Title	APPLICABILITY OF FILAMENTOUS FUNGI TO TREATMENT OF CASSAVA STARCH PROCESSING WASTEWATER CONTAINING CYANIDE
Author(s)	Truong, Quy Tung; Miyata, Naoyuki; Iwahori, Keisuke
Citation	Annual Report of FY 2003, The Core University Program between Japan Society for the Promotion of Science (JSPS) and National Centre for Natural Science and Technology (NCST). 2004, p. 256-260
Version Type	VoR
URL	https://hdl.handle.net/11094/13101
rights	
Note	

APPLICABILITY OF FILAMENTOUS FUNGI TO TREATMENT OF CASSAVA STARCH PROCESSING WASTEWATER CONTAINING CYANIDE

Truong Quy Tung*, Naoyuki Miyata**, and Keisuke Iwahori**
*Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422 - 8526, Japan
**Institute for Environmental Sciences, University of Shizuoka, 52-1, Yada, Shizuoka 422 - 8526, Japan

ABSTRACT

Evidence from the available literature identified the potential use of filamentous fungi for the treatment of the cassava starch processing wastewater. Experimentation was also done on eight strains of filamentous fungi, which were known as producers of amylase and glucoamylase for digesting raw starch. *Aspergillus oryzae* strains showed high efficiency in the removal of COD and TOC, starch hydrolysis and production of fungal biomass in the medium of the synthetic cassava starch processing (SCSP) wastewater containing pure cassava starch. With *A. oryzae* KCC F0010, treatment efficiency obtained 72% (TOC), 81 % (COD), 94 % (starch) and 2.4 g/l of biomass after 14-day incubation. The presence of cyanide caused inhibition for the growth of filamentous fungi. The toleration of *A. oryzae* IF0 30113 to cyanide was associated with its ability to remove cyanide. Ammonia was indicated as the end product of biodegradation of cyanide. Volatilization and microbial metabolism could be considered as the main mechanisms in the cyanide removal of the aerobic treatment process by filamentous fungi under the acidic condition.

Key words: *Asperillus oryzae,* cassava starch processing wastewater, cyanide biodegradation, filamentous fungi

INTRODUCTION

Cassava (*Manihot esculenta Crantz)* is the third most important source of calories in the tropics, after rice and corn. According to FAO, more than 750 million people depend on the cassava in Africa, Asia and Latin America (Pandey et al., 2000). The majority of cassava is used for human food in the form of fresh and processed products. Besides a variety of human food, starch and starch-derived products also represents an importance in the use of cassava. The processes of the industrial cassava starch production require large quantities of water resulting in the release of a significant quantity of wastewater (Hien et al., 1999). It is common for factories to discharge the effluents into the nearby rivers, crop field or to the land adjacent to the factories. The efforts are necessary to diminish the pollution problems caused by the cassava starch processing (CSP) wastewater, which have posed a serious threat to the environment and quality of life in rural areas. For this reason, the goal in our work is to find on the characteristics of wastewater, biodegradability of pollution substances by microorganism, biotechnological feasibility and experimentations to estimate the applicability of filamentous fungi to treatment the CSP wastewater.

Characteristics of the CSP wastewater

The wastewater generated from cassava starch production factories is combined from throughout almost sections of the manufacturing process: roots washing, starch extraction, dewatering, starch washing and refining, as well as equipment washing. However, the tuber washing water and the supernatant from the settling tanks are majority of the effluent. The common characters of the CSP wastewater are large volume with high concentration of the organic pollution compounds (Table 1).

Table 1. Characteristics of the CSP wastewater (Tung et al., 2002)

Parameter	Range*	Average*
pH	3.4 - 5.6	4.4
COD, mg/L	3800 - 25000	9400
BOD_5, mg/L	3600 - 13200	6200
Free sugar as glucose, mg/L	640 - 2070	1380
Total reducing sugar, mg/L	1100 - 2800	1780
Total suspended solids, mg/L	500 - 4100	2300
Total dissolve solids, mg/L	800 - 8000	3970
Total nitrogen, mg N/L	62 - 187	97
Total phosphate, mg P/L	5.8 – 6.4	6.1
Total cyanide, mg CN^-/L	3.2 – 27.4	9.3

* Data was collected and treated from reported literatures

In general, the CSP wastewater is acidic, highly organic but has relatively low nitrogen and phosphorus concentrations. The ratio of BOD_5 to COD estimated is 0.6 – 0.8, indicating that the CSP wastewater has, although, a high load of pollution substances but it is easily biologically degradable. One of the most worth attention for the CSP wastewater is the presence of cyanide compounds with a relatively high concentration. The large amounts of wastewater containing the high cyanide concentration and organic loading will be a menace to the quality of life in rural areas where the factories are located (Balagopalan et al., 1998).

