Overview across Green Production of Citric Acid

Abstract

Citric acid has a prominent position between the most highly pertinent biotechnological products in industrial demand. Multi-functional citric acid is well known of its applications, mainly in the food and nanotechnology drug delivery sectors. Improper disposal of the agro-waste causes a serious ecological threat by encouraging growth of microbial pathogens. The alternatives for utilization of such cellulosic biomass are therefore of great importance for cleaner production to reduce the cost of bioactive compounds production. Hence, pulps, seeds and peels wastes were used as feedstocks carbon sources for a citric acid-production. This review provides a brief summary describing microorganisms, morphology, self-immobilization, bioreactors variability, metabolic engineering, parameter optima and product recovery in order to highlight microbial citric acid production.

Keywords*: A. niger; glycolytic flux; biological carriers; Rotating Biological Contactor (RBC); immobilization; genetic engineered strains.*

Introduction

Citric acid (CA) possesses many important qualities as a green solvent, palatable, highly soluble and an extremely low toxic to human and mammals. Chemical synthesis of citric acid is more costly than fermentation, the second largest fermentation product. Industrial citrus processing or cashew apple juice extraction for example generates tons of wastes of peels and segment membranes that produce [1,2] odor and result in pollution from harmful chemicals which were used conventionally, and hence its handling is the most demanding target [3-5].

SUBSTRATES FOR CITRIC ACID PRODUCTION

Lignocellulosic biomass is the most abundant raw material on earth and could be used as renewable substrate for citric acid fermentation because it contains proteins, fats, fibers, and polysaccharide necessary for good acid fermentation like wheat bran for example, [6]. The chemical hydrolysis of cellulose and hemicellulose or the use of cellulolytic enzymes for the degradation of lignocellulosic biomass is an effective approach. “The cellulolytic liberation of sugars or pretreatment of solid substrate release peptides and amino acids from proteins and a mixture of fermentable sugars including glucose, mannose, xylose and arabinose”, [7], which could promote growth but dislike Ly generate in some substrates furfurals that inhibit biomass production.

Fruit wastes like shea nut shell, yam, sweet lime, banana and pineapple peels contain soluble sugar and pectin [8,9], Groundnut pod, pomegranate, rice bran [10,11], cassava, white star apple [12], cotton waste, pretreated milled peel moistened with co-substrate sucrose medium, banana pseudo-stem …etc. [13-19] are other examples for potential fermentation substrates using *A. niger* . “Similarly, *A. niger* as a world’s leading choice attractive production host excrete citric acid after growth on corn substrates because it is good source of nutrient sugary substrates for citric acid. Many microorganisms are able to ferment bagasse and blackstrap molasses in liquid-solid phase culture” [6] but fermenting beet and cane molasses result in lower citric acid production mostly due the presence of furfural and hydroxymethyl furfural that have been shown to be the main important fermentation inhibitors. Furfural inhibits central enzymes in glycolysis, e.g., hexokinase phosphofructokinase and triosephosphate dehydrogenase, [20]. Also, molasses contains high level of impurities so, pretreatment by centrifugation, potassium

ferrocyanide, cation exchange resins or activated charcoal in order to compete with cultures depend on utilizing simple refined sugars efficiently in glucose or sucrose synthetic medium.

Certain conditions of drastic nutrient imbalance in fungi, yeast, and bacteria produce CA in excessive amounts [21,22]. Traditionally, *A. niger, Penicillium janthinellum*, P. *restrictum, Trichoderma viride, Mucor pirifromis and Ustulina vulgaris,* produce the acid when cultivated on various traditional biobased feedstocks for fermentation. both natural and transformed strains of *Yarrowia lipolytica* able to produce CA using transesterification of oils obtained from seeds and glycerol-containing wastes of biodiesel industry [23]. Among other species that are reported to produce CA are *Bacillus licheniformis, and Corynebacterium* sp. Among Solid state fermentation (SSF) bioconversions, agricultural processing coproducts are coconut husk, coconut cake, sunflower cake, wet corn distillers' grains, or autoclaved grape pomace …etc., [24-26]. SSF aim to energy saving, and lower wastewater generation [27-29], taking into consideration low moisture levels reduced substrate availability to the fungus causing citric acid reduction while the high moisture content resulted in susceptibility to microbial attack. Enhanced aeration facilitates O2 and CO2 exchange between substrate matrices. Despite turnout *on A. niger* solid-state fermentation, commercial synthesis of citric acid has relied on submerged or surface fermentation, [15].

SCALE UP PROCESSING OF FERMENTATION

"As the submerged fermentation process is commonly employed technique for citric acid production, stirred tank bioreactor and airlift bioreactor both are generally preferred for this process" [30]. However, "different types of SSF bioreactors, including tray, packed-bed, rotating/stirred-drum, fluidized-bed, rocking-drum, and stirred-aerated bioreactors are used" [31].

