C H A P T E R - II

REVIEW OF LITERATURE

C H A P T E R – II

REVIEW OF LITERATURE

Carl Wehmer, the German Botanist, is credited for initiating the knowledge about fermentative production of carboxylic acids by molds. His researches (1891, 1893, 1918) have shown that several species of Aspergillus and Penicillium possessed this property. Felix Ehrlich (1911) found Rhizopus nigricans with fumaric acid producing potentialities. These researches represent pioneering studies and have been the basis for industrial production of certain carboxylic acids, using specific molds for fermentations. Since then, several investigations have been done to search source organisms, determine optimum conditions for fermentations at physical and physiological levels and also to improve the fermentation technology. Important contributions particularly those related to citric and fumaric acids have been reviewed below.

For the production of citric acid (CA), Aspergilli were first employed by Thom and Currie (1916); and it was only after the publication of Currie's excellent paper on the production of CA by A. niger in 1917 that the real work on CA production by fungal fermentations began.

The formation of these acids in substantial amounts has been reported in the case of only a limited number of fungi. The fungal species, in which one or more strains have been reported to accumulate CA, as given by Foster, 1949, include: *Citromyces sp.*, *C. pfefferianus*, *C. glaber*, *C. citricus*, *Aspergillus carbonarius*, *A. glaucus*, *A. clavatus*, *A. cinnamomens*, *A. fumaricus*, *A. awamori* Nakazawa, *A. awamori* var. *fumeus*, *A. aureus* Nakazawa, *Penicillium sp.*, *P. arenarium*, *P. olivaceum*, *P. divaricatum*, *P. sangifluus*, *P. glaucum*, *Mucor sp.*, *M. pyriformis* and the wood destroying fungus *Coniophora cerebella*. Some other fungi reported were *Penicillium luteum*, *P. citrinum*, *P. hannoli*, *P. citrogenum* and the plant parasite *Botrytis cinera* (Chrzaszcz and Leonhard, 1936); *A. luchensis* (Agnihotri, 1965); *A. gorakhpurensis* (Kamal and Shrivastava, 1976); *A. foetidus* (Kristiansen and Sinclair, 1979); the yeasts *Canadida lipolytica*, *C. citrica* and *Saccharomyces sacchari* (Toshiro and Toru, 1977; Masuda *et al.*, 1974; Briffaud and Engasser, 1979); and now *A. japonicus* (Karkashe and Sonar, 1983).

Organisms reported to produce fumaric acid (FA) similarly include *Rhizopus stolonifer* (Ehrlich, 1911); *Aspergillus fumaricus* (Wehmer, 1918); *R. niveus*, *R. shanghaiensis* and *R. tritici* (Takahashi and Sakaguchi, 1925); *R. japonicus* (Takahashi *et al.*, 1926); *Mucor stolonifer* (Butkewitsch and Federoff, 1929); *A. fumaricus* (Thies, 1930); *R. pseudochinensis* (Takahashi and Asai, 1933); *Penicillium griseo-fulvum* (Raistrick and Simonart, 1933); R. *oryzae* (Lockwood *et al.*, 1936; Lubowitz and La-Roe, 1958; and Soner and Karkashe, 1983); R. *nigricans* (Waksman and Foster, 1938 and Kane *et al.*, 1943); R. *japonicus* (Meder, 1952); and R. *arrhizus* (Rhodes *et al.*, 1959).

The potential organisms afterwards were investigated in detail to give the maximum yield of the product. These investigations were concerned with the determination of the optimum cultural factors, convertible substrates, conditions of fermentation and strain development aspects. The literature related to the studies of cultural conditions such as the nature and concentration of the carbon and nitrogen sources, mineral salts, pH, incubation temperature and period, has been extensively reviewed from time to time by Wells and Herrick (1938), Von Loesecke (1945), Perlman (1949), Prescott and Dunn (1959), Noyes (1969), Lockwood (1973), and Smith *et al.* (1974). The literature concerning the fungal production of *FA* has, similarly, been reviewed by Birkinshaw (1937), Lockwood and Moyer (1938), Foster (1949), Foster (1954) and Prescott and Dunn (1959).

The *CA* and *FA* producing organisms have been found to show different preferences in respect of the carbon and nitrogen sources. Foster and Waksman (1939-1943), Kane *et al.*, (1943) and Qureshi and Chughtai (1976) have observed that R. *nigricans* utilized many carbohydrates for *FA* production excepting the sucrose and sucrose containing raw materials. Same was the observation also of Lubowitz and La-Roe (1958) and Rhodes et al. (1959) who studied the production of FA by R. oryzae and R. arrhizus, respectively. Foster (1954) and Bilgrami and Verma (1981) suggested that, non-utilization of sucrose was due to the lack of biosynthetic ability for invertase in these organisms, which according to them is found through out the Mucorales. Among the nitrogen sources the Mucoraceous organisms have been shown not to utilize the nitrate nitrogen (Casida, 1968; Bilgrami and Verma, 1981). Rhodes et al. (1959) arrived at a general conclusion that the mineral salts such as $MgSO_4$ and KH_2PO_4 were required in lower amounts.