Filamentous fungi involved in cassava starch conversion and detoxification of cassava products

Almost cassava cultivars contain cyanogenic glucosides, which under some circumstances lead to human toxicity problems. Hence, cassava for food use has to be processed to remove cyanide-containing substances. The ability of filamentous fungi to grow on cassava cultivation in the presence of cyanide has been identified in the literatures (Table 2). Furthermore, a variety of filamentous fungi played an important role in the use using cassava starch as well as the residues of cassava crops as the sole carbon sources for growth and conversion into high-value products (Table 3). Correspondingly, it could be recognized that cassava starch and cyanogenic compounds present in the CSP wastewater are not too recalcitrant to be degraded by microorganisms, particularly, filamentous fungi.

Table 2. Filamentous fungi involved in detoxification of cassava products (Tung et al., 2002)

Microorganism	Preculture medium
Aspergillus nidulans	Glucose and cassava-root extract
Aspergillus niger	Vermiculite and bran
Aspergillus oryzae	Czapek broth and pure linamarin
Fusarium oxysporum	Glucose and cassava-root extract
Neurospora sitophila	Malt extract
Penicillium sp.	Glucose and cassava-root extract
Rhizopus oryzae	Potato dextrose agar and rotten cassava tuber, with and without linamarin and KCN
Rhizopus stolonifer	Malt extract agar, Yeast

Table 3. List of filamentous fungi using cassava starch as a substrate (Tung et al., 2002)

Microorganism	Process [a]	Product [b]
Cassava bagasse		
Aspergillus niger	SSF	Citric acid
Ceratocystis fimbriata	SSF	Aroma compound
Rhizopus arrhizus	SmF	Ethanol
Rhizopus formosa	SmF, SSF	Fumaric acid, feedstuff, SCP
Rhizopus oryzae	SSF	Aroma Compound
Rhizopus oryzae	SmF	Fumaric acid
Rhizopus sp.	SmF	Glucose
Cassava peel		
Aspergillus niger	SSF	Feedstuff, SCP
Rhizopus sp.	SSF	Feedstuff, SCP
Trichophyton sp.	SSF	Feedstuff, SCP
Cassava starch		
Aspergillus niger	SmF, SSF	Glucose, Protein
Aspergillus sp.	SmF, SSF	Protein, Glucose
Neurospora sitophila	SmF	Protein
Rhizopus delemar	SSF	Protein
Rhizopus oryzae	SSF	Protein
Rhizopus sp.	SmF, SSF	Glucose, Ethanol
Trichoderma viride	SmF	Glucose

(a) SmF: Submerge fermentation,
SSF: Solid substrate fermentation
(b) SCP: Single cell protein

Potential of filamentous fungi for biotechnological treatment of starch processing wastewater

In the starch processing wastewater, the major pollution components consist of starch residue free sugars and the sugars, which can be used as substrates for the generation of microbial biomass rich in protein. The aeration biotreatment of food processing wastewater can produce valuable products such as single cell protein, besides purifying the effluent (Manilal et al., 1991). Filamentous fungi have widely been used in industries for commercial production because of its ability to hydrolyze starch almost completely (Jin et al., 1999). The feasibility of starch wastewater treatment using pellets in submerged cultures of filamentous fungi has reported (Sekikawa et al., 2001). The conversion of waste using filamentous fungi not only reduces the pollution load, recovers valuable resources in reuse but also reduces the treatment costs due to the filamentous nature permits low-cost separation and recovery of biomass from the culture media.

Base on the attraction of the CSP wastewater to microorganism and the potential of filamentous involved in the degradability of cassava starch and cyanide compound, the way to diminish pollution substances in the CSP wastewater by filamentous fungi is of promises.