In order to work up product isolation immobilization was used. "The main methods employed in fungi immobilization are self-immobilization in pellets and mycelium adhesion to a fixed or porous surface. The first method isbased on the natural tendency that some filamentous fungi species in submerged cultures develop morphology in pellets" (32f,33). Exopolysaccharides, which function as an adhesive substance, are a preferred method of fixation to a surface or supporting material in other fungal species. Encasing the fungal mycelium in the pores or intersections of fibrous materials such as calcium alginate gel, polyacrylamide gel, or on polyurethane foam using immobilized cell techniques generally leads to the reduction of their main drawback of central autolysis caused by diffusional limitations, improving nutrient and oxygen transfers, and enabling repeated batch and continuous processes. Immobilization physically trapped within porous solids or matrices allowing repetitive use of the immobilized biofilm without loss of bioactivity is also performed. Such techniques have several advantages, as higher volumetric production and, protects the cells from shear forces and imparts a special stability to the microorganism against environmental stresses in the waste stream. Mycelial pellets biocarriers are used for enhanced large-scale fermentation to decrease culture viscosity and improve oxygen and mass transfer efficiency. "As biomass density is inhomogeneous and is inversely proportional to pellet porosity leading to a limited accessibility for nutrients and consequently hinder substrate uptake, the diameter of the mycelial pellets or clumps should be controlled within an appropriate range to prevent cell lysis. Electrostatics, hydrophobicity and interactions between spore wall components are main triggers for pellet formation, [32]as in immobilized *A. nigr* cells on using date palm syrup" [33].

When the process was operated in a packed-bed reactor solid-state fermentation, the bed loading, the airflow rate, substrate and particle size were the most important operational parameters. "Output in pilot citric acid production is controlled by the mixing characteristics of the bioreactor. In tray type bioreactor or bio-fermenter, large quantity of inoculum is required but increasing biomass concentrations result in highly viscous fermentation media, resulting in issues with gas−liquid mass transfer, liquid mixing resulted in a complex rheology in *A.terreus"* [34,35]. Using rotating biological contactor that obtained with a stirred-tank fermenter improve stirrer rotational speed, and oxygen flow rate [36].

"RBC–PUF is a bio-functions integrating filter system capable of retaining considerable amounts of attached *A. niger* biomass with self-immobilized and biofilm growth. RBC disks coupled to PUF porous biomass support when coupled with a good oxygen transfer capability of the system, could provide

successful performance" [35]. RBC provides both acceptable effluent quality and a removal efficiency at a comparatively very low cost. Short contact periods are required because the large active surface biomass generally has good settling characteristics.,

The fluidized bed biofilm reactor (FBBR) represents a recent innovation in biofilm processes. When biomass was repeatedly used, the immobilized filamentous fungal pellet as small fluidized particles of the medium results in higher liquid throughputs and absence of biomass wash-out, [37].

A significant number of engineered strains have been developed showing higher performance, [1]. With the development of molecular biology techniques, it is possible to control the mycelial morphology at the desired molecular level [38]. Nowadays, more sophisticated techniques as genomic editing has been explored in order to improve their special capabilities or metabolic of *A. niger* to endure stressful conditions. Metabolic engineering has emerged as a powerful and widespread method for genome editing due to its advantages of transient genome editing and reduced off-target effects, ribonucleoprotein-based (CRISPR–Cas9) complex genome editing system consisting of Cas9 protein [39] has been shown to revolutionize strain construction faster and more reliable [38]. Also, genetically engineered strains by knocking out genes or gene silencing [40]. The silencing of chitin synthase gene (chsC) in *A. niger* interferes with morphogenesis and citric acid production as it was demonstrated that chsC [39] silencing caused the compactness of the mycelial pellets was obviously reduced in the morphological mutants, with lower proportion of dispersed mycelia, leading to improvement in citric acid production. "Genetic manipulation of the chitin synthase gene chsC might represent an effective method to obtain excellent traits for improving the production of the desired product during industrial filamentous fungal fermentation of economic significance" [41].

NUTRITIONAL PARAMETER FOR CITRIC ACID FERMENTATION BY A NIGER

"Citric acid concentration will rise to appreciable amounts only under conditions of substantive metabolic imbalances, membrane transport in *A. niger* emphasizing the roles of glycolytic flux and its control excretion of citric acid from the mitochondria and the cytosol, within critical fermentation variables" [20]. "Statistical models predict optimization under Response Surface Methodology (RSM) which is widely used for maximizing production. The highest levels of the extraction parameters from single factor experiments were considered to be the basal point for the design of the final citric acid production and the fermentation optimization experiment through Box-Behnken design (BBD)" [42-44]. Models main advantage is ability to reduce xxx (d)xxx number of experimental runs needed to provide sufficient information for statistically acceptable results due to multitudinous factors that jointly determine the acid yield, and their complex interactions using Response Surface Methodology (RSM).

Central composite design and, RSM were applied to determine the effects of nutritional limiting factors C, N, P sources, fermentation time and their reciprocal interactions for acquiring better fermentation efficiency, beside other physicochemical factors as volume, age of inoculum, incubation temperature, moisture process.(xxxx(and surface area to volume ratio)xxxx). Additionally, **other** biotechnological processes such as, agitation which induce acid production, type of inoculum, and culture pH to boost mycelium formation in submerged cultures hence the morphology of the fugus were also studied.

