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Citric Acid Production by *Aspergillus Niger* through Submerged and Solid-State Fermentation: An Overview

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Abstract

Currently, citric acid is produce through microbial fermentation using various microorganisms in three different techniques, viz. submerged culture fermentation (SmF), solid-state fermentation (SSF) and liquid surface fermentation (LSF). Majority of the present commercial production of citric acid is through submerged culture fermentation by using A. niger on by-product of sugar industry as substrate. However, lately, exploitation of solid state fermentation has demonstrated some prospective in becoming a substitute to submerged fermentation for the commercial production of citric acid. In view of finding an alternative fermentation technique for citric acid production which is more effective, fuel-efficient, labour-saving and economical than the existing ones, this review discusses a comparative account of solid-state fermentation and submerged fermentation.

Keywords: *Aspergillus Niger*, Citric Acid, Submerged Fermentation (Smf), Solid-State Fermentation (SSF)

Abbreviations: SSF: Solid-State Fermentation; LSF: Liquid Surface Fermentation.

Introduction

Citric acid, a weak organic tri-carboxylic acid is naturally present in all citrus fruits and many other fruits and vegetables. Being one of the most important products which have broad applicability in food and beverage, pharmaceutical, cosmetic and various other industries, its demand is continuously growing in global market. The global market value for citrus extract was USD 2.6 Billion in 2014 and is anticipated to grow further, as predicted by West TP [1]. Citric acid and its derivatives are used as preservative, tenderizer, emulsifier, acidulant, flavorant, antioxidant, buffering agent, sequestrant, synergistic agent, chelating agent and plasticization in many food and other industries Auta HS, et al. [2], West TP [1]. Because of its environmental-friendly characteristics citric acid has also been used for removing solder flux residue Robin N, et al. [3] and used in manufacturing cleansers and detergents West TP [1]. Recent studies explored the possible use of citric acid as biopolymers for drug delivery and cell culture and implantation engineering [4,5].

Citric acid can be extracted from natural sources such as citrus fruits and synthetic sources such as chemical reaction and microbial fermentation [6]. Citric acid production through microbial fermentation is one of the most primitive techniques and still it is the most commonly used technique for commercial production. Since 1893, when Wehmer first observed the possibility of citric acid production by microbial fermentation, a great number of studies have shown that various fungi and bacteria are capable of producing citric acid through fermentation. However, the major breakthrough was made by James Currie in 1916, where large amount of citric acid was obtained through fermentation using diverse strains of *Aspergillus niger* [6]. This study inspired the industrial exploitation of A. niger for citric acid production which ultimately led to the development of modern-day effective large scale industrial production Dhillon GS, et al. [7], Show PL, et al. [6]. Numerous microorganisms have been appeared to yield citric acid from sugars and n-paraffins yet, A. niger has continually given the best outcomes in modern-day citric acid manufacturing Ho L, et al. [8] Papagianni M [9]. Characteristics such as capability to ferment diverse raw materials, easy to handle, and high yielding that makes A. niger dominant to other citric acid producing microorganisms [6,7]. Furthermore, researchers have also developed improved strains of A. niger through mutagenesis and strain selection specifically for overproduction [6,9]. Such strains are reported to produce more than 70% of the theoretical yield on the carbon source Papagianni M [9]. Microbial fermentation is a multifaceted biochemical reaction that needs absolutely customized and modulated condition. Several factors such as concentration and type of carbon source, pH of the medium, phosphate and nitrogen limitations, aeration, morphology of the microorganism used and concentrations of the trace elements affects the process. Furthermore, low or limited trace elements like manganese, phosphate and nitrogen and high oxygen and sugar content in the medium also favours the fermentation process Show PL, et al. [6], Soccol CR, et al. [10], Max B, et al. [11].

On account of its broad implementations in various industries, the demand for citric acid is ever increasing. In pursuance of meeting this requirement, many great efforts have been made by researchers all over the world to increase citric acid production West TP [1]. Resulting from these efforts, over the years, the world's citric acid production has significantly increased. However, in the present scenario, there is a pressing need to find an alternative technique which is more efficient than the existing ones, environmental friendly and cost-effective. Although, 80% of the world's citric acid production is yielded through SmF technique, this technique is not environmental friendly and cost-effective. On the one hand, SSF has the edge over the SmF technique West TP [1], Dhillon GS, et al. [7], Behera BC [12], as it can utilize diverse agro-industrial by-product or wastes as substrates, which have already poses threat to environment by their disposal, low operational cost, release inconsequential amount of effluent, etc. SSF could be the alternative technique that the current situation needs, as this technique is far more effective, economical and environmentally safe West TP [1], Dhillon GS, et al. [7], Behera BC [12]. However, consistent endeavors in screening and selection of suitable substrates and strains, generating new efficient strains through genetic engineering or mutation breeding, optimization of various biochemical and process parameters, etc., would be required to create SSF as a sustainable technique for citric acid production by *Aspergillus niger*. Making a deep comparison between these two types is the main goal of this review.

