CDD: 661

PRODUCTION OF *TRICHOSPORON SP* BIOMASS – SURFACE FERMENTATION WITH DEXTRIN AS A SOURCE OF CARBON

CÁSSIO L. KIRCHNER[1,2]
MARECI M. ALMEIDA[1,2]
GILVAN WOSIACKI [1,3]

1 Zootechny and Food Technology Department, Ponta Grossa State University
2 CNPq/RHAE Agribusiness Program
3 CNPq Research Fellow

RESUMO

O resíduo líquido gerado no processamento de mandioca, especialmente aquele conhecido com o nome de “manipueira”, pode causar muitos problemas no binômio economia x ecologia devido ao seu conteúdo em amido e em linamarina. O tratamento correto pode ser muito dispendioso e assim o seu impacto ecológico nas redondezas é bastante forte. Algumas pesquisas relacionadas à utilização desse resíduo agro-industrial ainda estão em desenvolvimento visando transformá-lo em subproduto passível de ser usado em um processo economicamente viável. A possibilidade de usá-lo como ingrediente na composição de um meio de cultura para uma cepa de *Trichosporon* sp. visa à produção de biomassa, tanto protéica [SCP] quanto lipídica [SCL], unicelulares. O agente microbiano é reconhecidamente produtor de gordura e pode respirar na presença dos glicosídios cianogênicos. Como leveduras não são, usualmente, amilolíticas, algumas investigações sobre a assimilação de carboidratos precisam ser feitas. Uma cepa isolada de resíduo industrial está sendo estudada em experimentos em nível de laboratório com fermentação em superfície com meios de composição definida. Na fase estacionária a biomassa produzida foi de 11,60 g/L e ao final do experimento o nível de carboidrato consumido foi de 99,25%. A taxa de conversão [biomassa produzida por açúcar consumido] foi de 0,67 [adimensional]. Na presença de dextrina obtida a partir de amido comercial de mandioca, a produção de biomassa e a utilização de carboidrato foram atípicas. A cepa estudada foi capaz de crescer de maneira atípica em um meio contendo amido hidrolisado, e o perfil de produção de biomassa mostrou ser dependente do grau de hidrólise dos açúcares. Os resultados demonstram ser necessária uma hidrólise preliminar do amido se o consumo total de açúcar for desejável.

Palavras-chave: *Trichosporon* sp, biomassa, resíduo líquido, fermentação em superfície

1. Introduction

Cassava roots have been used both as food and as raw material to be processed into several products of economic value. Nowadays, the production of cassava roots shows a remarkable growth in this agricultural sector. In the processing of cassava flour or starch, several wastes can be generated and among them, the *manipueira* [in the Brazilian Indian language, tupi – guarani, this means “can be extracted”].

This industrial waste water is highly inconvenient considering environmental pollution and toxicity related to starch and linamarin, respectively. The amount of starch in the industrial waste is varies according the traditional batch processing and to the technological level. The amount of linamarin, a cyanide glycoside that can be found in all parts of the plant, from the roots up to the leaves, is dependent on the raw material and is especially high in some varieties considered bitter (Cereda, 1994).

It has already been said that manipueira contains from 5% to 7% [w/w] as a sediment fraction and even more in a colloidal suspension, from 44 to 120 g/L of the total sugar; after decanting the level of sugar may be reduced to around 35 g/L. The reduction of the amount of sugar is the main responsible for the high chemical oxygen demand – COD – in the industrial wastes, which are dispensed into rivers in the neighborhood without any treatment or exploitation (Cereda, 1972).

In order to equilibrate the economy and the ecology losses it is necessary to increase the knowledge about this waste water and to study some possibilities focusing its use as by-products in the industry. The possibility of using it as a basis for a fermentation process aiming the microbial production of protein (Single Cell Protein - SCP) or lipids (Single Cell Lipids - SCL) for human or animal nutrition, or even for energy purpose, seems to be particularly interesting.

There are several academic scientists involved in this kind of research and many experiments were conducted in order to study the utilization of manipueira as a fertilizer (Fioretto, 1994), a herbicide and a nematocide (Ponte & Franco, 1994) and as a basis to promote microbial growth and/or secondly in the production of metabolites (Wosiacki et al., 1994); as examples of biotechnological opportunities it is possible also to include biogas (Kennedy et al., 1987; Cereda, 1994; Ratledge, 1979; Ratledge & Boulton, 1985) or the production of aroma (Damasceno, 1998).

