Biotechnological production of itaconic acid and its biosynthesis in *Aspergillus terreus*

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Abstract More than 80,000 tons of itaconic acid (IA) is produced worldwide each year and is sold at a price of around US$ 2/kg. The IA production yield from sugar is higher than 80 g/l. The widespread use of IA in synthetic resins, synthetic fibers, plastics, rubbers, surfactants, and oil additives has resulted in an increased demand for this product. However, at present, the IA production capacity exceeds the demand because this product has a restricted range of applications. Studies have been actively conducted in different biomedical fields—dental, ophthalmic, and drug delivery—to extend the range of applications of IA. Recently, many researchers have attempted to replace the carbon source used for microbial production of IA with cheaper alternative substrates. However, there is still a need for new biotechnology innovations that would help to reduce the production costs, such as innovative process development and strain improvement to allow the use of a low-quality carbon source. In this short review, we discuss the following aspects of IA production: strain improvement, process development, identification of the key enzyme *cis*-aconitic acid decarboxylase (CAD) in the IA metabolic pathway, metabolic importance of CAD, and new applications of IA.

Keywords Itaconic acid · *Aspergillus terreus* · *cis*-Aconitic decarboxylase · Biorefinery

Introduction

Itaconic acid (IA) is a promising organic acid. It is a white crystalline unsaturated dicarbonic acid in which one carboxyl group is conjugated to the methylene group and has a molecular weight of 130.1. IA is used worldwide in the industrial synthesis of resins such as polyesters, plastics, and artificial glass (Kin et al. 1998) and in the preparation of bioactive compounds in the agriculture, pharmacy, and medicine sectors. There is continued interest in developing biological methods to produce compounds with double bonds that are suitable for the manufacture of various polymers. IA also provides possibilities for selective enzymatic transformations to create useful polyfunctional building blocks (Ferraboschi et al. 1994).

IA was originally discovered as a product of pyrolytic distillation of citric acid. Kinoshita (1932) observed that an osmophilic strain of green *Aspergillus* species, which had been isolated from dried salted plums, formed IA. *Aspergillus terreus* was isolated as an IA-producing microorganism, and one strain (NRRL 1960 = ATCC 10020) was isolated by extensive screening. In 1955, industrial IA production by submerged fermentation was initiated by Pfizer Co. Inc. in their Brooklyn plant (Pfeifer et al. 1952). Subsequently, other plants were established in England, France, Russia, and Japan. Kobayashi (1967) and Kobayashi and Nakamura (1964) established a complete process for IA manufacture using *A. terreus* derived from ATCC 10020. Since the discovery of

IA by Kinoshita, many attempts have been made during this century to improve the economics of this process, and as a result, optimized industrial processes have been established. The main development in IA production was batch fermentation with free suspended biomass. However, the economic and environmental circumstances around IA production have drastically changed in recent times due to increasing concerns regarding sustainability, environmental conservation, cheaper alternative substrates, and rising energy costs. Therefore, new biotechnological methodologies involving fermentation processes and technologies that use alternative cheap substrates as the carbon source are currently under investigation and development.

Strain improvement of *A. terreus* by mutagenesis

Several types of microorganisms have been used for IA production, as shown in Table 1. To date, there have been a few reports on strain improvement for IA production. Kobayashi and Nakamura (1964) reported that IA production was suppressed during cultivation since the growth of *A. terreus* was significantly inhibited by the IA produced. The IA production rate of *A. terreus* IFO 6365 drastically decreased in the presence of IA concentrations higher than 20 g/l (Yahiro et al. 1995). To overcome such product inhibition, it is preferable to select an IA-resistant mutant strain that would give high IA yields. A high IA-yielding strain was isolated on an IA concentration-gradient agar plate after *N*-methyl *N*′-nitro-*N*-nitrosoguanidine (NTG) treatment. Six hundred and seventy colonies that appeared in a high IA concentration region were picked, and their IA

85 productivity was evaluated by a series of screening procedures. The mutant strain that
86 produced more than 65 g/l of IA was selected as the most promising high IA-yielding
87 producer, and it was designated TN-484 (Yahiro et al. 1995). This mutant strain was
88 evaluated as shown in Table 2. For commercial IA production, the yield based on the
89 amount of glucose consumed is a very important economic consideration because the
90 cost of the carbon source is reported to be more than 25% of the total production cost
91 (Kobayashi and Nakamura 1964; Rober and Kubicek 1996). The IA yield as a function
92 of the amount of glucose consumed remained at more than 0.54 (g IA/g glucose
93 consumed) in spite of the increase in the initial glucose concentration, which seems to
94 be advantageous for the commercial production of IA. Moreover, the morphology of
95 TN-484 was different from that of the parental strain; the size of the pellet mycelium
96 was smaller than that of the parent strain, as a result of which the viscosity of the culture
97 broth was maintained at low levels and IA productivity was improved. Industrially,
98 more than 85 g/l of IA was produced by this strain in a 100 kl-scale fermentor using a
99 simple medium consisting of glucose, corn steep liquor, and small amounts of minerals
100 (Role 1997).

