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STUDY OF DIFFERENT FORMS OF MICROBIAL FUEL CELL TECHNOLOGY (MFC) FOR GENERATION OF ELECTRICITY FROM DAIRY EFFLUENT USING A MICROBIAL CONSORTIUM

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ABSTRACT

Dairy industry is one of the prominent industries in India. It produces huge amount of effluent rich in organic matter with very high biochemical oxygen demand (B.O.D.) and chemical oxygen demand (C.O.D.). Conventional aerobic treatments are not affordable to small scale dairies. Hence, for bio-augmentation, a facultatively anaerobic microbial consortium was prepared using indigenous bacteria which were identified as *Enterococcus casseliflavus* and *Enterococcus faecium* and yeast identified as *Kluyveromyces lactis* mixed with exogenous bacteria *E. coli* and *Bacillus amyloliquifaciens*. After confirming bioaugmentation efficiency of the consortium it was used in microbial fuel cell (MFC) for generation of bio electricity. MFC was constructed using a salt bridge to connect cathode to anode and cathode was used un-aerated to make it cost effective. In the current study, different variations of anode and cathode chambers in basic two chambered model of MFC were studied for 10 consecutive days. Initially, use of sterile and non-sterile effluents in anode chamber of MFC were compared for 5 days. Using sterile effluent average voltage obtained was 116.3mV while using non-sterile effluent it was 90.68mV. When the cathode chamber was filled with sterile aerated distilled water and sterile phosphate buffer, pH7, the maximum voltage generated were 375mV and 370mV respectively. When cathode chamber was filled with sterile hydrogen peroxide, maximum voltage of 523mV was generated. Further use of immobilized anode with graphite granules and sterile hydrogen peroxide in cathode chamber resulted into steady voltage generation right from the beginning. The maximum voltage generated was 313mV. There was no initial drop in voltage as observed in other combinations of MFC. Significant reduction in B.O.D. of effluent in the range of 52% to 71% after 8- 10 days was obtained in various forms of MFC.

KEY WORDS : Dairy effluent, Microbial consortium, Microbial fuel cell (M.F.C.), Bioelectricity, Biological Oxygen Demand (B.O.D.).

INTRODUCTION

There are plenty of small scale and large scale dairies in India. Dairy industry is highly polluting food industry due to huge amount of water consumed. (Vourch *et al*., 2008).

Dairy effluent is rich in organic matter and has very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The waste water of dairy contains several organic components such as casein, lactose, fat, inorganic salts, and some detergents and sanitizers used for washing. (Sharma *et al*, 2013). Composition of dairy effluent varies from time to time and from place to place but is highly organic and all components are bio-degradable.

Microbial consortia are being widely employed in effective degradation of organic wastes (Garcha *et al*., 2014). Microbial consortium has several advantages over single organism. In consortium, synergism among microbial communities is observed. Robust nature of consortium helps it to survive under unfavourable conditions.

It has been estimated that energy demand will grow more than 50% by 2025 (Raguskas *et al.,* 2006). Conventional fossil fuels are getting depleted and they cause massive pollution. This is the current global problem. Microbial fuel cell (MFC) has become a popular technology to overcome both these problems. Energy produced by this technology is regarded as green energy (Ravinder Kumar *et al*, 2017). Production of renewable energy is a sustainable way to overcome the problem of global warming (Chaudhari *et al*., 2017)

Microbial fuel cell (MFCs) technology represents a promising approach for generating electricity from biomass using various micro-organisms. (Yong Luo *et al*, 2013). A MFC is a bioreactor that converts the energy stored in the chemical bonds of organic compounds directly into electric energy through electro-catalytic reactions of microorganisms under anaerobic conditions. A typical MFC consists of the anode and cathode chambers, physically separated by a proton exchange membrane (PEM) or a salt bridge. (Logan *et al*, 2006). Microorganisms in the anode oxidize the organic substrates and produce electrons and protons. Protons are conducted to the cathode chamber through the PEM or a salt bridge, and electrons through the external circuit. Protons and electrons are consumed in the cathode chamber with parallel reduction of oxygen to water, which is cathode reaction usually encountered in MFCs. (Antonopoulou *et al*, 2010). Unlike conventional fuel cells, MFCs have certain advantages like high energy-conversion efficiency and mild reaction conditions (Barua, 2010).

