



(12) **United States Patent**
Strittmatter et al.

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(54) **PROCESS FOR THE PREPARATION OF RECOMBINANT PROTEINS IN *E. COLI* BY HIGH CELL DENSITY FERMENTATION**

FOREIGN PATENT DOCUMENTS

WO 9215683 9/1992

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(73) Assignee: **Merck Patent Gesellschaft**, Darmstadt (DE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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§ 371 (c)(1),

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(51) **Int. Cl.**⁷ **C12N 15/00**; C12N 15/63; C12N 1/21; C12P 21/00; C07H 21/04

(52) **U.S. Cl.** **435/69.6**; 435/69.1; 435/320.1; 435/252.33; 435/252.3; 436/548; 536/23.1; 536/23.533

(58) **Field of Search** 435/69.1, 320.1, 435/252.3, 252.33, 69.6, 41, 91.4, 471; 436/548; 536/23.1, 23.53

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(57) **ABSTRACT**

The invention relates to a fed-batch fermentation process which uses special *E. coli* host/vector systems for the purpose of efficiently forming recombinant proteins, in particular recombinant antibody molecules, preferably antibody fragments such as miniantibodies. Under the given conditions, the *E. coli* cells are able to grow at a maximum specific growth rate up to very high cell densities. After the recombinant product formation has been switched on, it is only the formed product which restricts growth; there is no growth restriction due to substrates or metabolic by-products. High space-time yields of recombinant proteins can be achieved in this manner.

