INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

FERMENTATION, MEDIA OPTIMIZATION STUDIES FOR COENZYME Q10 PRODUCTION BY *Saccharomyces cerevisiae*

Narendra Kumar S*, Puspha Agrawal, Sujata A S and Bhavana B K
Department of Biotechnology, R. V. College of Engineering, Bangalore-560059, Karnataka, India

Article Received on: 12/11/11 Revised on: 18/12/11 Approved for publication: 19/01/12

*Email: mnsnandu@gmail.com

ABSTRACT
To establish the fermentation process for CoQ10 production by using *Saccharomyces cerevisiae* with subsequent bioprocess media optimization studies. Coenzyme Q10 (CoQ10) is a vitamin-like nutrient that plays a vital role in cellular energy production. Coenzyme Q10, apart from playing an important role in electron transfer for ATP synthesis, also acts as antioxidant. Therefore it is preferred as a potential therapeutic supplement to many critical diseases besides being used as dietary supplement in the energy drinks, anti-aging etc. This has driven the demand for Coenzyme Q10 supply to meet the market requirements. The method adapted for Coenzyme Q10 is batch fermentation route and preferred over synthetic method due to low production cost, safe environmental issue. The optimization of media for better yield of CoQ10 during fermentation and it was found that a maximum CoQ10 5mg/600ml is obtained at 4% of limiting substrate, 150 rpm at 30°C.
Key Words: *Saccharomyces cerevisiae,* Ubiquinone (CoQ10), Neutraceuticals, Packed Cell Volume (PCV) and Thin Layer Chromatography.

INTRODUCTION

The USP refers to Coenzyme Q10 as Ubidecarenone and it defined as fat-soluble vitamin-like substance present in every cell of the body and serves as a coenzyme for several of the key enzymatic steps in the production of energy within the cell. Coenzyme Q10 is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III) as well as enzymes in other parts of the cell. Coenzymes are vital participants in many of the chemical reaction in our bodies that are the very essence of life, moreover the electron and proton transfer functions of the quinone ring are of fundamental importance to all life forms; Coenzyme Q10 is a powerful antioxidant that aids in metabolic reactions (Nohl et al, 2001). It also known as ubiquinone because its chemical structure is that of a quinone and it is ubiquitously distributed in nature (Michael et al 2007). Coenzyme Q10 is marketed in the United States and Japan as a dietary supplement (Pravst et al 2010)

Commercial Importance of CoQ10

The research shows that from the age of 20, production of CoQ10 slows down. Hence CoQ10 is considered to be a "conditionally essential nutrient". Modern medicine seems to be based on an "attack strategy", a philosophy of treatment formed in response to the discovery of antibiotics and the development of surgical/anesthetic techniques. Like the vitamins, CoQ10 is an essential element of food that can now be used medicinally to support the sick host in conditions where nutritional depletion and cellular dysfunction occur. Surely, the combination of disease attacking strategy and host supportive treatments would yield much better results in clinical medicine. All metabolically active tissues are highly sensitive to a deficiency of CoQ10 (Revisson et al 2011, Hyson et al 2010, Hathcock et al 2006). CoQ10's function as a free radical scavenger only adds to the protein manifestations of CoQ10 deficiency (Crane et al 2001).

CoQ10 as a Therapeutic Supplement

CoQ10 is used extensively as a therapeutic supplement as well as a pharmacological active agent. It has wide use and acceptance in the treatment of cardiovascular disease in traditional as well as alternative medicine. It is used successfully in treating ischemic heart disease, chronic heart failure, toxin induced cardiomyopathy, hypertension and hyperlipidemia. Its action as an additional pharmacological agent in treatment of such cardiovascular disease processes may be to improve function of the involved tissues that are ischemic or pathologically altered by providing an increased energy source, by acting as a free radical scavenger. In addition, CoQ10 is found in high concentrations in healthy hearts and at low levels in people with congestive failure leading to the suggestion that supplementation with the coenzyme would be of help in the treatment of heart disease. It is theorized that Coenzyme Q10 might work in the heart in two ways; as an antioxidant to help thwart damage from free radicals that contribute to arterial blockage, and to help boost heart muscle action by improving energy efficiency. Additionally coenzyme Q10 may boost the effects of vitamin E, also a potent antioxidant with some potential beneficial heart effects. Other of the disease states which have received attention is cancer. Low levels of CoQ10 in the blood of some cancer patients have been noted, but overall, there is little data regarding cancer (Lockwood et al 1995). The best work to date documents a significant reduction in the cardiac toxicity of the chemotherapy drug, Adriamycin (Judy et al 1983). The cardiac toxicity of Adriamycin and related drugs may well relate to free radical generation and this might explain the benefit of CoQ10 in its capacity as a free radical scavenger. It is reasonable to assume that optimal nutrition (which would include optimal levels of CoQ10) is generally beneficial in any disease state, including cancer (Muralikrishnan et al 2005).