Submerged Fermentation

Submerged fermentation (SmF) is the process of growing microorganism in liquid broth (substrate) containing nutrients for the production of enzymes or organic acids. It requires meticulous selection of suitable microorganism and cultivation in nutrient rich fermentation medium with high oxygen concentration in closed containers. Currently, citric acid is commercially produced in large-scale through submerged fermentation (SmF) technique utilizing A. niger. SmF is generally the most favoured fermentation technique as it needs minimum space and labour to run and gives high yield Mattey M, et al. [13]. It has been reported that 80% of world's citric acid production is through SmF West TP [1], Behera BC [12], Vanderberghe LPS, et al. [14]. However, Darouneh E, et al. [15] reported that as far as yield and efficiency, surface fermentation is better than SmF.

Substrate

Generally, beet and cane molasses, fruit pulp, polysaccharides, pomegranate and sugars are utilized as substrate (carbon source) for citric acid production in SmF Papagianni M, et al. [9], Roukas T, et al. [16]. However, there is report of production of citric acid using other substrate such as orange peel through autohydrolysis Rivas B, et al. [17], Aidynova R, et al. [18]. Substrate consisting of a mixture of cane molasses and pumpkin also established to be a better and promising medium for the production of citric acid Aidynova R, et al. [18], Majumdar L, et al. [19].

Jianlong W, et al. [20] created a novel method of citric acid production using beet molasses combined with in-situ product separation by ion-exchange resin adsorption. In comparison to ordinary batch technique, this new method enhanced the final output from 0338 g/l to 0.543 g/l and the sugar conversion from 82.2% to 94.8%. Dhillon GS, et al. [21] described that apple pomace ultrafiltration sludge-1 (APS-1) used as substrate provide greater citric acid production by A. niger NRRL 567 (9.0 ± 0.3 g/l substrate) and NRRL 2001

(8.9 ± 0.3 g/l substrate) in SmF. Furthermore, augmenting the APS-1 with 3% ethanol and 4% methanol (v/v) further increased the A. niger NRRL 567 yield up to 18.2 ± 0.4 g/l and 13.9 ± 0.4 g/l substrate, respectively.

Comparison to other substrates, molasses is preferred because, being a waste product it is low cost, and the required minerals and organic and inorganic compounds for the fermentation process are also present. However, before fermentation the molasses is required to be pre-treated by appropriate methods as the organic and inorganic constituents may hinder the fermentation process Aidynova R, et al. [18], Angumeenal AR, et al. [22]. Some of the molasses pre-treatment for increased production of citric acid comprises of treatment with ferrocyanide Baby EA, et al. [23], sulphuric acid, tricalcium phosphate, tricalcium phosphate with HCl, tricalcium phosphate with HCl followed by sephadex fractionation Kundu S, et al. [24], ammonium oxalate followed by diammonium phosphate treatment Angumeenal AR, et al. [25], Mehyar GF, et al. [26] used date molasses strengthen with whey, methanol and tricalcium phosphate for acetic acid fermentation by two A. niger strains.

For procurement of a favourable output, it is vital to provide an appropriate culture medium that includes sucrose (approximately 200 g/l) and limited supply of mineral salts such as iron, zinc, copper, magnesium, manganese and phosphate. In addition, the fermentation process should be carried out in an optimal temperature (25-27ºC) and provide continuous aeration Ho L, et al. [8], Aidynova R, et al. [18].

Nutrient Optimization

The final yield is determined by the constituents of the medium and the strain of the microorganism that has been used. Many studied had been made regarding optimization of various constituents of fermentation medium according to the specific strain of the microorganism used. Owing to excessive oxygen requirement throughout the fermentation process for citric acid production, the concentration of dissolved oxygen in the medium is a key factor that affects the final yield. Jianlong W [27] and Yu D, et al. [28] described that use of n-dodecane as an oxygen-vector with a final concentration of 5% (v/v) and methanol and sweet potato vine hydrolysate as enhancers, enhanced citric acid production by A. niger by a factor of 1.4-fold. Furthermore, it also stimulated the mycelial growth and increased mycelial dry weight by 15% Yu D, et al. [28].