EXPERIMENTATION OF FILAMENTOUS FUNGI FOR TREATMENT OF THE CASSAVA STARCH PROCESSING WASTEWATER CONTAINING CYANIDE

Materials and methods

Eight strains of filamentous fungi used in this study include *Aspergillus awamori* IFO4033, *A. niger* IFO6428, IFO6661, KCC F0086, *A. oryzae* CZGLU, IFO30113, KCC F0010 and *Rhizopus javanicus* IFO5441. Cultures were maintained on potato-dextrose agar (PDA) slants at 4°C. Fungi were subcultured on the Czapek-Dox agar slant according to Sekikawa et al., 2001. With *R. javanicus* IFO 5441, subculture was carried out on malt extract agar slant. Spores were harvested in sterilized after incubation at 28°C for 4 days (*A. awamori* and *niger*) and for 6 days (*A. oryzae* and *R. javanicus*). Colleted suspension adjusted to 1.0 of the optical density at 600 nm was stored at 4°C and used as an inoculum.

Synthetic cassava starch processing (SCSP) wastewater was made of cassava starch obtained from the commercial source in Vietnam. Cassava starch was suspended in water by heating at 80-90oC in water bath until homogeneous solutions were obtained. The SCSP wastewater was supplemented with $NaNO_3$ and K_2HPO_4 and sterilized by autoclaving at 121°C for 15 min. The initial pH of wastewater was adjusted to 4.0 by sterilized NaOH or HCL 0.5N solution before inoculation. Characteristics of the SCSP wastewater consist of following: COD: 5300-5600, TOC: 4000-4400, starch conc.: 9550-9820, T-N: 125-130 and T-P: 8.4-8.8 (unit in mg/l). Cyanide was added from stock of 1M KCN solution, which was sterilized using filter with 0.2μm pore size (Advantec, Tokyo). Duplicate cultivations were carried out in 300-ml Erlenmeyer flasks containing 100ml of the SCSP wastewater. The flasks were inoculated with 1 % (v/v) of the spore suspensions (OD_{600}= 1) and were incubated in a rotary shaker with a shaking rate of 120 rpm at 28°C. At 1-day interval, 1–ml sample was withdrawn and centrifuged at 15000 × g at 4°C for 10 min. The supernatant was used to analyze the concentration of glucose, reducing sugar, COD and TOC after being diluted appropriately. The cultivation and sampling procedures were carried out under aseptic conditions.

COD was determined by permanganate method using COD meter (HC–507 CKC). TOC was measured with TOC analyzer (TOC-5000A, SHIMADZU Co.). Reducing sugar was determined spectrophotometrically at 490nm according to the phenol-sulfuric acid method. Glucose was determined by glucose oxidase method with commercial kits (Eiken Chemical Co.). Starch was calculated upon the equation: starch = (reducing sugar – glucose) x 0.9. Cyanide was quantified spectrophotometrically at 578 nm according to SMEWW. To analyze cyanide content accumulating on biomass, the samples were pretreated by distillation. Ammonia was determined in accordance with SMEWW using the phenate method after distillation. Fungal biomass expressed in gram of dry biomass per liter of the culture medium was harvested by centrifugation at 15000 × g at 4°C for 10 min, washed twice with deionized water, and then dried at 105°C for 24 h.

Results and discussion

Growth and organic-removing abilities of fungi in the SCSP wastewater medium

During the shaking incubation, the growth in terms of pellets took place for studied fungal strains except *Rhizopus javanicus* IFO 5441, which were present in a pulp form. All strains were able to hydrolyze cassava starch in the SCSP wastewater; especially, the rapidly transformation of cassava starch into glucose occurred in cultures of IFO 4033, 6661, 6428 and KCC F0086 just after the initial 3 day cultivation (Fig.1D). However, the efficiencies of COD and TOC reduction by *A. oryzae* KCC F0010, IFO 30113 and CZGLU strains were obviously higher, compared with that by *A. awamori* and *A. niger* strains. Figure 1 shows a obviously separation of the fungi into 2 groups in the formation of glucose, starch hydrolysis, TOC and COD removal, which seem to have two different ability in the metabolism of cassava starch.