30 Claims, 4 Drawing Sheets

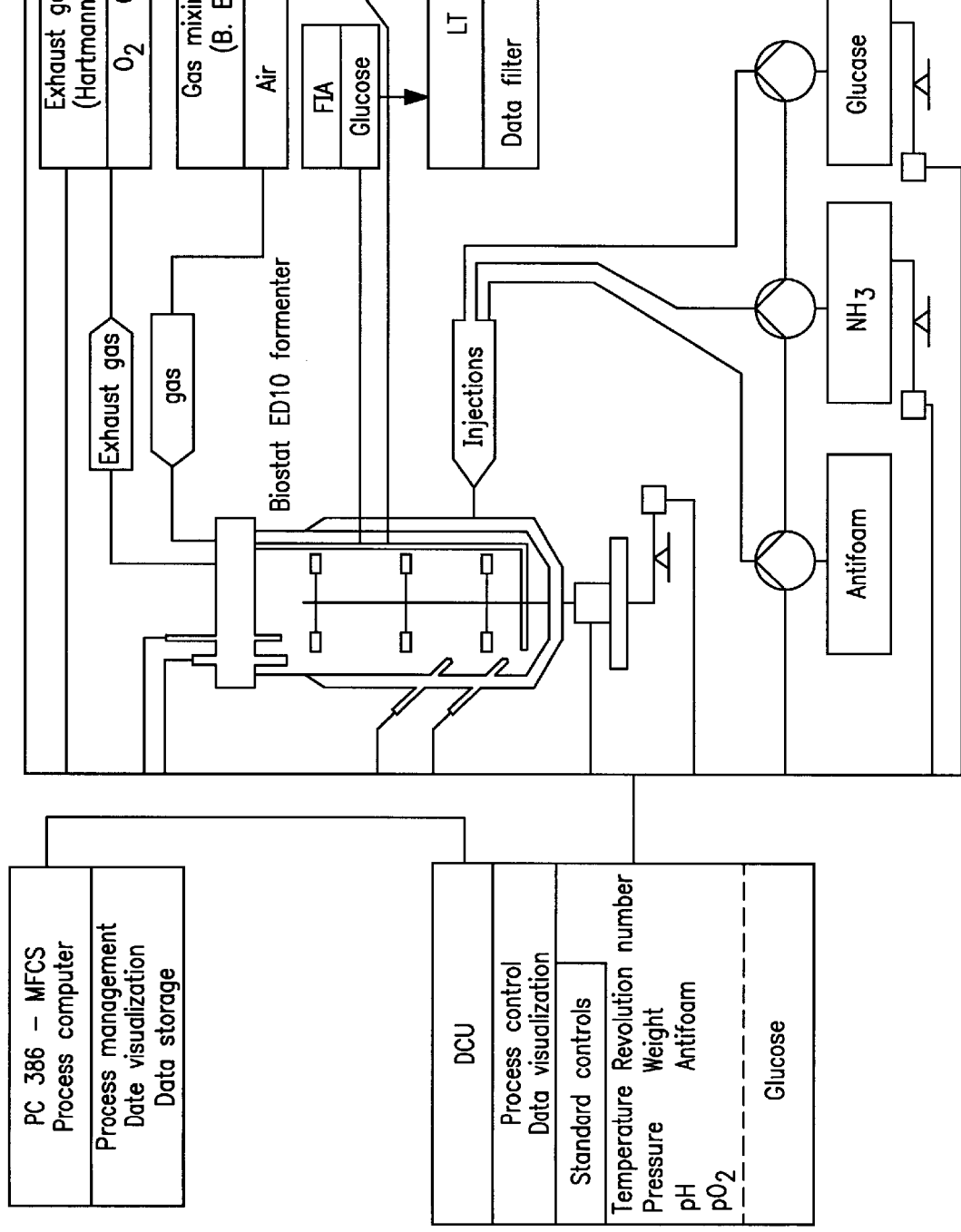


FIG. 1

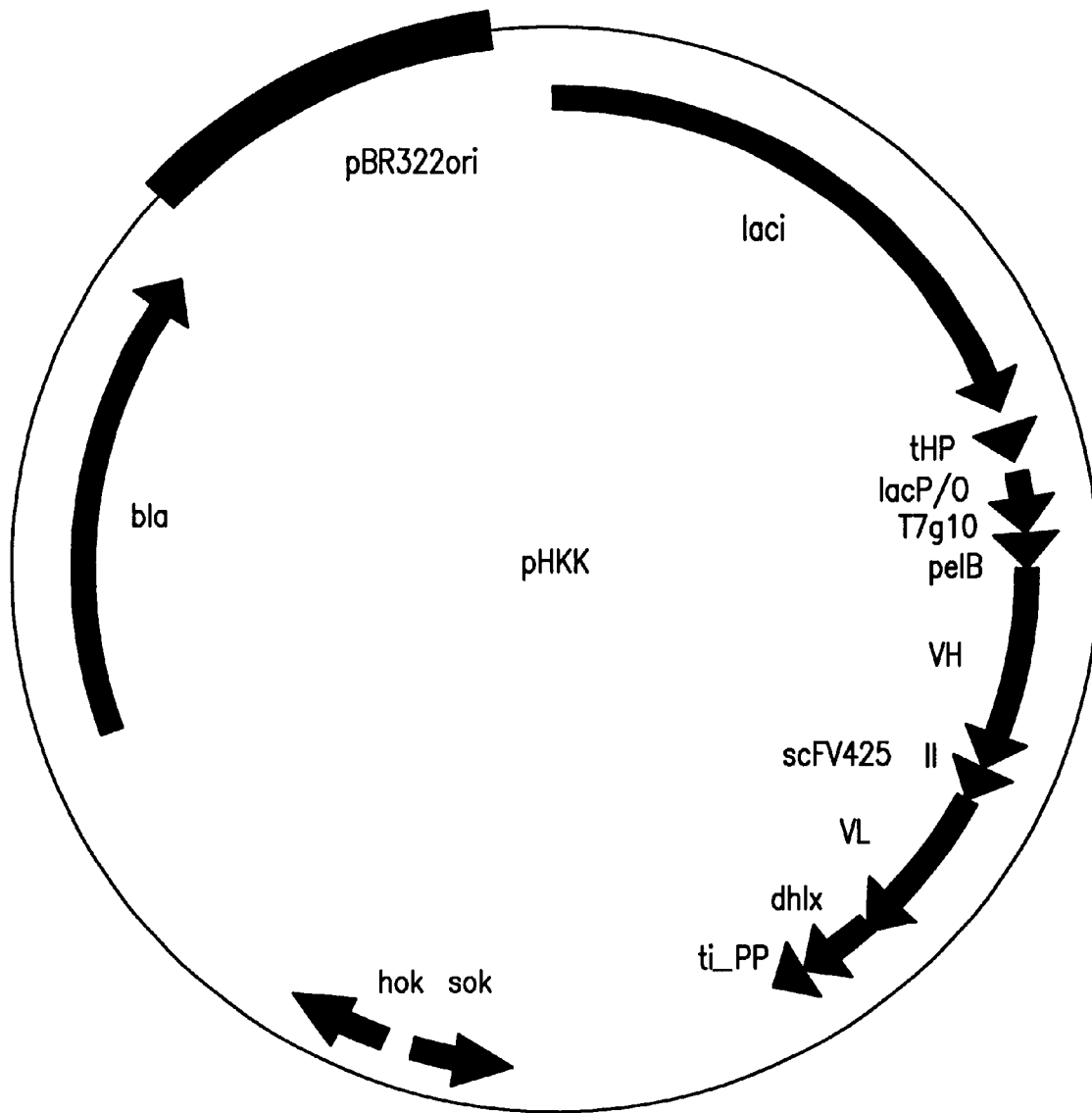


FIG. 2

