Plant Archives Volume 20 No. 2, 2020 pp. 5591-5595 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

VITAMIN B_{12} PRODUCTION FROM SAGO WASTE – A SHORT REVIEW

J. Manjunathan[1], D. Saravanan[2], Shymala Gowri[3], Amutha Swaminathan[4], R. Karthiyayini[5] and Sowmya Hari[6]

[1,2]Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS).
[3]Department of Botany, PachaiyappasCollege, Nungambakkam, Chennai.
[4,5],Department of Botany, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.
[6]Department of Bio-Engineering, B.TechBiotechnology, Vels Institute of Science, Technology and Advanced Studies (VISTAS).

Abstract

Vitamin B_{12} is very important in the diet because they are of great value in growth and metabolism of the living cell. As the demand for vitamin B_{12} increased, fermentation processes were developed with higher yielding strains. Commercial production is currently carried out easily by fermentation. Vitamin B_{12} is produced exclusively by microorganisms. Microorganisms most commonly exploited for the *Pseudomonas freudenreichii* and *Pseudomonas denitrificans*.

Key words: *Fermentation process, Pseudomonas Sp, Sago waste, Vitamin* B_{12}.

Introduction

Cobalamin (vitamin B_{12}) is the largest B vitamin and was the last one to be isolated in 1948 by Dr. E. Lester Smith in the UK from liver. It is a red crystalline substance. It had been known as early as 1926, that something in raw liver was a treatment for anaemia. There are various forms of the cobalamin (so called due to the presence of cobalt) molecule, some of these are; methyl-, cyano, adnosyl- and hydroxocobalamin (B_{12}b). There are also nitrit (B_{12}c), sulphito and aquacobalamins. The human body can normally convert from one from to the other. The human body typically contains 5000-10000 µg of B_{12} distributed about equally between the liver, kidneys and nervous system. Indeed the liver can store enough B_{12} for many years of supply, so that daily ingestion of B_{12} is not required. Most of the B_{12} present in animal tissues is in one of the two coenzyme forms, adnosylcobalamin or methylcobalamin and not actual vitamin B_{12} (cobalamin), which may be present due to diffusion from gut bacteria or active transport using intrinsic factor. Vitamin B_{12} is also water soluble and therefore easily lost, whereas cobalamin coenzymes will remain in the liver and nerve cells and can be effectively recycled.

Vitamin B_{12} deficiency and diseases

Vitamins play a major role in human for their normal metabolic and other activities. Deficiency of vitamins leads to adverse effects. Vitamin B_{12} was first isolated by E. Lester Smith in U.K. from human liver. It had been known as early as 1926, that something in raw liver was a treatment for anemia. According to Mervyn, pig's liver contains 25mg/100g of B_{12} vitamin. Chemical synthesis of vitamin B_{12} is practically impossible (Yongsmith *et al*., 1982).

The deficiency of vitamin B_{12} causes many a health problems to human beings such as blood disorders (Sengupta *et al*., 2001; Dguric *et al*., 2001.) and neurological disorders (French, 2000; Weir *et al*., 1999). The symptoms of vitamin B_{12} deficiency include disturbed sense of coordination, paraesthesiae, loss of memory, abnormal reflexes, weakness, loss of muscle strength, exhaustion, confusion, low self confidence, spasticity, incontinence, impaired vision, abnormal gait, frequent need to pass water and physiological deviances. Non anemic deficiencies play a role in disease such as multiple sclerosis, fibrinomyalgia, diabetes and chronic fatigue syndrome. Schizophrenia was also being successfully treated with B_{12} plus other supplements and

cardiovascular disease is linked to B_{12} deficiency while Herpes zoster used to be treated with vitamin B_{12} injection back in the 1950's (Carmel, 2000; Weir *et al*., 1999; Carmel *et al*., 1999).

