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PRECONCENTRATION BY FLOCCULATION USING CHEMICAL COAGULANTS: A COST AND ENERGY EFFICIENT ALTERNATIVE FOR BIOMASS HARVESTING FROM MICROALGAE-*Chlorella vulgaris*

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Abstract—*High energy input in harvesting biomass makes the currently applied micro algal bioenergy production techniques economically not feasible. Harvestingmicroalgaeusingmetalsaltsormoringa oliferaisonly marginallylessexpensivethancentrifugation,whichis currentlythemostcommonlyusedmethodforharvesting microalgae. The flocculation performance study of different flocculent salts were done while harvesting microalgae species chlorella vulgaris. Flocculation experiment using $ZnCl_2$ and $ZnSO_4$ salts at different concentrations were carried out under different pH conditions. Harvesting was done by maintaining different pH conditions, (6, 10 and 12.5) in different containers. The result showed that maximum efficiency was exhibited by $ZnCl_2$ under acidic conditions. Effect of $ZnSO_4$ was not negligible but remained less at lower concentrations and non-uniform settling was found at higher concentrations. Experiment setup was also carried out under light and darkness and it was found that the flocculation efficiency decreased in darkness. Hence the present workrevealed that $0.6gl^{-1}$ of $ZnCl_2$ gave the maximum flocculation efficiency in harvesting of micro algal cells of chlorella vulgaris.*

Keywords: *chlorella vulgaris, flocculation, centrifugation.*

1. INTRODUCTION

The current world's strategy shows initiatives to move from a fossil fuel energy driven economy in to a bioenergy based economy where the main attraction lies in biomass which replaces petroleum as a source for transportation fuel as well as feedstock for chemical industry. As a result of the growing population and an increase in living needs in developing countries, demand for biomass for various utilities mainly energy fuel source, food, animal feed is expected to increase by more than 50%in the next few decades[1]. Microalgae forms a promising source of bioenergy which includes biodiesel, syngas for thermal power plants, biomass etc.[2],[3],[4]. One of the major challenge in obtaining biomass from microalgae lies in harvesting, which requires the separation of a low amount of biomass consisting of small individual cells from a larger volume of culture medium.

Tominimizethecostfor mechanicaldewatering, itisimportantthatflocculation resultsinalowalgalsludge volume[5]. Harvestingmicroalgaeusingmetalsaltsisonly marginallylessexpensivethancentrifugation, whichis currently the most comm only used method for harvesting microalgae even though contamination is a major drawback.[6], [7], [8], [9],[10].Here we have done an overviewed study on challenges and possible solutions for flocculating microalgae under various conditions.

2. METHODS AND MATERIALS

2.1 Organism and culture medium

Chlorella vulgaris species, obtained from National Environmental Engineering and Research Institute (NEERI), Nagpur, Maharashtra (India), was grown in sterile Tris Acetate Phosphate (TAP) medium. The filtered sterilized distilled water was enriched with required quantity of TAP medium containing (g L-1): Tris-HCl (2.42 g L-1), TAP salts [NH4Cl (0.375 g L-1), MgSO4. 7H2O (0.1 g L-1), CaCl2. 2H2O (0.05 g L-1)], phosphate buffer [K2HPO4.3H2O (0.0108 g L-1), KH2PO4.7H2O (0.0054 g L-1)], micronutrients [EDTA disodium salt (0.05 g L-1), ZnSO4.7H2O (0.022 g L-1), H3BO3 (0.0114 g L-1), MnCl2.4H2O (0.005 g L-1), CoCl2.6H2O (0.0016 g L-1), CuSO4.5H2O (0.00157 g L-1), (NH4)6Mo7O24. 4 H2O (0.0011 g L-1), FeSO4. 7 H2O (0.05 g L-1)] and Glacial acetic acid (1 ml L-1). The medium was adjusted to pH 8 and autoclaved at 1210 C for 20 min. The filter sterilized vitamins were added after cooling. The contents were later introduced into a 250-ml Erlenmeyer flask. Mixing was provided by continuous shaking of the flask with culture. Lighting was supplied by four cool-white fluorescent tubes with an intensity of 5000 lux.

2.2 Flocculation Experiment

Particlessuspendedinwaterusuallycarryapositiveornegativesurface charge. Tomaintainelectricalneutrality, such charged particles will attractions with anopposite charge from the solution (counterions).