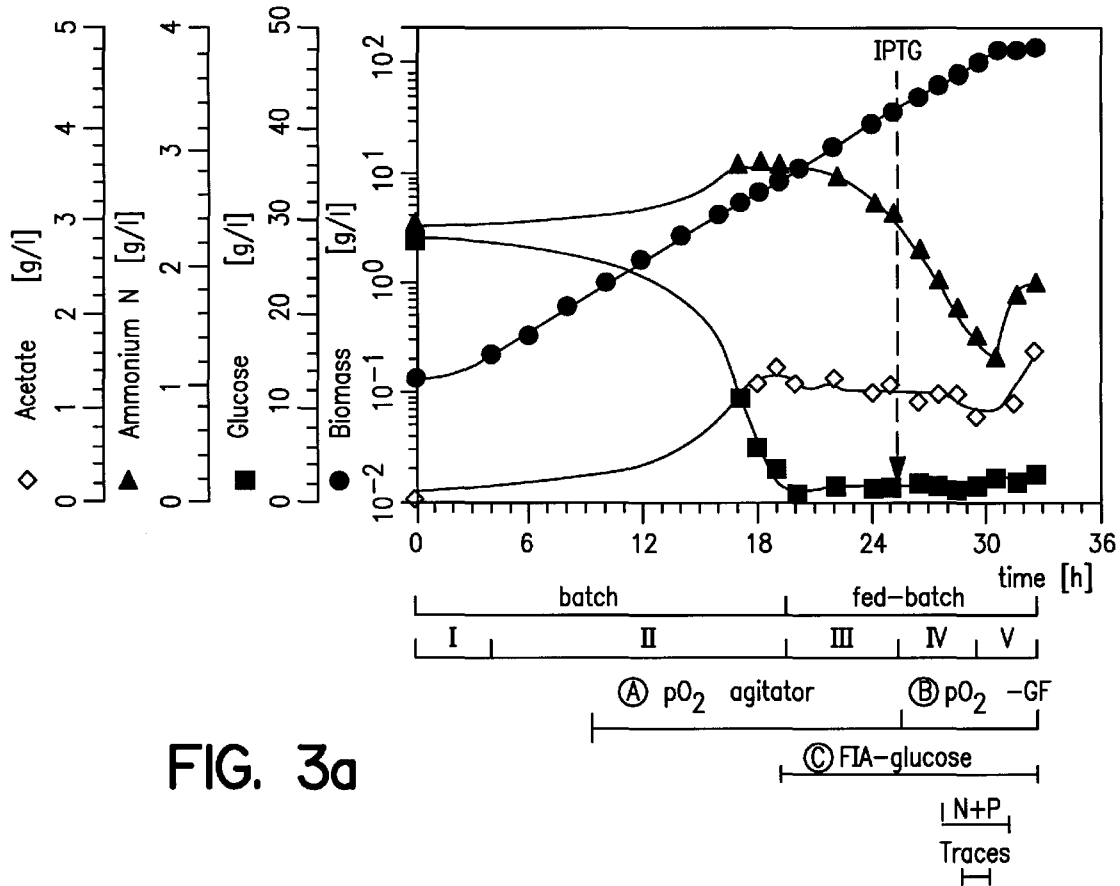


FIG. 3a

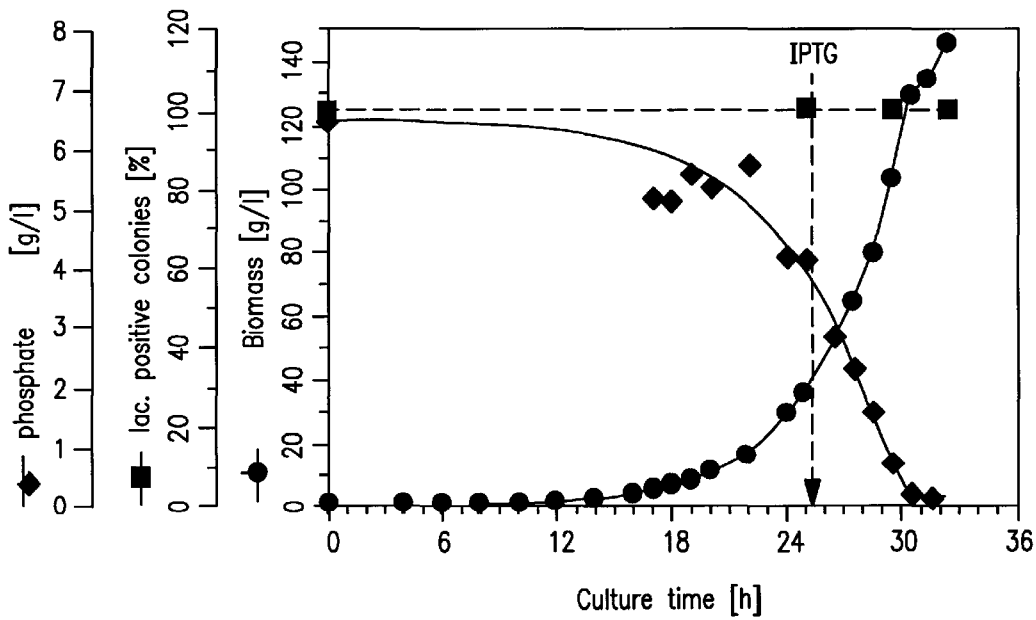


FIG. 3b

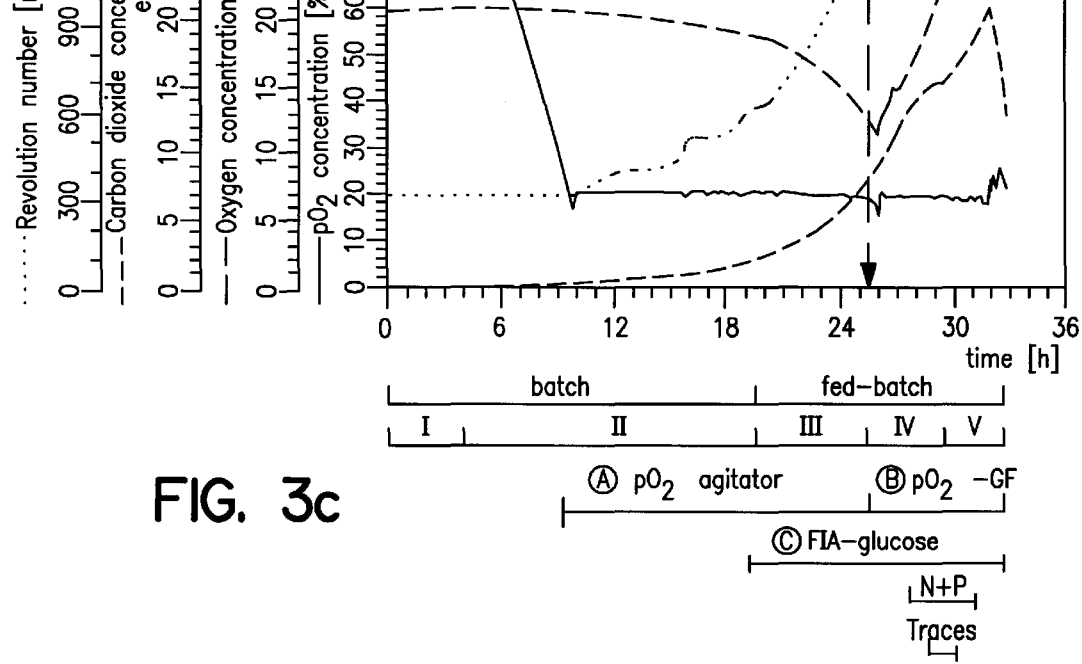