The production of a microbial biomass with starchy raw material, as waste or by-products from cassava processing lead the academic investigation to process and product engineer aiming to contribute in the agricultural industrial sector with technological innovations. This is done mainly in order to equilibrate the binomial ecology/economy but also to get information about the quality and commercial impact of new products. Kennedy et al. (1987) listed more than 400 yeast strains as potential microbial agents able to produce biomass; 100 strains were indeed able to grow by using starch as the sole carbon source. These selected strains were able to synthesize amylase, to reduce sugar through fermentation and to produce unicellular proteins.

Yeasts from the genus ***Trichosporon***, recognized lipogenic microorganisms (Ratledge, 1979), in this case isolated from manipueira, were able to utilize a special cyanide insensible respiratory pathway (Brasil et al., 1982; Cereda et al., 1981). This fact enables the strains to generate the energy necessary for primary and secondary production of metabolites using soluble sugars from the wastewater. However, as yeasts are, usually, non-amylolytic microorganisms, a previous starch hydrolysis step seems to be necessary to allow its utilization as a source of carbon (Fioretto et al., 1985; Sichieri et al., 1986; Wosiacki et al., 1994).

The microbial growth in this waste is due to the capability of some microorganisms to assimilate carbohydrate even in the presence of cyanide and to ferment starch dextrins or free sugars obtained either by its microbial enzyme system or by enzymes produced in the environmental microbial system. On the other side, according to the results of several fermentation procedures, like the cultivation of the mold in a semi solid substance containing manipueira and cassava flour or in a submerged or surface fermentation, the best results were found in the latter procedure (Fioretto, 1987) which strongly suggests the use of this technique in laboratory conditions.

Due to the experimental facilities and also because it represents the natural conditions, this investigation was conducted in a medium of synthetic growth with surface fermentation conditions aiming at the identification of the effect of several starch hydrolysis degrees in the production of biomass and in the consumption of carbohydrates.

2. Material and methods

Microorganism and culture conditions. As a fermentative microbial agent was used a strain isolated from soil typified as ***Trichosporon sp***. (Lodder, 1970), stored in a solid agar medium at 8º C in the presence of 20 ppm KCN. To start the experiment, the strain was adapted to the liquid medium during 24 hours and then the assays were conducted at 20ºC in stationary flasks. Details of the experimental procedure are available in the literature (Efing, 1991; Efing and Wosiacki, 1998).

Culture media. A synthetic culture medium was used with a chemical composition similar to that of manipueira (Sichieri, 1986), containing 16.52 g/L ammonium sulfate, 3.71 g/L magnesium sulfate, 3.19 g/L potassium chloride, 0.48 g/L potassium phosphate, 0.62 g/L calcium chloride, 0.11 g/L iron sulfate, 0.01 g/L zinc sulfate, 0.008 g/L cobalt chloride, 0.005 g/L manganese sulfate, 0.004 g/L copper sulfate and 0.14 g/L potassium cyanide (Efing, 1991), enriched with 5.0 g/L of yeast extract. The solution was adjusted to pH 5.0 and sterilized (1 atm; 121ºC; 20 min.). As a carbon source commercial dextrin and cassava starch were used. Commercial dextrin, with 13.38g% of reducing sugar, was hydrolyzed according to the process enzyme : enzyme and the sugar level were adjusted to 20.0 g/L. Commercial cassava starch was hydrolyzed with 0.1 N HCl in water at boiling temperature and 6 samples were collected during the experimental period which reached up to 330 minutes in order to obtain dextrin with different degrees of hydrolysis. After thermal treatment, the samples were adjusted to 30.0 g/L.

Surface fermentation. The assays, each one with three repetitions, were incubated at 20 ºC and the samples were similarly collected, followed by filtering through a paper filter; the liquid fraction was used to analyze sugars and the solid one, to analyze biomass production, according to Efing & Wosiacki (1998).