A study on deficiency of vitamin B_{12} in 225 patients (mean age of 43.08±3.8 and at a ratio of M:F = 3:6.1) demonstrates the clinical features such as neurological-neuropathy or myleopathy -47%, mental symptoms -14%, anemia related symptoms -14%, pallor -42%, oral ulcers-38%, hyper pigmentation -25%, diarrhoea -24%, vomiting-19% and epigastric pain -28%. Mean duration of the symptoms was 7 months.

Isolation of vitamin B_{12} producing organisms

Several microorganisms produce vitamin B_{12} Many species of bacteria, fungi and actinomycetes are being used for the production of vitamin B_{12}. The bacteria isolated from the sago waste produce minerals in the lowest amount followed by protein, vitamin B_{12} or riboflavin. Thus the vitamin production by bacteria from the sago waste appears to compliment vitamin concentration in sago waste (Ha *et al.,* 1996).

The sago waste is most pronounced with short rods, including the members of genere Pseudomonas, Flavobacterium, Alcaligens and it may be these group that are most active in vitamin production.

Fermentation

Vitamin B_{12} is exclusively synthesized by fermentation. The complexity in the structure led vitamin B_{12} to be rightly regarded as an extreme challenge to the synthetic chemist. Yet microorganisms achieve this synthesis *in vivo* with complete control of region and stereochemistry (Blanche *et al*., 1995).

Number of seperative fermentation processes are involved in the production of vitamin B_{12}, they are cross flow filtration (Hatanata *et al.*, 1988), fermentation coupled with activated charcoal adsorption column (Nikano *et al.*, 1996), extractive fermentation (Lewis *et al*., 1982; Yang *et al*., 1995), electro dialysis culture (Zhang, 1993) and immobilized cell culture (Youngsmith *et al*., 1982; Yang *et al*., 1995) have been developed to remove propionate and acetate from fermentation broth in the fermentation with propionic acid bacteria.

The *Propioni bacteria* produce vitamin B_{12} intracellularly and excrete propionic acid and acetic acid as a major fermentation product, especially propionate inhibits cell growth (Hsu *et al*., 1991). *Propioni bacterium freudenreichii* synthesize vitamin B_{12} more effectively while undergoing aerobic, anaerobic and periodic fermentations (Ye *et al*., 1996). During the past two decades several microorganisms including *Propioni bacterium* (Yongsmith and Chutima, 1983), Methanosarcina (Mazumdav *et al*., 1987) and *Butri bacterium* (Zeikus, 1980) have been investigated and shown to produce vitamin B_{12}.

Although *Propioni bacteria* are anaerobic, they are not sensitive to oxygen and can grow in aerated media without forming propionic acid (Hettinga *et al*., 1972). But oxygen is found to have an influence on the cells grown in aerated medium for a long time (Vries *et al*., 1972). Therefore, a two stage fermentation process was developed to improve the production of vitamin B_{12} namely anaerobic fermentation in the first stage and aerobic fermentation in the second stage for decomposition of propionic acid (Quesaele-Chanto, 1994). The microorganisms most commonly used in the industrial production of vitamin B_{12} are *Propioni bacterium freudenreichii, Propioni bacterium shermanii* and *Pseudomonas denitrificans* (Bykhovsky *et al.,* 1991).

Mixed cultures are more effective in the production of vitamin B_{12} by using raw and cheap materials as carbon source. In the case of waste, tomato pomace, the mixed culture of *Trichoderma reisei* and *Propioni bacterium shermanii* were used (Haddadin *et al.,* 2000). Sugars produced during the degradation of cellulose of tomato pomace by *Trichoderma reisei* was utilized as carbon source by *Propioni bacterium shermanii* (Haddadin *et al*., 2000). Another system of mixed culture of *Propioni bacterium freudenreichii* and *Ralstonia entropha* was employed to keep the propionic acid in low level, noting that the propionic acid produced can be simulated by the *Ralstonia entropha* (Byrom, 1987; Holmes, 1985; Doi, 1990; Anderson and Dawes, 1990).