In their studies Diveker et al., (1971), Singh and Mehrotra (1972) and Kobun (1974) found that maximum yield of CA could be had with approximately 15% concentration of sugar and a lower concentration of nutrient salts. However, Uchio et al. (1978) observed that sugar hydrolysates gave better yields of CA than the pure sugars. Addition of manganese, iron and zinc enhanced CA production, as reported by Clark (1966), Smith (1974) and Wold and Suzuki (1976). However, Wold and Suzuki were able to regulate the production of CA by simply varying the concentration of zinc in the fermentation broth. It has been found that the trace element requirement varies with the fermenting organisms and the nature of the product of fermentation.

With a view to making the process commercially feasible, a number of crude carbohydrate sources have been tried. The crude carbohydrates studied and found suitable for fermentation in the recent past include: waste pineapple juice (on dried bagassae) (Kobun, 1974), brewery spent grain liquor (Hang et al., 1975), fructocoline (Macris, 1975), dried cane molasses (Vadehara et al., 1977), corn steep liquor and black strap molasses (Hamissa et al., 1977), sugar beet and date molasses (Tehrani and Fazeli, 1975), date extracts (Popov et al., 1977), bagassae (Chaudhary et al., 1978) and rye and wheat bran (Ilczuk, 1979).

A recent development in the production of organic acids is the use of emulsified hydrocarbons as the substrates. The organisms used for this purpose are, however, yeast or yeast like organisms (Lockwood, 1975). 72% yield of industrial grade CA from n-paraffin has been reported (Anonymous, 1976). Similarly, the possibilities of utilizing dodacane or kerosene has been examined (Nette, 1975).

CA fermentations presently are conducted both by sumberged and the stationary culture techniques. The latter though much older, is still widely practised, as the submerged aerated fermentation has not yet equalled the high level conversions of sugar to CA provided by the stationary fermentation. Very little is actually known about the commercial stationary tray-fermentation, for, the principal industrial concerns have not even obtained patents that might reveal the process details (Casida, 1968). Nevertheless, enough idea can be gained from the vast amount of published work dealing with the significant factors that can influence the yield of CA in surface or submerged cultures.

To improve the efficiency of fermentation was a prime concern of the industrial microbiologists. Lecin et al. (1978) have come out with a new method of sterilization of molasses where, a 30 min. pasteurization is followed by 30-45 min. treatment with 1% CaClOCl + 1% NH_4Cl, at 40-50°C.

Similarly, to reduce the period of incubation, either continuous culture method (Kristiansen and Sinclair, 1978) or replacement culture methods (Foster, 1954) have been recommended. In replacement cultures, used with great success in FA production particularly, after the growth has reached a peak, or the nutrient medium is exhausted, the mycelium is separated from the bulk of the liquid by decantation or by centrifugation (in case of submerged culture) and a fresh sterile sugar solution is supplied to the fermentation vessel and incubated in the normal way. Production of FA begins at once and at a much faster rate. Since only a small part of the substrate is converted to cell material a very high acid conversion yield resulted. Further, the single lot of mycelium can be used repeatedly with very little reduction in efficiency (Foster, 1954). Also, Feyrath (1966) has described a method in which the use of vermiculite granules has been found reducing the incubation time to about half the usual.

As the high cation impurities present in the crude carbohydrates often make it unsuitable for fermentation, a number of purification methods have been found. The crude carbohydrates can be purified and freed from the trace metals by ion exchange resins (Noguchi and Johnson, 1961) or by the addition of metal chelating agents such as ferrocyanide (Gerdhardt et al., 1946 and Suzuki et al., 1966) and EDTA (Choudhary and Pirt, 1966). Alternatively mutants relatively insensitive to high levels of trace metals could be developed (Perlman and Lin, 1960; Trumpy and Millis, 1963). Also certain organic compounds such as low molecular weight aliphatic alcohols (Moyer, 1953; Dhankhar et al., 1974; Hamissa, 1977), unsaturated lipids i.e. natural oils, olic acid etc. (Millis et al., 1963) and adenocyne 3'5'-cyclic monophosphate (CAMP) (Wold and Suzuki, 1976) could be used.

Efforts have also been made to solve some of the problems related to fermentation by developing mutants of desirable characters. Using UV-rays and ethylene amine as the mutagenic agents, Shcherbakova (1970) developed strains resistant to adverse environmental conditions. Mutants able to tolerate high levels of trace elements have similarly been developed, using nitroso-guanidine, by Chaudhary et al. (1974). Recently, Hannan et al. (1976) reported that high yielding strains of A. niger could be obtained by a stepwise √-ray induction. Also, Das and Nandi (1969) have reported that superior mutants, than those obtained by UV-irradiation, nitrogen mustard or ethylene amine treatment, could be obtained by -irradiation. Certain criteria of selecting the high yielding mutants from among the huge lot of substrains can also be found. Thus substrains showing high reduction activity (Kasatkina and Zheltova, 1963), showing slow growth rate (Bilgrami and Verma, 1981) or stains that are diploid (Ilczuk, 1979) can be expected to be superior to others.

In few cases certain uncommon treatments also have resulted in increased yields of CA. Two quarternary ammonium compounds, Triburon (trichlobisonium chloride) at the concentration level of 40 µg/ml has been found giving maximum yield of CA (Raman and Shanmugasundaram, 1963). Amongst the various volatile compounds present in molasses, HOAC was found stimulating the production of CA (Lakowska et al., 1977). Recently Shrivastava and De (1980) have found that ultrasonic waves in presence of 0.5% NH_4Cl could increase the yield of CA to as much as 52.4% in comparison to the control.