101 **Development of an economic process for IA production**

102 Due to increasing production costs, the fermentation industry is finding it difficult to
103 produce antibiotics, amino acids, and organic acids at internationally competitive costs.
104 Therefore, it is necessary to reduce the costs of the fermentation process. One strategy is

105 to use a new type of fermentor that can replace the conventional stirred tank reactor
106 (STR). The STR is considered to be the workhorse of the fermentation industry;
107 however, it is expensive to construct and operate and is difficult to maintain due to its
108 complex construction. Furthermore, the STR is not suitable for filamentous
109 microorganisms such as fungi and *Streptomycetes* because shear stress is generated by
110 the mechanical agitation.

111 Therefore, various types of reactors, including the bubble column (Yoshida 1988),
112 packed bubble column (Abraham and Sawant 1990), tubular reactor (Moser 1991), and
113 air-lift reactor (ALR) (Siegel et al. 1986), have been examined in detail. The ALR has
114 been widely studied because it does not require mechanical agitation and therefore does
115 not have moving parts. Moreover, its energy demand is considerably lower than that of a
116 STR. An ALR can be easily constructed and requires only approximately one-third of
117 the energy needed for an STR (Träger et al. 1989). Consequently, *Candida utilis* (Kiese
118 et al. 1980), *Pseudomonas fluorescens* (Onken and Jostmann 1984), *Thiobacilli* (Helle
119 and Onken 1988), *Penicillium chrysogenum* (König et al. 1982), and *Saccharomyces*
120 *cerevisiae* (Wu and Wu 1991) have been tested in the ALR. Träger et al. (1992)
121 compared gluconic acid production by *Aspergillus niger* in the ALR and STR and found
122 that the ALR had good reliability and a low power requirement for pilot-scale
123 production of gluconic acid. *A. terreus* (IFO-6365) was used for IA production in the
124 ALR using a modified draft tube (Okabe et al. 1993). When this type of ALR reactor
125 was used, the IA production rate (0.66 g/l/h) increased to double the value from the STR

due to morphological changes of the fungus from the filamentous form to the pellet type. Park et al. (1994) reported that repeated IA production in the ALR was possible in 21 d, and an IA production rate of 0.37 g/l/h was achieved.

As noted earlier, the IA production rate in the STR was significantly lower than that in the ALR even if the dissolved oxygen (DO) concentration was maintained at a higher level than that in the ALR. To evaluate the economic efficiency of the reactor, the power input per volume for operating both reactors was compared (Yahiro et al. 1997a). In the case of the STR, the power input was a summation of agitation and aeration (Matsushima et al. 1972). On the other hand, in the case of the ALR, the power input was used only for aeration. The power input per unit volume (P_g / V) for the ALR was calculated as follows:

$$\frac{P_g}{V} \propto \Delta P \cdot Q,$$

where ΔP and Q indicate the pressure drop between the inlet and outlet gas (kg/cm^2) and the gas flow rate (l/min), respectively. The IA production rate was higher in the ALR than in the STR at each power input per unit volume (Fig. 1). In comparison to the STR, the ALR showed a higher IA production rate at less power input per unit volume.

IA producers have also been evaluated on the shake-flask scale. Even when the same strain of *A. terreus* was used, the IA concentration differed between the flask culture and ALR, with a slightly higher concentration of IA being produced in the flask culture than

145 in the ALR (Okabe et al. 1993; Park et al. 1994; Yahiro et al. 1997a). This might be due
146 to oxygen limitations in the ALR because mixing in the ALR is milder than that in the
147 rotary shaker.

148 Several workers have tried to immobilize *A. terreus* in order to improve the
149 performance of various fermentation systems (Table 3). Polyacrylamide (Horitsu et al.
150 1983), polyurethane foam (Kautola et al. 1990; 1991), calcium alginate (Kautola et al.
151 1985), celite R-626 (Kautola et al. 1985), and porous disks (Naihu and Wang 1986)
152 have been used to immobilize the mycelia. The production rates of IA in immobilized
153 cell bioreactors with porous disks or celite R-626 were relatively higher than those on
154 the other materials, although the IA concentrations were still lower than 20 g/l. In the
155 case of batch cultures, the IA production rate was similar and ranged between 0.26 and
156 0.32 g/l/h. The production rate in continuous cultures was 2-fold higher than that in
157 batch cultures. However, the IA concentration (18 or 26 g/l) was too low for industrial
158 purposes. Although, there are many reports on repeated batch culture, the IA
159 concentration was too low. In the ALR, the IA production rate in repeated batch culture
160 was 0.37 g/l/h, which was 40% higher than of the rate in batch cultures. Repeated batch
161 culture without the loss of IA formation activity may be expected in the ALR.