Propinic acid could be degraded and its concentration could be decreased when the culture condition was switched from anaerobic condition to aerobic condition where DO concentration was controlled in between 1 and 5ppm (Shijo and Shimizn, 1996). An another method of intermittence fermentation with 84 hours anaerobic and 84 hours aerobic culture at 30°C was successfully conducted by using whey lactose as a carbon source by Marwaha *et al.,* (1983). Microorganisms producing vitamin B_{12} from methanol or n-paraffin are well known, for instance *Protaminobacter rubber* (Toraya *et al*., 1975), *Bacterium* FM-027 (Ymane *et al*., 1976), *Pseudomonas* AM1, *Klebsiella* (Nishio *et al*., 1977), *Nocardiagardeneri* (Fujii *et al*., 1966) and *Acetobacteriuim* spp.

Media used for the production of Vitamin B_{12}

A number of chemical and modified media has been

suggested for the production of vitamin B_{12}. Ye *et al.*, (1996) suggested a chemical medium for *Propionibacteriumfreudenreichii* in their experiment with periodic variation of dissolved oxygen concentration in the fermentation system. Another chemical medium was recommended for *Acetobacterium* spp. for the production of vitamin B_{12} (Inone *et al.*, 1992).

Some others used modified media for the production of vitamin B_{12} with less cost by using raw sugar sources. Waste materials from other industries like tomato pomace (Haddadin *et al.*, 2000), whey permeate (Marwaha *et al.*, 1983), waste products of dairy industry (Marwaha and Sethi, 1984) are used as media for vitamin B_{12} production. The supplementation of amino acids, betaine and choline into whey lactose permeate medium improve the biosynthesis of vitamin B_{12} (Marwaha *et al*., 1983). The use of tomato pomace as a substrate for the synthesis of vitamin B group, for example vitamin B_{12}, could have much to recommend it (Brock, 1984).

Culturing of *Propionibacterium shermanii*, *Propionibacterium petersoni* and *Propionibacterium freudenreichii* (only in mixed culture) in a cheese-whey medium for vitamin B_{12} biosynthesis has been attempted successfully (Pedziwilk *et al.*, 1970; Janika *et al.*, 1976) but with various cultured conditions and biosynthetic capabilities. The incubation period of 168 hours was considered optimum for the Vitamin B_{12} production since thereafter a decreased productivity was recorded (Yongsmith *et al*., 1979). In a study conducted by Marwaha and Sethi, (1980) the time of fermentation was shortened by a day as compared with other workers (Pedziwilk *et al*., 1970).

The strains of *Propionibacterium shermanii* other than P.S.566 in cheese whey medium have produced maximum quantities of product when fermentation was carried out at 37°C for 4 days followed by 30°C for 4 days (Sankar *et al*., 1974) and at 37°C for 2 days and then 30°C for 5 days (Vorob'eva and Kozyreva, 1967). δ - aminolevalinic acid synthase and δ - aminolivalinic acid dehydrase are the two critical enzymes required for vitamin B_{12} production and 48 hours old inoculum of *Propionibacteriumshermanii* has been found to be optimum for fermentation.

Extraction of Vitamin B_{12}

In early days, some methods were used for the extraction of vitamin B_{12} from animal tissue and this include, digestion with pancreatin or trypsin at neutral pH and at a temperature of 37°C for 20-48 hours. Autolysis in presence of KCN, autoclaving in water for 5 or 30 minutes at 121°C, autoclaving in phosphate buffer of neutral reaction and autoclaving in acetate buffer of pH 4.5 (Shenoy and Ramasarma, 1953). The same authors demonstrated he use of bisulfite in order to prevent the destruction of vitamin B_{12} activity during the extraction procedure.

