MICROALGAL FLOCCULATION OF CHLORELLA VULGARIS BY ALTERATION OF pH

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ABSTRACT

Flocculation of chlorella vulgaris using NaOH in the pH range 8.5 to 11.00 was studied and the cell counts of 399 x 10^{-4} with a turbidity of 0.353 OD_{660}. The flocculation efficiency is very sensitive to pH and the cell concentration. The results of the algal flocculation by pH alteration were very effective at 10.38 pH on 14th day of the incubation of the culture and reached the flocculation efficiency upto 98.50 ü 0.34 % with recovery of total lipid upto 94.44 %.

KEY WORDS: Flocculation, Chlorella, Flocculation efficiency, Flocculation activity, Microalgae**.**

I. INTRODUCTION

The flocculating reactions of an algal biomass are particularly sensitive to the pH **(1 & 14)**. The removal of algae from the mass culture by flocculation has been investigated. In this present study the flocculation of the chlorella cells by altering the pH with NaOH was studied. However use of this flocculated microalga has several applications including the utilization of cultivated cells for the production of biodiesel and other nutroceutical products. Thus similar to other species of algal cultivation this method of cost effective harvest of microalgae by flocculation process will be effective and practical for removing the suspended cells from the culture broth.

Microalgal cells carry negative charge that inhibits aggregation of cells in a culture broth. Ideally, the flocculants used should be inexpensive, nontoxic, and effective in low concentration. In addition, the flocculant should be selected so that further downstream processing is not adversely affected by its use **(4)**. Effect of alkali NaOH on cell removal by flocculation from suspensions of Chlorella is been investigated in this work **(16).**

II. MATERIALS AND METHODS

A. Culturing media

Molar concentration of f/2 Medium and Derivatives in final medium for major nutrients $NaNO_3$ - 8.83 x 10^{-4} M, $NaH_2PO_4 \cdot H_2O$ - 3.63 x 10^{-5} M, $Na_2SiO_3 \cdot 9H_2O$ - 1.07 x 10^{-4} M; Trace metal solution of $FeCl_3 \cdot 6H_2O$ - 1 x 10^{-5} M, $Na_2EDTA \cdot 2H_2O$ - 1 x 10^{-5} M, $CuSO_4 \cdot 5H_2O$ - 4 x 10^{-8} M, $Na_2MoO_4 \cdot 2H_2O$ - 3 x 10^{-8} M, $ZnSO_4 \cdot 7H_2O$ - 8 x 10^{-8} M, $CoCl_2 \cdot 6H_2O$ - 5 x 10^{-8} M, $MnCl_2 \cdot 4H_2O$ - 9 x 10^{-7} M; vitamin Solution of Vitamin B_{12} (Cyanocobalamine) - 1 x 10^{-10} M, Biotin - 2 x 10^{-9} M, Thiamine·HCl -3 x 10^{-7} M. **(7 & 8).** The culture was grown in Erlenmeyer's flask - 2000ml under cool white fluorescent illumination of approximately 563 ft-c intensity at 25· C.

B.Growth Determination

The growth of the batch culture was measured tubidometrically using UV visible double beam spectrophotometer (Thermospectronic) at the wavelength of 660 nm. **(10)**. The growth of the batch culture was also measured by cell counts using Neubaeur's cell counter under microscope **(6 &13)**

C. Sampling procedure

A microalgae culture 100 ml aliquots of culture of chlorella was taken into 250 ml baker and the pH was adjusted by the addition of 1M NaOH solution and the pH was measured with a bench top pH meter (Thermo Orion) with 0.01 accuracy. In This study the chlorella culture has been adjusted the pH 8.50, 9.50, 10.38, 10.50 & 11.00. The pH adjusted culture of chlorella was then transferred immediately into a 100 ml class-A measuring cylinder. The aliquots of samples were retrieved from the 100 ml measuring cylinder for every 10 minutes and measured for turbidity in spectrophotometer at OD_{660}. Algal cell concentrations were determined turbidometrically by spectrophotometer and cell counts/ ml using Hemocytometer.