CoQ10 as Neutraceuticals Supplement

Another interesting topic is the relationship between the immune system and CoQ10. Immune function is extraordinarily complex and undoubtedly is influenced by numerous nutritional variables. There are some encouraging preliminary data from the study of AIDS patients. End stage AIDS, like other overwhelming illnesses, has been associated with a significant deficiency in CoQ10. Adequate CoQ10 supplementation (with close attention to plasma CoQ10 levels) is analogous to adequate hydration, and any treatment of critically ill patients should not ignore this easily measured

and correctable deficiency (Ernster et al 1995, Langsjoen et 1991)

The antioxidant or free radical quenching properties of Coenzyme Q10 serve to greatly reduce oxidative damage to tissues as well as significantly inhibit the oxidation of LDL cholesterol (much more "efficiently than vitamin E) (Bowry et al 1995). In keeping with the free radical theory of aging, these antioxidant properties of Coenzyme Q10 may have clear implications in the slowing of aging (Lenaz et al 2002, Rafael de Cabo et al 2007) and age related degenerative diseases such as Parkinson disease (Kieburtz K et al 2002).

CoQ10 Production

No cost-effective chemical processes for synthesizing Q10 are known. There are only a few natural sources in which the Q10 content is sufficiently high e.g. a few microorganisms or vegetable oils (Meganathan et al 2001, Weber et al 1997). At present Commercial production of CoQ10 is largely by way of fermentation technology using microbes and to a smaller extent by semisynthetic (Negishi et al 2002, Lipshutz et al 2002). Ubiquinone production is usually carried out by yeast (Jin-Ho Choi et al 2005 & Nakao K et al 1979). Takeda chemical Industries, Japan claims that upon extensive research, microorganisms of genus Saccharomyces or genus Oosporidum were able to accumulate Coenzyme Q10 intracellularly in large amounts and further several process developed for improved yield of CoQ10 from improved strains and fermentation process (Yajima et al, 2005, Yoshida et al, 1998, Rupert et al, 2004).

MATERIALS AND METHODS

The major chemicals are Yeast Extract (HIMEDIA.RM-027), Malt Extract (HIMEDIA.RM-004), Peptone (HIMEDIA.RM-001), Tryptone (HIMEDIA.CR-014), Hexane-HPLC and Absolute alcohol used in this study.

Growth Medium

Culture Maintenance Medium: The producing culture was maintained in the form of working stock in test tube slant having a composition of 1 % of glucose; 0.3 % yeast extract; 0.3% malt extract; 0.5 % of peptone and 2 % agar in DM-water 1 L. The pH of the medium was adjusted to 6.2 with 3 N Sodium hydroxide solutions.

Seed Medium: The YM (Yeast Malt) seed medium contains 1 % Glucose, 0.3 % yeast extract, 0.3 % malt extract and 0.5 % peptone and DM-water1L. The pH of the medium was adjusted to 6.2 with 3 N Sodium hydroxide solution.

Production Medium: contains 3 % Glucose, 0.5 %yeast extract, 0.5 % malt extract; 1 % peptone; 3 % glycerin; 3 % tryptone and DM-water1L. The pH of the medium was adjusted to 7-7.2 with 3 N Sodium hydroxide solution.

Shake Flasks Kinetics

The seed culture was grown in a 500 ml Erlenmeyer flask containing 150ml of YM basal medium under agitation of 150 rpm at 30˚C for 24 hrs on a rotary shaking incubator. The shake flask kinetics were performed in a five Erlenmeyer flasks (l Liter) containing 100 ml of the production medium at 30 ˚C, 150 rpm for 48 hrs after inoculating with 5% (v/v) of the seed culture. The samples are aseptically withdrawn at different time interval from each flask for analysis of pH, PCV and Wet Biomass.