162 **Production of IA from cheaper alternative substrates**

163 Microbial production of multifunctional organic acids has been of interest due to their
164 possible applications in the food industry and potential as raw materials in the

manufacture of biodegradable polymers (Tsao et al. 1999). The highest IA yield is achieved when glucose is used as the substrate, but crystalline glucose is too expensive to use as a raw material for the commercial production of IA. Therefore, other raw materials that are cheaper than crystalline glucose, such as starch, molasses, hydrolysates of corn syrup or wood, and other combinations, were also tested. The most frequently used substrates are beet or sugarcane molasses (Nubel and Ratajak 1964), which are pretreated by ion exchange or ferrocyanide (Batti and Schweiger 1963). Among the various carbohydrates available, corn starch is one of the best carbon sources since it is very pure, inexpensive, and stable in a mass supply. However, corn starch is not a popular fermentation raw material because it is very difficult to sterilize due to gelatinization upon heating. The problem of gelatinization of corn starch upon heat sterilization was solved by hydrolyzing the starch using acid or enzymes. Hydrolysis using glucoamylase (5000 AUN/ml) resulted in IA yields of up to 0.36 g/g starch, whereas hydrolysis with nitric acid at pH 2.0 yielded 0.35 g/g starch. When the corn starch was hydrolyzed with hydrochloric or sulfuric acid, the *A. terreus* cells required an additional nitrogen source for IA production even though the corn starch itself contained a small amount of the nitrogen source. However, when the starch was hydrolyzed with nitric acid, the cells grew and produced IA without any additional ingredients. These results indicate that nitric acid acts not only as an acid for the hydrolysis of corn starch but also as a nitrogen source for *A. terreus*. When raw corn starch was used for IA production, the production medium consisted of only corn starch

that had been pretreated by partial hydrolysis with either with glucoamylase or nitric acid at pH 2 prior to autoclaving at 121°C for 20 min. More than 60 g/l of IA was produced by *A. terreus* TN-484 in a 2.5- l air-lift bioreactor from a medium consisting of 140 g/l of corn starch with no nitrogen source or other ingredients (Yahiro et al. 1997b). The IA yield based on the amount of corn starch consumed was more than 50% and was similar to that from crystalline glucose. In the case of sago starch, the medium containing nitric acid for both hydrolysis and IA production from sago starch was optimized, and 48.2 g/l of IA was produced with a yield of 0.34 g/g sago starch (Dwiarti et al. 2007). Market refuse, apple, and banana were also used as substrates for IA production, and IA yields of 28.5 and 31.0 g/l were obtained using acid- and α-amylase-hydrolyzed corn starch.

cis-Aconitic acid decarboxylase (CAD) in IA biosynthesis

The pathway for IA biosynthesis in fungi has been studied by several groups (Kinoshita 1932; Eimhjellen and Larsen 1955; Shimi et al. 1962). Bentley and Thiessen (1957a, 1957b, 1957c) showed that *cis*-aconitic acid, which is produced in the tricarboxylic acid (TCA) cycle, could be a substrate for an *A. terreus* crude enzyme preparation that contained *cis*-aconitic acid decarboxylase (CAD; E. C. 4.1.1.6) and could lead to the formation of IA. Bonnarme et al. traced ^{14}C-labelled metabolites and concluded that CAD catalyzed the decarboxylation of *cis*-aconitic acid to IA in the cytoplasm (1995). These results suggest that CAD is an essential enzyme for IA biosynthesis. However,

206 until then, the enzyme had not been purified to homogeneity due to its instability.
207 Dwiarti et al. (2002) investigated the purification conditions for this enzyme and
208 purified a 55-kDa protein with CAD activity to homogeneity from the high
209 IA-producing strain *A. terreus* TN484-M1. The protein was stable in a buffer containing
210 30% glycerol and was identified as the essential metabolic enzyme for IA production in
211 the fungus.

