FACULTY OF ENGINEERING & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

Creation/procedures for developing mutants

Selection of induced mutants

The selection of induced mutants synthesizing improved levels of primary metabolites:

- ✓The levels of primary metabolites in micro-organisms are regulated by **Feedback control systems**.
- ✓The major systems involved are **feedback inhibition and feedback repression**.

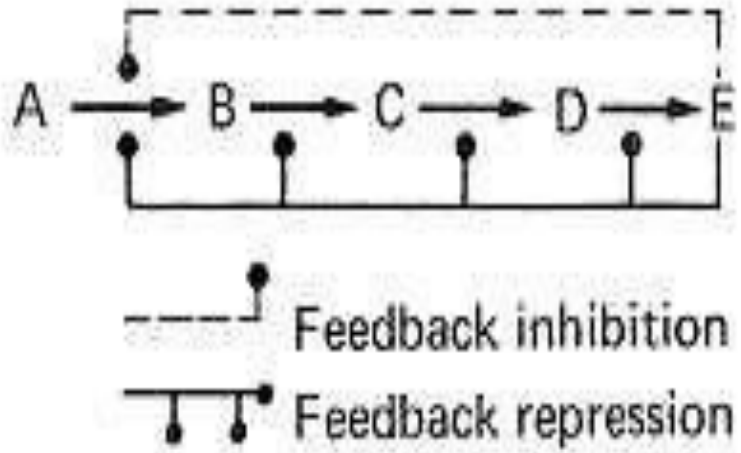


FIG.1. The control of a biosynthetic pathway converting precursor A to end product E via the intermediates B, C and D.

Concerted or multivalent feedback control:

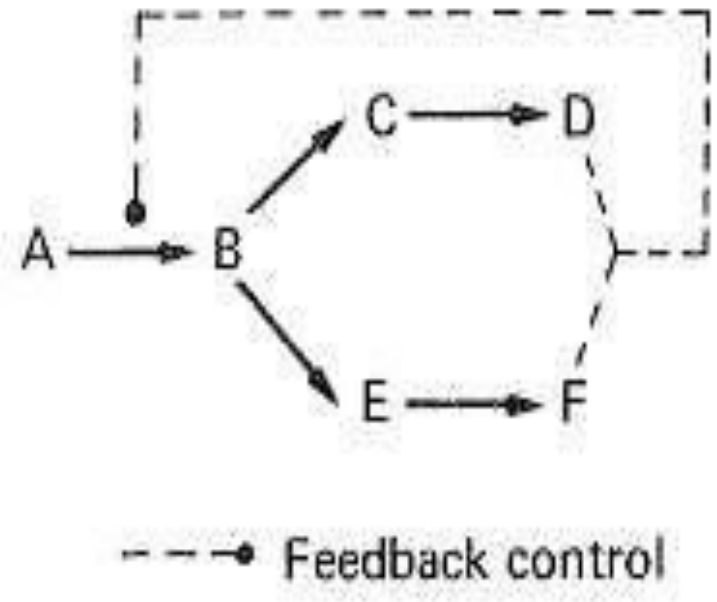


FIG.2 The control of a biosynthetic pathway by the concerted effects of products D and F on the first enzyme of the pathway.