Variations in MFC mainly include presence of various chemicals in cathode chamber like phosphate buffer, aerated water, hydrogen peroxide, potassium permanganate etc. (Ucar *et al.,* 2017). To increase efficiency of micro-organisms at anode, an immobilization of microorganisms with graphite and calcium alginate has been tried by some researchers (Wahab *et al.,* 2018).

Reviews have been published elaborating early models of MFCs, latest modifications in the models, potential applications of MFC and limitations of MFC technology (Ashley and Kelly, 2010), (Barua *et al*, 2010).In a detailed review (Pant *et al.,* 2009), use of various substrates used in MFC has been mentioned . Successful use of food processing wastewater, starch processing waste water have been elaborated. However according to these researchers, comparison of various substrates could not be done because of different conditions employed in MFC.

Drysia *et al*., (2017) had constructed a Continuous flow type two chambered microbial fuel cell for the treatment of dairy effluent using organisms present in cow dung. Un-treated effluent was collected from the equalization tank of local dairy. Various combinations of anode and cathode were tried. They got maximum voltage of 502 mV with the use of stainless steel electrode. Deval *et al.* in 2013, had constructed a two chambered glass microbial fuel cell. The chambers were connected with 15 ml of salt bridge. They used artificial waste water and distillery waste water for generation of electricity. Maximum voltage generated using *Bacillus megaterium* was 419mV.They have mentioned that use of salt-bridge is cheaper than use of membrane in MFC models (Tamakloe, 2018).

Borah *et al.* in 2013, had isolated several organisms from tea garden soil and screened for their efficiency of bioelectricity generation from household waste. They had constructed a two chambered microbial fuel cell .The potential isolate was identified as *Bacillus megaterium*. It was used in microbial fuel cell for generation of bio electricity. Maximum voltage generated was 440mV by this technique.

A combined use of the membrane aerated biofilm process and MFC process was proposed by Yu *et al.* (2011) for simultaneous nitrification, denitrification and organic carbon removal in a single two-chambered MFC system.

A two chambered MFC was constructed in which the anodic chamber was connected to cathodic chamber by a proton exchange membrane. With the use of this membrane more voltage and electricity could be obtained. Maximum voltage recorded was 856mV. (Mansoorian *et al.,* 2014). However use of the membrane makes the MFC technology quite expensive. Rabaey *et al*., in 2010 had used ferricyanide in cathode and reported more electricity generation. Kim and Lee (2010), examined various experimental factors to obtain the maximum power output in a dual-chamber mediator-less microbial fuel-cell (MFC) using *Geobacter sulfurreducens* and acetate as an electron donor in a semi-continuous mode.

Mostafa *et al*., 2011 had used MFC in batch and continuous modes. They had observed continuous mode having some advantages over batch mode.

Conventional aerobic treatments are not affordable to small scale dairies. Hence, in the current study, a microbial consortium was developed consisting of indigenous bacteria isolated from the dairy effluent, and identified as *Enterococcus casseliflavus* and *Enterococcus faecium* using VITEK 2 automated system and yeast which was identified as *Kluyveromyces lactis* using 18S r-RNA sequencing technology at NCMR, Pune, mixed with exogenous bacteria *E.coli* and *Bacillus amyloliquifaciens*. All organisms in the consortium selected were able to work under anaerobic conditions in anode chamber. After confirming bioaugmentation efficiency of the consortium it was used in microbial fuel cell (MFC) for generation of bio electricity. Further, variations in microbial fuel cell were carried out and compared to establish the best combination of cathode and anode for generation of electricity from dairy effluent. Effect of immobilization of anode was also studied. Decrease in biological oxygen demand of effluent was also measured and was seen to be effective.

MATERIALS AND METHOD

Sampling - 2 L untreated effluent was collected from a small scale dairy, in clean plastic can. It appeared white in colour and was significantly turbid. It was thoroughly mixed and within two hours, subjected to B.O.D estimation and used in MFC. In case of delay, it was refrigerated.

Analysis of dairy effluent

The dairy effluent was subjected to estimation of initial B.O.D. and B.O.D after treatment in MFC for 8 to 10 days. Protocol for analysis of dairy effluent was as per 'APHA manual' (American public health analysis) using iodometric titration. All tests were carried out in triplicates.

