

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 12-153951- -4	FECHA: 2014-08-26 09:15:19
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 379 FASE NACIONAL INCLUIDO CAPITULO II
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 14
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
SILVANA MARIA LOPEZ DIAZ

Asunto: Radicación: 12-153951- -4
 Trámite: 11
 Evento: 379
 Actuación: 519
 Oficio: 10806
 Folios: 14

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I		Capítulo II	X
No. Solicitud Internacional	PCT/US2010/025371				
Fecha de Presentación Internacional	25/02/2010				

1. Documentos estudiados

Descripción:	Radicado N°12–153951–00000–0000	Septiembre 7 de 2012
Reivindicaciones	Radicado N°12–153951–00000–0000	Septiembre 7 de 2012

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga

más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Se realizará el estudio de las reivindicaciones 1 a 20 presentes en el Radicado N°12-153951-00000-0000, porque se realizó el pago respectivo por las reivindicaciones adicionales 11 a 20, de acuerdo con el recibo presente en la página 58 del Radicado N°12-153951-00000-0000.

Título de la solicitud: “Utilización de sustratos agro – residuales para la producción fermentativa de L – arginina”.

El problema planteado en esta solicitud consiste en lograr un proceso alternativo para obtener L – arginina, a partir de fuentes de carbono diferentes a los azúcares puros.

Para resolver este problema técnico, la solicitud en estudio plantea un proceso para obtener L – arginina mediante producción microbiana utilizando *Corynebacterium glutamicum*, donde la fuente de carbono inicial es un hidrolizado de sustratos de origen agrícola, particularmente de bagazo de yuca y semillas de panapén.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C12P13/10.

4. De la Solicitud de la Patente

Título

El título de la solicitud no es claro, porque este sugiere que el objeto de la invención es el empleo de sustratos agrícolas mas no el proceso para obtener L – arginina. Se recomienda el siguiente título: “Obtención fermentativa de L – arginina a partir de sustratos de origen agrícola como fuente de carbono”

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara, porque un proceso para obtener un producto debe ser definido por una serie de pasos, donde cada uno de estos debe contener las cantidades y/o proporciones de las sustancias o elementos que intervienen y las condiciones a las cuales se lleva a cabo cada uno de los pasos del proceso.

Adicionalmente, al mencionar expresiones “agro residuo”, se incluyen muchísimos productos residuales de material agrícola pero que no están sustentados en la descripción, por lo que se solicita mencionar específicamente que materiales de origen agrícola intervienen en el proceso para obtener arginina. Además, la expresión “en presencia de por lo menos una de *Corynebacterium glutamicum*” no es clara, porque la expresión “al menos” se considera imprecisa (no permite diferenciar el objeto de la invención con respecto al estado de la técnica) y la expresión “una de *Corynebacterium glutamicum*” puede hacer referencia a especie, cepa, enzima, entre otros. Se solicita realizar las aclaraciones requeridas. También se requiere indicar las etapas para obtener el licor que contiene L – arginina por el proceso de fermentación.

Para la reivindicación 3 se solicita mencionar el proceso de fermentación del sustrato agrícola, así como se solicita mencionar las enzimas específicas para la hidrólisis del almidón.

Las reivindicaciones 8 y 9 referentes al pH se consideran como resultados a alcanzar, porque no solo se menciona la solución propuesta en la solicitud, sino que se incluyen las alternativas presentes y futuras que conduzcan a obtener este resultado. Se solicita eliminar estas reivindicaciones.

Para la reivindicación 12 se solicita mencionar el agente betalactámico y la cantidad del mismo.

Se sugiere eliminar la reivindicación 13 del pliego reivindicatorio e incluir el objeto de la misma dentro de la reivindicación 1, debido a que el proceso relacionado con la hidrólisis enzimática de los sustratos de origen agrícola constituye un paso fundamental para el proceso de la reivindicación 1, mas no se considera como un proceso independiente y aislado como lo pretende ilustrar la reivindicación 13.

Se solicita eliminar las reivindicaciones 14 a 20 porque replican la materia de las reivindicaciones anteriores. Se solicita incluir dentro de la reivindicación 1 el proceso relacionado con la hidrólisis enzimática del sustrato agrícola descrito en la reivindicación 13.

Descripción (Art 28 D 486)

Debido a que la solicitud se refiere a material biológico, es necesario presentar los Certificados de Depósito para la cepas de *Corynebacterium glutamicum* ATCC 21831 y *Corynebacterium glutamicum* ATCC 21831.

Se solicita corregir la expresión “hidrosilatos” por la expresión “hidrolizados”.

5. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de presentación internacional: Febrero 25 de 2010.

Consultadas las fuentes de información con que se cuenta, se encontraron los siguientes documentos que anticipa el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación
D1	US3849250	“Process for producing L – arginine by fermentation”	19/Noviembre/1974
D2	US5196326	“Method for concurrent fermentation of basic amino acid and acidic amino acid”	23/Marzo/1993
D3	<i>Bioresource Technology</i> . 2000; 74: 81 – 87	Biotechnological potential of agro – industrial residues II: cassava bassage	01/Agosto/2000

El documento D1¹ divulga un proceso de obtención de L – arginina por fermentación mediante una bacteria productora de L – arginina (Abstract).

El documento D2² divulga un proceso de obtención de L – aminoácidos básicos por fermentación mediante bacterias productoras de dichos aminoácidos (Abstract).

El documento D3³ divulga el empleo de residuos agroindustriales, particularmente el bagazo de yuca como fuente de almidón para procesos de bioconversión mediante fermentación (Abstract).

