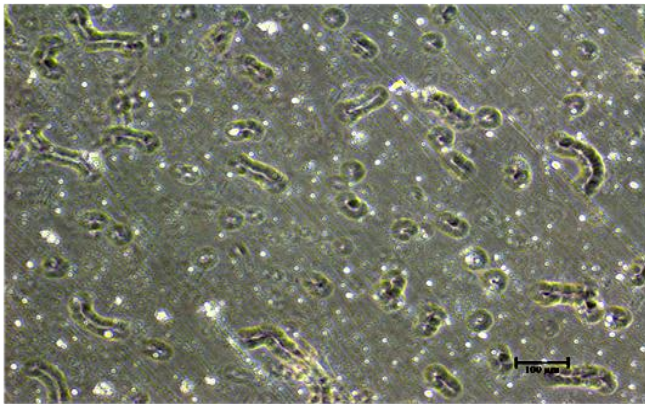
### Cell viability in neutral red stain using alkalis



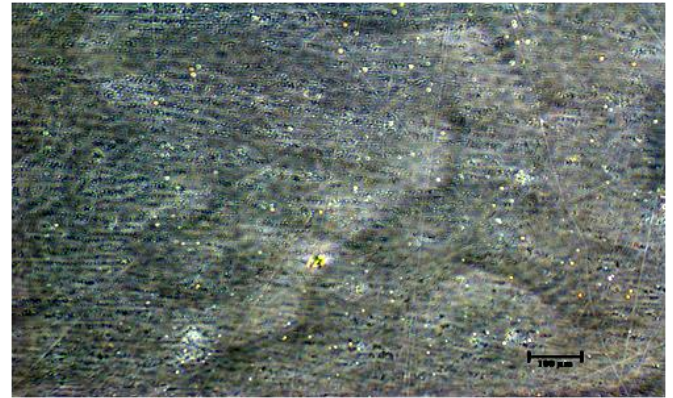
Neutral red control



Ca(OH)

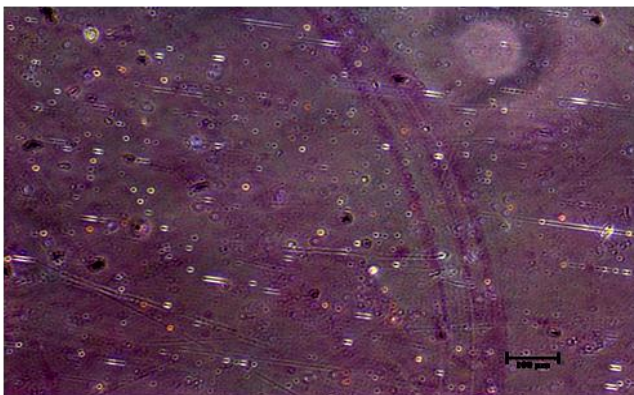


KOH

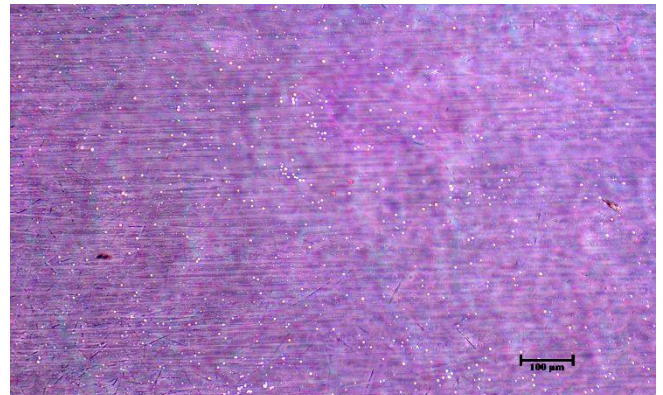


NaOH

### Cell viability in Rodamine B stain using alkalis

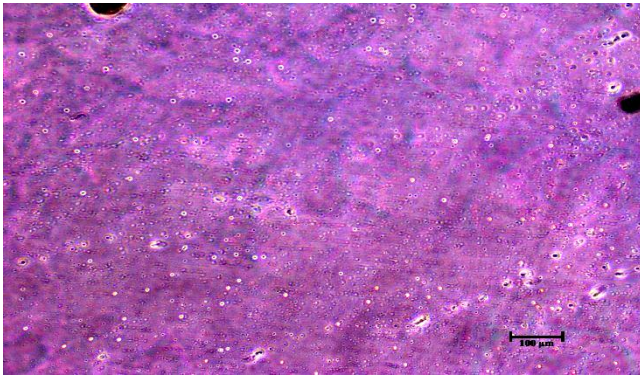


Control

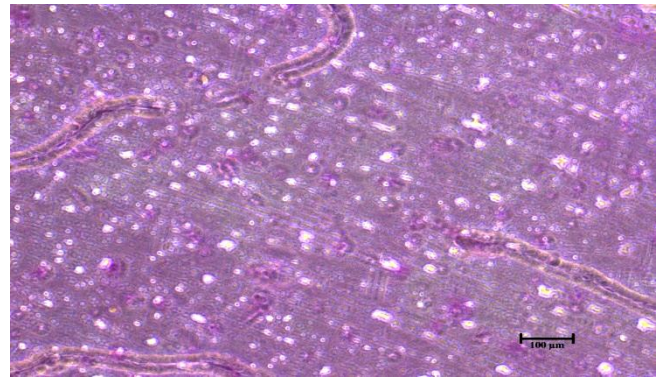


Ca(OH)



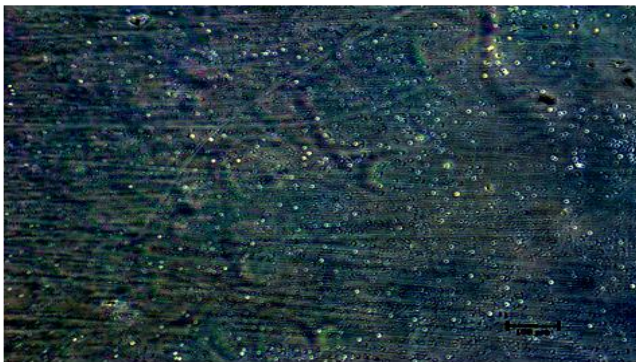


KOH

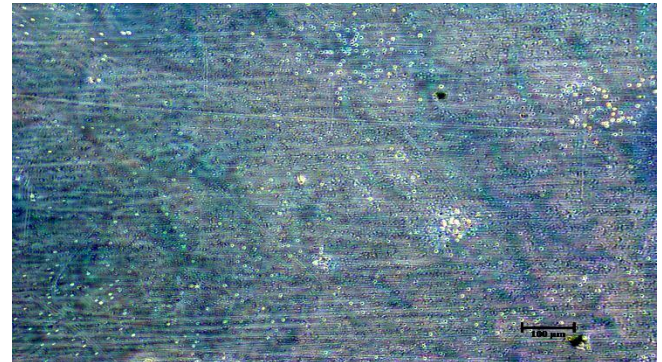


Na(OH)

### Cell viability in Trypan blue stain using alkalis



Control



Ca(OH)



KOH



Na(OH)

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