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Copolymer Polyacrylate Polyalcohol – Hydrolytic Characteristics and Influence on Process Variables as an Immobilization Agent

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Abstract: Copolymer Polyacrylate polyalcohol (PP) was investigated in terms of its physical and functional characteristics in addition to as an immobilization agent in suspended cultures. Hydrolytic characteristics of the copolymer were observed via its behavior in water. The copolymer addition in suspended cultures as an immobilization agent was found to have positive impact on the process variables such as pH and DO thereby increasing the process efficiency. The copolymer induction in water resulted in the increase of water pH by around 34%, and that it had no negative effect on the water DO. The hydrolytic behavior as exhibited by the copolymer suggested that the particles were first involved in a series of functions which included expansion, coalescence and colonization in the initial passage of hydrolysis reaction. This was followed by the process of particle dissipation due to the effect of rocking phenomenon, which resulted in the breaking up of induced bonding between the particles followed up by their settlement at the bottom under the influence of reduced particle density.

Keywords: Hydrolysis, copolymer, Polyacrylate polyalcohol, pH, DO, immobilization

1. INTRODUCTION

Copolymer Polyacrylate polyalcohol (PP) is a cationic long-chain organic compound, which is a macromolecule that is composed of thousands of similar or repeating units or building blocks namely monomers with a high molecular mass in millions (Chertin *et al*., 2010; Asadi, 2007 and Madigan *et al*., 1997). PP is a water insoluble hydrophobic cross-linked copolymer containing many polyfunctional crosslinking agents in its structure such as aldehydes, ketones, isocynates or epoxides (Yilmaz *et al*., 2010). PP can absorb water up to thousands of times of their dry weight and form chemically stable or biodegradable gels (Kusch *et al*., 2015). An immobilization agent is used to physically confine the materials of interest to a certain defined region limiting the free movement of the cells while retaining their desired catalytic activity. Cell immobilization in polymeric materials such as ethylene-vinyl acetate copolymer alcohol and polymethyl methacrylate have been successfully used before, because of their low cost, high mechanical strength and durability with ease of handling (Tata, *et al*., 2015).

Photosynthetically oxygenated processes are often limited by the slow growth of microalgae and the risk of microalgal inhibition due to settling of algae cells usually near the bottom of the reactor during free cell cultivation, receiving reduced amount of incident light for energy and growth of the cells (Rehman, 2011). Hence algae cells are immobilized by different ways for suspension in the medium near the surface of water (Munoz *et al*., 2009). The use of entrapped cells in biological wastewater remediation is drawing attention as it offers several advantages over free cells, including relatively easy cell separation, reuse of the same cells for a prolonged period of time due to continuous cell regeneration. However, the major advantage of cell entrapment is that immobilized cells do not suffer from physical or chemical changes occurring in the bulk medium during the immobilization process. Thus, permeability, null toxicity and transparency of the immobilized matrices create a very growth-conducive environment for immobilized cells (Morino-Garrido, 2008). The green algae such as *Chlorella Vulgaris* are mostly non-motile and known to behave in water in a static way particularly during the initial period of the cell growth as they lack flagella, a part of the cell structure responsible to make the cells moving in the medium. Therefore, mobilizing agents, either chemical or solid substances, are used for algae flocculation in water to keep the cells in suspension near the surface of water for maximizing the photosynthetic activity of the cells (Richmond, 2004).

The objective of this research is to identify the characteristic properties of relatively untested copolymer Polyacrylate polyalcohol when used in medium waters for immobilization purposes. Based on this baseline research, influence of copolymer particles on the process variables was then investigated during the course of cell culturing immobilized with the copolymer particles.

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2. EXPERIMENTAL

2.1 Polyacrylate polyalcohol

Copolymer Polyacrylate polyalcohol (PP), also known as Dialyzorb with a product number of P7588, was purchased from Sigma Aldrich (United Kingdom) as analytical grade substance. The copolymer was comprised of small particles of transparent white colour with a size range between 100 and 850 µm with a typical molecular weight of higher than 10^8. The PP particles were applied directly in the given medium with relevant proportion.