Downstream Processing of CoQ10

Biomass Separation: At the end of fermentation period, cells were harvested from the whole broth by centrifuging at 10000 rpm for 20 minutes. The supernatant was discarded and the wet biomass was stored at 4˚C.

Extraction of CoQ10 : The moist cells were suspended in the (1:5) with ethanol and extracted by warming at 60 ˚C for one hour. A total of 3 extractions were carried out in a similar manner and the extracts were pooled, diluted with water and further processed with hexane for total extraction of CoQ10.

Detection: Thin Layer Chromatography (TLC) was carried out to detect the presence of the desired compound i.e. Coenzyme Q10 in the extracted samples with mobile phase was Hexane: ethyl acetate (90:10).

Quantification: High Performance Thin Liquid Chromatography was performed. The standard Coenzyme Q10 dissolved in Hexane HPLC grade at known concentration was used at the beginning and at the end to compare the chromatograms of samples to identify the position of the compound and to check the consistency of retention time. Standard compound was run at different concentration to check for the linearity in the obtained chromatogram area.

RESULT AND DISCUSSION

Microorganism

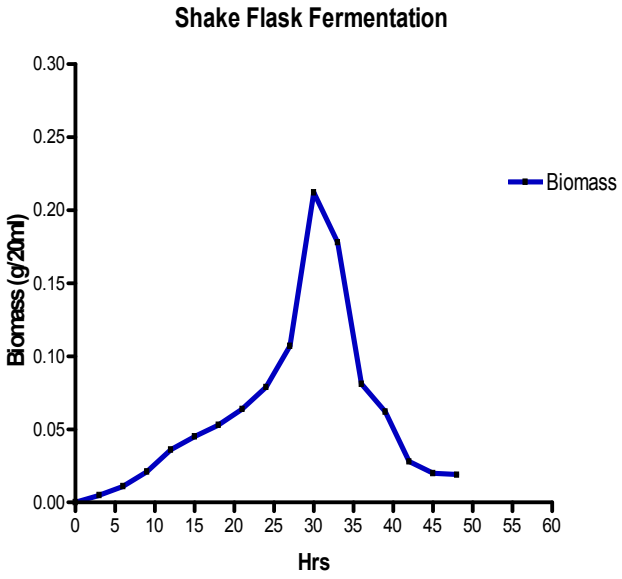
The producing culture *Saccharomyces cerevisiae* was grown in YM (Yeast Malt) broth and the stock culture was maintained in the slant form at 4 ˚C.

Shake Flask Kinetics

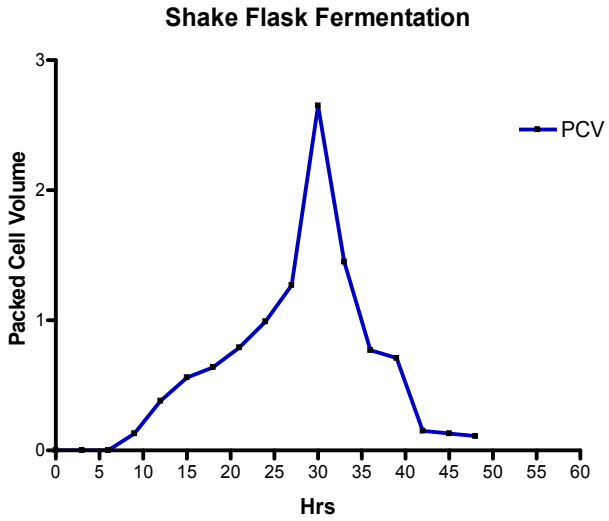
The growth kinetics of the culture was carried out in 1 L Erlenmeyer flask with 150 ml broth on a shaker run at 150 RPM at 30 ˚C for 104 hours. Periodical sampling in a frequency of once 3hrs was carried out to check pH, PCV, Wet biomass .