1[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=3849250A&KC=A&FT=D&ND=&date=19741119&DB=&locale=en_EP)

CC=US&NR=3849250A&KC=A&FT=D&ND=&date=19741119&DB=&locale=en_EP

2[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=5196326A&KC=A&FT=D&ND=&date=19930323&DB=&locale=en_EP)

CC=US&NR=5196326A&KC=A&FT=D&ND=&date=19930323&DB=&locale=en_EP

3<http://www.sciencedirect.com/science/article/pii/S0960852499001431>

6. Examen de Patentabilidad.

Novedad (Art 16 D 486)

La reivindicación 1 divulga “Un método para producir arginina mediante la fermentación de agro – residuos que comprende: someter el agro – residuo en presencia de por lo menos una de *Corynebacterium glutamicum* ATCC 21831 o *Corynebacterium glutamicum* ATCC 21493 para producir un licor fermentado que contiene arginina y recuperar la arginina del licor fermentado” (Pág. 22 del Radicado N°12–153951–00000–0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D1
Proceso para obtener arginina	Proceso para obtener L – arginina (Col. 4, reiv. 3)
Se fermenta un agro – residuo en presencia de <i>Corynebacterium glutamicum</i> (ATCC 21831 ó 21493)	Se fermenta harina de soya hidrolizada (obtenida por tratamiento con H ₂ SO ₄ 6N y neutralizado con amoníaco) en presencia de <i>Corynebacterium glutamicum</i> ATCC 21831 (Col. 1, .ln. 48 – 64; col. 2 – 3, ej. 2; col. 4, reiv. 3)
La arginina obtenida se separa del licor fermentado	Recuperación de la L – arginina obtenida (Col. 4, reiv. 3)

Las características técnicas esenciales del proceso de la reivindicación 1 han sido divulgadas previamente en el documento D1 (D1: Col. 1, .ln. 48 – 64; col. 2 – 3, ej. 2; col. 4, reiv. 3), donde se menciona un proceso para obtención de L – arginina mediante la fermentación de un material vegetal pretratado como fuente de carbono (harina de soya hidrolizada) en presencia de *Corynebacterium glutamicum* ATCC 21831, seguido de la recuperación de la L – arginina obtenida, de acuerdo con las características técnicas esenciales descritas en la tabla anterior.

A pesar de que la solicitud menciona un residuo agrícola, al no estar debidamente definido, un residuo agrícola puede ser cualquier tipo de material vegetal, el cual el documento D1 divulga la harina de soya, por lo que se considera que este material vegetal es el residuo de un producto agrícola y por lo tanto, se tiene en cuenta como características técnica esencial para anteceder el objeto de la solicitud en estudio.

En consecuencia, la reivindicación 1, de acuerdo a lo divulgado en la reivindicación 1, carece de Novedad.

Las reivindicaciones 3 y 5 a 11, dependientes de la reivindicación 1, no menciona características técnicas adicionales que otorguen novedad al proceso mencionado en la reivindicación 1. Port lo tanto, estas reivindicaciones también carece de Novedad

Nivel Inventivo (Art 18 D 486)

La reivindicación 1 divulga “Un método para producir arginina mediante la fermentación de agro – residuos que comprende: someter el agro – residuo en presencia de por lo menos una de *Corynebacterium glutamicum* ATCC 21831 o *Corynebacterium glutamicum* ATCC 21493 para producir un licor fermentado que contiene arginina y recuperar la arginina del licor fermentado” (Pág. 22 del Radicado N°12–153951–00000–0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D1	D3
Proceso para obtener arginina	Proceso para obtener L – arginina (Col. 4, reiv. 3)	No se menciona
Se fermenta un agro – residuo en presencia de <i>Corynebacterium glutamicum</i> (ATCC 21831 ó 21493)	Se fermenta una fuente de carbono y una fuente de nitrógeno en presencia de <i>Corynebacterium glutamicum</i> ATCC 21831 (Col. 1, .ln. 48 – 64; col. 2 – 3, ej. 2; col. 4, reiv. 3)	Fermentación de bagazo de yuca para obtención de azúcares mediante microorganismos (Pág. 85, col. der., ln. 42 – 53)
La arginina obtenida se separa del licor fermentado	Recuperación de la L – arginina obtenida (Col. 4, reiv. 3)	No se menciona

El documento D1 se considera el estado de la técnica más cercano al proceso de la presente solicitud de acuerdo con la reivindicación 1, porque se divulga un proceso para obtener L – arginina que consiste en fermentar una fuente de carbono en presencia de un microorganismo como *Corynebacterium glutamicum* ATCC 21831, seguido de la recuperación de la L – arginina (Col. 1, .ln. 48 – 64; col. 2 – 3, ej. 2; col. 4, reiv. 3).

La diferencia entre el proceso descrito en la solicitud con respecto al proceso mencionado en el documento D1, radica en que para la solicitud el producto de partida para fermentación es un residuo agrícola como fuente de carbono, mientras que el proceso descrito en el documento D1 el producto de partida para la fermentación es un carbohidrato o un ácido orgánico.

Esta diferencia al parecer no le confiere un efecto técnico inesperado o superior al proceso desarrollado en la solicitud con respecto al proceso desarrollado en el documento D1, porque el desarrollo de la solicitud menciona la obtención de L – arginina en una concentración máxima de 1.41mg/ml y 1.72mg/ml partiendo de bagazo de yuca y semillas de árbol de pan respectivamente después de 120 horas pe proceso (tablas 4 y 5), mientras que el proceso divulgado en el documento D1 menciona la obtención de L – arginina en concentración de 17.7mg/ml partiendo de glucosa 2% y harina de soya hidrolizada después de 24 horas de proceso (Ej. 2).

El problema técnico objetivo que se pretende resolver es el siguiente: ¿Cómo modificar el proceso divulgado en el documento D1 para obtener un proceso alternativo para obtener L – arginina por proceso fermentativo?

El documento D3 divulga un proceso de obtención productos hidrolizados como fuentes de carbono a partir de bagazo de yuca por un proceso de fermentación donde intervienen microorganismos (Pág. 85, col. der., ln. 42 – 53).

Por lo tanto, una persona normalmente versada en la materia y conocedora de los conceptos mencionados en los documentos D1 y D3, sin ningún esfuerzo, modificaría el proceso descrito en el documento D1, sustituyendo la fuente de carbono descrita en el documento D1, incluyendo como fuente de carbono el hidrolizado de bagazo de yuca sugerido en el documento D3, para obtener el proceso propuesto en esta solicitud, de manera obvia o evidente.

En consecuencia, la reivindicación 1, de acuerdo con los documento D1 y D3, carece de Nivel Inventivo.

Las reivindicaciones 2 a 20, dependientes de la reivindicación 1, no divulgan características técnicas adicionales que confieran nivel inventivo a los compuestos

de la reivindicación 1, porque el documento D1 menciona las condiciones de pH 7.2 y temperatura de 30°C para obtener la L – arginina de acuerdo con el ejemplo 2 (Col. 3, ln. 3 – 6; 17 – 19); adicionalmente, el documento D3 menciona la presencia de amilasa para hidrolizar el almidón del bagazo de yuca. Por lo tanto, estas reivindicaciones también carecen de Nivel Inventivo.