Co-operative feedback control

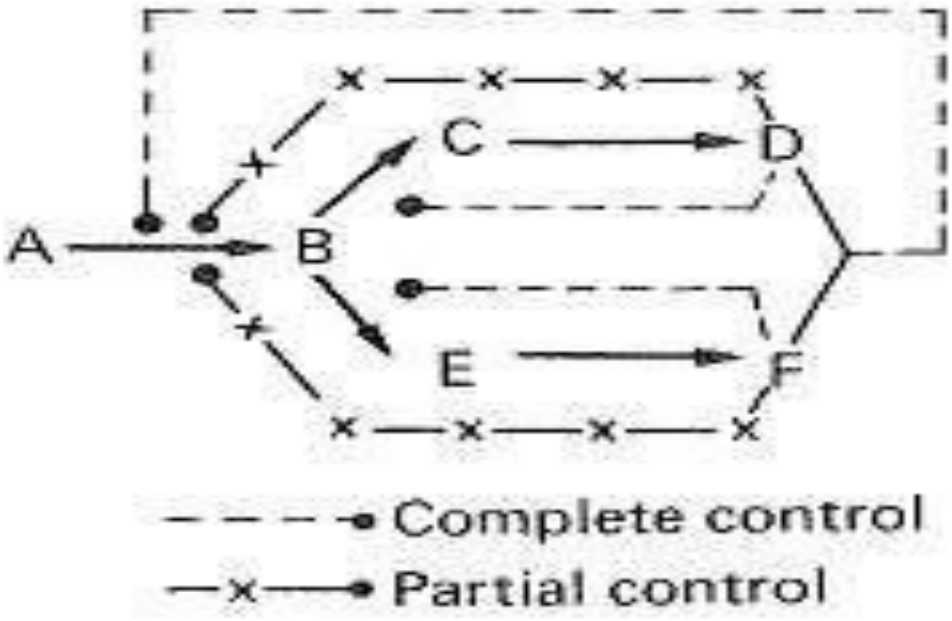
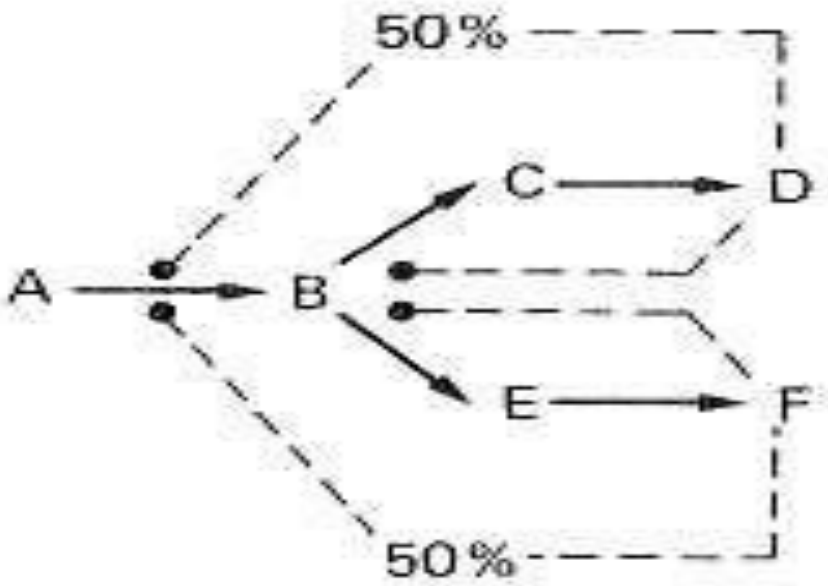


FIG. 3 The control of a biosynthetic pathway by the cooperative control by end products D and F.

Cumulative feedback control



--- 50% ---- Inhibition of 50% of the activity of the enzyme
------ Total inhibition of enzyme activity
FIG.4 The control of a biosynthetic pathway by the cumulative control of products D and F.