Table (1): Growth kinetics of the culture

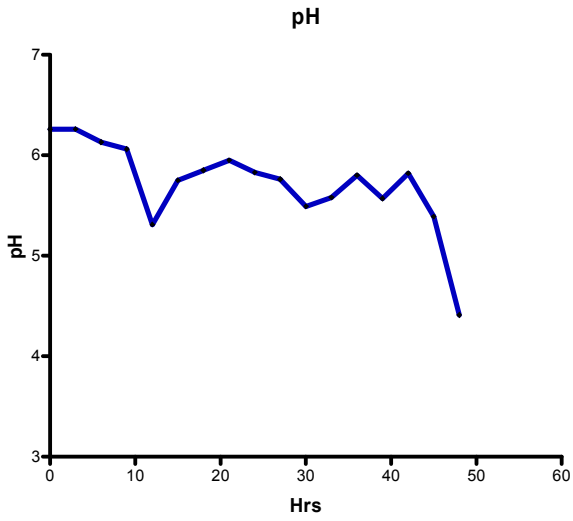
Log Hrs	pH	PCV in %	Wet Biomass g/20ml
0	6.26	0	0
3	6.26	0	0.005
6	6.13	0	0.011
9	6.06	0.13	0.021
12	5.31	0.38	0.036
15	5.75	0.56	0.045
18	5.85	0.64	0.053
21	5.95	0.79	0.064
24	5.83	0.99	0.079
27	5.76	1.27	0.107
30	5.49	2.65	0.212
33	5.58	1.45	0.178
36	5.8	0.77	0.081
39	5.57	0.71	0.062
42	5.82	0.15	0.028
45	5.39	0.13	0.02
48	4.41	0.11	0.019



(A)



(B)



(C)

Figure(1) : Time verses Wet Biomass(A), PCV(B), pH(C) of Shake Flask Kinetics

The yields of the Biomass/PCV are directly proportional to CoQ10 production due and biomass yield are considerably low in Shake flask fermentation process. Further experiments are underway to determine most crucial parameters affecting CoQ10 productivity with respect to growth condition of sachharomyces cultures.

The PCV and Biomass concentration were lower initially when the initial pH of the medium was high. The pH, PCV and Wet Biomass trend during the growth is also indicated in Figure (1). It is found that there is some acidic metabolite is getting produced during early phase of the growth. At this point of time a clear cut inference can't be made on the influence of pH on the production of CoQ10.

MEDIA OPTIMIZATION

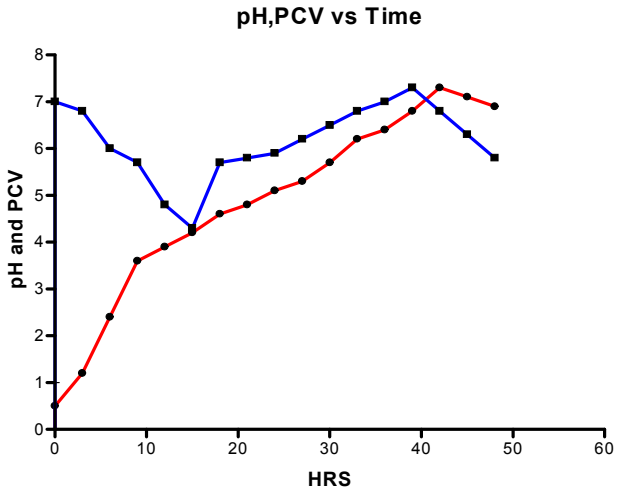
The major carbon source in the media is glucose. An attempt is made to change the glucose concentration to see its effect on Biomass/CoQ10 production. From the glucose standard graph the substrate consumption was calculated and tabulated.

Effect of Carbon Source

The media was prepared with different glucose concentrations - 2.0%, 2.5%, 3.0%, 3.5% & 4.0% to see its effect on Biomass and PCV. The data obtained are tabulated, plotted for individual pH and PCV. The uptake rate for glucose at different hours was estimated and plotted in graph. Further growth of yeast at different substrate concentrations was analyzed and resulted in graph.