212 The N-terminal sequence and four internal amino acid sequences of purified CAD
213 were determined, and the gene was cloned by referring to the *A. terreus* genome
214 database provided by the Broad Institute (http://www.broad.mit.edu) (Kanamasa et al.
215 2008). The gene was classified as ATEG_09971 in the database and is represented as
216 *CAD1*. A fragment containing *CAD1* was amplified from the *A. terreus* IFO6365
217 genome by PCR and then sequenced (accession number AB326105). The predicted
218 *CAD1* gene encoded a polypeptide of 490 amino acid residues with a calculated
219 molecular mass of 52,721 Da. This was consistent with the experimentally determined
220 molecular weight of purified CAD (55 kDa on SDS-PAGE). The *CAD1* gene was
221 functionally expressed in yeast, and the results proved that the obtained *CAD1* gene
222 encoded the *A. terreus* CAD protein (Kanamasa et al. 2008).

223 The CAD protein contains a conserved domain of the MmgE/PrpD family of
224 proteins of bacteria and fungi, which includes several 2-methylcitrate dehydratases of
225 bacteria that are involved in propionate catabolism. The protein that showed the highest
226 identity (53%) with CAD in the DNA Data Bank of Japan was an unnamed protein from

227 *Aspergillus oryzae* that possessed a conserved region of the PrpD family (accession no.
228 AP007175).

229 Regarding the localization of CAD, there has been some debate as to whether it is
230 present in the mitochondria or in the cytoplasm because *cis*-aconitic acid is produced in
231 the TCA cycle while IA is finally secreted into the culture broth. The WoLF PSORT
232 (Horton et al. 2006) algorithms predicted that this protein would be localized in the
233 cytoplasm, suggesting that *cis*-aconitic acid was transported from the mitochondria to
234 the cytoplasm in *A. terreus*.

235 No typical sequence for the TATA box exists in the 5′-untranslated region of *CAD1*,
236 while consensus binding motifs for the HAP complex (CCAAT), a global transcription
237 activator identified in eukaryotes including filamentous fungi (Goda et al. 2005; Kato et
238 al. 1998; Xing et al. 1993), are present upstream of *CAD1*, suggesting that it is a highly
239 transcribed gene. The inhibitory effect of IA on IA production by *A. terreus* was
240 reported by Lockwood and Reeves (1945). This phenomenon could be caused by
241 feedback inhibition by IA at the transcriptional level of *CAD1*. However, it was found
242 that the transcription of *CAD1* was not inhibited in the presence of IA (Kanamasa et al.
243 2008).

244 To clarify the role of *CAD1* in the high-producing strain TN484-M1, the *CAD1*
245 gene was sequenced. There were no differences in the nucleotide sequences of *CAD1*
246 from the wild-type and TN484-M1 strains, but the *CAD1* transcription level of the
247 TN484-M1 strain was 5-fold higher than that of the wild-type strain (Kanamasa et al.

2008). This suggests that high IA productivity was not caused by the substitution of the amino acid sequence of CAD but was caused by the higher expression levels of *CAD1* in the high-producing strain in comparison to the wild-type strain. The *CAD1* will provide a way for enhancement of the IA productivity by biotechnological methods.

Process for the industrial production of IA

The process for the industrial production of IA from the culture broth consists of five steps, as shown in Fig. 2. The culture broth is filtered to remove mycelia and other suspended solids. The filtrate of the IA culture is concentrated to a value higher than 350 g/l and crystallized at 15°C. This crystallization process is carried out twice in series. The IA crystals from the two crystallization processes are decolorized by active carbon treatment at 80°C. However, this step can be omitted in the case of the industrial-grade product. The decolorized broth is evaporated and recrystallized. The recrystallized IA is dried and packaged. If IA of high purity is required, further purification steps such as solvent extraction, ion exchange, and re-decolorization are required. The IA recovery yield is 95% in the filtration process, 98% in the concentration process, and 95% in the crystallization and drying processes. The total IA recovery yield from cultivation to final packaging is approximately 80%.

To reduce the manufacturing costs, waste starch may be used in IA production. When sago starch was used as the carbon source, the IA recovery yield was almost the same as that obtained when glucose was used; however, the purity was slightly lower

than that obtained when glucose was used as the carbon source (Dwiarti et al. 2006; 2007). IA purified from glucose and sago starch had a purity of 99.0% and 97.2%, respectively. The melting points of these two samples were 166°–169°C and 166°–167°C, respectively. Although the form and whiteness of both crystal products from sago starch were the same as those of the authentic IA standard, an extra purification step might be required to obtain higher purity.

Application trends

IA has been used in a wide range of industries (Table 4). During the 1950s, IA was used in industrial adhesives. Overall, during this period, IA was used at an industrial scale, and large amounts of it were required. The alkali salt or sulfonated form of poly IA is used as a detergent and in shampoos. The polymerized methyl, ethyl, or vinyl esters of IA are used as plastics, adhesives, elastomers, and coatings. In the textile industry, IA was employed in nonwoven fabric binders.