Sequential feedback control

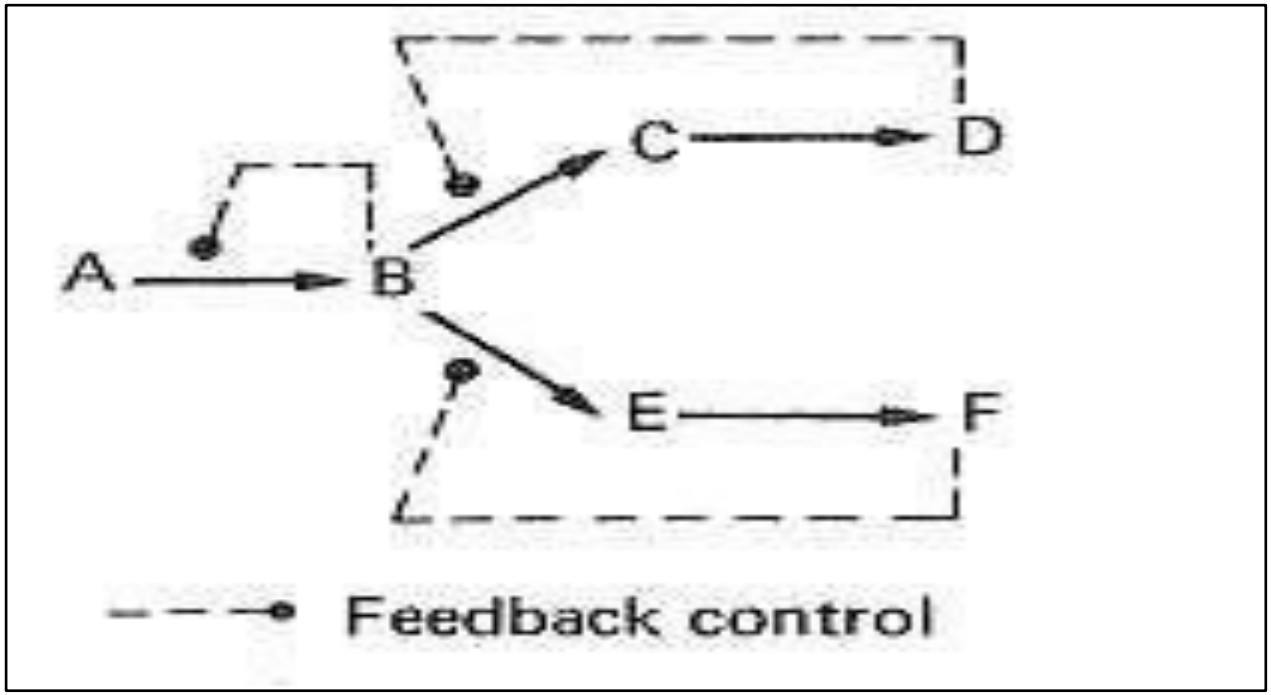


FIG.5 The control of a biosynthetic pathway by sequential feedback control.

Isoenzyme control

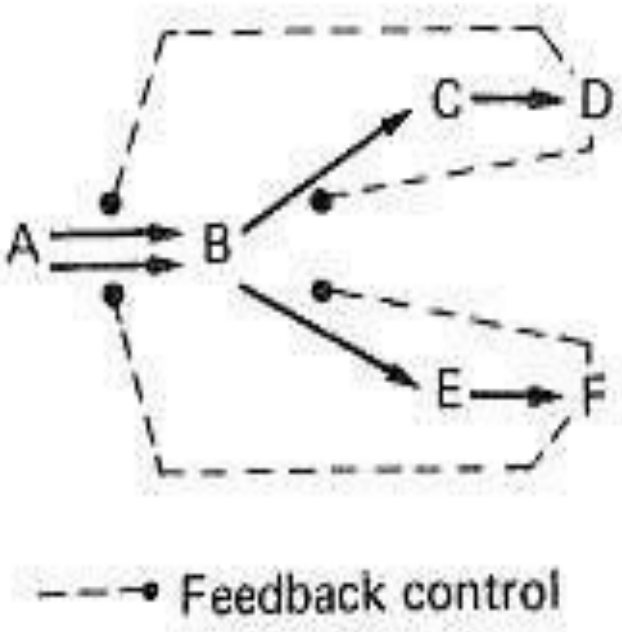


Fig.6 The control of two isoenzymes (catalysing the conversion of A to B) by end products D and F.

The isolation of mutants which do not produce feedback inhibitors or repressors:

