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A simple and economical algal culture system for stable isotopic labelling

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SUMMARY

An alternative method for culturing algae for production of stable isotopically ^{13}C,^{15}N-labelled growth media is presented. The culturing principle relies on a closed system connected to a chemical carbon dioxide generator. The system enables economical and labor-inexpensive production of stable isotopically labelled extracts

In recent years there has been a rapid development in multi-dimensional NMR methods aiming at the study of larger proteins. It is a prerequisite for these studies that the protein of interest can be stable isotopically labelled with ^{2}H, ^{15}N, ^{13}C or a combination (for recent reviews see Oppenheimer and James, 1989). Double-labelling with ^{13}C and ^{15}N has become a particularly important methodology with regard to the new multidimensional NMR techniques (Kay et al., 1990; Clore et al., 1991). Currently, two strategies are employed for ^{13}C,^{15}N double-labelling proteins. The protein under investigation is expressed in minimal media supplemented with either ^{15}N-labelled ammonium salts and ^{13}C-labelled glucose (Kay et al., 1990; Clore et al., 1991) or a ^{13}C,^{15}N double-labelled algal hydrolysate (Wang et al., 1990). As the carbon source (ultimately provided by photosynthetic microalgae in both cases) is the main determinant for the cost of double-labelling a protein, the economy of this kind of experiments is in reality determined by the availability of an efficient algal culture system. In conventional closed algal culture systems, inorganic carbon is supplied by bubbling the cultures with CO_2-enriched gas (Lee and Pirt, 1984; Radmer et al., 1987). Unfortunately, these systems demand delicate and expensive equipment to ensure proper dosage of the gas and involve a risk of leakage.

We describe here an alternative approach based on a simple and economical chemical carbon dioxide supply.

Culture conditions

Scenedesmus obliquus cultures were double-labelled by culturing in inorganic medium contain-

ing per liter: 0.18 g $Na_2HPO_4 \cdot 2H_2O$, 0.49 g NaH_2PO_4, 0.46 g NaCl, 0.25 g $MgSO_4 \cdot 7H_2O$, 6 mg $CaCl_2 \cdot 2H_2O$, 6.5 mg $FeSO_4 \cdot 7H_2O$, 8 mg EDTA, 2.9 mg H_3BO_3, 1.8 mg $MnCl_2 \cdot 4H_2O$, 0.22 mg $ZnSO_4 \cdot 7H_2O$, 0.08 mg $(NH_4)_6Mo_7O_{24} \cdot 2H_2O$, 0.08 mg $CuSO_4 \cdot 5H_2O$, 0.08 mg $CoCl_2 \cdot 6H_2O$, and 0.81 g $K^{15}NO_3$ (Isotec Inc.). The medium was prepared in water extensively degassed to remove any dissolved CO_2. The final pH of the medium was 6.5. $^{13}CO_2$ was supplied to the culture system from a chemical generator as described below. The cultures were illuminated with fluorescent light (15 W, cool white). All experiments were carried out at 32°C. Algal hydrolysates were prepared as described by Brodin et al. (1989).

The culture system

The algal culture system we have developed is shown in Fig. 1. The principle is of a closed system consisting of a bank of serially connected air-lift flasks each containing 900 ml. The mixing of the cultures was carried out by a small membrane pump with a capacity of 2.5 liters per minute (ex. of the aquarium type) which could maintain the mixing of up to 15 liters of culture. However, the most efficient mixing was obtained with four flasks per pump.

Each pump was connected to a chemical ^{13}C-carbon dioxide generator. This consisted of a 3:1 solution of $NaH^{13}CO_3/Na_2{}^{13}CO_3$ (Isotec Inc.) and from this solution $^{13}CO_2$ was produced according to the equilibrium: $CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$. By keeping the pH of the solution at 8.5 by addition of either $Na_2{}^{13}CO_3$ or HCl, production of CO_2 is favored. In order to recycle non-assimilated CO_2, the last flask in the bank was connected back to the carbon dioxide generator. Overpressure in the system due to evolving vapor and oxygen was prevented by placing a safety valve downstream of the pump.

Because the cultures in this set-up are grown under carbon-limiting conditions, a culture density gradient is initially created over the bank of cultures. However, this gradient tends to decrease towards the end of a culturing period. Alternatively, the flasks may be rearranged in sequence during the culturing to provide a more even and optimal carbon dioxide distribution.

The productivity of this culture system is 2 g dry weight cells per liter per week for an input of 1 g carbon per liter. Although the growth rate of algaes cultured this way is lower compared to

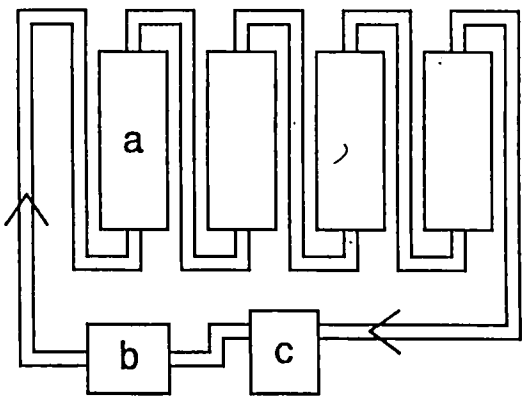

Fig. 1. Diagram of the closed algal system. (a) air-lift flask; (b) gas recycle pump; (c) chemical carbon dioxide generator containing a 3:1 solution of $NaH^{13}CO_3/Na_2{}^{13}CO_3$. Arrows indicate flow direction of the gas.

conventional systems, it should suffice for most lab-scale purposes. With these characteristics, the cost of double-labelled extracts can be reduced by up to 10 times compared to commercially available extracts.

The system we have described here has the advantage over previously described systems that it is of low cost, easy to set up and handle, and it should enable lab-scale production of stable isotopically labelled biopolymers in a much more economical fashion..

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