Microalgal cells possess negative charges that prevent assemblage of cells in suspension. Infact the aim behind this study is ideally, the flocculants used should be inexpensive, nontoxic, and effective in low concentration. Moreover, the flocculant chemical which is been to be used is selected so that further downstream processing is not adversely affected by its use **(4).**

D. Flocculation efficiency

Flocculation efficiency as calculated by the following equation, flocculation efficiency (%) = (1 - A/B) × 100, A: OD_{660} of sample, B: OD_{660} of reference (without flocculant). **(3 & 9). The flocculation of batch settling is shown in the form of settling curve** Fig. (4 - 11) at different

concentrations and at different pH conditions. Settling curve is indicated as the ratio of the final volume (*V*) to the initial volume (V_0) **(10).**

$$Settling\ ratio = V/V_0$$

where V= Final volume and V_0 *= Initial volume*

E. Total lipid estimation

The flocculated microalgal cells were macerated in a tissue homogenizer with chloroform-methanol (2:1, v/v) and transferred to a separating funnel. Lipids were extracted, and separated into chloroform and aqueous methanol layers by the addition of methanol to give a final solvent ratio of chloroform : methanol of 2 : 1 is a modification of the method by Bligh and Dyer. The chloroform layer was washed with 20 ml of 0.6% NaCl solution, and evaporated to dryness, total lipids were measured gravimetrically **(2).**

III. RESULTS

The flocculation of microalgae was not effective under the acidic PH condition. However by the increasing the pH remarkably floc formation occurred and it rapidly started settling. A batch settling experiment using chlorella culture with the same batch culture was investigated on 2nd, 4th, 6th, 8th, 10th, 12th, 14th and 16th day at different cell concentration levels. The settling was completed with an hour. During the 2nd and 4th day at Ph 8.50, 9.50, 10.38 &10.50there algal flocs did not occur and pH 11.00 started forming floc. But the cell culture when it reached the 16th day the cell count was 398.5x 10^{-4} cells/ml of culture. There was no floc formation or settling were observed at pH 8.50 and pH 9.50 of algae, at pH 11.00 also there was floc formation but they remain suspended in the media. At pH 10.50 also there was floc formation nevertheless the settling velocity was slower than settling by pH 10.38. It is suggested that flocculation leads to the high recovery of micro algal cells for effective utilization.

IV. Discussion

The rise in the culture pH using NaOH accelerated the mass settlement of algae which made the harvest of algae easier. The removal efficiency increased as the cell concentration increased **(5)**. Thus the algal flocculation with NaOH is most effective at high pH 10.38.A distinct stoichiometric relationship is investigated between algal cell concentration and requisite pH with time. The optimal microalgal flocculation is late log phase and the early declining growth phase **(12)**. The flocculation efficiency upto 98.50 ü 0.34 % which was higher than the 72 % and 78 % produced by aluminium sulphate and polyacylamide **(9)**. Thus the NaOH can be used effectively to harvest chlorella vulgaris from large-scale cultures**.**

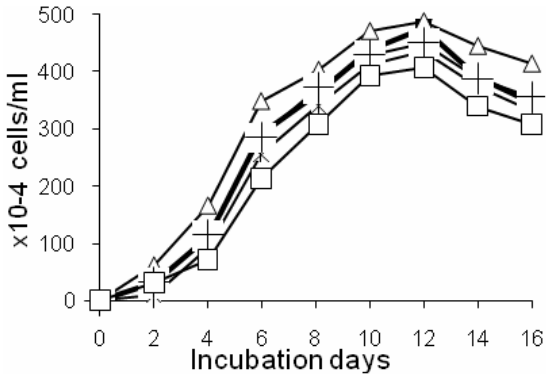


Fig. 1. Growth curve of chlorella vulgaris was observed in seven set of batch culture.

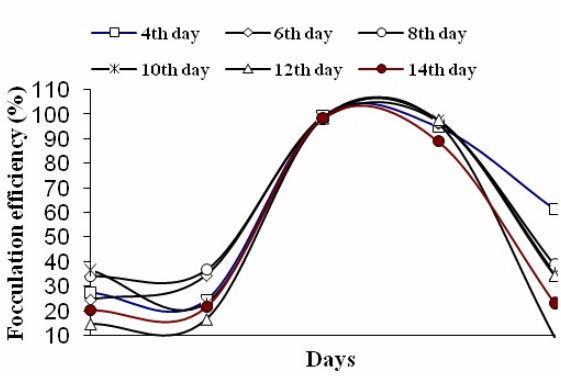


Fig. 2. Flocculation efficiency curve, Flocculation efficiency (%) = (1 - A/B) × 100, A: OD_{660} of sample, B: OD_{660} of reference