Chemical Analysis. Dry weight of mycelium was determined gravimetrically. The carbohydrate contents were determined considering sugar totals (ART) - with the phenol - sulfuric acid technique (Dubois et al., 1956), reducing sugars (AR) with Somogyi and Nelson colorimetric procedures (Plummer, 1981) and specifically glucose (GLC) with the enzyme technique (Dalquist, 1961). All the results were expressed as glucose, in g/L. The parameter used to evaluate fermentation performance was the ratio of the mycelium dry weight and the residual sugar, both in g/L. The magnitude of these adimensional figures reveals both the biomass production and the residual sugar and it rises when biomass production is high and/or when residual sugar is low (Efing, 1991). Other parameters can be used in order to define the fermentation process more precisely and investigate the yield [amount of biomass produced by the amount of carbohydrate consumed], in the consumption of carbohydrate. These results were analyzed through simple statistical descrition so that each parameter could be discriminated; when necessary the statistical zero was used [0.01].

3. Results and discussion

The programmed investigation aiming at identifying nutritional requirements in toxic conditions concerning quality and quantity of carbohydrate by a ***Trichosporon sp.*** strain was done in a synthetic culture medium with several sources of carbon, including monosaccharide and dextrin, all derived from cassava starch. It was considered a scientific report about the utilization of glucose (Sichieri, 1986) and also the information that the products from enzymatic hydrolysis of starch were partially utilized, which suggests that the selected microorganism is not able to completely utilize cassava starch as the carbon source (Efing, 1991).

Figure 1, structured with results obtained from the specialized literature (Efing & Wosiacki, 1998), shows the results of a surface fermentation experiment with enzyme : enzyme hydrolyzed starch and clearly shows that on the 10th day the process is already over, leaving a residual sugar level of around 10% of the initial content. This residual sugar level strongly suggests the presence of dextrin still not used in the fermentation process and also that apparently the original starch was not completely hydrolyzed with the enzyme: enzyme procedure.

Fermentation with hydrolyzed dextrin. The results of the fermentation conducted with commercial dextrin completely hydrolyzed with amylolytic enzymes are shown in Table 1, including the figures concerning biomass production, residual sugar and the proposed indicator of fermentation performance. The results related to sugar consumption and the degree of conversion (biomass/consumed sugar) were also taken to account.

The first sample was collected after 113 hours of fermentation, when it was possible to see biomass in the flask. The increase of biomass was practically observed in the period from 113 up to 185 hours of incubation. By graphic interpolation it was possible to observe that microbial growth began after the 3rd day, which means, around 72 h. The stationary phase, characterized by the stabilization of biomass production [shadowed area A], could be observed after 185 hours; the mean value of biomass production was 11.6 ± 0.3 g/L, with a variation coefficient of 2.58%, very stable if compared with the variability of all the processing (46.73%). According to the level of residual sugar, a rapid utilization can be observed from 161and 185 h. – just 24 h. [shadowed area B], when around 52.9% was consumed. After this step, the sugar level decreased to 0.2 g/L, which represents 99.25 % from initial figures. It is interesting to observe that the ratio of amount of biomass produced by the amount of sugar consumed shows the great variability of the fermentation (73.68%) but if only the results concerning the stationary phase were considered this ratio would have low figures, low variability, a mean value of 0.67 ± 0.07 (w/w), which indicates a variation coefficient of 10.0% [shadowed area C].

In Figure 2 it is possible to observe the experimental data considering biomass production and decrease in the sugar level. The results obtained in this experiment strongly suggest that this microbial strain is able to metabolize all the reducing sugar available.

Fermentation in the presence of dextrin. In the experiment planned to observe the utilization of commercial dextrin as a source of carbon, the culture media contained 20.0 g/L of total sugar, including 13.83% of reducing sugars; in this amount there are 2.13% of glucose. The results are shown in Table 2, including the same parameter.

Mycelium appears on the surface of the culture media only after the 7th day of incubation, making evident the long lag phase and showing an atypical fermentation profile. The stationary phase was very long and began only after the 10th day – in the last experiment it began after seven days. The results shown in this experiment comprehend a set of irregular figures.

The mean value concerning biomass production was 6.62 ± 1.90 g/L, which explains a variation coefficient of 28.63% [shadowed area D], too high when compared with the 2.52% from the former experiment. The results concerning the production of biomass may be explained practically because the biomass fell down in the flask many times during all the 18 days of the experiment; after each precipitation, the production of biomass went on, always with the formation of new colonies. The residual sugar level decreased slowly before reaching a minimum value of 11.11 g/L at the end, which indicates a maximal utilization of 44.5%.