2.2 Bioreactor for cell culturing

Both free and immobilized cell culturing was carried out in a designed bioreactor in prepared culture medium. The bioreactor is made of Perspex material and has a rectangular shape with flat bottom. The bioreactor is bifurcated into two compartments of equal width to give it the shape of a lagoon comprising both ingoing and outgoing streams **(Fig. 1)**. A lighting unit (Boss lighting, UK), with two fluorescent tubes of 38 wattage, is attached at the top of the bioreactor. One tube with blue spectrum emits the light in the range of 400-500 nm, which efficiently excites fluorescence in algae cells (Gregor *et al*., 2008); while the other tube with cool white luminance discharges the photons in the range of 400-700 nm.

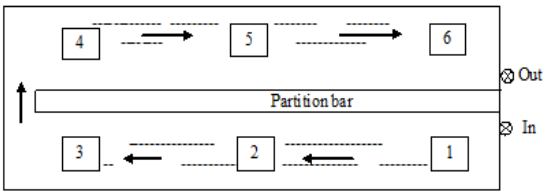

Fig. 1: Inoculation points in bioreactor
Legends: 1 = Front Right, 2 = Front Middle, 3 = Front Left, 4 = Back Left,
5= Back Middle, 6 = Back Right.

2.3 Algae culturing

Green algae *C. Vulgaris* cells were used for their culturing, based on their recognized rapid growth pattern, mixotrophic characteristics and biodegradation capability in organic rich conditions (Ono and Cuello, 2006). *C. Vulgaris* is one of the dominant green algae species, which are also used in oxidation pond systems (Sahinkaya and Dilek, 2009; Oilgae, 2009). Two *C. Vulgaris* strains CCAP No. 211/79 were purchased from Culture Collection for Algae and Protozoa, Scotland, UK and received the same in 10 ml liquid solution of the medium carrying 2-3 ml of *C. Vulgaris* cells in each tube. Upon delivery from the supplier, the vials were lyophilized by keeping them in a freezer at -80 ^{0}C before being revitalized and used for culturing. Preculturing of algae cells was carried out in a prepared rig, which contained two 30-W cool white fluorescent tubes (BLT Direct, UK) with wavelength of 400-700 nm, placed in a horizontal position slightly above the lab bench-top. The culturing bottles were evenly placed in front of the lights in culturing cabin, which was hooded from the top by the contiguous extractor to emit out the possible generation of micron size aerosol particles to keep them from being airborne (Kumar *et al*., 2008). The sides of the cabin were wrapped with aluminium foil to enclose the surroundings to retain the temperature. The fluorescent lighting was also covered from both sides with cardboard material for safety reasons leaving only the central part as exposed for the culturing bottles. The distance between the lights and the bottles was dependent on the amount of heat being transferred to the bottles by the light source as well as the light flux in Lux being irradiated towards the bottles. The distance between the bottles and the light source was maintained at 11" to satisfy the required culturing conditions of temperature (28^{0}C) and incident light (2800 Lux). (**Table 1)** shows the growth conditions for culturing of algae cells.

For mixing and as a CO_2 feed the culture contents were supplied with air via Algarde 1000 Aquarium air pump, filtered with Pall Acro 0.2 µm in-line air filter, through peristaltic santoprene tubing. The air supply was regulated by the attached stopcock valve to supply the air at low to moderate flow rates in the range of 0.5 to 1 l min^{-1} to avoid the buildup of shear force causing damage to algae cells.