La reivindicación 1 divulga “*Un método para producir arginina mediante la fermentación de agro – residuos que comprende: someter el agro – residuo en presencia de por lo menos una de Corynebacterium glutamicum ATCC 21831 o Corynebacterium glutamicum ATCC 21493 para producir un licor fermentado que contiene arginina y recuperar la arginina del licor fermentado*” (Pág. 22 del Radicado N°12–153951–00000–0000).

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Solicitud	D2	D3
Proceso para obtener arginina	Proceso para obtener L – arginina (Col. 9 y 10, ej. 5; col. 11 y 12, reiv. 1 – 3)	No se menciona
Se fermenta un agro – residuo en presencia de Corynebacterium glutamicum (ATCC 21831 ó 21493)	Se fermenta una fuente de carbono y una fuente de nitrógeno en presencia de Corynebacterium glutamicum y Brevibacterium flavum ATCC 21493 (Col. 2, ln. 18 – 20, 31 – 36; col. 9 y 10, ej. 5; col. 11 y 12, reiv. 1 – 3)	Fermentación de bagazo de yuca para obtención de azúcares mediante microorganismos (Pág. 85, col. der., ln. 42 – 53)
La arginina obtenida se separa del licor fermentado	Recuperación de la arginina obtenida (Col. 10, ln. 3 – 8)	No se menciona

El documento D2 se considera el estado de la técnica más cercano al proceso de la presente solicitud de acuerdo con la reivindicación 1, porque se divulga un proceso para obtener L – arginina que consiste en fermentar una fuente de carbono en presencia de un microorganismo como Corynebacterium glutamicum ATCC 21493, seguido de la recuperación de la L – arginina (Col. 2, ln. 18 – 20, 31 – 36; col. 9 y 10, ej. 5; col. 11 y 12, reiv. 1 – 3).

La diferencia entre el proceso descrito en la solicitud con respecto al proceso mencionado en el documento D2, radica en que para la solicitud el producto de partida para fermentación es un residuo agrícola como fuente de carbono, mientras que el proceso descrito en el documento D2 el producto de partida para la fermentación es un carbohidrato o un ácido orgánico.

Esta diferencia le confiere el efecto técnico obtener L – arginina en una concentración máxima de 1.63mg/ml y 2.05mg/ml partiendo de bagazo de yuca y semillas de árbol de pan respectivamente después de 48 horas de proceso (tablas 4 y 5), mientras que el proceso divulgado en el documento D2 menciona la obtención de L – arginina en concentración de 1.05mg/ml.

El problema técnico objetivo que se pretende resolver es el siguiente: ¿Cómo modificar el proceso divulgado en el documento D2 para obtener un proceso alternativo para obtener L – arginina por proceso fermentativo?

El documento D3 divulga un proceso de obtención productos hidrolizados como fuentes de carbono a partir de bagazo de yuca por un proceso de fermentación donde intervienen microorganismos (Pág. 85, col. der., ln. 42 – 53).

Por lo tanto, una persona normalmente versada en la materia y conocedora de los conceptos mencionados en los documentos D2 y D3, sin ningún esfuerzo,

modificaría el proceso descrito en el documento D2, sustituyendo la fuente de carbono descrita en el documento D2, incluyendo como fuente de carbono el hidrolizado de bagazo de yuca sugerido en el documento D3, para obtener el proceso propuesto en esta solicitud, de manera obvia o evidente.

En consecuencia, la reivindicación 1, de acuerdo con los documento D2 y D3, carece de Nivel Inventivo.

Las reivindicaciones 2 a 20, dependientes de la reivindicación 1, no divulgan características técnicas adicionales que confieran nivel inventivo a los compuestos de la reivindicación 1, porque el documento D2 menciona las condiciones de pH 7.2 y temperatura 30°C para obtener la L – arginina de acuerdo con el ejemplo 5 (Col. 9, ln. 46 – 48, 65 – 68); adicionalmente, el documento D3 menciona la presencia de amilasa para hidrolizar el almidón del bagazo de yuca y adicionalmente, el documento D2 menciona la inclusión de un antibiótico betalactámico dentro del proceso de fermentación (Col. 12, reiv. 2 y 3). Por lo tanto, estas reivindicaciones también carecen de Nivel Inventivo.

7. Conclusión

Los requisitos de Patentabilidad de esta solicitud se ven afectados de la siguiente manera:

Item	Documento	Reivindicaciones	Requisitos que afecta
D1	US3849250	1, 3 y 5 a 11	Novedad
		1 a 20	Nivel Inventivo
D2	US5196326	1 a 20	Nivel Inventivo
D3	<i>Bioresource Technology</i> . 2000; 74: 81 – 87	1 a 20	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



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El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-09-02

Elaboró: Dayron Alexander Ramirez Reyes
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Review paper

Biotechnological potential of agro-industrial residues. II: cassava bagasse

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Abstract

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues such as cassava bagasse. Cassava bagasse, which is a fibrous material, is the by-product of the cassava-processing industry. It contains about 30–50% starch on dry weight basis. Due to its rich organic nature and low ash content, it can serve as an ideal substrate for microbial processes for the production of value added products. Attempts have been made to produce several products such as organic acids, flavour and aroma compounds, and mushrooms from cassava bagasse. Solid-state fermentation has been mostly employed for bioconversion processes. This paper reviews the developments in processes and products developed for the value addition of cassava bagasse through biotechnological means. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Cassava bagasse; Submerged fermentation; Solid-state fermentation; Biotechnological applications

1. Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues such as cassava bagasse, sugar cane bagasse, sugar beet pulp, coffee pulp/husk, apple pomace, etc. Several processes have been developed that utilize these as raw materials for the production of bulk chemicals and value-added fine products such as ethanol, single-cell protein (SCP), mushrooms, enzymes, organic acids, amino acids, biologically active secondary metabolites, etc. (Pandey, 1992, 1994; Pandey et al., 1988; Nampothiri and Pandey, 1996; Pandey and Soccol, 1998). Application of agro-industrial residues in bioprocesses on the one hand provides alternative substrates, and on the other hand helps in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization. In this article,

we intend to discuss the biotechnological potential of cassava bagasse for value addition.