Since the 1990s, the applications of IA have been extended to biomedical fields, such as the dental, ophthalmic, and drug delivery fields. A major problem in ophthalmic drug delivery is retention of an adequate concentration of the therapeutic agent in the pre-corneal area. Polycarboxylic carriers such as polyacrylic acid and polyIA in a subcolloidal nanoparticulated hydrogel-form (De et al. 2004; Stanojevic et al. 2006) have high potential uses in sustained drug release during ocular delivery. Therefore, poly(*N*-isopropylacrylamide/IA) (Tasdelen et al. 2004) and poly (*N*-vinyl

2-pyrrolidone/IA) (Sen and Yakar 2001) were tested for the delivery of lidocaine and terbinafine hydrochloride, respectively. Further, poly(acrylamide(A)-co-monomethyl itaconate) hydrogel was used for the dermal delivery of a bupivacaine-loaded formulation that could be used as a dressing against wound pain (Blanco et al. 2003).

Another potential application of IA is in the preparation of glass ionomer cement (GIC). GICs were introduced 30 years ago and have been shown to be very useful adjuncts in restorative dentistry. GIC is composed of a calcium-aluminosilicate glass powder and an adequate solution of an acrylic acid homo- or copolymer. These cements possess certain unique properties that make them useful as restorative and adhesive materials: they adhere to the tooth structure and base metals, exhibit anticariogenic properties due to release of fluoride, are thermally compatible with tooth enamel, and are biocompatible (Nagaraja and Kishore 2005). Crisp and Wilson (1980) synthesized a copolymer of acrylic and IA that proved to be indefinitely stable in aqueous solution. This copolymer was the first commercial marketable cement. Recently, an *N*-vinylcaprolactam-containing copolymer of acrylic-IA (Moshaverinia et al. 2009) and poly(acrylic acid-co-IA) (Culbertson 2006) was developed for use in functional and mechanical GICs. These materials are finding increasing applications in clinical dentistry.

Supply and demand

IA is an important intermediate in polymer production. It is extremely useful in the

industrial production of synthetic resins, synthetic fibers, pesticides, plastic, rubbers, surfactants, ion-exchange agents, and lubricating oil additives. The applications of IA have been extended to the production of special glass fiber reinforced plastics, special optical lens, artificial dental cements, and drug delivery.

China is one of four IA-producing countries in the world and has become increasingly important in terms of the global IA supply (Table 5). China plays a key role in maintaining the supply and demand of IA from the viewpoint of production, manufacture, and worldwide competitiveness. In the early 1990s, the IA output was very low and China mainly relied on imports to meet the domestic demand. After 1993, China began to set up IA-producing units in order to satisfy the domestic demand for IA. In 2000, China had 10 normally functioning enterprises that had a combined total output of 20,000 tons, and it became the second-largest IA producer after the USA. Currently, unofficial statistics estimate that the annual IA production capacity of China has reached 30,000 tons. IA consumption can be roughly accounted for as follows: 40% is used in the production of nitrilon, which is an acrylonitrile-based synthetic fiber that contains 93% acrylonitrile, 5.7% methyl acrylate, and 1.3% IA (Gong and Wang 2002); 30% is consumed in the ion-exchange resin sector; 10% in papermaking; 10% in the water treatment sector; and 10% in other sectors.

In 2005, China's IA production capacity exceeded the demand. Ten years ago, the price of this product ranged between US$ 4/kg (Willke and Vorlop, 2001) and US$ 4.3/kg (Bressler and Braun 2000). However, at present, it is US$ 2/kg. The

329 domestic price in China has fallen to below US$ 1.5/kg. The main reason for the low
330 utilization of the capacity is the restricted range of applications of IA, with the main
331 consumption being in the nitrilon and ion-exchange resin sectors.

332 **Concluding remarks**

333 IA is a promising organic acid that has been categorized as one of the “top twelve”
334 building block molecules from sugars in advance biorefineries (Kurian 2005). The
335 market for IA and its derivatives is still growing. Moreover, investigations into new
336 properties of this compound have opened up possibilities for novel applications in the
337 fields of polymer chemistry, pharmacy, and agriculture. For ensure efficient supply of
338 IA, further studies on reducing the production costs are essential. Sugar, used as the
339 carbon source, should be replaced by cheaper alternative substrates such as cellulolytic
340 biomass because most starch is used in food. Moreover, innovations by which the
341 process becomes more energy-saving are necessary. Strain improvement by genetic and
342 metabolic engineering is also an important aspect since it would allow cheaper
343 alternative substrates to be utilized. In this regard, the development of an IA producer
344 that is capable of utilizing lignocellulosic biomass as the carbon source is highly
345 recommended.
346