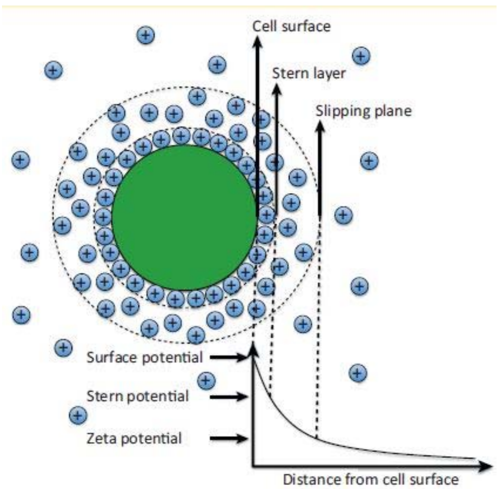


Fig. 1: Structure of the electrical double layer of charged ions in solution surrounding a negatively charged microalgal cell.

Micro algalcell suspensionsarestabilized by the surface charge of the cells from carboxylic(-COOH)andamine(-NH_2) groups on the cell surface. This results in anetnegativesurfacechargeabovepH4 – 5. Stationary growth was obtained in 12 days when chlorella vulgaris species was grown in TAP medium. 50 ml volume of samples were taken in measuring cylinders and different concentrations of chemical flocculants were added at zero time keeping one without coagulant as control. Different pH conditions, say 6, 10, 12.5 were maintained. Two different flocculants were used (ZnCl2, $ZnSO_4$), at varying dosages which ranged in between 0.2 g L-1 to 1.0 g L-1. The flocculation efficiency was measured for each parameter at different time intervals like 0-360 min after start and continuous monitoring was done up to 24 hrs in ½ hr interval. This experiment was done in triplicates each time.

2.3 Flocculation Efficiency

After addition of flocculants, each tube was kept in orbital shaker and stirring speed was maintained at 250 rpm. The initial microalgal biomass concentration in the tubes was estimated from the optical density of 680 nm (OD 680) [11], in UV-Visible Spectrophotometer (Cintra 6, Australia). At every 30 minutes, the optical density of the supernatant was measured at $3/4^{th}$ the height of the clarified culture [12]. Flocculation efficiency was calculated by [9],[10]

Flocculation Efficiency (%) = 100 (1- A/ B), where, A= OD 680 value of sample and B=OD 680 value of control.

2.4 Results and Discussion

Effect of coagulants on micro algal cells were studied on two different salts namely, $ZnCl_2$ and $ZnSO_4$at different concentrations for a period of 4 hours. Efficient flocculation of the microalgae cells were observed in $ZnCl_2$after 360 min. The flocculation of microalgae salts are showed in Fig. 2.

Fig. 2: Settling on effect of coagulants.

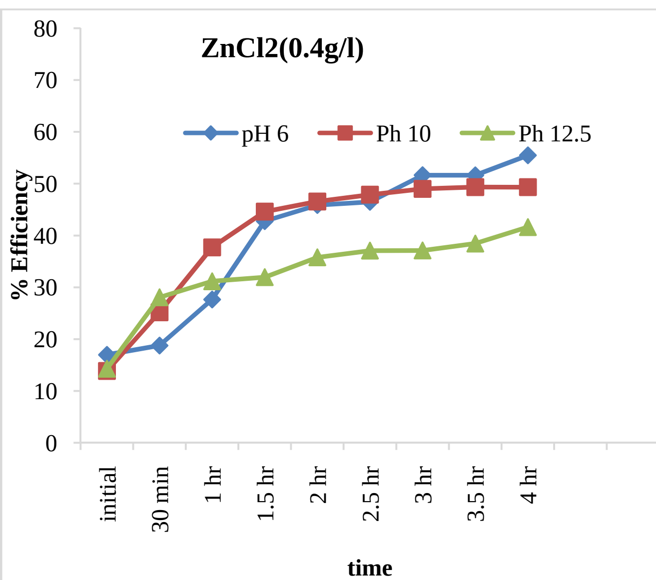
ZnSO4 was comparatively ineffective resulting in efficiency of 33.74, 36.24 and 49.85% at 0.6 gl^{-1} and 55.12, 56.92 and 57.80% at 0.8gl^{-1} at pH conditions 6, 10 and 12.5 respectively in 4 hours whereas $ZnCl_2$ (0.6 gl^{-1}) at pH 6 was best having 91.55% efficient in 4 hours.

