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Palm petiolar felt-sheath as a new and convenient material for the immobilization of microalgal cells

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This communication reports the use of the petiolar felt-sheath of palm as a novel biomatrix for the immobilization of microalgal cells. Immobilized cells, as compared with free cells, were observed to have significantly higher biomass and polysaccharide production after 27 days of culture growth. Immobilized cells were successfully maintained through 12 successive batch cultures over 96 days. Extracellular polysaccharide production during this period ranged from 382 to 440 mg L^{-1}. The new immobilization material is cheap, stable and easily available, and the procedure developed for entrapment of the microalga is simple, reliable and practical.

Keywords: biomatrix; immobilization; *Porphyridium cruentum*; extracellular polysaccharide

Introduction

Immobilized biocatalysts such as enzymes, microorganisms, organelles, and plant and animal cells have added new dimensions to the application potential of the rapidly advancing frontiers of biotechnology [7,9,22]. Among these, immobilized whole-cell systems in particular have been well investigated in view of the numerous advantages they can offer [8,21,25]. A variety of matrices have been used for cell immobilization, such as natural polymeric gels (agar, carrageenan, calcium alginate) and synthetic polymers (polyacrylamide, polyurethane, polyvinyl). Entrapment in natural polymeric gels has become a preferred technique for cell immobilization due to the toxicity problems associated with synthetic polymeric materials [5,10,24,27]. The use of natural gels is, however, limited by their mechanical strength and the lack of open spaces to accommodate active cell growth resulting in their rupture and cell release into the culture medium [4,6]. Their efficiency is also limited by diffusion restrictions [28] and Ca-alginate gels are unstable in contact with various complexing anions such as phosphate and citrate which are frequently used in media [2].

The use of the natural structure of vegetable sponge, obtained from the matured dry fruit of the dishcloth gourd (*Luffa cyclindrica*, family Cucurbitaceae), for cell entrapment has added another dimension to the variety of immobilization matrices [12,13]. The advantages accruable from such a biostructure are reusability, freedom from toxicity problems, mechanical strength for necessary support, and open spaces within the sponge for the growing cells thus avoiding rupture [1,13]. These have suggested the need to search for other types of structures from diverse plant sources that may be used for cell entrapment. The petiolar felt-sheath common in the Fan-palm, the major group of genera within the family Palmae [17], was accordingly tested as an immobilization biomatrix. The petiolar felt-sheath is a reticulate fibrous network found as a dense matting around the bases of young leaves of palm [19]. The felt-sheath used in the present study was obtained from the palm *Livistona chinensis* which is widely cultivated on the East African coast and coastal islands, throughout East and South-East Asia spreading from Afghanistan, Iran, the Indo-Malaya region, China and Japan to the Australian continent [17,19].

The present work describes for the first time the use of this easily available and cheaper source of biomatrix for immobilization and the technique used for entrapment of microalgal cells. Microalgae have great potential in commercial applications as a source of food, pharmaceuticals, industrial chemicals and a variety of carbohydrates [3]. Many species of microalgae have been considered for biotechnological exploitation [20] and among these, *Porphyridium cruentum* used in the present study is one of the most commercially viable. *P. cruentum* produces a variety of compounds such as extracellular polysaccharides, polyunsaturated fatty acid (arachidonic acid) and the proteinaceous coloured pigments phycoerythrin and phycocyanin [26] which are highly valuable due to their potential applications in food, chemical, pharmaceutical and cosmetic industries [3,20].

Materials and methods

Organism and culture medium

The unicellular red alga, *Porphyridium cruentum*, strain IAM-R-I, was obtained from the Culture Collection of Algae and Microorganisms, Institute of Applied Microbiology, University of Tokyo, Japan. Axenic cultures of *P. cruentum* were grown in 70 ml artificial sea water (ASW) medium [14] contained in 250-ml Erlenmeyer flasks shaken at 100 rpm and maintained at $25 \pm 2°C$ under continuous illumination with cool white light at an intensity of 50 $\mu E\ m^{-2}\ s^{-1}$.