347 **Table 1** Itaconic acid producers

Strain	IA concentration (g/l)	Reference
Ustilago zeae	15	
Ustilago maydis	53 (5 d)[a]	(Tabuchi and Nakahara 1980)
Candida sp	35 (5 d)[a]	(Tabuchi 1981)
Candida mutant	42 (6 d)[a]	(Hashimoto et al. 1989)
Rhodotorula sp	15 (7 d)[a]	(Kawamura et al. 1981)
Aspergillus terreus SKR10	20	
Aspergillus terreus TN-484-M1	82 (6 d)[a]	(Yahiro et al. 1995)

348 [a] Culture time.

349

350 **Table 2** Itaconic acid productivity of TNH-484

Initial glucose concentration (g/l)	140	150	160
Dry cell weight	7.1	7.9	7.9
Residual sugar	2.6	3.3	1.8
IA	75.4	81.3	82.4
IA yield (g/g) of based on consumed glucose	0.57	0.57	0.54
Cellular yield (g/g) based on consumed glucose	0.054	0.055	0.052
IA yield (g/g) based on dry cell weight	10.6	10.3	10.4

351

352

353 **Table 3** Comparison of IA production rate among various bioreactors

Culture method		Immobilized on	Substrate	IA conc. (g/l)[a]	IA PD (g/l/h)[b]	Yield (g/g)	Operation time (d)	Reference
Batch	Flask	Free cells	Glucose	51.0	0.31	0.44	7	(Park et al. 1994)
	Flask			82.0	0.57	0.54	6	(Yahiro et al. 1995)
	Fermentor			30.0	0.32	0.55	4	(Kautola et al. 1985)
	Fermentor			49.4	0.31	0.43	7	(Park et al. 1993)
	Air-lift reactor			44.0	0.26	0.45	7	(Okabe et al. 1993)
	Air-lift reactor			63.7	0.64	-	4	(Yahiro et al. 1997a)
Continuous	Disk bioreactor	Porous disk	Glucose	18.2	0.73	0.46	30	(Naihu and Wang 1986)
	Column bioreactor	Polyacrylamide gels		-	Max. 0.60	-	15	(Horitsu et al. 1983)
	Column bioreactor	Polyurethane foam cube		26.0	0.14	-	18	(Kautola et al. 1990)
Repeated batch	Flask	Agar gels	Xylose	14.0	0.12	0.23		(Kautola et al. 1985)
		Calcium alginate	Xylose	7.0	0.06	-	17	(Kautola et al. 1985)
		Celite R-626	Glucose	11.5	1.20	0.18	24	(Kautola et al. 1985)
		Polyurethane foam cube	Glucose	Max. 51.0	Max. 0.15	0.34	14	(Kautola et al. 1991)
		Free cells	Glucose	56.0	0.47	0.49	45	(Park et al. 1994)
	Air-lift reactor	Free cells	Glucose	48.0	0.37	0.48	21	(Park et al. 1994)
		Cell-recycling	Glucose	46.0	0.39	0.39	16	(Park et al. 1994)

354 [a,b] Values in batch, continuous, and repeated batch cultures denote final, steady state and average, respectively.

355 **Table 4** Application of itaconic acid

Materials	Application	Reference
Vinylidene chloride containing below 2% IA	Improved adhesion to paper, cellophane	(Pitzl 1951)
Alkali salt of poly IA	detergent	(Lancashire 1969)
Rubber-like resin (Copolymer of IA)	Electrical insulation	(Smith et al. 1974)
N-substituted pyrrolidones (IA with amines)	Thickeners in lubricating grease, detergents, shampoos	(Gordon and Coupland 1980)
Imidazoline derivative	shampoos	(Christiansen 1980)
Polyacrylonitrile copolymer incorporating low level of IA	Efficient dying and deep shade in textile industry	(Tate 1981)
Copolymer of acrylic acid and IA	Scale inhibitor in boiler	(Walinsky 1984)
IA monoester compounds	Dental adhesives, dental fillers	(Saitoh et al. 1993)
Hardening agent	Contact lens	(Ellis et al. 1994)
Pigmented dispersion resins containing 0.1-1.5% IA	Wet abrasion resistance	(Zhao et al. 1999)
Styrene butadiene lattices containing 1-5% IA	Carpet backing or paper coating	(Willke and Vorlop, 2001)
Acrylic lattices supplemented with IA	Nonwoven fabric binder	(Willke and Vorlop, 2001)
Sulfonated poly IA	Industrial cleaner	(Willke and Vorlop, 2001)
N-vinyl 2-pyrrolidone/IA hydrogels	Antifungal drug	(Sen and Yakar 2001)
Poly(acrylamide-co-monomethyl itaconate) hydrogels	Transdermal therapy	(Blanco et al. 2003)
IA	Inhibitor of fructose 2,6-bisphosphate synthesis	(Sakai et al. 2004)
N-isopropylacrylamide/IA copolymeric hydrogels	Drug release	(Tasdelen et al. 2004)
Polycarboxylic acid nanoparticles	Ophthalmic drug delivery	(De et al. 2004)
Poly(acrylamide-co-IA) hydrogels	Drug delivery	(Stanojevic et al. 2006)
Poly(acrylic acid-co-IA)	Glass-ionomer cements	(Culbertson 2006)
N-vinylcaprolactam-containing copolymer of acrylic-IA	Glass-ionomer dental cements	(Moshaverinia et al. 2009)