Table 1

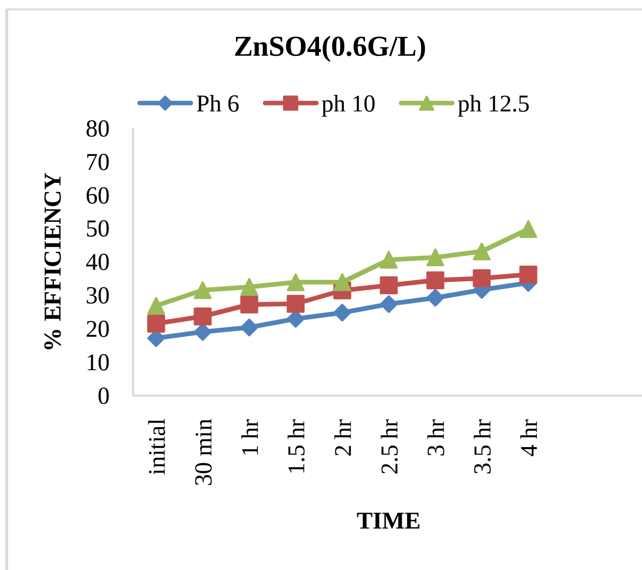
					ZnCl2(0.4 g/l)						ZnSO4(0.6 g/l)			
time	control	percentage	Ph 6	percentage	Ph 10	percentage	Ph 12.5	percentage	Ph 6	percentage	Ph 10	percentage	Ph 12.5	percentage
initial	1.2978	0	1.0774	16.9825859	1.118	13.8542148	1.1123	14.29341963	1.0745	17.206041	1.018	21.55956234	0.9486	26.90707351
30 min	1.2849	0.99398983	1.0436	18.7796716	0.9613	25.1848393	0.9242	28.07222352	1.0398	19.0754144	0.9803	23.70612499	0.8792	31.57444159
1 hr	1.2762	1.66435506	0.9232	27.6602413	0.7948	37.7213603	0.8783	31.17849867	1.0157	20.4121611	0.9281	27.27628898	0.8611	32.5262498
1.5 hr	1.2695	2.18061335	0.7265	42.7727452	0.7032	44.6081134	0.8637	31.96534069	0.9776	22.9933045	0.9205	27.49113824	0.8386	33.94249705
2 hr	1.2676	2.32701495	0.686	45.8819817	0.6773	46.5683181	0.8139	35.79204796	0.9529	24.8264437	0.8686	31.47680656	0.8371	33.96181761
2.5 hr	1.2675	2.3347203	0.6783	46.4852071	0.6607	47.8737673	0.7974	37.0887574	0.9203	27.3925049	0.8492	33.00197239	0.752	40.67061144
3 hr	1.2638	2.61981815	0.6111	51.64583	0.6444	49.0109194	0.7947	37.11821491	0.8937	29.2846969	0.8277	34.50704225	0.7408	41.38313024
3.5 hr	1.2619	2.76621976	0.6104	51.6284967	0.6392	49.3462239	0.7767	38.44995641	0.8627	31.6348364	0.8188	35.11371741	0.7172	43.16506855
4 hr	1.241	4.37663739	0.5527	55.463336	0.6289	49.3231265	0.7243	41.6357776	0.8222	33.7469782	0.7913	36.23690572	0.6224	49.84689766
20 hr	1.1364	12.4364309	0.238	79.0566702	0.4864	57.1981697	0.3224	71.62970785	0.3898	65.6986976	0.5055	55.51742344	0.3059	73.08166139
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Table 2