In the culture of *A. niger* KCC F0010, the efficiencies of TOC and COD reduction and cassava starch hydrolysis were 72%, 81% and 94%, respectively. The significant results were also achieved by *A. oryzae*

CZGLU (67%, 76%, and 89%) and followed by *A. oryzae* IFO 30113 (61%, 70%, and 86%). *A. oryzae* KCC F0010 and IFO 30113 also yielded the highest biomass concentrations of 2.4 g/l (data was not shown). *A. oryzae* strains have also been suggested as potential strains for the production of microbial biomass protein and reclamation from wheat- and corn-starch processing wastewater (Jin et al., 1999).

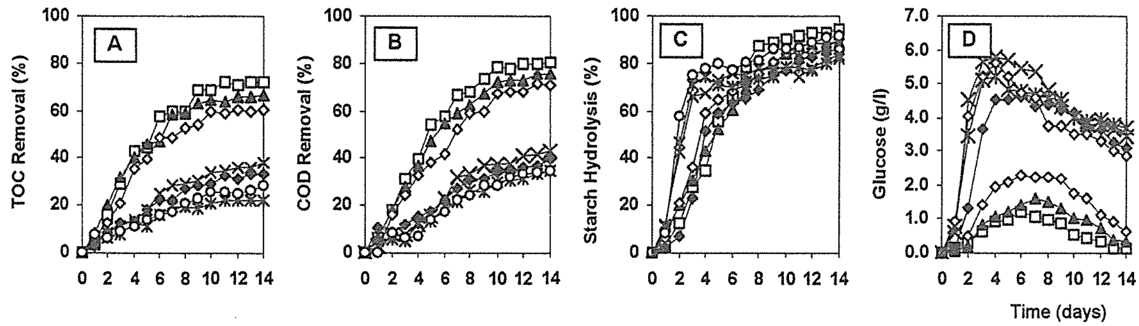

Figure 1. Profiles of TOC (A) and COD (B) removal; starch hydrolysis (C) and glucose concentration (D) during 14-days cultivation of filamentous fungi in the SCSP wastewater. Symbols: ⊠. KCC F0010, π. CZGLU, ♪. IFO 30113 ⊗. KCC F0086, ↯. IFO 6661, ⊕. IFO 4033, and ▣. IFO 6428.

Toleration of *A. oryzae* strains in SCSP wastewater containing cyanide

Filamentous fungi suitable for treatment of the CSP wastewater have not only ability of removing the organic compounds but also good toleration to cyanide that is well known as inhibitor for microbial growth. The experiment was carried out for *A. oryzae* strains in the SCSP wastewater medium with cyanide concentration of 20 mg/l.

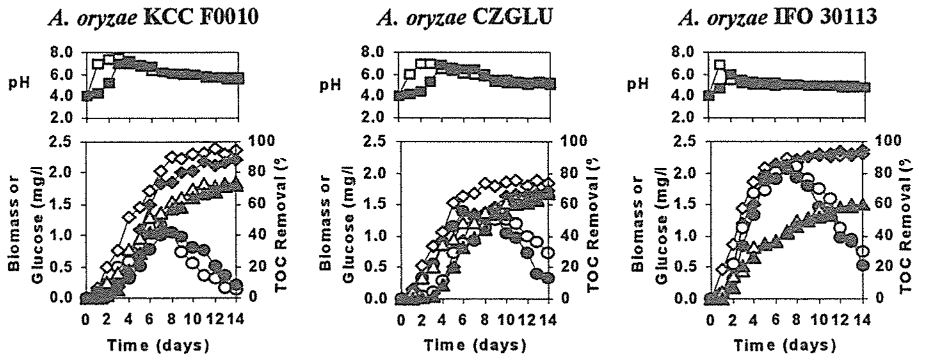

Figure 2. Profiles of pH (⊠/▣), biomass concentration (♪/●), TOC removal (ρ/π) and glucose concentration (▣/★) during incubation of *A. oryzae* strains in the SCSP wastewater without (blank marker) and with (fill marker) cyanide (20mg/l).

Cyanide inhibited the growth of selected fungi through the change of pH profile at the beginning period of time, reduction of TOC and COD removal rate, and delay of biomass and glucose production. During cultivation the effect of cyanide on the growth and metabolisms of *A. oryzae* IFO 30113 was much smaller than the effect of those of *A. oryzae* KCC F0010 and CZGLU. This proved that *A. oryzae* IFO 30113 was able to tolerate in the SCSP wastewater medium containing cyanide.