In Figure 3 the surface fermentation process is illustrated, when commercial dextrin was used as the carbon source, with emphasis on biomass production, residual sugar. It is possible to observe the long lag phase and the instability of all the fermentative process, already shown in the Table 2 by the high variation coefficients, although each assay in this experiment has been conducted three times. The results suggest that the microbial strain is unable to promote both the substrate hydrolysis and the dextrin utilization.

Effect of the degree hydrolysis of cassava starch. The influence of the hydrolysis was investigated and the data related to the input variable - the time of cassava starch hydrolysis and the degree reached, and the results of the effect in biomass production, in carbohydrate consumed, in the degree of conversion, in the level of residual sugar and in the indicator are shown in the Table 3.

The culture medium composed with ions and with native cassava starch had also reducing sugars although there was no hydrolysis treatment. The hydrolysis degree for each assay was defined as the ratio between reducing and total sugar content. The mean value of total sugars in each assay was 31.60 ± 1.70 g/L at the beginning and the content of reducing sugar was dependent on the degree of hydrolysis, and automatically on the reaction time. The correlation of the reaction time and the degree of hydrolysis is high (+ 0.97) and statistically significant at a 5% level. In the assays the biomass production reached an average value of 13.36 ± 2.54 g/L. (C.V.= 19.01%) and it is visible that these figures increase proportionally with the degree of hydrolysis; the correlation between them is – 0.94 statistically significant to the 5% level. The mean value of residual sugar found in the assay was 19.78 ± 7.23 g/L (C.V. = 36.55%) and the results are inversely correlated (– 0,93) to the level of 5%; so the higher the degree of hydrolysis, the lower the residual sugar; this statement implies that mold utilizes more frequently soluble carbohydrate with low molecular weight.

The performance of the fermentation process shows that the lowest value [0.40] is directly related to the minimum starch hydrolysis [42.56%] and the highest value [1.73], to the maximum starch hydrolysis [100%]. A statistical analysis shows a positive correlation of 0.92 significant at 5% level (Figure 4). The results clearly indicated the preference of the microorganism for low molecular weight soluble sugar, which can be seen by comparing initial and final values. The figures are shown in Table 4, where initial and final contents of total sugar can be seen, reducing sugar and glucose, specifically.

The results allow us to observe the consumption of different kinds of dextrin in the culture media, from starch [observed by extrapolation] down to glucose; they show that sugar consumption is directly influenced by the degree of starch hydrolysis. The consumption of glucose reaches the mean value of 80%, while reducing sugar including glucose is around 70%.

The degree of hydrolysis of cassava starch positively influences the fermentative process both increasing biomass production and decreasing residual sugar; this influence can also be observed in the conversion of sugar in biomass, which decreases when the availability of reducing sugar increases.

The behavior of ***Trichosporon*** sp. considering the utilization of dextrin was irregular and atypical: the microorganism shows a cyclical behavior concerning growth and the consumption of sugar was dependent on both the quality and quantity of carbohydrate available in the culture media. This situation reflects low figures of the processing quality indicator.

This strain of ***Trichosporon*** sp. shows the ability to utilize the source of carbon available in manipueira but a previous hydrolysis step is necessary to increase the consumption of sugar. The results of the programmed experiment showed that the selected strain has no ability to hydrolyze dextrin and has a preference for low molecular weight soluble sugar.