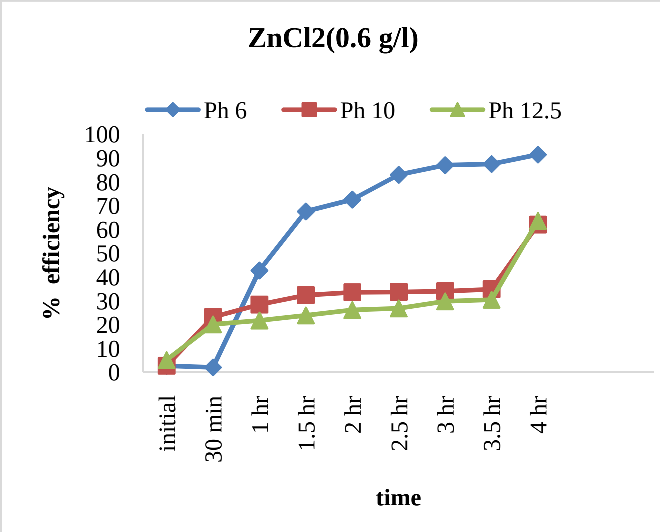
					ZnCl2 (0.6 g/l)						ZnSO4(0.8 g/l)			
time	control	percentage	Ph 6	percentage	Ph 10	percentage	ph 12.5	percentage	Ph 6	percentage	Ph 10	percentage	Ph 12.5	percentage
initial	1.2978	0	1.2616	2.7893358	1.2616	2.7893358	1.2304	5.193404223	1.1252	13.2994298	1.2472	3.898905841	1.2328	5.008475882
30 min	1.2849	0.99398983	1.2581	2.08576543	0.9864	23.2313799	1.0251	20.21947233	1.1094	13.6586505	1.0571	17.72900615	1.165	9.331465484
1 hr	1.2762	1.66435506	0.7297	42.8224416	0.9126	28.4908322	0.9977	21.82259834	1.0661	16.4629368	1.0519	17.57561511	1.034	18.97821658
1.5 hr	1.2695	2.18061335	0.4108	67.6408035	0.8572	32.4773533	0.9649	23.99369831	1.0112	20.3465931	1.0089	20.52776684	1.036	18.39306814
2 hr	1.2676	2.32701495	0.3471	72.617545	0.8404	33.7014831	0.9352	26.22278321	0.9532	24.8027769	0.9618	24.12432944	0.9063	28.50268223
2.5 hr	1.2675	2.3347203	0.2149	83.0453649	0.8391	33.7988166	0.9265	26.90335306	0.8632	31.8974359	0.9775	22.87968442	0.8933	29.52268245
3 hr	1.2638	2.61981815	0.1633	87.0786517	0.8319	34.1747112	0.8865	29.85440734	0.7541	40.3307485	0.9689	23.33438835	0.8878	29.75154297
3.5 hr	1.2619	2.76621976	0.1569	87.5663682	0.8208	34.9552262	0.8764	30.54917188	0.6489	48.5775418	0.9621	23.7578255	0.8758	30.59671923
4 hr	1.241	4.37663739	0.1049	91.5471394	0.4698	62.1434327	0.4502	63.72280419	0.5569	55.1248993	0.5346	56.92183723	0.5237	57.80016116
20 hr	1.1364	12.4364309	0.0987	91.3146779	0.4327	61.9236184	0.4388	61.38683562	0.0997	91.2266807	0.5388	52.58711721	0.5149	54.69024991
24 hr	1.1007	15.1872399	0.0831	92.4502589	0.4151	62.2876351	0.4281	61.10656855	0.0949	91.3782139	0.4681	57.47251749	0.4657	57.69056055



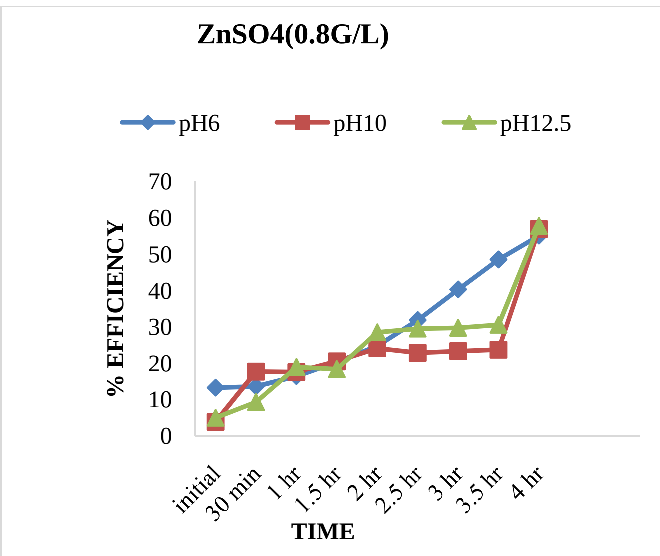
Graph 1



Graph 2



Graph 3



Graph 4

3. CONCLUSION

The overall study revealed that Zinc chloride were the most efficient flocculants causing no damage to the microalgal cells. Zinc sulphate also showed good efficiency at higher concentration but in larger time duration. But as concentration increases, cells were found to be affected. Illumination was a major criterion for flocculation, increasing the efficiency. From this study, to harvest microalgae for biodiesel production, ZnCl2 at pH 6 was found to the most effective chemical flocculants which could flocculate at a shorter time, 360 min., at a minimum concentration of 0.6g L-1.

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