Table (2): Growth kinetics of the culture at 2% Glucose

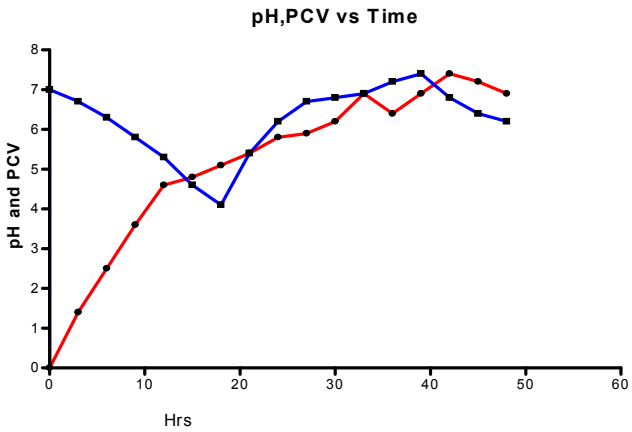
BATCH FOR 2.0 % GLUCOSE								
Log Hrs	pH	OD	PCV	Wet Biomass	% of glucose consumed	d[PCV]	dt	d[PCV]/dt
0	7	0.019	0	0.1253	100	-	-	-
12	4.8	0.031	3.9	0.0868	73.98	0.3	3	0.1
24	5.9	0.0402	5.1	0.1103	25.87	0.3	3	0.1
48	5.8	0.09	6.9	0.0036	0.00	0.2	3	0.06



Figure(2) : Time verses pH and PCV(B) of 2% glucose

Table (3): Growth kinetics of the culture at 2.5% Glucose

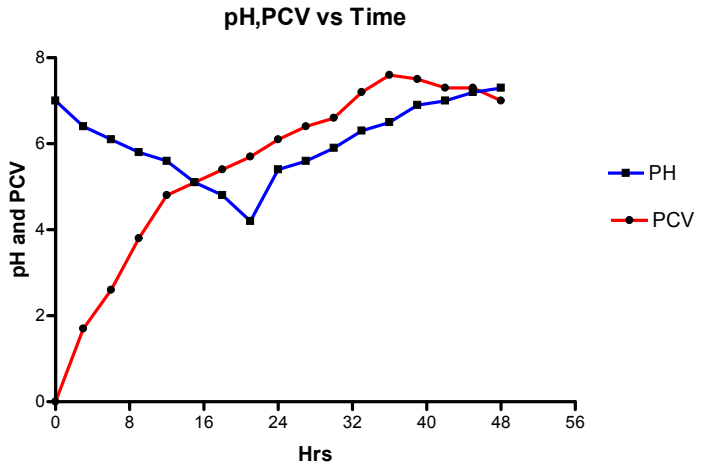
BATCH FOR 2.5% GLUCOSE								
Log Hrs	pH	OD	PCV	Wet Biomass	glucose consumed %	d[PCV]	dt	d[PCV]/dt
0	7	0.026	0	0.0062	100	-	-	-
12	5.3	0.039	4.6	0.1946	82.43	1.0	3	0.33
24	6.2	0.032	5.8	0.1368	46.76	0.4	3	0.13
36	7.2	0.78	6.4	0.0147	0.110	0.5	3	0.16
48	6.2	0.06	6.9	0.0060	0.000	0.3	3	0.10



Figure(3) : Time verses pH and PCV(B) of 2.5% glucose

Table (4): Growth kinetics of the culture at 3% Glucose

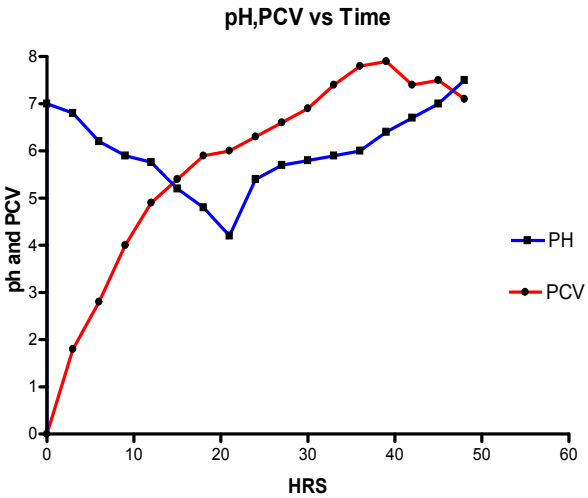
BATCH FOR 3% GLUCOSE								
Log Hrs	pH	OD	PCV	Wet Biomass	glucose consumed %	d[PCV]	dt	d[PCV]/dt
0	7	0.039	0	0.1107	100	-	-	-
12	5.6	0.039	4.8	0.3013	84.23	1.0	3	0.033
24	5.4	0.007	6.1	0.0867	58.76	0.4	3	0.13
36	6.5	0.847	7.6	0.0449	19.76	0.4	3	0.13
48	7.3	0.118	7	0.0189	0.01	0	3	0