2. Cassava

Cassava (*Manihot esculenta* Cranz) is considered an important source of food and dietary calories for a large population in tropical countries in Asia, Africa and Latin America. It is known as tapioca in Asian countries, as aipin, castelinha, and macaxeira in Brazil, as yuca in Spanish-speaking countries of Latin America, and as manioc in French-speaking countries in Africa (Soccol, 1996; Rosenberg 1957; Laukevics et al., 1985). Cassava is considered to have originated in Venezuela during 2700 B.C. (Soccol, 1996). In a significant research published during May 1999, biologists from the Washington University in St. Louis discovered that cassava originated from the southern border of the Amazon River basin in Brazil. They used a sophisticated DNA sequencing technique that traced variation in a single gene (*G3pdh*) found in cultivated and wild cassava (Sachal and Olsen, 1999). It was introduced in Africa during the 16th century and from there into Asia during the 18th century. It is a bushy plant producing tubers

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and is made up of an aerial part and an underground part. The aerial part can be as high as 2–4 m with a trunk and branches on it. The underground part consists of two types of roots: the ones responsible for the plant nutrition, and the others with axial disposition surrounding the trunk. These are called tubers and are the edible parts of the plant. Each plant may have 5–20 tubers, and each tuber may attain a length of 20–80 cm and a diameter of 5–10 cm. The fresh weight of each tuber may vary between a few hundred grams and 5 kg. Table 1 shows the chemical composition of cassava tubers. As is evident, the cassava tubers are rich in starch but poor in protein.

Cassava ranks as the world's sixth most important food crop and is the basic food for more than 700 million people in several countries (Soccol, 1996; Kato and Souza, 1987; Cereda et al., 1996). It has the remarkable capacity to adapt to various agro-ecological conditions. It is also considered as a low-risk crop. In view of its drought-resistant nature and non-requirement of any specific growth conditions, much attention has been paid in the past 15–20 years to its agricultural aspects, for increasing its production all over the world, which has been well achieved. World production of cassava has steadily increased from about 75 million ton in 1961–1965 to 153 million ton in 1991. During 1994, the world production touched approximately 167 million ton (FAO, 1995), but fell slightly in 1998 to 162 million ton (FAO/GIEWS, 1999). Africa is the largest producer with about 53% of the world's production, followed by Asia with about 29% and Latin America with about 18%. Although cassava is cultivated in about 88 countries, only five countries account for about 67% of the production. These are Nigeria (approximately 31 million ton), Brazil (23 million ton), Thailand, 19 million ton), Zaire (18 million ton), and Indonesia (16 million ton)

Table 1
Physico-chemical composition of cassava tubers (100 g basis)^a

Composition	Fresh weight	Dry weight
Calories	135	335
Moisture (%)	65.5	15.7
Proteins (g)	1.00	1.4
Lipids (g)	0.2	0.5
Starch (g)	32.4	80.6
Fibres (g)	1.1	1.2
Ash (g)	0.9	1.8
Calcium (mg)	26	96
Phosphorus (mg)	32	81
Iron (mg)	0.9	7.9
Sodium (mg)	2	–
Potassium (mg)	394	–
Vitamin B2 (mg)	0.04	0.06
Vitamin C (mg)	34	0
Niacine (mg)	0.6	0.8
Cyanide (%)	–	1.6

^a Source: Hohnholz, (1980), Soccol (1996) and Cereda and Takahashi (1996).

(Soccol, 1996). Brazil ranks first in the production in Latin America. Although India ranks third among the Asian countries in the production (about 5.0–5.5 million ton fresh roots), the average yields on a per hectare basis are highest in India, and are about 20 ton/ha in comparison to 9–10 ton/ha, the world average (Pandey and Damodaran, 1991).

About 60% of the cassava produced all over the world is used for human consumption. It is consumed in natural form as flour or in fermented forms such as *gari*, *fufu*, etc. The most important consumer is Africa with an average of about 102 kg/person/year or 220 kcal/person/day (Giraud, 1993). Another large consumer of cassava is the animal food industry, using about 33% of the world production. The remaining 7% is used by industries such as textile, paper, food and fermentation. With the advent of biotechnological approaches, focus has shifted to widening the application of cassava and its starch for newer applications with the aim of value addition.

2.1. Industrial processing of cassava

Industrial processing of cassava is done mainly to isolate flour (which generates more solid residues) and starch (which generates more liquid residues) from the tubers. Most of the industries are of small or medium size. Fig. 1 shows the processing of cassava tubers for isolating starch with the mass balance. Two types of

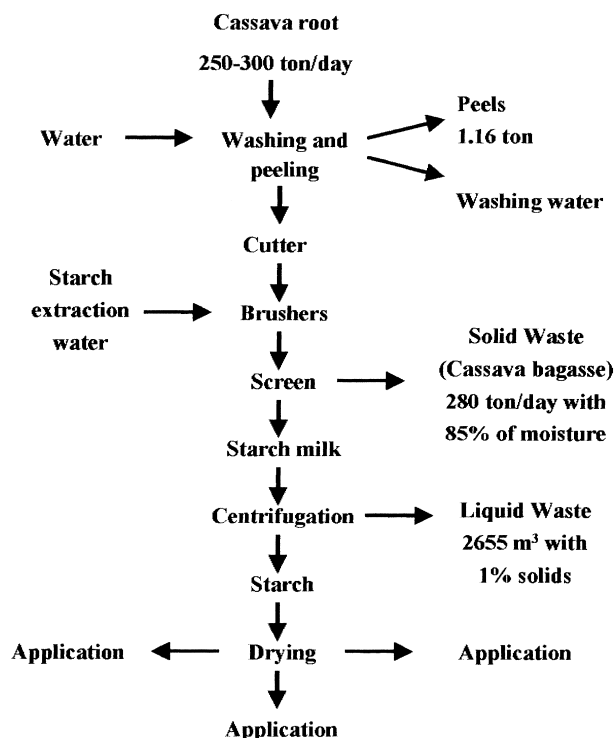


Fig. 1. Industrial processing of cassava.

wastes are generated: solid and liquid. Solid wastes include peels and bagasse. As is evident, processing of 250–300 ton of cassava tubers results in about 1.6 ton of solid peels and about 280 ton of bagasse with a high moisture content (85%). Liquid wastes include wastewater (about 2655 m³) with about 1% solids. Solid wastes are generally discarded in the environment as landfill without any treatment. Their disposal is a serious concern to the environment.

2.2. Cassava bagasse

Cassava bagasse is a fibrous residue, which contains about 50% starch on a dry weight basis (Carta et al., 1999). Table 2 shows the composition of cassava bagasse as determined by various authors. These analyses (Table 2) were conducted on the bagasse samples obtained from different processing units at different times in the State of Parana, Brazil. The composition shows variation probably due to the fact that most of the processing is done under poorly controlled technological conditions. In addition, the composition may also differ due to the use of different crop varieties. Starch is the main component determined as carbohydrates. Cassava bagasse does not show any cyanide content. However, its

poor protein content makes it unattractive as an animal feed.