FIG. 3c

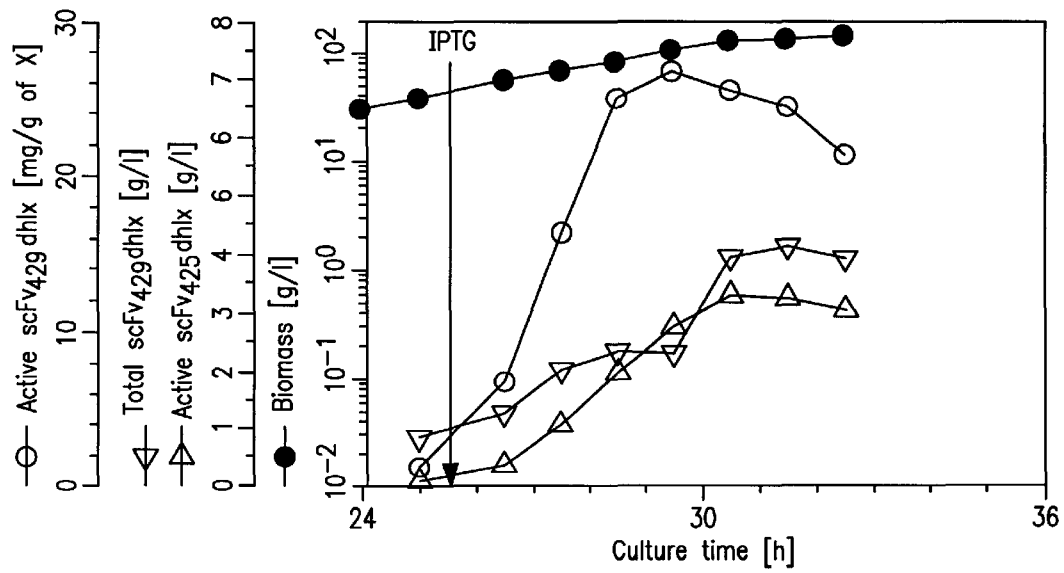


FIG. 3d

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