Figure(4) : Time verses pH and PCV(B) of 3% glucose

Table (5): Growth kinetics of the culture at 3.5% Glucose

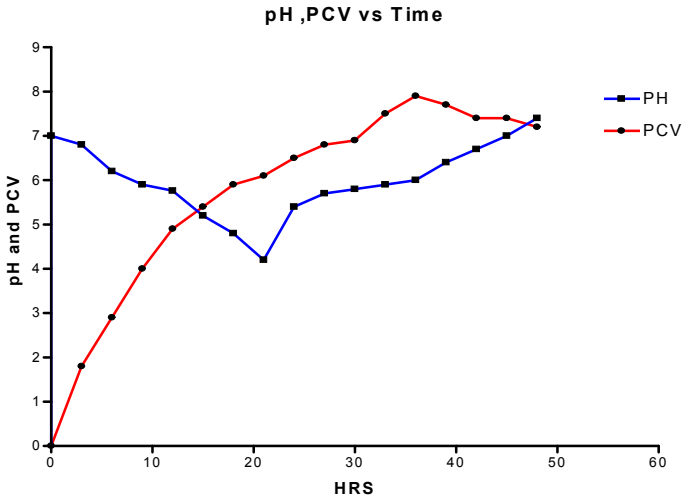
BATCH FOR 3.5% GLUCOSE								
Log Hrs	pH	OD	PCV	Wet Biomass	glucose consumed %	d[PCV]	dt	d[PCV]/dt
0	7	0.992	0	0.0506	100	-	-	-
12	5.76	0.995	4.9	0.2407	81.67	0.9	3	0.30
24	5.4	0.62	6.5	0.0400	56.32	0.4	3	0.13
36	6.0	0.729	7.9	0.0646	27.89	0.4	3	0.13
48	7.2	0.14	7.2	0.0160	0.010	0.1	3	0.03



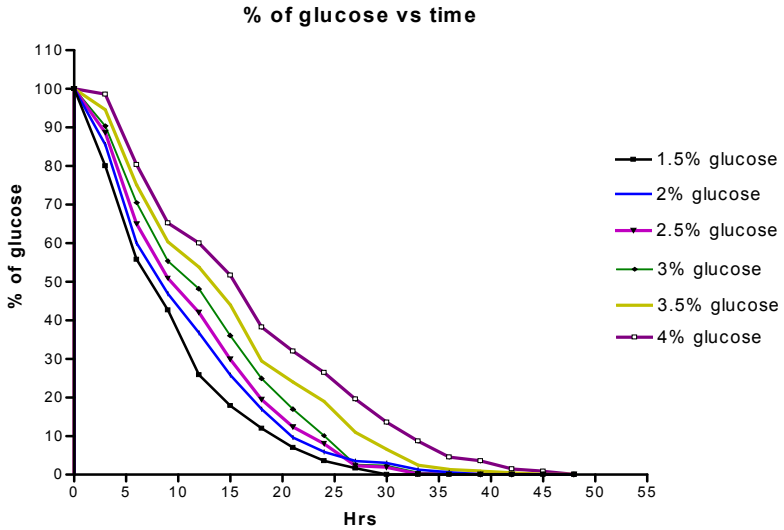
Figure(5) : Time verses pH and PCV(B) of 3.5% glucose

Table (6): Growth kinetics of the culture at 4% Glucose

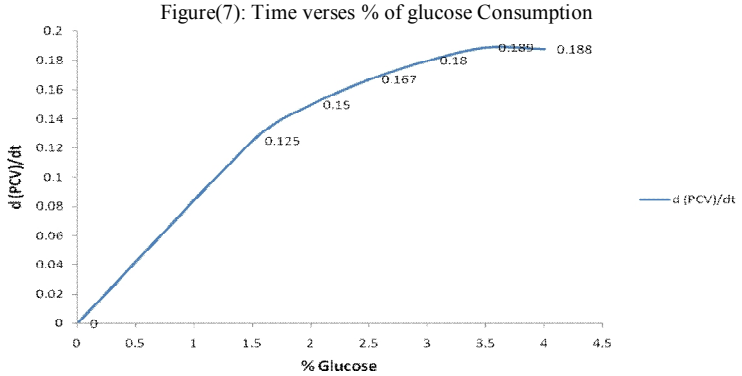
BATCH FOR 4% GLUCOSE								
Log Hrs	pH	OD	PCV	Wet Biomass	glucose consumed %	d[PCV]	dt	d[PCV]/dt
0	7.0	0.067	0	0.2125	100	-	-	-
12	5.7	0.069	4.9	0.2403	76.23	0.9	3	0.30
24	5.4	0.042	6.5	0.2125	51.98	0.4	3	0.13
36	6.0	0.842	7.9	0.2160	36.87	0.4	3	0.13
48	7.4	0.126	7.2	0.0470	0.003	0.1	3	0.03