Development of microbial consortium

Two indigenous bacteria isolated from dairy effluent identified as *Enterococcus casseliflavus* and *Enterococcus faecium* by VITEK-2 automated system were grown in 100 ml MRS broth separately for 24 hours at 20 ^{0}C. Fastest growing indigenous yeast identified as *Kluyveromyces lactis* by 18S-rRNA sequencing at NCMR, Pune, isolated from dairy effluent was grown in 100 ml of Sabouraud's broth for 24 hours at 20 ^{0}C. Exogenous bacteria *E.coli and Bacillus amyloliquifaciens* were grown in 100 ml sterile nutrient broth for 24 hours at 37 ^{0}C and 20 ^{0}C respectively. After incubation, all cultures were diluted with sterile saline to 0.1 optical density (O.D.) at 540 nm using spectrophotometer. Equal volumes of diluted cultures were mixed and centrifuged at 4000 rpm for 10 minutes. The pellet was washed with sterile saline twice to avoid incorporation of any organic material from the medium into the effluent and re-suspended in the volume of sterile saline equal to initial volume of medium.

Construction of microbial fuel cell and generation of bioelectricity

A basic two chambered model of microbial fuel cell (MFC) was constructed with anodic chamber and cathodic chamber. Flat graphite electrodes were used in both the chambers. Electrodes were cleaned by soaking in distilled water overnight before use. Wiped, dried and wrapped with copper wires. Ends of Copper wires were drawn out from the lids of both the chambers. Both the chambers were wiped with alcohol and dried. Anodic chamber was filled with 500 mL sterile dairy effluent. It was properly sealed to make it anaerobic. Effluent in the chamber was inoculated with 5% consortium having 0.1 O.D.at 540 nm. Cathodic chamber was filled with various agents as electron acceptors like phosphate buffer, aerated distilled water, hydrogen peroxide. The chambers were connected to each other by a sterile salt agar bridge (2.5% NaCl and 2.5% agar). Voltage generated was measured after every ½ hour for 8 to 10 days (10 readings were taken at the same time on each day) using a multi-meter (DM3540A model).

Different forms of MFC

Anode chamber was filled with 500 ml non-sterile effluent and inoculated with 5% microbial consortium, O.D., 0.1 at 540 nm. Cathode chamber was filled with 500 ml non-sterile phosphate buffer, pH 7. Ten readings of Voltage were taken at the same time for 5 days using a multimeter.

Anode chamber was filled with 500 ml sterile effluent and inoculated with 5% microbial consortium, O.D.,0.1 at 540 nm. Cathode chamber was filled with 500 ml sterile phosphate buffer, pH 7, 500 ml sterile aerated distilled water, 500 ml diluted sterile hydrogen peroxide solution (Original 30% hydrogen peroxide was diluted to 80% and sterilized) (Tamakloe, 2018). Ten readings of Voltage were taken at the same time for 10 days using a multimeter.

An immobilized anode was prepared. 25 ml consortium was mixed with 1.5 g graphite powder and kept on shaker overnight. 0.75 gm sodium alginate was added to it. Slowly with constant stirring, using a syringe the mixture was added drop by drop in 3% chilled sterile $CaCl_2$ solution to make beads. They were left undisturbed for 1 hour for hardening, washed with sterile distilled water thrice, inoculated into 500 ml sterile effluent in anode chamber (Wahab *et al.*, 2018). The cathode chamber was filled with sterile dilute hydrogen peroxide solution. 10 readings of Voltage were taken at the same time for 8 days using a multimeter.

RESULTS AND DISCUSSION

Effluent collected from a small scale dairy looked significantly turbid. Majority of small scale dairies have milk as the principal product. Some dairies also have curd, butter milk and ghee as their secondary products. None of these dairies have sophisticated treatment plant for the effluent.

Initial B.O.D of the effluent was 4220 mg/l indicating presence of high organic matter present in it. With different combinations of anode and cathode, significant reduction in B.O.D. in the range of 52% to71% after 8- 10 days was obtained. Maximum reduction of 71% in 8 days was obtained with Immobilized anode and hydrogen peroxide solution in cathode chamber (Fig. 1).