Table 1: Specifications for culturing algae cells

Description		Observed/Standard value
Temperature of lighting unit at the source, ^{0}C		35
Culturing bottle temperature, ^{0}C		28
Incident Light at the skin of the bottle, Lux		2800
Distance between light and culturing bottles, cm		24
pH of the culture		7-7.2
Initial culture composition:	DW	100
BG-11 medium,	ml	25
Starter algae culture,	ml	5

2.4 Experimental protocol

Free and immobilized runs of *C. vulgaris* cultivation in prepared sugar water were separately held in the bioreactor. During the course of experiments, different operational parameters were analyzed on diurnal basis. pH was determined by Jenway 370 pH meter (Fisher Scientific, UK), DO and temperature were monitored by Accumet AP74 DO meter (Fisher Scientific, UK), incident light was measured in Lux units by LX-319 light meter (Farnell, UK). Photosynthetic efficiency of *C. vulgaris* was expressed in terms of the grown wet mass by collecting 100 ml

sample from the same point in bioreactor after every 24 hr. The collected sample was centrifuged at 40 rpm for 30 min followed by removal of the supernatant water before weighing the separated algae cell wet mass. The measured *C. vulgaris* mass result was then converted from g 100 ml^{-1} to g l^{-1}. The surrounding temperature in the lab was set at 25^0C via heating and cooling unit. All the experiments were repeated three times to optimize the homogeneity of the results. The results are presented as the means of the three replicates in relation to respective range of standard deviation.

3. RESULTS AND DISCUSSION

3.1 Hydrolytic characteristics of copolymer

Copolymer Polyacrylate polyalcohol (PP) was investigated as to its physical characteristics with respect to its expansion or swelling in water leading to the variation in size and its influence on water pH and DO. To do this, a visual experiment in a beaker, containing 100 ml tap water, was carried out. It was estimated by counting that there are about 100 particles of varying sizes in 1 mg PP or 10^5 or more particles in 1g PP. For hydrolytic analysis of PP, 1 mg PP was poured into a beaker containing 100 ml tap water. **Table 2** presents the observations made during this analysis. After the particles' introduction in water, the particles tended to integrate with each other which was likely caused by the gravity factor and due to the fact that the particles got heavier in weight after being soaked in water, and thus inducing particle collisions forming the resultant copolymer agglomerates.

Table 2: Polyacrylate polyalcohol characteristics in water

Time after PP addition in Water, min	Observation description	Explanation
1	Some particles suspend at the surface and some near the bottom	Probably the larger size particles suspended at the surface, while the smaller size ones took to the bottom
5	Surface particles start coalescing	After water absorption, the particles due to their heavier weight collided with each other
10	Particles below the surface start rising to the surface in colonies	The particles showed the tendency to move upwards while forming associations after water absorption and collision with each other
20	All the particles suspend at the surface coalesced and fully expanded (800 - 1000 µm)	

3.2 Copolymer induction kinetics and influence on water characteristics

To know the physical behavior of copolymer at the larger scale, PP was added in 13 l of distilled water (DW) in the bioreactor tank at the rate of 700 mg l^{-1} with 1500 mg at each of the six points of the bioreactor for a total copolymer addition of 9000 mg. **Fig. 2** shows the pH, DO and temperature results during DW lagooning with PP for 24 hr in the bioreactor. The pH of DW was increased by 34% due to copolymer addition in the bioreactor, which remained unchanged after 24 hr of DW lagooning. The graph also shows that there was no negative effect of copolymer addition on DW DO as the value showed increase of around 10% due to the possible effect of water recirculation. However, DO at the surface was slightly higher than at the bottom likely due to heat transfer and retention at the bottom than its neutralization at the surface, where the temperature might have been equilibrated by the water movement. However, the surface temperature at the surface was slightly higher by 2% than at the bottom during the course of lagooning due to direct exposure to light.

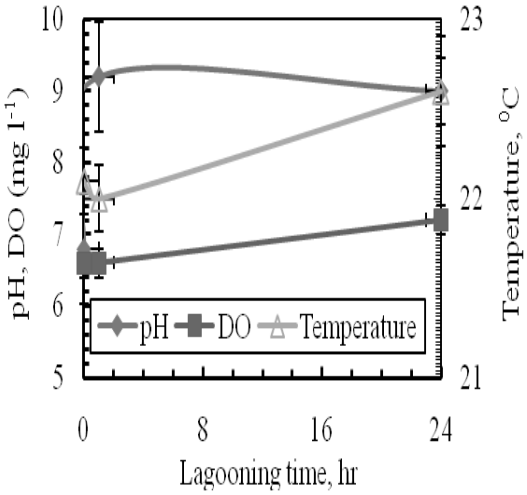

Fig. 2: pH, DO and temperature profile during distilled water lagooning with copolymer Polyacrylate polyalcohol. All the results are represented by their mean ± SD; n=3.