According to collaborative test reported by Shenoy and Ramasarma, (1953), highest and most consistent values were obtained on treatment with bisulfite or 25% alcohol owing to their eluting and (or) stabilizing properties. Protection of vitamin B_{12} activity by nitrite has also been reported by the authors mentioned above. Vitamin B_{12} has been extracted from the cells by adjusting the pH of the medium to 3 using concentrated H_2SO_4 and then boiling for 30 minutes. The resulting solution was centrifuged. Dead cells were discarded and the centrifugate was used to determine vitamin B_{12} content (Janicks and Pedziwilk, 1966). At the end of fermentation, the cell biomass was centrifuged off and used for vitamin B_{12} extraction by adjusting the pH to 5.0 with 0.75N H_2SO_4 and warming in a water bath for 30 minutes (Pediziwilk, 1966).

Assay of Vitamin B_{12}

Determination of vitamin B_{12} is of relevance to various fields such as clinical analysis, food processing and fermentation processes (Kothouse *et al.*, 1977). Methods used frequently for the vitamin B_{12} determination are microbiological assay (Schneider *et al*., 1987; Sato, 1983, 1996; radio isotope assay (Bain *et al.*, 1982; Sahni *et al*., 2001), high performance liquid chromatography (HPLC) (Li *et al*., 2000), chemiluminecence assay (Wentworth *et al*., 1994) and flourimetric assay (Li and Chen, 2000).

Vitamin B_{12} is also determined either by direct absorbance measurements on aqueous solution or, indirectly and more frequently by measuring the cobalt it contains by atomic absorption spectrometric, chromatographic or catalytic kinetic (Manuela *et al.*, 1983) methods.

Another method for the detection of vitamin B_{12} is the competitive binding assay, which was first described by Herbert in 1958 (Herbert and Colman, 1988). This technique was subsequently employed for use in determining the amount of vitamin B_{12} in serum and plasma (Barakat and Ekins, 1961; Rothenberg, 1961) and was later applied to foods by a number of investigators (Richardson *et al.*, 1978; Beck, 1979; Marcus *et al*., 1980; Casey *et al*., 1982; Bennink and Ono, 1982; Osterdahl *et al*., 1986).

Calibration standards employed in competitive binding assays are most often prepared from cyanocobalamin, whilst the radioactive vitamin B_{12} and the binding protein utilized are usually cyanocobalamin and hog intrinsic factor respectively (Richardson *et al.*, 1978; Beck, 1979;

Marcus *et al.*, 1950; Casey *et al.*, 1982; Nexo and Oleson, 1982). The extraction and determination of vitamin B_{12} by treating with a mixture of phosphate buffer and KCN followed by spectrophotometry at a wave length of 367 nm (Nakano *et al.*, 1996) has been reported. In another method of spectrometry the vitamin B_{12} was converted into dicyano form and measured at 580nm (Marwaha *et al*., 1983).

Referencces

Anderson, A.J. and E.A. Dawes (1990). Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates, *Microbiol.*, **54:** 450-472.

Azuma. R., K. Ogimoto and K. Suto (1971). Anaerbic culture method with steel wool, Jpn. *J. Bacteriol*., **17:** 808-806.

Bain, B., G.N. Broom, J. Woodside, R.A. Litwinczuk and S.N. Wickansingle (1982). Assessment of a radioisotopic assay for vitamin B_{12} using an intrinsic factor preparation with R-protein blocked by vitamin B_{12} analogs, *J. Clin. Pathol. Lond*., **35, I1:** 101-113.

Barakat, R.M. and R.P. Ekins (1961). Assay of vitamin B_{12} inblood. *A simple method Lancet*, **II:** 25-6.

Blanche, F., B. Cameron, J. Crouze, L. Debussche, D. Thibaut, M. Vuilhorgne, F.J. Leeper and A.R. Battersby (1995). Vitamin B_{12}: How the Problem of its Biosynthesis was solved by Angew. *Chem., Int. Ed. Engl*., **34:** 383-411.