Because of its low ash content, cassava bagasse could offer numerous advantages in comparison to other crop residues such as rice straw and wheat straw, which have 17.5% and 11.0%, respectively, ash contents, for usage in bioconversion processes using microbial cultures. In comparison to other agricultural residues, cassava bagasse can be considered as a rich solar energy reservoir due to its (cassava's) easy regeneration capacity. When compared with sugar cane bagasse, it offers advantages, as it does not require any pretreatment and can be easily attacked by micro-organisms.

3. Microbial strains cultivated on cassava bagasse

Micro-organisms which utilize starch as the substrate for growth and activity have generally been preferred for bioconversion processes utilizing cassava bagasse because of its high starch content. Some yeasts and fungi have been used for cultivation on cassava bagasse. However, filamentous fungi have been most widely employed. Table 3 cites different micro-organisms cultivated on cassava bagasse for various purposes.

Table 2
Physico-chemical composition of cassava bagasse (g/100 g dry weight)

Composition	Soccol (1994)	Cereda (1994)	Sterz (1997)	Vandenberghe (1998b)
Moisture	5.02	9.52	10.70	11.20
Protein	1.57	0.32	1.60	1.61
Lipids	1.06	0.83	0.53	0.54
Fibers	50.55	14.88	22.20	21.10
Ash	1.10	0.66	1.50	1.44
Carbohydrates	40.50	63.85	63.40	63.00

Table 3
Bioprocesses involving cassava bagasse^a

Micro-organism	Process	Application	Reference
<i>A. niger</i> LPB 21	SSF	Citric acid	Kolichski et al. (1995)
<i>A. niger</i> NRRL 2001	SSF	Citric acid	Vandenberghe et al. (1999)
<i>A. niger</i> CFTRI 30	SSF	Citric acid	Shankaranand and Lonsane (1994)
<i>Candida lipolytica</i>	SmF	Citric acid	Vandenberghe et al. (1998b)
<i>C. fimbriata</i>	SSF	Aroma compounds	Christen et al. (1997)
<i>C. fimbriata</i>	SSF	Aroma compounds	Bramorski et al. (1998a)
<i>K. marxianus</i>	SSF	Aroma compounds	Medeiros (1998)
<i>L. edodes</i>	SSF	Mushroom	Beux et al. (1995)
<i>P. sajor-caju</i>	SSF	Mushroom	Barbosa et al. (1995)
<i>Rhizopus</i> sp.	SSF	Biotransformation	Soccol et al. (1995a,b,c)
<i>R. arrahizus</i>	SmF	Fumaric acid	Carta et al. (1999)
<i>R. ciriciensis</i>	SmF	Fumaric acid	Carta et al. (1999)
<i>R. delemere</i>	SmF	Fumaric acid	Carta et al. (1999)
<i>R. formosa</i>	SmF	Fumaric acid	Carta et al. (1998, 1999)
<i>R. oligosporus</i>	SmF	Fumaric acid	Carta et al. (1999)
<i>R. oryzae</i>	SmF	Fumaric acid	Carta et al. (1999)
<i>R. oryzae</i>	SSF	Aroma compounds	Bramorski et al. (1998b)

^a SSF: solid-state fermentation, SmF: submerged fermentation.

4. Cultivation systems

The processes involving cultivation of microbes on cassava bagasse can broadly be classified into two groups: processes based on liquid fermentation, and processes based on solid-state fermentation (SSF). Most of the work has been carried out in SSF systems (Soccol, 1996). High water retention capacity (85–90%) also makes it an ideal substrate for SSF processes. Submerged fermentation (SmF) processes have rarely been utilized due to obvious reasons of cost effectiveness.

5. Bioprocesses involving cassava bagasse

5.1. Production of aroma compounds

One of the important areas of cassava bagasse utilization in bioprocesses has been on the production of flavour and aroma compounds. Bramorski et al. (1998a) compared fruity aroma production by *Ceratocystis fimbriata* in solid cultures from several agro-industrial wastes: cassava bagasse, apple pomace, amaranth and soya bean. Cassava bagasse was used in combination with soya bean or apple pomace. All the media supported fungal growth. Media containing cassava bagasse with apple pomace or soya bean produced a strong fruity aroma. The aroma production was growth dependent and the maximum aroma intensity was detected a few hours before or after the maximum respirometric activity. Sixteen compounds were separated by gas chromatography of the components present in the headspace and 15 of them were identified as acid (1), alcohols (6), aldehyde (1), ketones (2) and esters (5). Christen et al. (1997) studied fruity aroma production from various agro-industrial residues; wheat bran, cassava bagasse and sugar cane bagasse. All the substrates were shown to be adequate substrates for the growth and aroma production by the mould *C. fimbriata*. Addition of glucose to the solid medium (200 g l⁻¹) resulted in the production of a fruity aroma, whereas addition of leucine or valine resulted in a strong banana aroma. In these studies also aroma production was dependent on growth and the maximum aroma intensity was detected at about the time of the maximum respirometric activity. Twentyfour compounds were separated by headspace analysis using GC and 20 were identified as aldehyde (1), alcohols (7), ketones (4) and esters (8). It was clearly demonstrated that the chromatographic profile of the headspace of the culture was dependent on the substrate used and on the eventual precursor added. When leucine or valine was added to the substrate, the production of total volatiles in the headspace was ten times higher than that for ripe bananas. The Gompertz model, a logistic-like equation, was used to fit the integrated CO₂ and volatiles production data.

Bramorski et al. (1998b) also studied the production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates. When *R. oryzae* was grown on a medium containing cassava bagasse plus soybean meal (5:5 w/w), CO₂ production rate was at its highest (200 ml/l), whereas the highest volatile metabolite production was with amaranth grain as the sole substrate (282.8 ml/l). In the headspace, ethanol was the most abundant compound (more than 80%). Acetaldehyde, 1-propanol, ethyl propionate and 3-methyl butanol were also present. CO₂ and volatile metabolite productions reached their maxima around 20 and 36 h, respectively.

