pullulanase, and then fractionated by a gel chromatography using Toyopearl HW-65F. About 10-15% of partial hydrolyses were appropriate to collect the shortish chains released from amylopectin. The chain distribution of the smallest fraction from outer-layer of amylopectin consisted of many short-chains (DP6～15). The comparative experiments among the debranching enzymes confirm that the partial hydrolyzed materials obtained under low enzyme activity of free isoamylase and pullulanase could be used for studying the outer-layer of amylopectin like as immobilized isoamylase (published on J. Appl. Glycosci., 48 (1), 2001).

On the second part: Short-amylose chains released from normal corn starch with isoamylase were studied to establish an analytical method for getting directly the structural information of amylopectin. The partial hydrolysates of normal corn starch by free isoamylase obtained without previous separation of amylose and amylopectin were fractionated into three fractions by gel chromatography of Toyopearl HW-50S. Although the short-amylose chains in the fr.3 came not from only amylopectin, but also branched amylose, it was estimated that the chains relating to the branched amylose were minor in the hydrolysates (will be published on J. Appl. Glycosci., 48 (2), 2001).

The analytical method conducting together by controlling the debranching reaction of starch and GPC on Toyopearl HW-50S is convenient for studying the structure of normal starch without previous separation of amylose and amylopectin, and will be worth using as the first screening for understanding the characteristic of normal starches.

General information of amylopectin structures obtained from Japan and South-east Asia regions cultivated starches, especially corn and rice starches, were applied on this method, and the results were shown on the third part of this research.

生物機能応用科学専攻

氏名	ドイエラ　エフィエルニ
学位記番号	生博　甲第111号
学位記授与の日付け	平成13年3月26日
学位論文題目	Studies on Hydrogen Production from Chitinous Biomass by *Clostridium paraputrificum* M-21. (*Clostridium paraputrificum* M-21. によるキチン含有バイオマスからの水素生産に関する研究)
論文審査委員	主査　教　授・大宮　邦雄 教　授・菅原　庸 教　授・久松　眞 助教授・粟冠　和郎 助教授・木村　哲哉 大阪大学大学院　教　授・宮本　和久

要　旨

The studies were carried out to convert chitinous biomass to valuable materials such as hydrogen gas and organic acids by an anaerobic microorganisms.

A strictly anaerobic, mesophilic, chitinolytic bacteria was isolated and identified as Clostridium paraputrificum M-21 (FERM P-16390). Optimum cultivation conditions of the bacterium for gas evolution in GS medium containing N-Acetyl-D-glucosamine (GlcNAc) as a main carbon source

had been determined in batch culture system using jar fermentor. Cultivation was continued until gas evolution was ceased. Of total gas 2.8 liter (65% H_2 and 35% CO_2) was evolved during 5 hours of cultivation from 9.4 g of GlcNAc at initial pH 6.5, 45℃, 250 rpm of agitation speed and 500 ml of working volume equal to the headspace in one liter jar fermentor. Maximum dry cell weight of C. paraputrificum 2 g was harvested. Major organic acids produced were acetic, propionic, butyric and lactic acids.

Since organic acids as by-product were produced simultaneously with hydrogen during cultivation of the strain M-21, medium pH was controlled by feeding NH4OH. When substrate was GlcNAc, hydrogen evolution was the highest at pH 5.8 with the yields of hydrogen of around 2.4 mol H2/mol GlcNAc, 1.3-fold of finding in the previous research. In case of ball-milled chitin, the hydrogen yield was 1.5 mol H2/mol GlcNAc equivalent at pH 6.0, the optimum pH of major chitinases of this strain M-21. In continuous culture, hydrogen production was maintained during 8 h, the highest level at dilution rate of D＝1.2/h.

The capability of C. paraputrificum M-21 to degrade natural substrates was tested by cultivating it on raw shrimp and lobster shells (chitinuous wastes) as the carbon sources in batch culture system. The hydrogen evolved were 11.4 mmol H2 from 2.6 g of the former and 7.8 mmol H2 from 1.5 g of the latter, respectively. Those amount of hydrogen evolved were enhanced two fold when both shells were pretreated by acid and alkali. Raw wastes from the starch industries such as corn fiber and gluten feed were also converted to hydrogen, i.e., 12.5 and 15.4 mmol H2/l medium, respectively.

Detection of major chitinase of C. paraputrificum M-21 were carried out on cultivation in insoluble materials. When the strain M-21 was cultivated on ball-milled chitin and ball-milled shrimp shell for 14 and 12 h, respectively, chitinases ChiA and/or ChiB were detected as the major chitinase species, in the supernatants of the cultures, suggesting that they play a critical role in degradation of chitinous materials.

From the studies, a clue of clean gas production from chitinous wastes was obtained by using Clostridium paraputrificum, isolated from soil at Mie University campus.

生物資源開発科学専攻

氏名	近藤　茂則
学位記番号	生博　甲第112号
学位記授与の日付け	平成13年7月18日
学位論文題名	海産魚類浮性卵の卵質評価法の開発に関する研究 －シロギスを例として－
論文審査委員	主査　教　授・柏木　正章
	教　授・宗宮　弘明
	教　授・前川　行幸
	助教授・吉岡　　基
	三重大学　名誉教授・日高　磐夫

要　旨

魚類の種苗生産においては，質の良い卵と悪い卵を判別し，良質卵だけをふ化管理することが重要である。ヒラメやマダイなど多くの有用海産魚類が産出する浮性卵については，一般に，産卵海水との相対的な比重の違いによって決まる浮上卵率が有効な指標として広く利用されているが，