Brock. T.D. (1984). Biotechnology: A Textbook of industrial microbiology, Science Tech. Inc., Madison, US.

Brown, D.E. and D.J. Halsted (1975). The effect of acid pH on the growth kinetics of *Trichodermaviride*. *Biotechnol. Bioeng*., **18:** 1199-1210.

Bullerman, L.B. and E.C. Berry (1966). Use of cheese whey for vitamin B_{12} production. III. Growth studies and dry weight activity. *Appl. Microbiol*., **14:** 358-60.

Burkholder, P.R. (1951). Determination of vitamin B_{12} with a mutant strain of *Escherichia coli. Sciente*, **114:** 459-60.

Bykhovskii, V.Y., N. Zaiseva and Eliseev (1991). A, Employment of anaerobic process in the biosynthesis ofvitamin B_{12}, Prikl. *Biokhim. Mikrobiol*., **27:** 467- 481.

Byrom, D. (1987). Polymer synthesis by microorganisms: technology and economics. *Trends Biotechno*., **5:** 246-250.

Carmel, R. (2000). Current concepts in cobalamin deficiency, *Annu. Rev. Med.*, **51:** 357-75.

Casey, P.J., K.R. Speckman, F.G. Ebert and W.E. Hobbs (1982). Radioisotope dilution technique for determination of vitamin B_{12} in foods, *J. Ass. Off. Anal.Chem*, **65(1):** 85-90.

Chahal, D.S. (1985). Solid state fermentation with *Trichodermareesei* for cellulase. *Appl. Environ. Microbiol*., **49:** 205-210.

Chaix, P. and C. Fromageot (1942). Les cytochromes de *Propionibacteriumpentosaceum*. *Bull. Soc. Chim. Biol*., **24:** 1125-1127.

Delwiche, E.A. and S.F. Carson (1953). A citric acid cycle in *Propionibacteriumpentosaceum*. *J. Bacteriol*., **65:** 318-321.

Drasar, B.S. and P.A. Barrow (1985). Intestinal microbiology, VanNostald Reinhold (U.K) Co., Workingham, 59-75.

Fujti, K., S. Shimizu and S. Fukui (1966). Studies on the formation of vitamins and their functions in hydrocarbon fermentation. I. Production of vitamin B_{12} using several kinds of bacteria in hydrocarbon media, *J. Ferment. Technol*., **44:** 185-191.

Holmes, P.A. (1985). Applications of PHB-a microbially produced biodegradable Thermoplastic. *Phys. Technol.*, **16:** 32-36.

Halver, J.E. (1989). The vitamins., Fish Nutrition, 2nd edition. Academic Press, San Diego, 31-109.

Haranaka, H., E. Wang, M. Taniguchi, S. Iijima and T. Kobayashi (1988). Production of vitamin B12 by a fermentor with a hollow fibre module, *Appl. Microbiol. Biotechnol*., **27:** 4730-4731.

Lambert, D., C. Adjella, F. Felden, S. Benhayoun, J.P. Nikolas and J.L. Gueant (1992). Identification of vitaminB_{12} and analogues by high performance liquid chromatography, *Journel of Chromatography*, **608:** 311-315.

Lessel, R. (198l). Microflorebacterienne du tube digestif des poissons. M. Fontaine (Eitor), Nutrition des Poissons. Centre National de la Recherche Scientifique, Paris, 89-100.

LewisYang, S.T. (1992). A novel extractive fermentation process for propionic acid production from whey lactose, *Biotechnol. Bioeng*, **8:** 104-110.

Louw, H.A. and Y.D.M. Weble (1959). A study of soil bacteria dissolving certain mineral phosphate fertilizers and related compounds, *Journal of Applied Bacteriology*, **22:** 227-233.

Manuela Marques, Manuel Silva and Dolorez Perez-Bendito (1990). Kinetic determination of vitaminB_{12} in pharmaceuticals by the continuous adition of reagent technique, *Journel Pharmaceutical and biomedical Analysis*, **8:** 563-567.