A strain of the yeast *Kluyveromyces marxianus* was used for the production of a fruity aroma in SSF using cassava bagasse as substrate (Medeiros, 1998). Experiments were performed with a 2⁵ statistical experimental design. The parameters studied were cultivation temperature, pH, initial water content and C/N ratio of substrate, and inoculum size. The volatile compounds were measured by the headspace analysis on a HP 5890 GC. A sensorial evaluation was employed to characterize the aroma of the cultures. The initial pH and the C/N ratio of the medium were statistically significant at 5% level for the production of volatile compounds. Aroma production increased in acidic pH (3.5) medium with a C/N ratio of 100. Sensorial evaluation of the produced volatiles revealed a fruity aroma, probably due to the production of isoamyl acetate and ethyl acetate. These compounds were identified by comparing with standard compounds. The results showed the feasibility of using cassava bagasse as a substrate to produce a fruity aroma with *K. marxianus* in SSF.

Flavour and aroma (including fragrance) compound synthesis by biotechnological processes nowadays plays an increasing role in the food, feed, cosmetic, chemical and pharmaceutical industries. This is mainly due to an increasing preference by the consumer for natural food additives and other compounds of biological origin. Attempts are being made to produce such compounds by fermentation of simple nutrients such as sugars and amino acids. In this regard, the works mentioned above have good implications. However, it will be necessary to develop efficient extraction processes for isolating these compounds from the fermentation media.

5.2. Biotransformation of cassava bagasse

In view of the high starch contents of cassava bagasse, an approach was applied to biotransform this into food and feed using edible fungal cultures. Soccol et al. (1995a, 1995b, 1995c) explored the possibilities of cultivating *Rhizopus* strains capable of attacking raw cassava starch present in cassava bagasse. They used 19 *Rhizopus* strains in SSF, but only three of them were capable of attacking significantly raw starch present in

cassava bagasse, six attacked moderately, seven weakly and three did not grow at all (Soccol et al., 1995a). Based on the results, a strain of *R. oryzae* 28627 was selected for further studies. Ideal conditions for biotransformation of cassava bagasse in SSF were determined. These were: temperature, 28–32°C, inoculation rate, 10^5 spores/g dry bagasse, substrate initial moisture and pH, 70% and 5.7–6.4, respectively, and C/N, 4.7–14. After 24 h of fermentation, the fermented matter showed 12 g protein/100 g cassava bagasse on a dry weight basis, which was almost sevenfold more than the initial protein content of cassava bagasse (1.67 g/100 g dry bagasse). The yield coefficient between consumed starch and synthesised protein was about 0.50 (Soccol et al., 1995b). The results on scale-up showed that tray-type bioreactors were most suitable. Six to eight cm thick substrate bed in the trays was good to ferment without any considerable loss in the growth of the fungus, as compared to initial experiments in the flasks. The biotransformed cassava bagasse showed a satisfactory quality on microbiological evaluation required by the existing law in Brazil. The final protein content in this case was about 13.5 g/100 g of dried cassava bagasse (Soccol et al., 1995c). These results are important, but it needs further studies to improve the protein content.

5.3. Production of mushrooms

Cassava bagasse has also been used for mushroom cultivation in SSF. Beux et al. (1995) compared the cultivation of *Lentinus edodes* on cassava bagasse and sugarcane bagasse, individually or in their mixture. Both the substrates were found suitable for mushroom production, but the best results were obtained when a mixture of cassava bagasse (80%) and sugarcane bagasse (20%) was used. Data on kinetics of starch consumption (present in cassava bagasse) showed that about 77% of the starch was used during the biotransformation process. The protein content of the substrate was improved three times. The results were claimed to be useful in providing a novel alternative technology for *shiitake* production. Barbosa et al. (1995) also compared cassava bagasse and sugarcane bagasse for mushroom production. They used a different fungal culture, *Pleurotus sajor-caju*. Cassava bagasse showed good potential for mushroom cultivation, but the best results were obtained when cassava bagasse was used in a mixture with sugarcane bagasse (8:2, dry weight basis). The results were claimed to be useful for upgrading the cassava bagasse for animal feed.

5.4. Production of organic acids

Among the various products produced through microbial cultivation on cassava bagasse, organic acids are important ones. Among these, citric acid production has

been well studied and reported. Citric acid is used in several industrial processes, such as food and pharmaceutical industries. It is also used in cosmetics and plastic industries. Most of the production of citric acid is by fermentation (submerged or liquid-surface methods), employing agro-industrial residues such as sugar beet molasses (Milson and Meers, 1985; Wang, 1998). Attempts have also been made to use alternative substrates such as carob pod extract (Roukas, 1998). In recent times, much attention has been paid to the production of citric acid from various other agro-industrial residues through the SSF route (Hang and Woodams, 1998; Pintado et al., 1998; Pintado and Lonsane, 1998). Kolicieski et al. (1995) studied citric acid production on three cellulosic supports in SSF. Out of the six strains of *Aspergillus niger* one, LPB 21, was selected for cultivation on cassava bagasse, sugarcane bagasse and vegetable sponge. Cassava bagasse was found to be a good substrate, giving 13.64 g citric acid per 100 g dry substrate. This corresponded to 41.78% yield. Under improved fermentation conditions, the citric acid production increased to 27 g/100 g dry substrate, which corresponded to 70% yield (based on sugars consumed). Shankaranand and Lonsane (1994) presented a comparative profile of citric acid production from various agro-industrial residues, such as cassava bagasse, wheat bran, rice bran, sugarcane pressmud, coffee husk, etc., using an indigenous strain of *A. niger* CFTRI 30. Cassava bagasse gave the highest yield of citric acid based on the total starch or sugars present initially in the medium (Shankaranand and Lonsane, 1994). Vandenberghe et al. (1999) used three substrates, sugarcane bagasse, coffee husk and cassava bagasse for citric acid production with *A. niger* NRRL 2001. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. The citric acid production reached a maximum (88 g/kg dry matter). The results were of significant importance for commercial production.

5.5. Hydrolysis of cassava bagasse and application of hydrolysate in bioprocesses

One alternative approach for utilizing cassava bagasse has been to subject it to enzymatic hydrolysis using amylolytic enzymes and then to use the hydrolysate for cultivation of micro-organisms for bioprocesses. Vandenberghe et al. (1998a) and Carta et al. (1997) conducted a study to optimize the hydrolysis parameters of cassava bagasse using commercially available α -amylase (Termamyl 120 L) and glucoamylase (AMG, Novo Nordisk). A statistical experimental design was used to obtain a hydrolysate with high concentration of reducing sugars, mainly glucose. Parameters studied were the concentration of enzyme, period of enzyme action and bagasse particle size. Best results were obtained when

hydrolysis was carried out using 100 µl of Termamyl 120 L/100 g of starch at 90°C for one h, and 471 µl of AMG/100 g of starch at 60°C for 24 h with a particle size <0.84 mm. Hydrolysates reached 110 g/l of reducing sugars, demonstrating a good conversion efficiency.