Kurbanoglu EB [29] Used ram horn hydrolysate as supplement (inducer) in fermentation medium for the enhancement of citric acid production by A. niger NRRL 330. A maximum value (94g/l) was obtained by adding ram horn hydrolysate with a final concentration of 4% (v/v). Ikram H, et al. [30] reported that used of 0.20 µg/l vermiculite as an additive into the fermentation medium (blackstrap molasses) resulted in higher yield of 146.88 g citric acid monohydrate/l by A. niger NGGCB-101. Based on statistical designs, Lofty WA, et al. [31] implemented an optimization strategy to increase citric acid production by A. niger in submerged fermentation using beet molasses and corn steep liquor. It has been reported that by this near ideal medium preparation the citric acid output was 5-folds higher.

The amount of trace metal ions present in the fermentation medium can also substantially affect the citric acid production by A. niger. As A. niger is highly sensitive to metals ions like zinc, manganese, iron and magnesium, concentration of these ions in the medium can affect the final yield, particularly in submerged fermentation West TP [1], Soccol CR, et al. [10], Hang YD, et al. [32], Adham NZ, et al. [33], Ali S, et al. [34], Ikram H, et al. [35], Jiang C, et al. [36]. Various metal ions augmented with fermentation medium act as stimulants to the metabolism A. niger and rendered better citric acid yield. Improved nutritional value of pre-treated molasses medium with transition metal ions such as Cr, Fe, Co, Ni, Cu, Mo, Cd and Pb triggered early germination and rapid multiplication of A. niger. Among these metal ions, nickel proved to be the best stimulant by yielding 269 mg/l citric acid Angumeenal AR, et al. [25]. Guilherme AA, et al. [37] reported that the optimum experimental condition for A. niger NRRL 2001 for higher production of citric acid was 7.0 mg/l of Fe+3 and 6.5 mg/l of Zn+2 in the absence of Mn+2.

Strain Selection

Selection of suitable strain through mutation breeding of citric acid producing microorganisms for maximum production is as important as optimization of components of fermentation medium. Over the years, many researchers have created, screened and selected suitable strains or mutant through mutation West TP [1], Behera BC [12], Roukas T, et al. [16], Aidynova R, et al. [18], Yu D, et al. [28], Ali S, et al. [34], Chetan DM, et al. [38], Wang B, et al. [39] produced three mutant strains through UV-induced mutagenesis of A. niger GCB 75, among them the mutant GCB 45 was observed to be the citric acid overproducer. GCB 45 has been further subjected to nitroglycerin (NTG) for mutation and obtained three deoxy-D-glucose-resistant mutants. Out of these three mutants, GCM 7 yielded 86.1 ± 1.5 g/l citric acid in black strap molasses which was pre-treated with potassium ferricyanide and sulphuric acid. Likewise, Ali S, et al. [40] reported that A. niger GCBT7 strain produced 99.56 ± 3.5 g/l citric acid in black strap molasses. Lofty WA, et al. [31] derived 15 mutant strains from preexisting strain A. niger UMIP 2564 through UV-radiation, ethyl methane sulfonate (EMS) and acridine orange (AO). Among which, 8 mutants were found

to be improved regarding citric acid overproduction in batch cultures using sugar.

A new mutant strain of A. niger HW2 from preexisting A. niger H4002 strain through carbon ion irradiation was obtained by Hu W, et al. [41]. It was reported that the new mutant HW2 cultivated in corn starch can accumulate 118.9 g/l citric acid, which was 18% higher in comparison to the original strain. Moreover, the sugar utilization in the fermentation process decreases up to 12.5%. Furthermore, it has also stated that physiological characteristics of conidia of A. niger induced by carbon ion irradiation were linked with citric acid accumulation. Haq IU, et al. [42] made a comparative study of two strains A. niger GCB-47 (parental strain) and A. niger GCMC-7 (mutant strain) in term of their citric acid producing capability using raw starch as fermentation medium. The result revealed that the mutant strain yielded 1.48-fold higher citric acid in comparison with the parental strain.