Similar copolymer characteristics were observed when it was added with a higher dose of 0.7 g/l in a large volume of 13 l in the bioreactor tank. **Fig. 3** is a combo camera picture containing four grabs exhibiting copolymer expansion process. The copolymer particles after their introduction in the bioreactor initially took to the bottom and started colonizing until forming copolymer associations of roughly 1 to 3 mm dia to influence their upward movement (grabs a and b). The copolymer agglomeration took 20 min to complete, when most of the particles were observed suspending at the surface in colonies (grabs c and d).

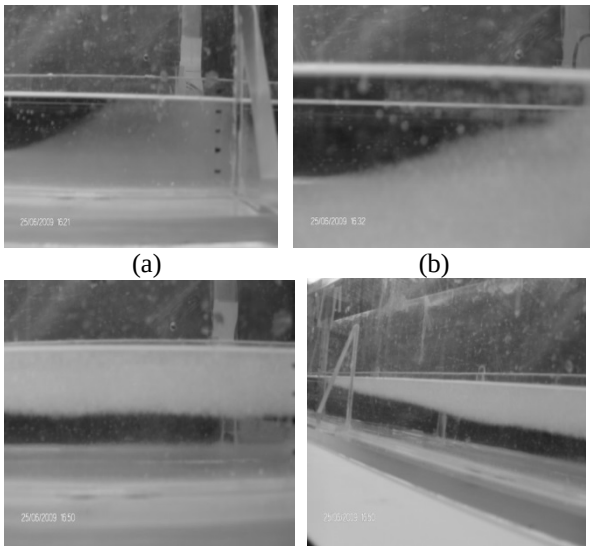

Fig. 3: Combination of camera images showing copolymer Polyacrylate polyalcohol (PP) characteristics in DW: (a) PP particles at the bottom of LPT after their addition into the tank; (b) agglomeration of PP particles and corresponding rise to the surface; (c and d) PP associations in colonies suspending at the surface within 20 to 30 min of hydrolysis reaction.

3.3 Incident light profile in the bioreactor

The incident light (IL) profile in the bioreactor was also observed during DW lagooning with copolymer Polyacrylate polyalcohol, which is presented in **Fig. 4**. The data in the graph suggested that the middle points 2 and 5 were the high intensity points, which were 18% higher with respect to the incidence of light than the side points 1, 3, 4 and 6. After the addition of copolymer, instant decrease in the IL was registered by 11% at the side points and 8% at the middle points, which remained same after 24 hr. This variation in IL between the middle and side points was probably due to the fact that in case of the middle points the striking impact of the light photons was direct and maximum; besides, they could also receive the light photons travelling from left and right regions of the tank. In contrast, the side points located at the left and right areas of the tank receive the insolation only within their respective regions and often in a scattered and diluted form, hence, keeping the striking impact likely indirect and minimum.