Figure(6) : Time verses pH and PCV(B) of 4% glucose



Figure(7): Time verses % of glucose Consumption



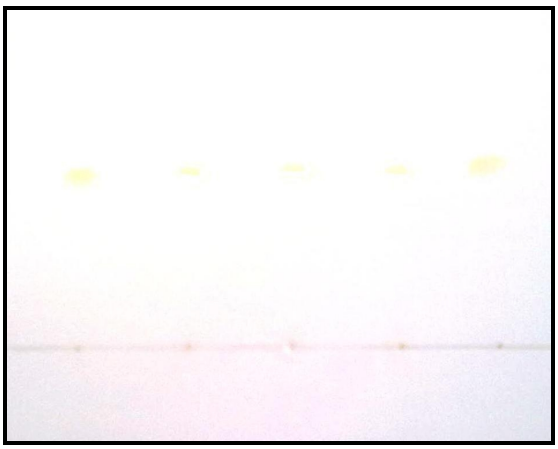
Figure(8): % of glucose verses % d(PCV)/dt

From the glucose pattern shown in the figure (2-6), it was observed that glucose was rapidly consumed and exhausted around 23 hrs. Traces of glucose concentration were left signifying the glucose consumed at faster rate. Further additional supply of carbon source at around 23 hrs would result in better yield of the biomass and CoQ10. The glucose consumption curves showed a similar decrease pattern at different hrs shown in figure (7). Overall the higher glucose (4%) concentration has a positive impact on the yield of biomass/CoQ10. The initially pH of the medium was high due to the high glucose solution in the production media. From the above results, it looks very clear that initial adjustment of media at pH and pH control will have beneficial effect on the growth kinetics and productivity. Further from graph (8), the specific growth rate was 0.178 hr^{-1} estimated by modified monad equation.

Quantative Estimation of CoQ10

High Performance Thin Layer Chromatography (HPTLC) was carried out to detect and quantify the desired compound i.e. Coenzyme Q10 in the total extracted samples as shown in the figure (9). The mobile phase was Hexane: ethyl acetate (90:10). Then around 5µl of the samples were spotted with robotic injection system and the spots were dried using drier. Standard coenzyme Q10 (0.3 mg/ml) dissolved in Hexane was used as the standard. The samples are spotted on the plate around 1.5 cm from the bottom of the plate. Then the

TLC plate was introduced into chamber saturated with mobile phase. After the mobile phase has reached 0.5 cm from the top of the plate, the plate was taken out of the chamber and was spots were observed.



Figure(9): HPTLC Plate used for detection of CoQ10

CONCLUSION

Overall, an attempt has been made to develop a process for CoQ10 production using a *Saccharomyces cerevisiae.* The same culture was used in process studies for media optimization. It was found that a maximum of 5mg/600ml of CoQ10 is obtained at 150 rpm performed in orbital at 30^{o}C. Further studies have to be continued in terms of strain manipulation (genetic or classical method) and process development in fermenter to make a commercially viable process for CoQ10.