Adeoye AO, et al. [43] screened five wild strains of A. niger and five mutant strains, which was generated from the wild strain, for their efficiency for greater citric acid production with optimized cassava peel substrate in submerged state. A. niger FUO 2 wild strain was observed to be the best with 1.93 g/l citric acid yield, among all the wild strains and A. niger FOU 110 mutant strain the best among the mutant strains screened with 9.4 g/l citric acid yield. Further, FOU 110 mutant strain with optimized substrate parameters that contributes to greater yield, gave 88.73 g/l citric acid yield, which was 45.97-fold than the yield of FUO 2 wild strain.

Submerged fermentation needs more advanced establishment, higher power cost and meticulous management, and development of foam. However, it gives higher output and is cost-effective by minimizing maintenance and manpower expenses, and has very little contamination risks Dhillon GS, et al. [7]; Show PL, et al. [6]. Besides, it is less sensitive to change in the medium constituent, whereby conferring a broad choice of substrates and appropriate management of substrates. These desirable characteristics of SmF make the preferred fermentation technique and molasses as the suitable substrate for the production of citric acid Max B, et al. [11]. Even though SmF can be performed in batch, fed batch or continuous method, batch mode is the most preferred technique. Generally, SmF is completed within 5-12 days, depended on the state of the fermentation process Dhillon GS, et al. [7], Soccol CR, et al. [10].

Solid-State Fermentation

Solid-state fermentation (SSF) is the process of growing microorganisms on solid substrates which is completely or almost devoid of free-flowing water for the production of organic acids, enzymes, secondary metabolites, etc. It concerns cultivating microorganisms on solid substrate that hold adequate amount of moisture, which also serves as carbon and energy source. SSF is considered as the easiest and straight-forward fermentation technique for citric acid production Kolicheski MB [44], Pallares J, et al. [45], Vandenberghe LPS [46]. In recent past, SSF, with its many advantages, caught the interest of many researchers as it provides an alternative technique from surface and submerged fermentation for the large-scale industrial production of organic acids, enzymes, etc. West TP [1], Behera BC [12], Roukas T, et al. [16], Aidynova R, et al. [18], Chetan DM, et al. [38], Wang B, et al. [39], Shojaosadati SA, et al. [47].

The most important benefits of SSF are its better yield and the potential to use economical and generally accessible agro-industrial wastes as substrates that is similar to the natural environment for many microorganisms, provides an alternative ecologically friendly approach in commercial citric acid production West TP [1], Gowthaman MK, et al. [48], Falony G, et al. [49]. In the process, SSF techniques transform a formerly low value material into a valuable one. Low water consumption, effective oxygen circulation, lesser operational expenses, not requiring elaborate and sophisticated setup, fewer post-recovery waste, lower contamination risk are some of the additional advantages of SSF technique Show PL, et al. [6], Durand A [50], Robinson T, et al. [51], Holker U, et al. [52], Susana RC, et al. [53]. Furthermore, unlike in SmF, substrate pretreatment with trace metal ions is not needed as it may not unfavorably influence the output of citric acid Soccol CR, et al. [10], Berovic M, et al. [54]. However, there are also some shortcomings in this method such as its inability to take advantage of utilizing the accessible nutrients present in the substrate thoroughly due to low heat and oxygen transfer Behera BC [12], Sangsurasak P, et al. [55].

Though, various microorganisms can be used in SSF for citric acid production, the most widely utilized and recognized to be the most suitable is A. niger Soccol CR, et al. [10]. In order to get an optimum yield, the solid substrate need to be 70% moistened (which depends on the absorption capacity of the substrate), a primary pH within 4.5 to 6.0, and incubation temperature of 28-30ºC (which depends on the microorganism used) Kolicheski MB [44]; Vandenberghe LP, et al. [56]; Vandenberghe LPS, et al. [57]. Usually, under favourable conditions, SSF process completes in 4 hours Drysdale CR, et al. [58].

Substrate

The solid substrates utilized in this fermentation process are mostly of organic raw materials comprising agricultural

and agro-based industrial waste, synthetic substance, etc. Pandey A [59]. By-products of agro-based industries like cotton waste, kiwi fruit peel, corn husks, date pulp, carob pods, wheat bran, soybean meal, pineapple waste, apple pomace, brewery spent grain, citrus waste, sphagnum peat moss, ultrafiltration sludge and solid waste, banana peel, orange peel, rice straw, sweet potato, potato, taro waste, and corn cob Max B, et al. [11], Roukas T, et al. [16], Aidynova R, et al. [18], Kiel H, et al. [60], Hang YD, et al. [61], Hang YD, et al. [62], Assadi MM, et al. [63], Alani F, et al. [64], Sauer M, et al. [65], Kareem S, et al. [66], Torrado AM, et al. [67], Yanti Y, et al. [68], Arshad Z, et al. [69], Addo MG, et al. [70] Although, inert medium can also be used as substrate, it need to be added with nutrients and carbon source Dhillon GS, et al. [21], Chetan DM, et al. [38].