Mansoorian *et al.* in 2016 had got 81.72% reduction in B.O.D. of dairy effluent using sophisticated MFC technology. With the inexpensive form of MFC, B.O.D. reduction obtained in the current study is definitely significant.

Khan *et al.*, 2013 used chemical oxygen demand (C.O.D.) as a parameter for checking efficiency of M.F.C. They had used glucose solution mixed with azo dye in anode chamber. Parkash A., 2016 also used C.O.D. as a parameter for treatment of dairy waste in M.F.C. 75% reduction in C.O.D. was obtained in 10 days and 99% reduction in 20days.

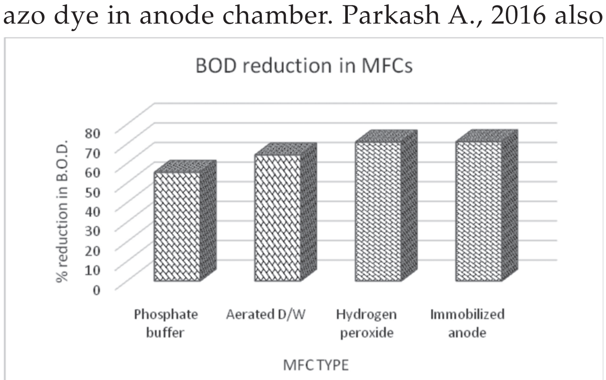

Fig. 1. Reduction in B.O.D. of effluent before and after treatment in different forms of MFC.

In the current study selection of B.O.D was considered as the most appropriate parameter to judge the efficiency of M.F.C because dairy effluent is highly organic in nature and contains all biodegradable constituents.

The microbial consortium developed using indigenous bacteria and yeasts combined with exogenous bacteria worked efficiently in MFCs. It could not only generate electricity but could reduce B.O.D. of the dairy effluent significantly.

Electricity generation using different forms of microbial fuel cell

When sterile dairy effluent was used in MFC, average voltage obtained in 5 days was 116.3mV while using non-sterile effluent it was 90.68 mV. The voltage generated using sterile effluent was significantly greater than when non-sterile dairy effluent was used (Fig. 2). Hence it was decided to use sterile dairy effluent in all other forms of MFC.

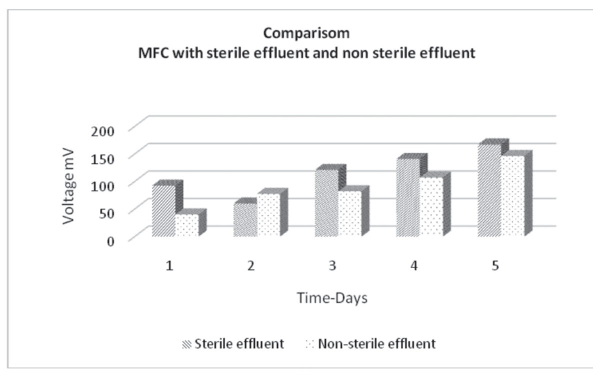

Fig. 2. Comparison of voltage generated using non-sterile dairy effluent and sterile dairy effluent in anode chamber inoculated with 5% microbial consortium and sterile phosphate buffer pH 7 in cathode chamber.

Greater voltage generation in sterile effluent may be due to absence of indigenous flora in the effluent who compete with the organisms in the consortium for available nutrients. Mansoorian *et al.* (2016) had used non sterile dairy effluent along with sewage sludge as inoculum. They could get maximum potential of 856 mV in 5 days. However, they had used sophisticated proton exchange membrane which is costlier and had aerated cathode chamber making it still more expensive. In current study the main objective was to develop a cost effective technique for small scale dairy owners. Hence, use of expensive membrane and aeration of cathode was avoided.

Fig. 3 indicates that maximum 370 mV potential could be generated using sterile phosphate buffer in cathode chamber in un-aerated state. Subsequently it was replaced by aerated distilled water to study the effect of aeration which makes oxygen available as electron acceptor in cathode chamber.

When phosphate buffer was replaced by aerated distilled water in cathode chamber, there was increase in maximum potential from 370mV to 375mV in 10 days (Fig. 3 & 4).