Table 5 Supply of IA

Company	Location	Since	Capacity (tons/year)
Pfizer Food Science	New York, USA; Sandwick, UK	1945-1995	5,000-7,000[a]
Iwata Chemicals	Kyogyo, Japan	1970	10,000[b]
Tianli Biological Fermentation Factor	Yunnan, China	1988	2,000[c]
Gansu Feipeng Biochemical Co. Ltd.	Gansu, China	1989	1,000[c]
Chengdu Lake Biology Engineering Industry	Sichuan, China	1993	4,000[c]
Nanjing Huajin Biologicals Co. Ltd.	Nanjing, China	1994	1,000[c]
Jiangsu Binhai Sanai Biological Co. Ltd.	Jiangsu, China	1994	1,200[c]
Rhodia	Melle, France	1995	10,000[b]
Zhejiang Guoguang Biochemical Co. Ltd.	Zhejiang, China	1995	1,000[c]
Cargill/Cultor Food Science	Eddyville, USA	1996	30,000[b]
Shandong Zibo Zhongshun	Shandong, China	1999	1,200[c]
Science & Technology Co. Ltd.	Zibo, China	1999	3,000[c]
Diversified Co. of Zibo Mineral Bureau			
Guangdong Leizhou Yueli IA Co. Ltd.	Guangdong, China	1999	1,500[c]
Qingdao Langyatai Group	Qingdao, China	2000	4,500[c]

[a] Stop IA production from 1996

[b] T. Udagawa (2009) Private communication

[c] Source: http://www.thefreelibrary.com/Itaconic+acid+supply+exceeds+demand.+(Market+Report).-a091473938

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550

551 **Figure legends**

552

553 **Fig. 1** Correlation between the power input per volume and IA production rate in STR

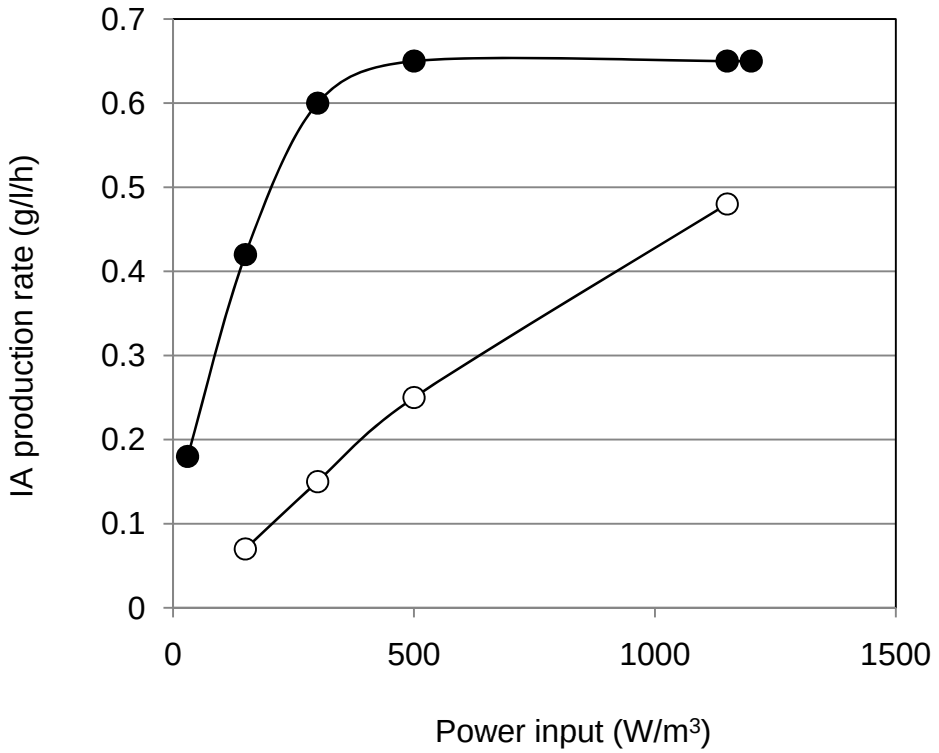
554 (open circles) and ALR (closed circles).