Selection for suitable substrate is an important feature of SSF, as the solid substrate provides physical support as well as nutrients to the growing microorganism. Generally, substrate with larger particles is used for greater citric acid yield as it facilitates better aeration, however, it offers less surface area to the microorganism to grow. On the other hand, substrate with smaller particles provides greater surface area but posed limitation to aeration due to lack of inter-particle space. Hence, it is essential to use a combination of larger, as well as smaller particles size for the optimization of substrate for higher yield. Substrates may or may not require appropriate value addition depending on the desired final product Pandey A [59].

Addo MG, et al. [70] improved corn cob substrate employing one-factor-at-a-time optimization method for greater production of citric acid by A. niger KA88. Maximum yield (138.24 g anhydrous citric acid/kg dry corn cob) was obtained by optimizing the substrate at 0.5-1mm and 3-5mm particle sizes, with 15% (w/v) sucrose, 4 g/l di-ammonium hydrogen phosphate as nitrogen source, and incubating at 28ºC for 6 days. Vandenberghe LPS, et al. [57] studied cane bagasse, cassava bagasse and coffee husk for their effectiveness as substrates in greater citric acid production by using A. niger. Among the three substrates studied, cassava bagasse proved to be the best substrate that is highly suitable for vigorous mycelium growth and gives highest citric acid yield. A. niger NRRL 2001 cultivated on corn husks pre-treated with dilute 0.5 mol/L NaOH and Rapidase Pomaliq yielded 259 ± 10 g/kg dry corn husks at 120 h incubation time at 30ºC Hang YD, et al. [62].

Roukas T [71] reported that A. niger ATCC 10577 cultivated on fig fruits as substrate produced 64 g/kg dry figs. Furthermore, incorporation of 6% (w/w) methanol into the substrate enhances the accumulation of citric acid 64 to 490 g/kg dry figs. Kumar D, et al. [72] investigated citric acid production by A. niger DS 1 utilizing cane bagasse with three different moistening agents viz. sucrose and clarified and non-clarified molasses. Sucrose was observed to be the best in term of citric acid production among the three moistening agents. It enables A. niger under optimum state to produce 20.2 g citric acid/100 g of dry solid which is 69.6% yield on the basis of consumption of sugar. It has been suggested that in comparison with wheat bran, bagasse is better suited for greater yield as it does not agglomerate following moistening, which results in appropriate heat and mass movement in the course of fermentation process.

In order to find an economical and new substrate, Dhillon GS, et al. [21] evaluated four different substrates viz. apple pomace, brewery spent grain, citrus waste and sphagnum peat moss for a feasible and sustainable production of citric acid by A. niger NRRL 567 and NRRL 2001 through SSF. It was reported that among all the substrates tested, apple pomace was found to be the most suitable substrate for citric acid production by *A. niger* NRRL 567 (66.0 ± 1.9 g/kg dry substrate) at an incubation period of 72 hours. *A. niger* NRRL 2001 followed closely with marginally lesser citric acid yield (61.0 ± 1.9 g/kg dry substrate) at similar incubation period. The citric acid yield of A. niger NRRL 567 was further significantly enhanced by supplementing 3% ethanol and 4% methanol (v/w) to the substrate (apple pomace) up to 127.9 ± 4.3 g/kg and 115 ± 3.8 g/kg dry substrate, respectively.

Nutrient Optimization

The selection and optimization of other process parameters for instance, humidity, substrate pre-treatment, aeration, nutrient supplements, incorporation inducers, inoculum age, dose and storage time, etc. is also an important step in fermentation process as these factors can significantly affect the citric acid yield West TP [1], Jiang C, et al. [73]. Hang YD, et al. [74] observed significant increase in citric acid yield (603.5 ± 30.9 g/kg dry corn cobs) by A. niger grown on corn cobs as substrate which was pre-treated with dilute NaOH and commercial enzyme Rapidase Pomaliq. Dhillon GS, et al. [75] reported A. niger NRRL 567 yielded 220.6 ± 13.9 g citric acid/kg dry solid when grown on optimized substrate (apple pomace) with 3% (v/v) methanol, 1 hour intermittent agitation at 2 rpm for every 12 hrs and 1 vvm of aeration at 120 hrs incubation period. Yadegary M, et al. [76] described that pre-treatment of cane bagasse with NaOH and acid (acetone) resulted to enhance citric acid yield, 97.81 g/kg and 87.32 g/kg dry cane bagasse, respectively.