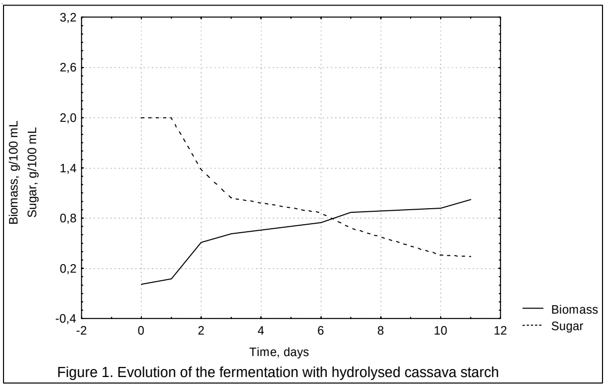

Figure 1. Evolution of the fermentation with hydrolysed cassava starch

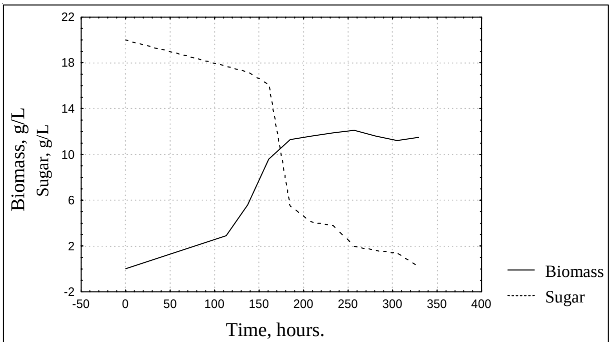

Figure 2. Evolution of fermentation with enzyme-hydrolysed dextrin

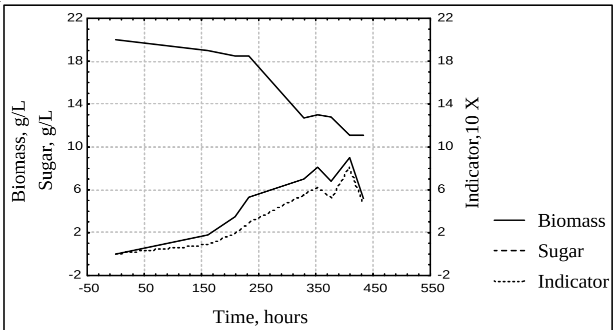

Figure 3. Evolution of surface fermentation with commercial dextrin.

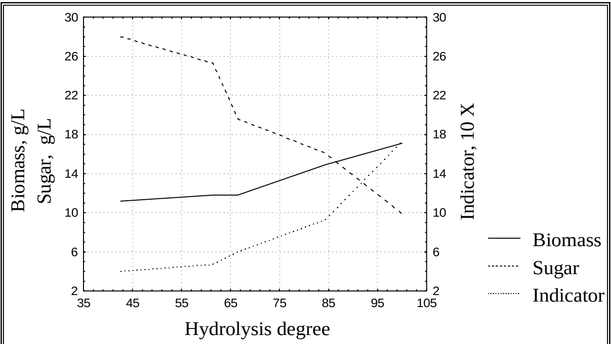

Figure 4. Effect of the hydrolysis degree in the fermentation proce

Table 1. Evolution of surface fermentation with hydrolyzed dextrin

Time, Hours	Biomass, g/L	Residual sugar, g/L	Performance indicator	Sugar consumption g/L	Yield	Consumed sugar %
0	0.01	20.00	0.01	0.01	0.01	0.01
113	2.90	17.70	0.16	2.30	1.29	11.40
137	5.60	17.20	0.33	2.80	1.99	14.00
161	9.60	B 16.10	0.60	3.90	2.47	19.35
185	A 11.30	5.50	2.05	14.50	C 0.78	72.25
209	11.60	4.10	2.83	15.90	0.73	74.70
233	11.90	3.80	3.13	16.20	0.73	81.15
257	12.10	2.00	6.05	18.00	0.67	89.90
281	11.60	1.60	7.25	18.40	0.63	91.80
305	11.20	1.40	8.00	18.70	0.60	93.25
329	11.50	0.20	[57.50]	19.80	0.58	99.25
Average	9.03	8.15	7.99	11.87	0.95	58.82
Deviation	4.22	7.80	16.67	7.81	0.70	38.83
V.C., %	46.73	95.70	-------	65.80	73.68	66.01

Table 2. Evolution of fermentation process with commercial dextrin.

Time, hours	Biomass, g/L	Residual sugar, g/L	Performance indicator	Sugar consumption g/L	Yield	Consumed sugar, %
0.01	0.01	20.00	0.01	0.01	0.01	0.01
161.00	1.80	19.00	0.09	1.00	1.80	5.00
209.00	3.50	18.50	0.19	1.50	2.33	7.50
233.00	5.30	E 18.50	0.29	1.50	3.53	7.50
329.00	D 7.00	12.70	0.55	7.30	0.96	36.50
353.00	8.10	13.00	0.62	7.00	1.16	35.00
377.00	6.80	12.80	0.53	6.80	1.00	34.00
409.00	9.00	11.10	0.81	8.90	1.01	44.50
433.00	~~5.20~~	11.10	0.47	8.90	0.58	44.50
Average	5.19	15.19	0.40	4.77	1.38	23.83
Deviation	2.96	3.70	0.27	3.67	1.04	18.37
V.C., %	57.03	24.36	67.50	76.94	75.36	77.09