REFERENCES

1. Nohl H, Kozlov AV, Stanick K, Gille L. (2001) The multiple functions of coenzyme Q. Bioorg Chem;29:1-13.
2. Michael V. Miles: (2007) "The uptake and distribution of coenzyme Q(10) Mitochondrion 7S S72–S77.
3. Pravst, Igor; Zmitek, Katja; Zmitek, Janko (2010). "Coenzyme Q10 Contents in Foods and Fortification Strategies".Critical Reviews in Food Science and Nutrition 50 (4): 269–80.
4. Revisson E, Dimauro S, Navas P, Salviati L (October 2011)."Coenzyme Q deficiency in muscle". Curr. Opin. Neurol. 24 (5) 449–56
5. Hyson HC, Kieburtz K, Shoulson I, et al. (September 2010). "Safety and tolerability of high-dosage coenzyme Q10 in Huntington's disease and healthy subjects". Mov. Disord. 25 (12): 1924–8
6. Hathcock JN, Shao A (August 2006). "Risk assessment for coenzyme Q10 (Ubiquinone)". Regul. Toxicol. Pharmacol. 45 (3): 282–8
7. Crane FL. (2001); Biochemical functions of coenzyme Q10. J Am Coll Nutr; 20(6):591-598. (PubMed)
8. Lockwood K., Moesgaard S., Yamamoto T., Folkers K. 1995 Progress on therapy of breast cancer with vitamin Q10 and the regression of metastases. Biochem Biophys Res Commun; 212(1):172-7.
9. Judy W.V. Hall J., H., Dugan W., Toth P.D., and Folkers K. (1983) Coenzyme Q10 reduction of Adriamycin toxicity. In: Biomedical and Clinical Aspects of Coenzyme Q, vol. 4 Folkers K., Yamamura Y., (Eds) Elsevier, Amsterdam, pp 231-241.
10. Muralikrishnan Dhanasekaran and Jun ren: (2005) "The Emerging Role of Co-enzyme Q-10 in aging, Neurodegeneration, cardiovascular disease, cancer and diabetes Mellitus. Current Neurovascular research, 2, 6-8.
11. Ernster L, Dallner G. (1995); Biochemical, physiological and medical aspects of ubiquinone function. Biochim Biophys Acta; 1271:195-204.
12. Langsjoen P. H., Langsjoen P. H., Folkers K., (1991) Richardson P. Treatment of patients with human immunodeficiency virus infection with coenzyme Q10. In: Folkers K., Littarru G.P., and Yamagami, T., (Eds) Biomedical and Clinical Aspects of Coenzyme Q, vol. 6, pp 409-415.
13. Bowry V.W., Mohr D., Cleary J., Stocker R. (1995) Prevention of tocopherol-mediated peroxidation in ubiquinol-10-free human low density lipoprotein. J Biol Chem 1995 Mar 17; 270(11):5756-63.
14. Lenaz G, Bovina C, D'Aurelio M, Fato R, Formiggini G, Genova ML, Giuliano G, Pich MM, Paolucci U, Castelli GP, Ventura B: (2002) Role of mitochondria in oxidative stress and aging. Ann N Y Acad Sci 959:199-213.
15. Placido Navas, Jose Manuel Villalba, Rafael de Cabo: (2007) The importance of plasma membrane coenzyme Q in aging and stress responses. Mitochondrion 7S S34-S40.
16. Shults CW, Oakes D, Kieburtz K, et al. (2002) "Effects of Coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline". Arch Neurol. 59(10):1541-1550.
17. Meganathan R (2001) Ubiquinone biosynthesis in microorganisms. FEMS Microbiol Lett 203:131–139
18. Weber C, Bysted A, Holmer G. (1997) Coenzyme Q10 in the diet-daily intake and relative bioavailability. Mol Aspects Med.; 18 Suppl: S251-254.
19. Negishi E, Liou SY, Xu C, Huo S (2002) A novel, highly selective, and general methodology for the synthesis of 1,5-diene-containing oligoisoprenoids of all possible geometrical combinations exemplified by an iterative and convergent synthesis of coenzyme Q10.Org Lett 4:261–264.
20. Lipshutz BH, Mollard P, Pfeiffer SS, Chrisman W (2002) A short, highly efficient synthesis of coenzyme Q10. J Am Chem Soc 124:14282–14283.
21. Jin-Ho Choi. Yeon-Woo Ryu. Jin-Ho Seo: (2005) Biotechnological production and applications of coenzyme Q10 Appl Microbiol Biotechnol 68: 9–15.
22. Nakao, Kitano, Imada, Morimoto, (1979); Method for producing ubiquinone-10 (US Patent 4070244).
23. Yajima et al. (2005) processes for producing Coenzyme Q10 (United States Patent 20050069996).
24. Yoshida H, Katani Y, Ochiai K, Araki K (1998) Production of ubiquinone-10 using bacteria. J Gen Appl Microbiol 44:19–26
25. Rupert et al (2004) Process for producing saccharomyces strain with improved Coenzyme Q10 Production. (United States Patent:20040209368)

Source of support: Nil, Conflict of interest: None Declared