Concentrations of nutrients contain in the nutrient solution which is used for moistening various dry substrate for SSF may also affect the final citric acid yield significantly. Kim JW, et al. [77] optimized the initial sugar (glucose), nitrogen, phosphorus and NaCl concentrations in nutrient solution which is utilized for moistening peat moss for better

production of citric acid by A. niger NRRL 567 strain. Highest citric acid yield (82 g/kg dry peat moss) was obtained with 967.9 g glucose/kg^{-1}, 15.4 g $(NH_4)_2SO_4$/kg^{-1}, 43.9 g KH2PO4/kg^{-1} and 4.0 g NaCl/kg^{-1} in the nutrient solution.

Strain Selection

Selection of specific efficient strain for a particular profitable product yield is a meticulous exercise. Choosing an appropriate strain for a certain purpose relies on various elements, specifically on the characteristics of the substrate and the ambient condition Aidynova R, et al. [18], Chetan DM, et al. [38], Pandey A [59]. Therefore, careful selection of strains is an imperative measure in citric acid production through SSF technique by using A. niger, because no two different strains respond similarly to the same condition.

Tran CT, et al. [78] reported that A. niger ACM 4992 strain produced greater citric acid yield (19.4 g/100 g dry fermented pineapple by-product) than the ACM 4993 and ACM 4994 strains. Alani F, et al. [64] studied a newly isolated A. niger strain and a mutant strain A. niger G4, which was selected from the new strain by giving four successive gamma ray radiation, for their efficiency for the production of citric acid using carob pods as substrate. It has been reported that after 7 days, the parental and mutant strain produced 34 and 64 g/kg citric acid, respectively which was considered as highest. However, it has been observed that after adding 2% methanol the yield was increased up to 42 and 65 g/kg for parental and mutant strain, respectively. Furthermore, incorporation of 0.01 mg/l Cu, 0.15 mg/l Fe and 0.1 mg/l Zn, further enhance the yield of parental strain to 46 g/kg and mutant strain to 73 g/kg.

Large-scale production of citric acid is very taxing then laboratory production. For large-scale or industrial scale production, a large amount of spore suspension or culture is needed as inoculum. The development of spore suspension in such substantial amount is not an easy task Alam MZ, et al. [79]. The quantity or doses of spore suspension or culture inoculum used can significantly affect the final output. Besides, there are other factors such as age and storage time of spore and culture inoculum that can also affect citric acid yield. Alam MZ, et al. [79], Ikram H, et al. [80] studied the above-mentioned factors of inoculum that can affect citric acid production by A. niger on oil palm empty fruit bunches as substrate. It has been described that 3 to 4 days old spore are ideal for making spore suspension for inoculation of substrates and the limited storage period of spore suspension is 2 days. For the preparation of optimum culture inoculum, 20% (v/v) of spore suspension was found to be the perfect quantity. Culture (biomass) inoculum of 48 hours old provides higher citric acid production, however, 7 days old culture inoculum at 0.21 g biomass/kg substrate, gives the highest (367.4 ± 2 g/kg substrate) citric acid yield.

Dhillon GS, et al. [7] suggested that citric acid production can be elevated significantly through improving bioreactor designs by introducing suitable automation to the processes of SSF. Moreover, the practical utilization of renewable and amply obtainable agro-based industrial waste and enhancement of various parameters of the SSF technique may culminate to an environmental friendly and cost-effective manufacture of citric acid West TP [1], Behera BC [12].

Conclusion

A careful evaluation of current available literatures on SmF and SSF by several researchers has shown that SSF technique of citric acid production has competitive advantages over SmF. SSF does not require sophisticated and elaborate setup, waste materials or by-products of various agro-industries can be utilized as substrates, low water consumption resulting in release of almost negligible amount of effluent, effective oxygen circulation, lower contamination risks, cost-effective, gives higher yield in comparison with SmF. SSF can be develop as an alternative efficient, profitable and eco-friendly fermentation technique. In order to achieve this, further investigations are needed for the selection of suitable A. niger strain from naturally existing isolates or generation of mutant strains for different substrates and optimization of various biochemical and physical parameters which is conducive to greater citric acid yield.

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