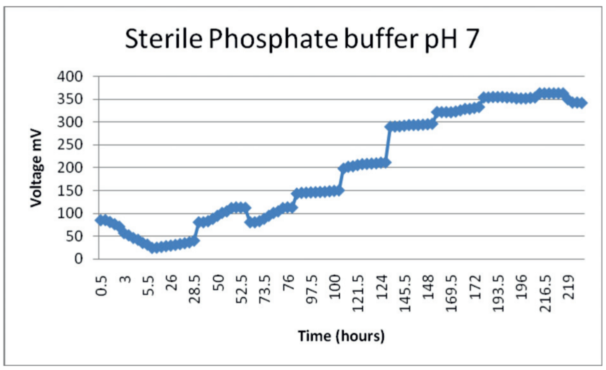

Fig. 3. Voltage generation using Sterile dairy effluent in anode inoculated with 5% microbial consortium and sterile phosphate buffer pH 7 in cathode chamber studied for 10 days.

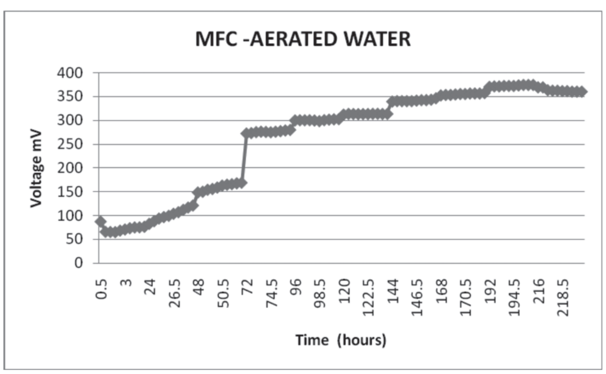

Fig. 4. Voltage generation byreplacing phosphate buffer by aerated distilled water in cathode chamber.

Strik *et al.*, 2010 have mentioned that oxygen is most widely used as an electron acceptor in cathode chamber due to its high oxidation potential and ready availability. However use of oxygen consumes electricity and is expensive. Hence different electron acceptors are being explored in cathode chamber. Gu *et al.*, 2007 suggested use of alternative electron acceptors which would increase the power generation and reduce operating costs. They used cholorophenol containing waste water in cathode chamber and achieved dechlorination of the compound along with energy generation. Hence, in the current study, various electron acceptors were tried in cathode chamber.

When cathode chamber was filled with hydrogen peroxide, a strong oxidizing agent, vigorous bubbling was observed in it (Fig. 5). Maximum voltage generated was 523 mV (Fig. 6). Tartakovsky and Guiot, 2006 compared oxygen and hydrogen peroxide as electron acceptor in a two chambered MFC model similar to one used in the current study. They noticed the use of hydrogen peroxide solution more suitable for keeping MFC for longer duration. In their experiment, anode chamber was filled with glucose solution. Maximum voltage generated was in the range of 340-350mV which is comparable to results obtained in the current study. Moreover in anode chamber instead of glucose solution, dairy

Fig. 5. Bubbling seen in cathode chamber of M.F.C. when sterile dairy effluent in anode inoculated with 5% microbial consortium and 80% of original 30 % hydrogen peroxide solution in cathode chamber.

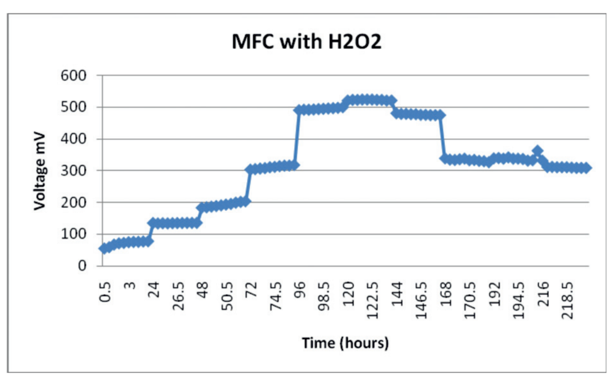

Fig. 6. Voltage generation using sterile dairy effluent in anode inoculated with 5% microbial consortium and 80% of original 30 % hydrogen peroxide solution in cathode chamber.

effluent was filled, making it more economical approach.