Table 3. Effect of the starch hydrolysis degree in the fermentation process

Hydrolysis time min	Hydrolysis degree %	Biomass g/L	Residual sugar, g/L	Performance indicator	Sugar consumption g/L	Yield	Consumed sugar %
50	42.56	11.20	28.00	5.60	16.67	2.00	0.40
100	61.45	11.80	25.30	7.90	23.80	1.49	0.47
150	66.48	11.80	19.60	10.90	35.74	1.08	0.60
200	84.37	14.90	19.60	15.90	49.74	0.94	0.93
330	100.00	17.10	9.90	21.90	68.87	0.78	1.73
Average		13.36	19.78	12.44	38.96	1.26	0.83
Deviation		2.54	7.23	6.54	20.90	0.49	0.54
C.V.%		19.01	36.55	52.57	53.64	38.88	65.06

Table 4. Effect of the degree of starch hydrolysis in the microbial utilization

Input variables		Initial values, in g/100 mL			Final Values, g/100 m			Sugar consumption, %		
Time, h.	Degree, %	ART	ARS	GLC	ART	ARS	GLC	ART	ARS	GLC
50	42.53	33.60	14.30	10.00	28.00	6.70	1.60	16.67	67.16	84.00
100	61.45	33.20	20.40	15.80	25.30	6.20	1.90	23.80	69.60	87.72
150	66.88	30.50	20.40	13.20	19.60	8.30	3.30	35.74	59.31	75.00
200	84.37	32.00	27.00	18.30	16.20	7.80	4.80	49.74	71.85	73.77
330	100.00	31.80	31.20	20.80	9.90	7.00	5.40	68.87	77.56	74.04

Recebido para publicação em 05/00.
Aceito para publicação em 05/00.

ABSTRACT

Cassava processing wastewater, known as “manipueira”, causes many problems concerning economy and ecology due to its starch and linamarin contents. The ecological impact on the agricultural industry is usually very strong, and a proper treatment is yet unknown and would be very expensive. Much research concerning the utilization of this industrial residue is still in progress, aiming at transforming it in a by-product that can be used in an economic process. The possibility of using it as a culture medium for *Trichosporon* sp. in order to produce single cell protein or single cell lipid is interesting because this microorganism is known to grow in the presence of cyanide glycoside and also to produce high amounts of intracellular lipids. This yeast is generally known to be a non-amylolytic microorganism, but some investigation about the assimilation of carbohydrate had to be done. A strain isolated from industrial wastewater was studied in laboratory experiments with surface fermentation in synthetic media. In the stationary phase the biomass produced was 11.60 g/L and at the end of the experiment the carbohydrate consumed was 99.25%. In the presence of commercial cassava dextrin the production of biomass and the utilization of carbohydrate were atypical. The strain under study was able to grow in a medium containing hydrolyzed starch and the growth profile proved that this strain is dependent on the degree of hydrolysis. The results suggest that a preliminary starch hydrolysis procedure is necessary if a total utilization of the carbohydrate from the wastewater is expected.

Key words: *Trichosporon* sp., biomass, liquid waste, agricultural technology, surface fermentation

Endereço para contato: wosiacki@centerline.com.br

Acknowledgements

The authors are deeply grate to the Ponta Grossa State University – UEPG, to the National Council of Scientific and Technological Development – CNPq, to RHAE/CNPq in the AGROINDÚSTRIA PROGRAM for scholarship, to Carla Cristine Kanunfre for reviewing the manuscript and to Maria Etelvina Madalozzo Ramos, the references.

REFERENCES

Brasil, O. G.; Cereda,M.P.; Fioretto,A.M.C. (1982).Indução da respiração resistente ao cianeto em microrganismo pela presença de inibidores de crescimento microbiano. **Phyton**,.Buenos Ayres, v. 42, p. 49-53.

Cereda,M.P.(1972) Água residual das fecularias. Estado de São Paulo, São Paulo, 18/06/1972, Supl. agrícola.

Cereda,M.P. (1994.) Resíduos da Industrialização da Mandioca no Brasil. Utilização e Tratamento. Paulicéia : São Paulo.

Cereda,M.P.; Brasil,O.G.; Fioretto,A.M.C. (1981) Microrganismos com respiração resistente ao cianeto isolados de líquido residual de fecularias. **Phyton**, Buenos Ayres, v. 41, p. 197-20.