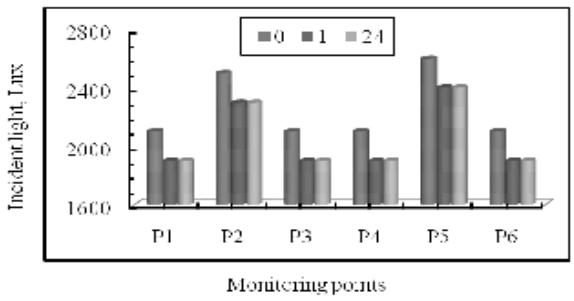

Fig. 4: 24-hr Incident light profile at all points in the bioreactor

3.4 Cell growth analysis

C. Vulgaris cell growth was determined by gravimetric analysis in terms of cell dry mass. 50 ml of cultivated cell mass was collected after every 24 hr at the same time from the middle point in 100 ml beaker. The collected biomass was first centrifuged at 40 rpm for 30 min followed by drying of separated *C. Vulgaris* cells in oven for 1 hr at 105^0C. The combined results for dry mass of *C. Vulgaris* obtained from both free and immobilized cell cultivation are presented in **Fig. 5** after adjusting the values obtained in g ml^{-1} to g l^{-1}. The graph suggests that *C. Vulgaris* growth obtained using lower copolymer dose of 80 mg l^{-1} was almost comparable to that observed during *C. Vulgaris* free cell cultivation with 100% BG-11 broth. The overall *C. Vulgaris* growth rate in g l^{-1} d^{-1} obtained with immobilization dose of 80 mg l^{-1} was 0.81 as compared to 0.41 with immobilization dose of 160 mg l^{-1}, 0.52 during free cell cultivation with 20% BG-11 broth and 1.02 during free cell cultivation with 100% BG-11 broth. The curvature trend in the graph suggests that rapid *C. Vulgaris* growth started to occur from day 2 onwards, with maximum growth occurring between days 3 to 5 of cultivation. To compare the cell mass growth during the log phase between day 2 to 5, 85% cell mass growth at 1.3 g d^{-1} was obtained during *C. Vulgaris* cultivation immobilized with lower copolymer dose of 80 mg l^{-1}. This was in contrast to 72% and 91% at 0.53 and 1.3 g d^{-1} during *C. Vulgaris* cultivation immobilized with higher copolymer dose of 160 mg l^{-1} and during free cell cultivation of *C. Vulgaris* with 100% BG-11 broth respectively.

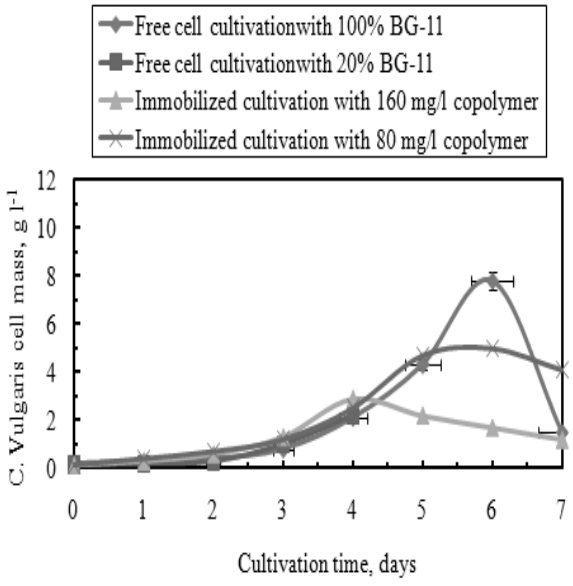

Fig. 5: *C. Vulgaris* growth profiles during free cell cultivation in sugar water with 100% and 20% BG-11 broth and immobilized cell cultivation with copolymer doses of 160 and 80 mg l^{-1} (mean, n=3).

4. CONCLUSIONS

Copolymer Polyacrylate polyalcohol addition in water was found to raise the water pH by 34%, and that it had no negative effect on the water DO. The copolymer particles showed their physical properties when immersed in water such as expansion, coalescence, colonization in the initial passage of hydrolysis reaction, which was followed by the process of particle dissipation under the influence of rocking phenomenon. The copolymer was also observed to enhance the hydroxyl ions concentration of the medium, which may be attributed to its macro structure saturated with such ions. The copolymer-induced increase in hydroxyl ion concentration resulted in pH increase of the cultivation medium cutting the need for pH regulation by other means. Immobilization of algae cells with copolymer particles proved to be effective in terms of biomass growth as comparable biomass weight was obtained with optimum copolymer dosage of 80 mg l^{-1} as compared to that obtained with free cell cultivation or with higher dosage of copolymer for cell immobilization.

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