Marcus, M., M. Prabhudesai and Wassef (1980). S, Stability of vitamin B_{12} in the presence of ascorbic acid in food and serum: restoration by cyanide of apparent loss, *Am. J. Clin. Nutr.*, **33:** 137-43.

Marwaha, S.S., R.P. Sethi and J.F. Kennedy (1983). Role of amino acids, betaine and choline in vitamin B_{12} biosynthesis by strains of *Propionibacterium, Enzyme Microb. Technol*., **5:** 185-191.

Menon, A. and D. Shemin (1967). Concurrent decrease of enzyme activities concerned with the synthesis of coenzyme B_{12} and of propionic acid in *Propionibacteri, Arch. Biochem. Biophys.*, **121:** 304-310.

Nakano, K., H. Kataoka and M. Matsumura (1996). High density culture of *Propionibacteriumfreudenreichii* coupled with propionic acid removal system with activated charcoal. *J. Frment. Bioeng*., **81:** 37-41.

Nexo, E. and H. Olesen (1982). Quantitation of cobalamins in human serum, In B_{12}, D. Dolphin, Wiley-Inter-science, New York, 87-104.

Nishio, N., Y. Tsuchiya, M. Hayashi and S.A. Naga (1977). Fed-batch culture of methanol- utilizing bacteria with pH stat, *J. Ferment. Technol.*, **55:** 151-155.

Osterdahl, B., K. Janne, E. Johansson and H. Johnsson (1986). Determination of vitamin B_{12} in gruel by a radio-isotope dilution assay, *Internat. J. Vit. Nutr. Res.*, **56:** 95-9.

Pedziwilk, F., I. Janicka and K. Nowakowska (1970). The biosynthesis of corrinoids by *Propionibacteriumshermanii. I. in* cheese whey medium. *Acta Microbiol. Pol. Ser. B.*, **2(19):** 229-36.

Quesada-Chanto, A., A.S. Afschar and F. Wagner (1994). Microbial production of propionic acid and vitamin B_{12} using molasses or sugar, *Appl. Microbiol. Biotechnol*, **41:** 378-383.

Ralph Carmel and Ralph Green *et al*. (1999). Serum cobalamin, homocysteine and methylmalonic acid concentrations in a multiethnic elderly population: ethnic and sex differences in cobalamin and metabolite abnormalities. *American Journal of Clinical Nutrition*, **70:** 904-910.

Richardson, P.F., D.J. Favell, G.C. Gidley and G.H. Jones (1978). Application of a commercial radioassay test kit to the determination of vitamin B, in food, *Analyst* (*London*), **103:** 865-8.

Rothenberg, S.P. (1961). Assay of serum vitamin B_{12} concen-tration using ^{51}Co-B_{12} and intrinsic factor, *Proc. Soc. Exp. Biol. ed*., **108:** 45-8.

Snedecor, Quesada-Chanto, A., A.S. Afschar and W. Wagner (1994). Microbial production of propionic acid and vitamin B_{12} using molasses or sugar. *Appl. Microbiol. Biotechnol*., **41:** 378-383.

Sachs, L. (1984). Applied Statistics-A Handbook of Tech. nigues, 2nd edn, translated by Reynarowych, Springer Verag, New York, 293-301.

Sato, K. (1983). *Assay methods of vitamin B_{12}, Vitamins* (*Japan*), **57:** 609-616.

Shankar, P.A., P.P. Bambha and V.K.N. Nambudripad (1974). Production of vitamin $B1_2$ by *Propionibacteriumshermanii* in whey. *Indian J. Dairy Sci*., **27:** 35-40.

Shewan, J.M., G. Hobbs and W. Hodgkiss (1960). A determinative scheme for the identification of certain genera of Gram-negative bacteria, with special references in Pseudomonada-ceae, *J. Appl. Bacteriol*., **23:** 379-390.