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Immobilizing material and technique

The reticulated fibrous network of petiolar felt-sheaths was obtained as peelings from the trunk of the palm tree *Livistona chinensis*. The felt-sheaths were washed thoroughly with tap water, soaked in boiling water for 15–20 min and placed in 3–4 changes of distilled water over 24 h. The washed felt-sheaths (Figure 1a) were cut into pieces (2 cm^2), oven-dried at 80°C for 24 h, individually weighed, and autoclaved at 120°C for 30 min. For immobilization, *P. cruentum* was cultured in 250-ml Erlenmeyer flasks containing 70 ml ASW medium and four pre-weighed sterilized felt-sheath pieces. Cultures were incubated at 25 ± 2°C for 14 days on a shaker set at 100 rpm under continuous illumination with cool white light at an intensity of 50 $\mu E\ m^{-2}\ s^{-1}$. The reticulated fibre pieces were then removed from the culture flasks and given several washings with sterile 2.5% saline solution. The pieces along with the immobilized algal cells were inoculated into 70 ml fresh ASW culture medium in 250-ml Erlenmeyer flasks and the biomass was measured at periodic intervals for 27 days. Immobilized cells grown in batch culture for 24 days were used for sequential batch culture studies. These stationary phase cells were transferred to 70 ml fresh production medium (ASW growth medium without nitrogen) and the medium replaced every 8 days for 12 sequential batch cultures. All experiments were carried out in triplicate.

Analytical methods

For biomass determination felt-sheath pieces containing immobilized cells were washed with distilled water and oven-dried at 80°C to constant weight. Levels of extracellular polysaccharide in the culture medium were determined using Alcian Blue reagent [18]. For electron microscopy, samples were fixed, dehydrated and dried according to the method of Lee and Tan [15]. The dried specimens were coated with platinum and viewed by scanning electron microscopy.

Results and discussion

The immobilization process

Immobilization of algal cells on the felt-sheath was observed to occur within 5–7 days of initial incubation. Immobilization at this stage, however, was not stable as reinoculation of felt-sheath pieces into fresh medium resulted in free cell suspension of algal biomass. It was found necessary to extend the entrapment period to at least 14 days so as to obtain a stable immobilized system. This system on reinoculation did not reveal, even on microscopic examination, any free algal biomass in the culture medium.

Light microscopy of the immobilized system showed that the initial entrapment of *P. cruentum* cells occurred within the felt-surface depressions and on the irregularly scattered fibrous outgrowths. With the passage of time (14–16 days), however, entrapped *P. cruentum* cells were observed to completely cover the felt-sheath pieces. Scanning electron microscopy of the immobilized system (Figure 1b) revealed that most of the cells tended to aggregate along the surface of individual fibrous bundles, and also along the fibrous outgrowths, resulting in scattered linear cellular clumps on the surface of the felt-sheaths.

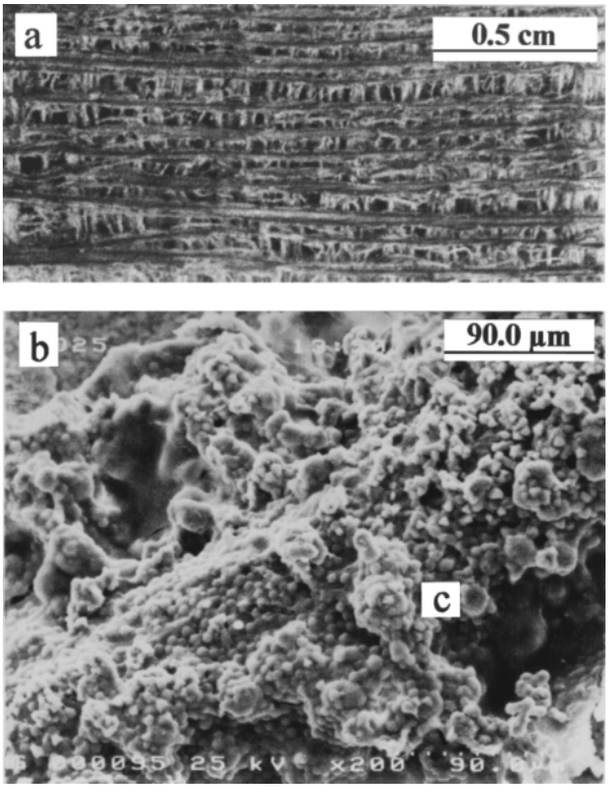

Figure 1 *Livistona chinensis* felt-sheath piece: (a) slightly magnified; (b) scanning electron micrograph of *Porphyridium cruentum* cells immobilized on the fibrous network; (c) immobilized cells aggregated in clumps.