Cassava bagasse hydrolysate was used for the production of citric acid by fermenting with a yeast culture of *Candida lipolytica* NRRL Y-1095 (Vandenberghe et al., 1998b). Batch fermentations were performed in two stages, a carbon-limited growth phase and a nitrogen-limited phase. In the first stage, growth was carried out at 28°C, pH 5.5 and 150 rpm for 40–48 h, and then the cells were separated from the medium by centrifugation. In the second stage, fermentation was carried out using these cells (28°C, pH 5.5, 200 rpm, 96 h). Citric acid yields were 10 g/l. However, 2.5 g/l of isocitric acid was also produced simultaneously.

Another important organic acid produced using cassava bagasse hydrolysate is fumaric acid (C₄H₄O₄). Fumaric acid has a wide range of applications. It is an interesting intermediate in chemical synthesis involving esterification reactions. It is non-toxic and non-hygroscopic in nature and due to these properties is also used as an acidulant in the food and pharmaceutical industries. Carta et al. (1998, 1999) studied the prospects of production of fumaric acid from cassava bagasse. First, cassava bagasse was subjected to enzymatic hydrolysis and the hydrolysate so obtained was used for fumaric acid production. Submerged fermentation was carried out using several *Rhizopus* strains. Six different media were constituted using different nitrogen sources and hydrolysate. An experimental design was used to optimize the media and cultivation conditions. The strain *Rhizopus formosa* MUCL 28422 was found to be the best fumaric acid producer, yielding 21.28 g/l in a medium containing cassava bagasse hydrolysate as the sole carbon source, KNO₃ as nitrogen source (C/N ratio of 168), 20 g/l of CaCO₃, 10 µg/l of biotin, 0.04 g/l of ZnSO₄·7H₂O, 0.25 g/l of MgSO₄·7H₂O, 0.15 g/l of KH₂PO₄, and 15 ml/l of methanol. The process was found feasible for commercial fumaric acid production.

6. Conclusions

It can be concluded that bioconversion of cassava bagasse could be economically useful in some cases, e.g. for the production of enzymes, organic acids, feed, etc. As cassava bagasse is degraded easily by micro-organisms without any pretreatment, it offers advantages in comparison with sugarcane bagasse. Production of microbial enzymes could be an area to be exploited using cassava bagasse. Biotransformed cassava bagasse could be used as cattle feed. However, these would require economical considerations too. In this regard, economic constraints may not favour liquid fermentation for such

processes. However, SSF may hold promise and focus should be made on developing SSF technologies. Development of efficient microbial strains, mainly fungal cultures, suitable for bioconversion of cassava bagasse is still a largely unexplored area. Efforts should be also made for improving cassava bagasse hydrolysis conditions; its effective conversion into fermentable sugars is an area which needs further inputs in terms of research and development. Cassava bagasse hydrolysate could serve as a good substrate for production of value-added products.

References

- Barbosa, M.C.S., Soccol, C.R., Marin, B., Todeschini, M.L., Tonial, T., Flores, V., 1995. Prospect for production of *Pleurotus sajor-caju* from cassava fibrous waste. In: Roussos, S., Lonsane, B.K., Raimbault, M., Viniegra-Gonzalez, G. (Eds.), *Advances in Solid State Fermentation*. Kluwer Academic Publishers, Dordrecht, pp. 513–525.
- Beux, M.R., Soccol, C.R., Marin, B., Tonial, T., Roussos, S., 1995. Cultivation of *Lentinus edodes* on the mixture of cassava bagasse and sugarcane bagasse. In: Roussos, S., Lonsane, B.K., Raimbault, M., Viniegra-Gonzalez, G. (Eds.), *Advances in Solid State Fermentation*. Kluwer Academic Publishers, Dordrecht, pp. 499–511.
- Bramorski, A., Soccol, C.R., Christen, P., Revah, S., 1998a. Fruity aroma production by *Ceratocystis fimbriata* in solid cultures from agro-industrial wastes. *Rev. Microbiol.* 29 (3), 208–212.
- Bramorski, A., Christen, P., Ramirez, M., Soccol, C.R., Revah, S., 1998b. Production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid state cultivation on tropical agro-industrial substrates. *Biotechnol. Lett.* 20 (4), 359–362.
- Carta, F.S., Soccol, C.R., Vandenberghe, L.P.S., Lebault, J.-M., 1997. Enzymatic hydrolysis of cassava bagasse for production of metabolites obtained by fermentation. Paper presented in Enzytec-97, Rio de Janeiro, Brazil.
- Carta, F.S., Soccol, C.R., Machado, L., Machado, C.M.M., 1998. Prospect of using cassava bagasse waste for producing fumaric acid. *J. Sci. Ind. Res.* 57 (10–11), 644–649.
- Carta, F.S., Soccol, C.R., Ramos, L.P., Fontana, J.D., 1999. Production of fumaric acid by fermentation of enzymatic hydrolysates derived from cassava bagasse. *Bioresource Technol.* 68, 23–28.
- Cereda, M.P., 1994. Caratecrização de resíduos da industrialização da mandioca. In: Cereda, M.P. (Ed.), *Industrialização da mandioca no. Paulicéia, SãoPaulo, Brazil*, pp. 11–50.
- Cereda, M.P., Takahashi, M., 1996. Cassava wastes: their characterization and uses and treatment in Brazil. In: Dufour, D., O'Brien, G.M., Best, R. (Eds.), *Cassava Flour and Starch*. CIAT, Cali, Columbia, p. 222.
- Cereda, M.P., Takitane, L.C., Chuzel, G., Vilpoux, O., 1996. Starch potential in Brazil. In: Dufour, D., O'Brien, G.M., Best, R. (Eds.), *Cassava Flour and Starch*. CIAT, Cali, Columbia, pp. 19–24.
- Christen, P., Meza, J.C., Revah, S., 1997. Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata*: influence of the substrate type and the presence of precursors. *Mycolog. Res.* 101, 911–919.
- Food and Agricultural Organization, 1995. Cassava. *Cassava News Lett.* 19 (1), 9.
- FAO/GIEWS, 1999. Cassava. *Food Outlook* (2), 6.
- Giraud, E., 1993. Physiologie et Enzymologie d' une Novele Souche de *Lactobacillus Plantarum* Amylolytique en Relation avec la Croissance e Degradation des Composes Cynogeniques du