Cereda,M.P.; Fioretto,A.M.C.; Wosiacki,G.; Sichieri,V.L.F.S. (1984). Parâmetros de crescimento de duas cepas de ***Trichosporon*** sp. SIMPÓSIO NACIONAL DE FERMENTAÇÃO. Fortaleza.

Dahlquist,A. (1961) Determination of maltase and isomaltase activities with a glucose oxidase reagent. **Biochem.J.** v. 80, p. 547-551.

Damasceno,S. Cultivo de Geotrichum fragrans em Manipueira. Botucatu, 1999. Tese de doutorado. Faculdade de Ciências Agronômicas – FCA, Universidade Estadual Paulista Júlio de Mesquita Filho. Campus de Botucatu.- UNESP. 114 p.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. (1956) Colorimetric method for determination of sugars and related substances. **Analytical Chemistry**, v. 28, p.350-356.

Efing,L.M.A.C. (1991) Biomassa de ***Trichosporon*** sp. Determinação das condições de cultivo em meio químico de composição definida semelhante à da manipueira. Curitiba, 1991. Dissertação de Mestrado. Departamento de Tecnologia Química. Universidade Federal do Paraná - UFPR.

Efing,L.M.C.; Wosiacki,G. (1998). Estabelecimento de condições de cultivo de uma cepa de ***Trichosporon*** *sp.*isolada de manipueira. **Bol. CEPPA**, Curitiba, v. 16, n. 1, p. 23-36, jan./jun.].

Fioretto,A.M.C. (1987) Viabilidade de cultivo de ***Trichosporon*** *sp.*em manipueira. Dissertação de Mestrado. Faculdade de Ciências Agronômicas – FCA, Universidade Estadual Paulista Júlio de Mesquita Filho,Campus de Botucatu – Unesp.

Fioretto, A.M.C.; Wosiacki,G.; Brasil,O.G.; Cereda,M.P. (1985.) Produção de Biomassa oleaginosa de ***Trichosporon*** a partir de amido. – Pré-tratamento enzimático. CONGRESSO BRASILEIRO DE MICROBIOLOGIA, jul. São Paulo.

Fioretto, R.A. (1994) Uso direto da manipueira em Fertirrigação. In: CEREDA,M.P. Resíduos da Industrialização da Mandioca no Brasil. Utilização e tratamento.: Paulicéia, São Paulo.

Kennedy, J.F.; Cabral, J.M.S.; Sá Correia, I. (1987). Starch biomass - a chemical feedstock for enzyme and fermentation process. In: Galliard, T. Starch: properties and potential. John Wiley e Sons : New York .

Lodder,J. (1970). The yeasts – A taxonomic study. 2. ed. North Holland Publishing Company, London pp.35-120, 1308-1352.

Plummer,D.T. (1981.) An introduction to Practical Biochemistry.: McGrawn Hil : London.

Ponte, J.; Franco, (1994.) O. uso da manipueira como defensivo agrícola. In: CEREDA,M.P. Resíduos da Industrialização da Mandioca no Brasil. Utilização e tratamento.: São Paulo Paulicéia.

Sichieri,V.L.F.S.(1986) Produção e caracterização dos lipídios da biomassa de ***Trichosporon*** penicillatum. Londrina. Dissertação de Mestrado. Departamento de Tecnologia de Alimentos e Medicamentos. Universidade Estadual de Londrina – UEL.

Sichieri, V.L.F.S.; Wosiacki,G. Silva, R.S.F.S.; Cereda, M.P. (1986.) Produção de biomassa de ***Trichosporon*** sp, isolado do líquido residual de fecularias. IV - Determinação da influência do binômio tempo x temperatura. SIMPÓSIO NACIONAL DE FERMENTAÇÃO, Londrina,

Ratledge,G.R. (1979). Resources conservation by novel biological processes. I - Grow fat from wastes. **Chem. Soc. Ver**.,.v. 8,p. 283-297.

Ratledge,C.; Boulton,C.A. (1985.) Fats and Oils. In: Industrial, chemical, biochemical and fuels. [s.n.], London

Wosiacki, G.; Fioretto, A.M.C.; Cereda,M.P. (1994). Utilização da manipueira para produção de biomassa oleaginosa. In: Cereda,M.P. Resíduos da Industrialização da Mandioca no Brasil. Utilização e tratamento. Paulicéia, São Paulo.