Batch culture studies

Changes in the dry weight of immobilized and free cell biomass during batch culture are presented in Figure 2. A statistically significant difference (Duncan's new multiple range test at $P = 0.05$) [23] in biomass production by the immobilized cells, as compared with free cells, was noted after 15 days of culture and continued until 27 days when the batch culture growth studies were discontinued. A significantly higher amount of extracellular polysaccharide

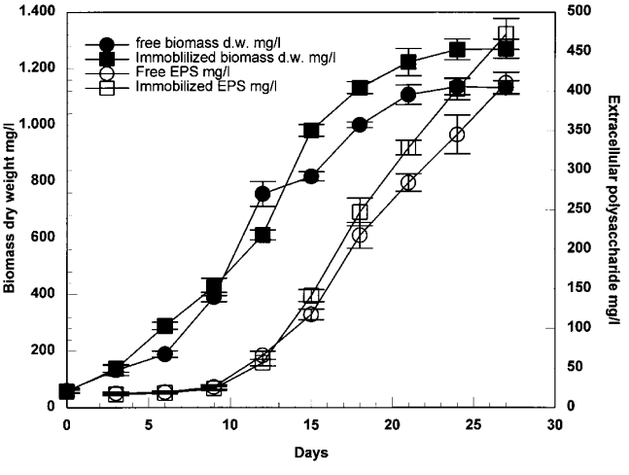

Figure 2 Growth and extracellular polysaccharide production by immobilized and free *Porphyridium cruentum* cells.

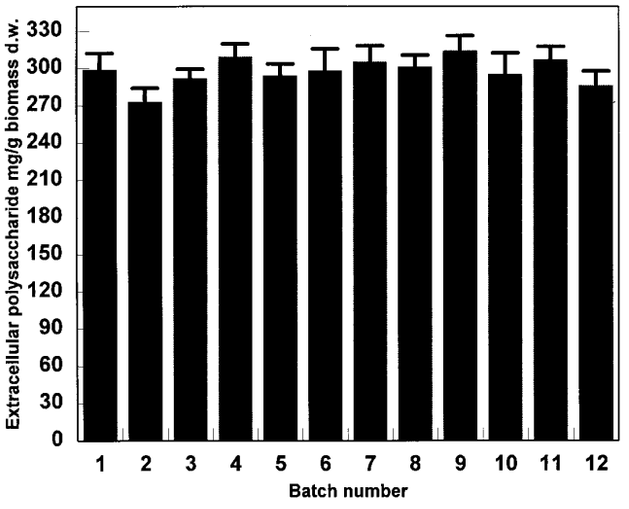

Figure 3 Sequential batch production of extracellular polysaccharide using immobilized cells of *Porphyridium cruentum*.

(EPS) production by immobilized cells, as compared with free cells (Figure 2) was also observed. These results compared favourably with the inhibitory effects noted with other immobilizing matrices, both natural and synthetic [10,16,24,27].

Sequential batch cultures

Figure 3 illustrates typical EPS production during 12 sequential 8-day batch cultures (a total of 96 days culture). EPS produced in these sequential batch cultures ranged between 382–440 mg L^{-1}. A statistical analysis of the data showed no significant difference in EPS production during the entire period of sequential batch culture studies.

Conclusions

The foregoing observations show that the use of the petiolar felt-sheath of *L. chinensis* as a matrix for microalgae has advantages over synthetic and natural immobilization materials in present use. It is simple to use, involving no reagents and complicated preparative procedures, unlike other commercially available matrices [2,11,24], and does not inhibit cell growth or EPS production. Felt-sheath has no significant commercial utility at present and is easily obtainable from the widely distributed numerous members of the palm family [17]. The felt-sheath of *L. chinensis* is only the second plant biostructure that has been successfully used as an immobilization matrix. The biostructure of felt-sheath, however, is much different in its morphological nature, constructional framework, and the type of available entrapment spaces as compared with the vegetable sponge of *Luffa cylindrica* whose use was previously reported [12,13]. The practicability and advantages of *Luffa cylindrica* [1,13] and *Livistona chinensis* dried supports should encourage not only a further search for additional plant sources as cell immobilization matrices but also further studies on the application of the felt-sheath of *L. chinensis* to a wider range of bioprocesses involving other types of immobilized cells.

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