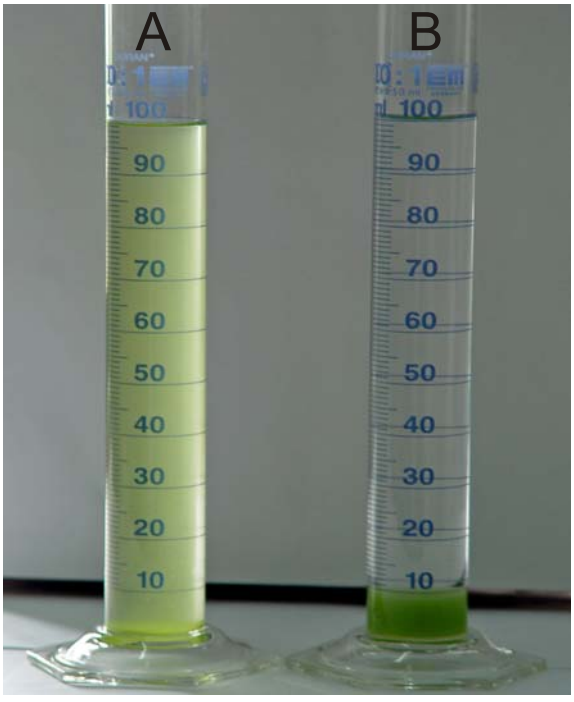


Fig. 3a. Flocculation of *C. vulgaris* (A) - Control without pH adjustment with NaOH (E) – Flocculated with NaOH at 10.38 pH

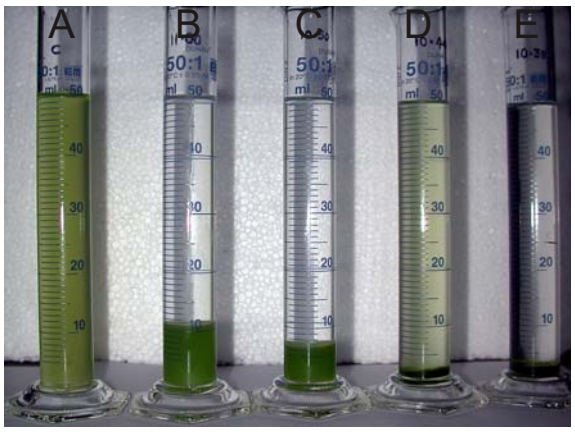
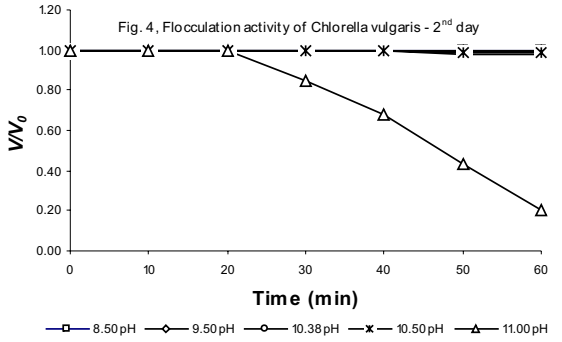
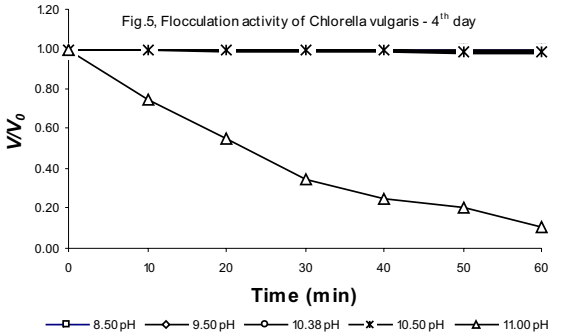
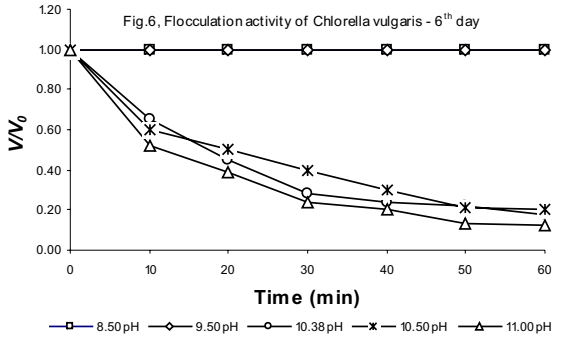
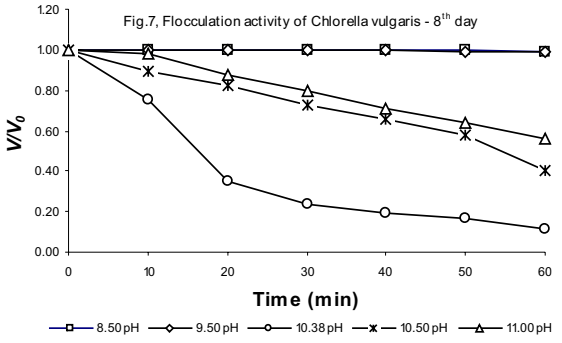