Ashley and Kelly (2010) in their review have mentioned that many researchers have used potassium ferricyanide in cathode chamber. However, its use should be prohibited due to its toxic effects. Hence, in current study hydrogen peroxide was preferred as an oxidizing agent in cathode chamber.

With immobilized anode in form of graphite beads filled in anodic chamber (Fig. 7) maximum voltage of 313mV was generated. There was no initial drop in voltage generation as observed with other combinations of anode and cathode. Generation of voltage was steady up to 148 hours, then it dropped down (Fig. 8).

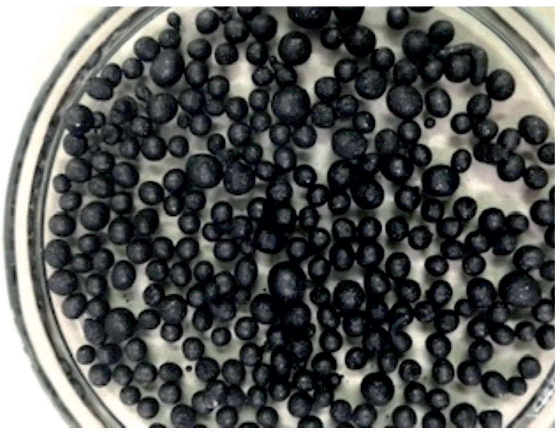

Fig. 7. Immobilized bio anode beads.

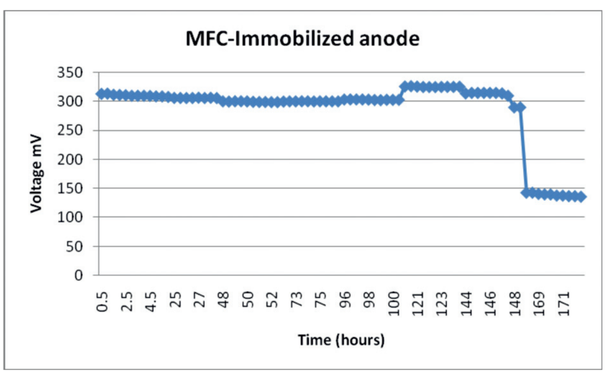

Fig. 8. Voltage generation usingimmobilized bio anode and hydrogen peroxide solution in cathode chamber.

Wahab *et al.* (2018) used dilute sludge as a source of inoculum and compared voltage generation in MFC with free cells and immobilized anode with graphite. They noted double voltage generation with immobilized anode than free cells using glucose solution. They observed maximum voltage of 300 mV after 15 hours. They have also observed no initial drop in voltage generated.

Mesran *et al.*, 2014 prepared alginate-cell-activated carbon immobilization and tested for power production using MFC. After a 200 hours operation of MFC, their immobilized system achieved 403 mV while the non-immobilized system achieved 217 mV indicating immobilization as an effective technique to achieve more and steady power generation.

CONCLUSION

The microbial consortium consisting of indigenous bacteria and yeast isolated from dairy effluents and exogenous bacteria worked efficiently in microbial fuel cells. Voltage generated in all forms of MFC was comparable with the voltage obtained by other researchers. Maximum voltage was obtained when the cathode chamber was filled with hydrogen peroxide solution which is a strong oxidizing agent. Use of immobilized anode resulted into steady voltage generation right from the beginning, without initial drop in voltage and maximum reduction in B.O.D. in 8 days. Aeration of cathodic chamber which has been carried out by many other researchers and use of sophisticated membrane, was avoided to make the process cost effective and affordable to small scale dairy owners.

There is a general tendency observed amongst small scale dairy owners that, unless something is gained from the treatment of effluent, they are not ready to treat it before disposal. They are not much aware about the massive pollution caused by the disposal of effluent in water bodies. The conventional aerobic treatments like activated sludge system are not affordable to them as they consume a lot of electricity for aeration.

In microbial fuel cell, dual goals are achieved. The organic matter is degraded reducing B.O.D. of the effluent and simultaneously electricity is generated which is a wealth from waste. This will definitely encourage small scale dairy owners to treat the effluent before discharging it in water bodies and avoid pollution caused by it. MFC unit could have been kept for more days as the electricity generation had not come down to zero and further reduction in B.O.D. could have been achieved. This technology can be scaled up and transferred to small scale dairy owners.

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