- Manioc et la Production d'acide Lactique. Ph.D. Thesis, University of Provence, France.
- Hang, Y.D., Woodams, F.F., 1998. Production of citric acid from corn cobs by *Aspergillus niger*. *Bioresource Technol.* 65 (3), 251–254.
- Hohnholz, J.H., 1980. *Appl. Geogr. Dev.* 16, 117–135.
- Kato, M.S.A., Souza, S.M.C., 1987. Informe Agropecuario 13, 9–16.
- Kolichski, M.B., Soccol, C.R., Marin, B., Medeiros, E., Raimbault, M., 1995. Citric acid production on three cellulosic supports in solid state fermentation. In: Roussos, S., Lonsane, B.K., Raimbault, M., Viniegra-Gonzalez, G. (Eds.), *Advances in Solid State Fermentation*. Kluwer Academic Publishers, Dordrecht, pp. 447–460.
- Laukevics, J.J., Aptise, A.F., Viesturs, U.S., Tengerdy, R.P., 1985. *Biotechnol. Bioengng.* 27, 1687–1691.
- Medeiros, B.P.A., 1998. Production and Composition of Aromatic Volatile Compounds by *Cluyveromyces marxianus* in Solid State Fermentation. Master's Thesis, Federal University of Parana, Curitiba, Brazil.
- Milson, P.J., Meers, J.L., 1985. Citric acid. In: Blanch, H.W., Drew, S., Wang, D.I.C. (Eds.), *Comprehensive Biotechnology*. Pergamon Press, Oxford, pp. 665–680.
- Nampoothiri, K.M., Pandey, A., 1996. Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp. *Biotechnol. Lett.* 16 (2), 199–204.
- Pandey, A., 1992. Recent process developments in solid state fermentation. *Process Biochem.* 27 (2), 109–117.
- Pandey, A., 1994. Solid state fermentation an overview. In: Pandey, A. (Ed.), *Solid State Fermentation*. Wiley Eastern, New Delhi, India, pp. 3–10.
- Pandey, A., Damodaran, A.D., 1991. Prospects of production of alcohol from tapioca. A report to the Government of Kerala, Trivandrum, India.
- Pandey, A., Soccol, C.R., 1998. Bioconversion of biomass: a case study of ligno-cellulosics bioconversions in solid state fermentation. *Brazilian Arch. Biol. Technol.* 41 (4), 379–390.
- Pandey, A., Nigam, P., Vogel, M., 1988. Simultaneous saccharification and protein enrichment fermentation of sugar beet pulp. *Biotechnol. Lett.* 10 (1), 67–72.
- Pintado, J., Lonsane, B.K., 1998. On-line monitoring of citric acid production in solid state fermentation by respirometry. *Process Biochem.* 33 (5), 513–517.
- Pintado, J., Torrado, A., Gonzalez, M.P., Murado, M.A., 1998. Optimization of nutrient concentration for citric acid production by solid state culture of *Aspergillus niger* on polyurethane foam. *Enzyme Microb. Technol.* 23 (1), 149–154.
- Rosemberg, J.A., 1957. *Arquivos de Fermentacao* 2, 85–93.
- Roukas, T., 1998. Citric acid production from carob pod extract by cell recycle of *Aspergillus niger* ATCC 9142. *Food Biotechnol.* 12 (1–2), 91–95.
- Sachal, B.A., Olsen, K.M., 1999. Cassava's origin. *Proceedings of National Academy of Sciences, USA*, 11 May 1999. Cited from Quadnet, 4 June 1999, Cassava6-4WU (www.quad.net.com/archive/CASSAVA6-4wu).
- Shankaranand, V.S., Lonsane, B.K., 1994. Citric acid by solid state fermentation a case study for commercial exploitation. In: Pandey, A. (Ed.), *Solid State Fermentation*. Wiley Eastern, New Delhi, India, pp. 149–152.
- Soccol, C.R., 1994. Contribuição ao Estudo da Fermentação no Estado Sólido em Relação com a Produção de Ácido Fumárico, Biotransformação de Resíduo Sólido de Mandioca por *Rhizopus* e Basidiomacromicetos do Gênero *Pleurotus*. Curitiba, 1994. Tese (Professor Titular), Universidade Federal do Paraná, pp. 228.
- Soccol, C.R., Stertz, S.C., Raimbault, M., Pinheiro, L.I., 1995a. Biotransformation of solid waste from cassava starch production by *Rhizopus* in solid state fermentation, 1. Screening of strains. *Arch. Biol. Technol.* 38 (4), 1303–1310.
- Soccol, C.R., Stertz, S.C., Raimbault, M., Pinheiro, L.I., 1995b. Biotransformation of solid waste from cassava starch production by *Rhizopus* in solid state fermentation, 2. Optimization of the culture conditions and growth kinetics. *Arch. Biol. Technol.* 38 (4), 1311–1318.
- Soccol, C.R., Stertz, S.C., Raimbault, M., Pinheiro, L.I., 1995c. Biotransformation of solid waste from cassava starch production by *Rhizopus* in solid state fermentation, 3. Scale-up studies in different bioreactors. *Arch. Biol. Technol.* 38 (4), 1319–1326.
- Soccol, C.R., 1996. Biotechnological products from cassava roots by solid state fermentation. *J. Sci. Indust. Res.* 55, 358–364.
- Stertz, S.C., 1997. Bioconversão da Farinha de Mandioca Crua (Manihot Esculenta, Crantz) por Fungos do Gênero *Rhizopus* em Fermentação no Estado Sólido. Tese Mestrado, Universidade Federal do Paraná, Curitiba.
- Vandenbergh, L.P.S., Soccol, C.R., Carta, F.S., Lebeault, J.-M., Milcent, P.F., Machado, L., 1998a. Enzymatic hydrolysis of liquid and solid wastes of cassava root industry for production of metabolites by fermentation. *COBEQ 98*, Porto Alegre, Brazil.
- Vandenbergh, L.P.S., Soccol, C.R., Lebeault, J.M., Krieger, N., 1998b. Cassava wastes hydrolysate an alternative carbon source for citric acid production by *Candida lipolytica*. Paper presented in Internatl. Congr. Biotech'98, Portugal.
- Vandenbergh, L.P.S., Soccol, C.R., Pandey, A., Lebeault, J.M., 1999. Solid state fermentation for the synthesis of citric acid by *Aspergillus niger*. *Bioresource Technol.*, in press.
- Wang, H., 1998. Improvement of citric acid production by *Aspergillus niger* with addition of phytase to beet molasses. *Bioresource Technol.* 65 (3), 243–247.