Snedecor, G.W. and W.G. Cochran (1967). Statistical Methods, Iowa State University Press, Ames.

Spalla, C., A. Grein, L. Garofano and G. Ferni (1976). Microbial production of vitamin B_{12} Stenberg. D, A method for increasing cellulose production by *Trichodermaviridea*, *Biotechnol. Bioeng*., **18:** 1751-1760.

Taylor, R.J. (1952). Chemical method for determining vitamin B_{12}, *Anal. Chem*., **24:** 1154-6.

Toraya, T., B. Yongsmith, A. Tanaka and S. Fukui (1975). Vitamin B_{12} production by a methanol-utilizing bacterium, *Appl. Miccrobiol.*, **30:** 477-499.

Udaka, S. (1960). Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicu,. J. of Bacteriology*, **79:** 754-755.

Vorob' eva, L.I. and L.F. Kozyreva (1967). Effect of temperature on the biosynthesis of vitamin B12 by *Propionibacieriumshermanii* culture. *Vestn. Mosk. Univ. Ser*., **22(2):** 52-4.

Vries, W.D., M.C. Wilhelmina and V. Wijck-Kapteijn (1972). Influence of oxygen or growth cytochrome synthesis and fermentation pattern in propionic acid bacteria. *Gen. Microbiol.*, **71:** 515-524.

Weir, D.G. and J.M. Scott (1999). Brain function in the elderly: role of vitamin B_{12} and folate, *Br. Med. Bull.*, **55(3):** 669-82.

Yamane, T., Kishimoto and F. Yoshida (1976). Semi-batch culture of methanol-assimilating bacteria with exponentially increased methanol feed, *J. Ferment. Technol*., **54:** 229-240.

Yang, S.T. and Y. Huang (1995). A novel recycle batch immobilized cell bioreactor for propionate production from whey lactose. *Biotechnol. Bioeng.*, **45:** 379-386.

Ye, K., M. Shijo and M. Shimizu (1999). Metabolic pathway of *Propionibacterium*growing with oxygen: enzymes, ^{13}C NMR. *Biotechnol. Prog.*, **15:** 201-207.

Ye, K., S. Jin and K. Shimizu (1996). Performance improvement of lactic acid fermentation by multistage extractive fermentation, *J. Ferment. Bioeng*., **81:** 240-246.

Ye, K., M. Shijo and K. Shimizu (1996). Efficient production of vitamin B_{12} from propionic acid bacteria under periodic variation of dissolved oxygen. *J. Ferment. Bioeng*., **82:** 484-491.

Yongsmith, B., K. Sonomoto, A. Tanaka and S. Fukui (1982). Production of vitamin B_{12} by immobilized cells of propionic acid bacterium, *Eur. J.Appl. Microbiol. Biotechnol*., **16:** 70-74.

Yongsmith, B., A. Tanaka and S. Fujji (1979). Microbial production of vitamins. *Ann. Report ICME*, **2:** 257-60.

Yongsmith, B. and K. Chutima (1983). Production of vitamin B_{12} by living bacterial cells immobilized in calcium alginate gels. *J. Ferment. Biotechnol*, **61:** 593-598.

Yongsmith, B., K. Sonomoto, A. Tanaka and S. Fukui (1982). Production of vitamin B_{12} by immobilized cells of a propionic acid bacterium, *Eur. Appl. Microbiol. Biotechnol*., **16:** 70-74.

Zeikus, J.G., L.H. Lynd, T.E. Tompson, J.E. Krzycki, P.J. Weimer and P. Hegge (1980). W, Isolation and characterization of a new methylotrophic, acidogenic anaerobe, the Marburg strain, *Curr. Microbiol.*, **3:** 381-386.

Zhang, S.T. and Matsuoka (1993). Production and recovery of propionic and acetic acid in electrodialysis culture of Propionibacteriumshermanii. *J. Ferment. Bioeng*., **75:** 276-282.