Biodegradation of cyanide by *A. oryzae* strains

In order to judge the cyanide removal efficiency of fungi, quantitative of cyanide degradation were determined. During the incubation on the rotary shaker, the loss of cyanide under the opened experimental conditions was identified (data is not shown). The SCSP wastewater was used in the acidic condition (pH 4.0), therefore almost all cyanides were present as HCN. The loss of cyanide due to the volatilization was reliable.

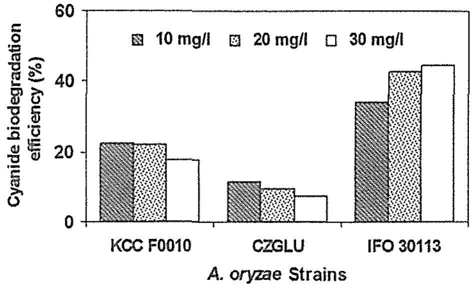

Figure 3. Comparison of cyanide biodegradation efficiency of *A. oryzae* KCC F0010, CZGLU and IFO 30113 at the different concentration of cyanide in the SCSP wastewater after 1-day cultivation.

Figure 3 shows biodegradation efficiencies of cyanide by the *A. oryzae* strains after the first day. The cyanide biodegradation efficiency by fungi was expressed in term of percentage of the biodegraded cyanide amount and the initial cyanide amount exclusive of the respectively lost cyanide amount causing by shaking without microorganism in control experiment *A. oryzae* IFO 30113 had a high ability to reduce the cyanide concentration in comparison with KCC F0010 and CZGLU strains. In the case of *A. oryzae* KCC F0010 and CZGLU, the cyanide removal efficiency decreased with the increase in the initial cyanide concentration and on the contrary with *A. oryzae* IFO 30113. Thus, the toleration of *A. oryzae* IFO 30113 to cyanide was associated with its ability to degrade cyanide.

Although, cyanide is highly toxic to organism, many microorganisms can grow in the presence of cyanide either by developing a cyanide-insensitive respiratory system or cyanide metabolism (Padmaja et al., 1985). Via the enzymes hydratase and amidase, the products of hydration (formamide) and hydrolysis (formic acid and ammonia) are considerably less toxic than cyanide and can be used as growth substances in certain cases (White et al., 2000). To confirm whether the formation of ammonia during the cyanide metabolism of *A. oryzae* IFO 30113, the concentration variation of ammonia in the cultures with 20mg/l cyanide was examined. Profile of ammonia concentration in Figure 4 showed that the processes of cyanide degradation and ammonia production occurred simultaneously. The formation of ammonia demonstrated that *A. oryzae* IFO 30113 could be transformed cyanide into ammonia, which served as the nitrogen source. Therefore, the reduction of cyanide was associated with the biodegradation process.

The inhibition of cyanide to the growth of fungi resulted in the extension of the lag phase observed on the profile of fungal biomass (Fig. 4). The fungus can grow rapidly after detoxification is completed. Furthermore, the effect of cyanide concentration on fungal biomass concentration and TOC removal by *A. oryzae* IFO 30113 was shown in Fig. 5. Although the presence of cyanide delayed the metabolism of organic compound by fungi, the fungal biomass concentration increased with the increase of cyanide concentration. The SCSP wastewater medium was poor in nitrogen nutrient, consequently, with the increase in cyanide concentration in the culture medium, biodegradation of cyanide might generate a certain amount of ammonia utilized as nitrogen source for the growth of fungi and resulted in the slight increase of the fungal biomass.

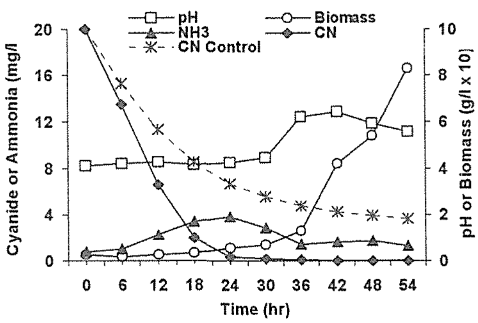

Figure 4. Ammonia formation during cyanide degradation by *A.oryzae* IFO30113 (initial cyanide concentration: 20 mg/l)