555 **Fig. 2** Schematic diagram of IA production and recovery process from *A. terreus*

556 culture.. A, Medium preparation; B, Pre-culture; C, Fermentor; D, Filter; E, Evaporator;

557 F, 1st Crystallization; G, Separator; H, 2st Crystallization; I, Decolorization; J, Heat

558 exchangher; K, Recrytallization; L, Drying shelves; M, Packaging.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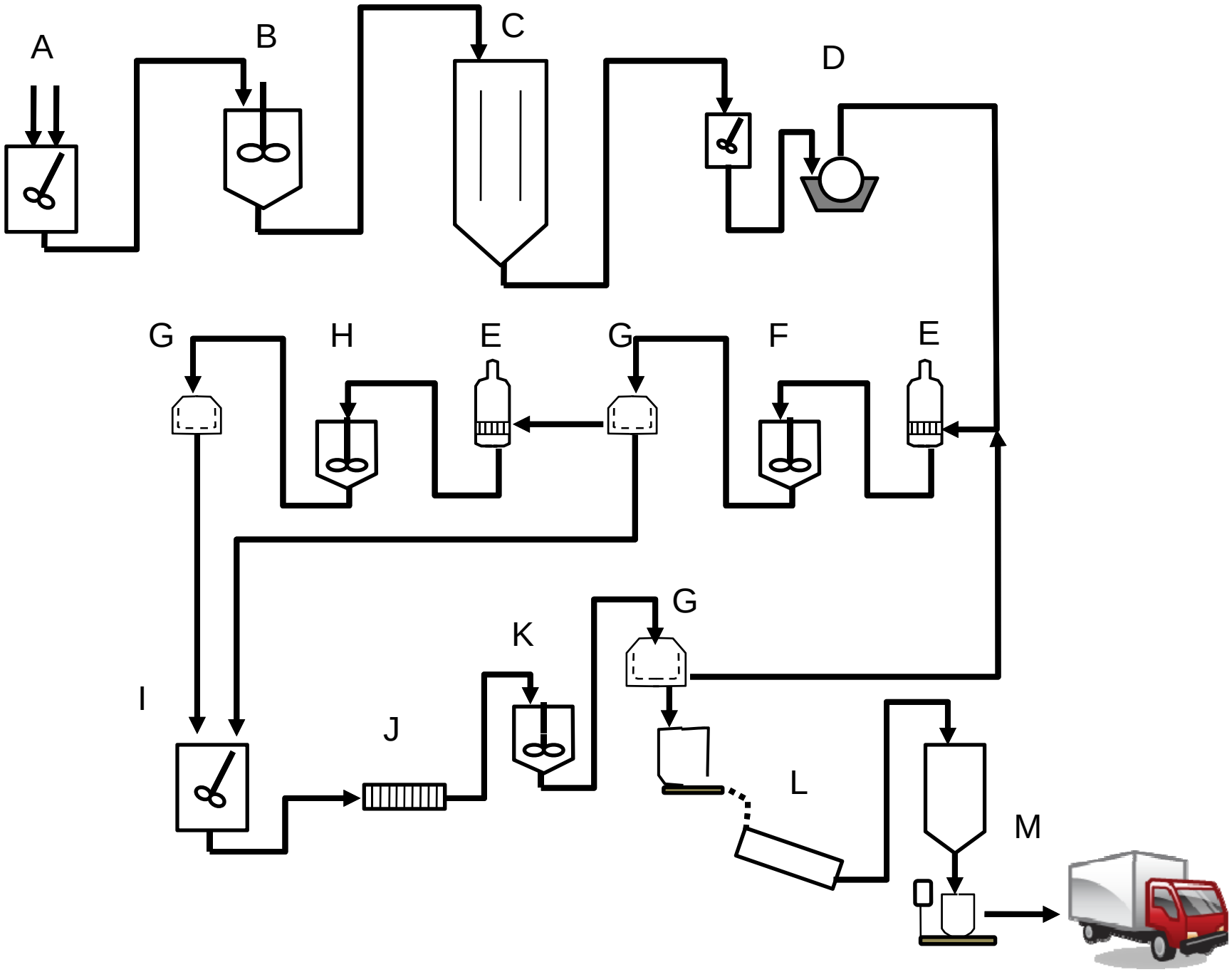
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