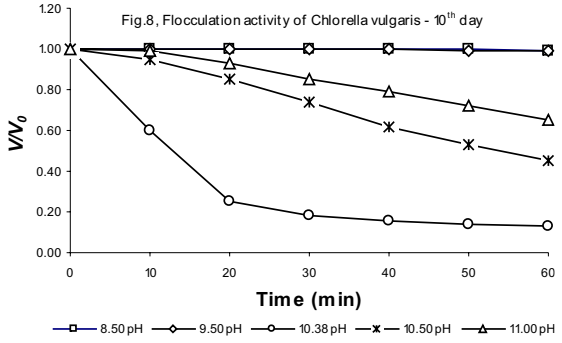
Fig. 3b. Flocculation of *C. vulgaris* at different pH adjustment with NaOH (A) – Control; (B) -11.00 pH; (C) - 10.50 Ph; (D) - 10.40 pH & (E) - 10.38 pH

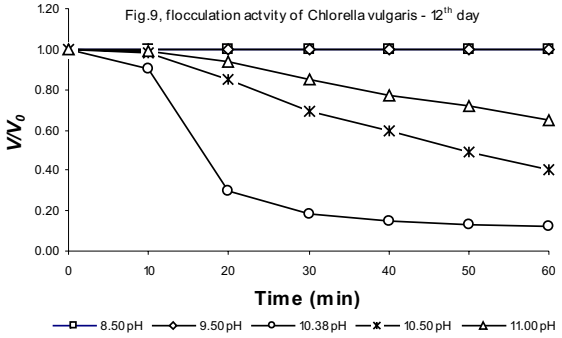


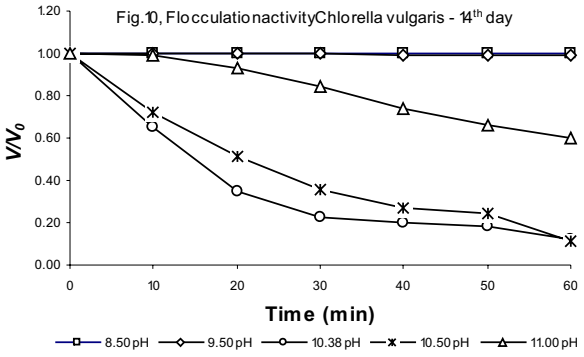












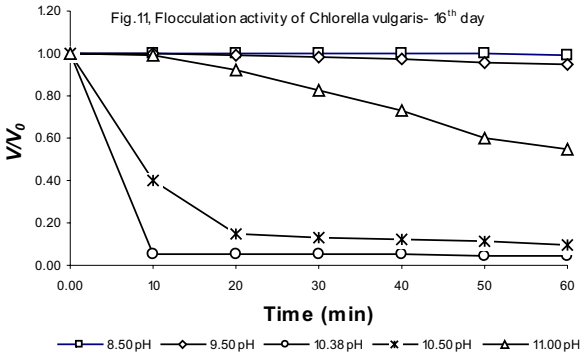


Fig. 4 - 11 Shows Settling curve of *Chlorella vulgaris* under various cell concentrations and at different pH conditions. Settling curve is indicated as the ratio of the final volume (V) to the initial volume (V_0).

Table 1. Percentage of total lipid content recovery at different pH

Sample	Total lipid (gms/100ml)	% of lipid recovery
Control	0.018 ü 0.005	100.00 %
pH 8.50	0.007 ü 0.005	38.89 %
pH 9.50	0.005 ü 0.005	71.43 %
pH 10.38	0.017 ü 0.005	94.44 %
pH 10.50	0.012 ü 0.005	70.59 %
pH 11.00	0.004 ü 0.005	33.33 %

Table 2. Flocculation efficiency (%) at various pH conditions

pH	Flocculation efficiency (%)
8.50	26.28 ü 7.57
9.50	26.09 ü 7.13
10.38	98.50 ü 0.34
10.50	95.46 ü 3.06
11.00	33.72 ü 5.82

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