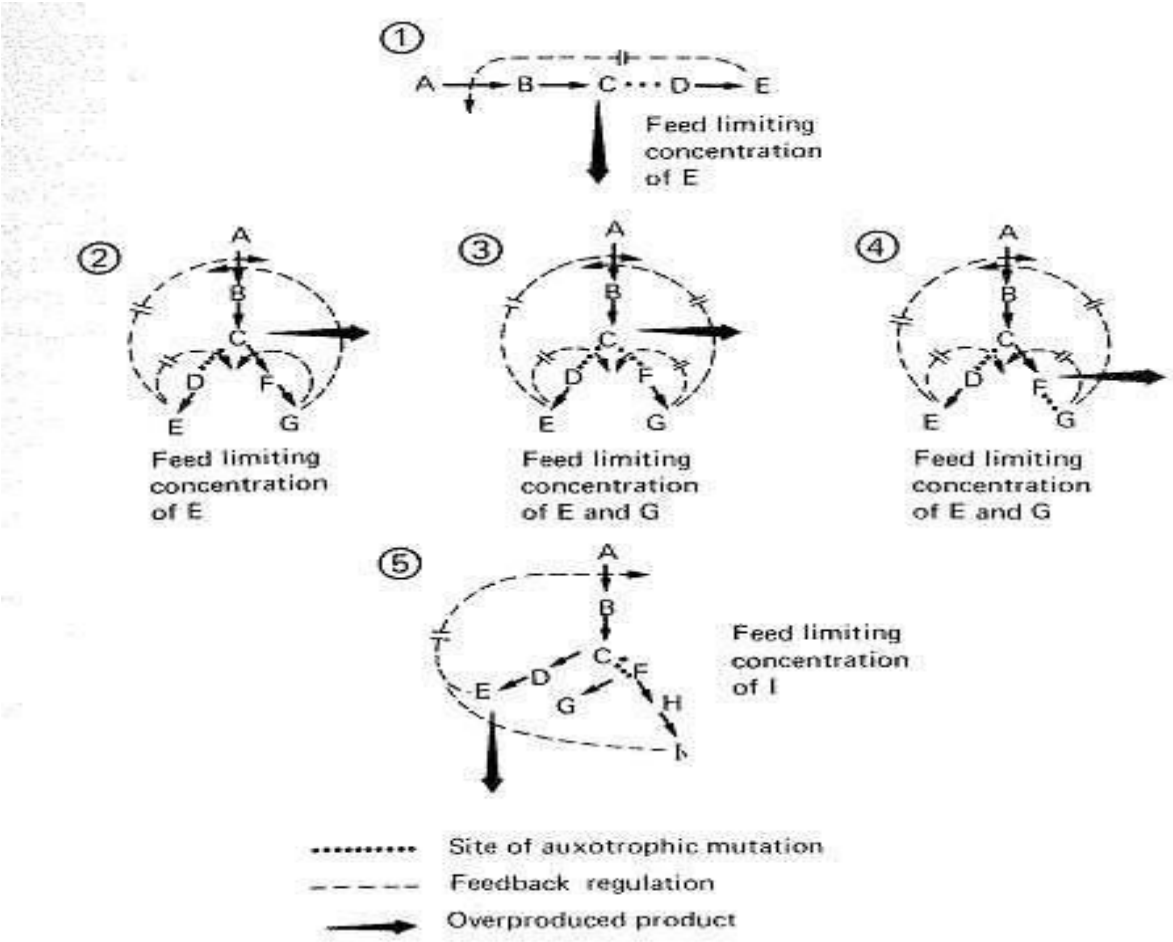


Fig.7 Overproduction of primary metabolites by decreasing the concentration of a repressing or inhibiting end product.