However, in industrial mass scale certain nutrients must be in excess as sugar and oxygen. Some studies report that limitation of the nitrogen source and deficiency of manganese or phosphate in the fermentation medium will inhibit the anabolism of *A. niger* resulting in protein degradation which, in turn, leads to a higher concentration of ammonium ion.

"Biomass production by the fungus can affect its citric acid production because the fungus consumes large quantities of sugar to support cell multiplication. The decrease in citric acid production was likely because of oxidation of citric acid upon exhaustion of the fermentable sugars". [45] Stated that sucrose can be readily attacked by the microbial intracellular enzymes, because of its low molecular weight in nutrient medium recording maximum citric acid production than the glucose added medium [17].

Divalent metal ion, pretreatment of fermentation medium and the addition of different low molecular weight alcohols affect the yield of citric acid. Ethanol resulted in inactivate crucial glycolytic enzymes and affect mitochondrial functionality. Furthermore, it also known to increase the permeability of the cell membrane and thus higher secretion of CA due to morphological changes and damaging the cell wall. Methanol addition stimulate a dehydrating action on the substrates, intensifies the outbound permeability of cellular walls for secretion of citric acid and reduce the inhibitory effects of some metal ions [46].

Many research reported the use of ANN in intelligent modeling for approximate media constituents in pilot scale citric acid production, [45,47] and it is thought that ANN and ANFIS have the capability to model and precisely predict the rapid changes and the final product yield [43].

Xxx (Methods used in biotechnology, including pH, temperature, inoculum type, moisture content, and other factors. methods used in biotechnology, including pH, temperature, inoculum type, moisture content, and other factors.) xxxxxxx

RHEOLOGICAL BEHAVIOR OF *A. NIGER*

The growth of *A.niger* was discovered to be diauxic, with spore germination followed by an exponential phase, a growth disturbance phase, and finally a secondary growth phase with a constant coefficient of growth decline. The chief increase in citric acid concentration took place in the last fermentation phase. The transient element act as nutrients should be in limiting concentrations Zn, Mn, Fe, ions and so are the heavy metals. Mn deficiency result in accumulation of citric acid by interfering with phosphofructokinase enzyme of TCA cycle. Oligo- elements contents affect mold morphology, e.g., copper ions have a direct influence on mycelial morphology, provoked pellet formation and increased volumetric productivity. Addition of some ions produce an antagonistic effect on deleterious action of iron and manganese ions.

EXTRACT RECOVERY OF CITRIC ACID

Although classical method is the most used technique on large scale processes, solvent extraction has been developed for the separation of citric acid. “The high energy cost, complex process, and heavy use of reagents hazardous to the environment in the conventional industrial separation, extraction, and production strongly call for modern technological innovation” [48]. Recovery technologies are processed by precipitation by typical downstream processing using calcium hydroxide to get calcium citrate and subsequent sulfuric acid to regenerate citric acid which then recovered and crystalized. Solvent extraction method as an alternative to citric acid classical large scale precipitation process has been developed. The reactive solvent extraction method from dilute aqueous broth by tri-n-butyl phosphate (TBP), tri-n-octylamine (TOA), and the surfactant Aliquat 336 (A336), is quite familiar [49,50]. Supercritical CO2 is another recommended solvent as a reactive extractant. Capturing supercritical CO2 used to convert calcium citrate to citric acid. Ion exchange, macro-porous adsorption process and the biological electrodialysis with bipolar membrane and ultrafiltration techniques are used for citric acid recovery to eliminate calcium sulfate [51]. Minimizing cost, saving experimental time, adjusting combined effect of the extraction process parameters; shaking speed, solvent ratio, and shaking time were done statistically with the help of experimental design BBD under RSM and a second order regression model [52].

CRYSTALLIZATION OF CITRIC ACID

The evolution of the crystal size and shape distribution during crystallization processes is an important task, as the latter decisively influences the physical and solid-state properties of crystalline material, i.e., crystallization kinetics nucleation and growth are the major steps which control the process [53]. The microstructures of crystalline citric acid from seeding in large seed population and the measuring the dimensions change of a single crystal in a microscopic cell at different supersaturation levels were reported using evaporative crystallization.i.e.CA crystallization **process in dense phase was done using a vibrated bed.** In de-supersaturation tank **, a complex cooling operation that involves hydrodynamic,**

thermal and mechanical phenomena take place. Both the measured de-supersaturation profiles and the time variations of the crystal size dimensions were explained by the simultaneous occurrence of nucleation and crystal growth. This involves two different populations of particles: seed crystals and particles issued from sustained secondary nucleation (53). The yield of citric acid crystals estimated gravimetrically and predictive models were characterized using X- ray diffraction, attenuated total reflection (ATR)- Fourier transform infrared (FTIR) spectroscopy [13].

CONCLUSION

In order to emphasize microbial citric acid production, this article offers a succinct outline of the following topics: microorganisms, morphology, self-immobilization, bioreactor variability, metabolic engineering, parameter optima, and product recovery.

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