Examples of the use of auxotrophs for the production of primary metabolites

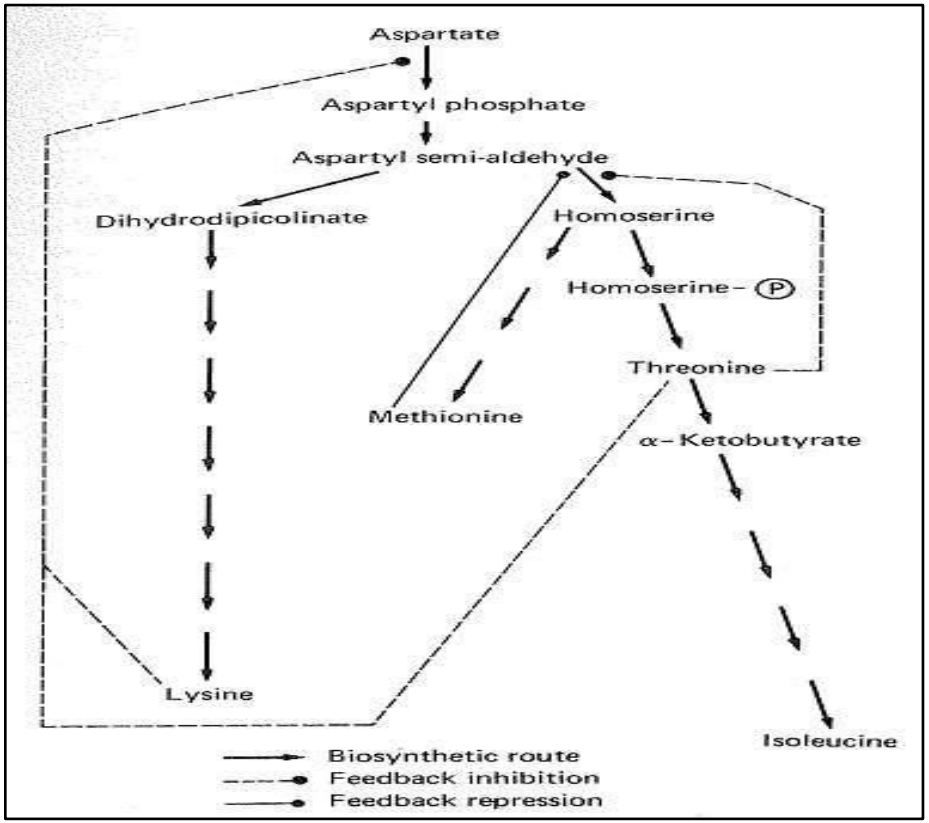


FIG. 8. The control of the aspartate family of amino acids in C. glutamicum.

The use of recombination systems for the improvement of industrial micro-organisms

• **Recombinant DNA techniques –** In simple words, rDNA technique can be explained as, bringing together in one organism, genes from several organisms, has the potential for not only increasing yields but also for producing entirely new substances.

• Recombinant DNA technology has resulted in organisms compounds which they were not able to produce previously.

The application of the parasexual cycle

•Many industrially important fungi do not possess a sexual stage and therefore it would appear difficult to achieve recombination in these organisms.

•However, Pontecorvo et al. (1953) demonstrated that nuclear fusion and gene segregation could take place outside, or in the absence of, the sexual organs.

•The process was termed the parasexual cycle and has been demonstrated in the imperfect fungi, A. niger and P. chrysogenum, as well as the sexual fungus A. nidulans.

The improvement of industrial strains:

•Although a strain may produce a very high level of a metabolite it would be unsuitable for a commercial process if its productivity were extremely unstable, or if the organism's oxygen demand were such that it could not be satisfied in the industrial fermenter available for the process.

•Therefore, characteristics of the producing organism which affect the process may be critical to its commercial success. Thus, it may be desirable to modify such characteristics of the producing organism which may be achieved by selecting natural and induced variants and recombinants.

Important characteristics of Strain improvement

- The selection of strains resistant to infection
- The selection of non-foaming strains
- The Selection of strains which are resistant to components in the medium
- The selection of morphologically favourable strains
- The selection of strains which are tolerant of low oxygen tension
- The elimination of undesirable Products from a production strain
- The development of strains producing New fermentation products

The selection of stable strains:

•The ability of the producing strain to maintain its high productivity during both culture maintenance and afermentation is a very important quality.

Example: Woodruff and Johnson (1970) selected a double auxotrophic mutant of **Micrococcus glutamicus requiring both** homoserine and threonine and compared its lysine-producing properties with those of a homoserine auxotroph.

The selection of strains resistant to infection:

Bacterial fermentations may be affected very seriously by phage infections, which may result in the lysis of the bacteria.

•A possible method for reducing of failure due to phage contamination is to select bacterial strains which are resistant to the phages isolated in the fermentation plant **(Hongo et al., 1972)**

The selection of non-foaming strains:

•Foaming during a fermentation may result in the loss of broth cells and product via the air outlet as well as putting the fermentation at risk from contamination.

• Thus, foaming is normally controlled either by the chemical or mechanical means, but this task may be made easier if a non-foaming strain of the commercial organism can be developed.

•The selection of morphologically favorable strains:

Backus and Stauffer (1955) recognized the influence of the genetic of a strain on the morphology of P. chrysogenum in submerged culture and its role in controlling foaming and broth filtration characteristics

The selection of strains which are tolerant of low oxygen tension:

Example, Mindlin and Zaitseva (1966) isolated a lysineproducing strain which maintained its productivity under aeration conditions which decreased the parental strain productivity by almost a half.

The elimination of undesirable products from a production strain:

• Athough an industrial micro-organism may produce large quantities of a desirable metabolite it may also produce a large amount of a metabolite which is not required, is toxic or may interfere with the extraction process.

• An example in the penicillin-producing strains is the elimination of the production of the yellow pigment, chrysogenein, selection of non-pigmented mutants which made the extraction of the antibiotic much simpler **(Backus Stauffer, 1955).**

- **The development of strains producing new fermentation products:**

- The isolation of organisms from the natural environment synthesizing commercially useful metabolites an expensive and laborious process.

- Therefore, means of producing novel compounds which may be some industrial significance have been attempted.