International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Study of Semiconductor Photocatalysed Oxidation of β-Hydroxybutyric Acid has Biological and Pharmaceutical Importance

Pushkar Raj Meena[1], **Dr. R. K. Khandelwal**[2]

[1]H.O.D. Chemistry, S.P.S.B. Govt. College-Shahpura, Bhilwara (Raj.)-India

Abstract: ***Titanium dioxide (TiO2), as a semiconductor photocatalyst has been intensively investigated since Fujishima and Honda studied the photocatalytic splitting of water on TiO2 electrodes in 1972.[1] Since then, many research efforts have been performed in understanding the fundamental processes and in enhancing the photocatalytic efficiency of TiO2. Titanium dioxide is when exposed to UV light, electrons in the uppermost valence band will jump to the conduction band and create conduction band electrons and valence band holes. In most instances, the valence band holes and conduction band electrons simply recombine liberating heat or light, a process known as recombination. Recombination is responsible for the low quantum yields.[2-4] TiO_2 also, due to its non-toxicity, long term photo stability, and high effectiveness, has been widely utilized in mineralizing toxic and non-biodegradable environmental contaminants as well as the novel organic synthesis. As a food additive[16-18], TiO2 (titanium dioxide) is approved for use in various countries like EU, USA ,Australia and New Zealand; it is listed by INS number 270 or E number E-270. The oxidized and polymeric derivatives of β-hydroxybutyric acid occur widely in nature. In humans, D-β-hydroxybutyrate can be synthesized in the liver via the metabolism of fatty acids e.g. β-hydroxy β-methylbutyrate, and ketogenic amino acids through a series of reactions that metabolize these compounds into acetoacetate, which is the first ketone body that is produced in the fasting state.[18] The concentration of β-hydroxybutyrate in human blood plasma, as with other ketone bodies, increases through ketosis.[19] The compound can be used as an energy source by the brain when blood glucose is low.[20] Diabetic patients can have their ketone levels tested via urine or blood to indicate diabetic ketoacidosis. In alcoholic ketoacidosis, this ketone body is produced in greatest concentration.In the present part of study, it is planned to investigate photo-oxidation of tartronic acid (hydroxyl-propandioic acid) by semiconductor sensitized in visible light and the photoproduct was mesoxalic acid. It is therefore planed to investigate photo-oxidation of tartronic acid by semiconductors. Hence, this part of reported work may help to arouse the interdisciplinary interest about the side effects of photoproducts, where these are being used.***

Keywords: Semiconductor, Titanium dioxide, Photo-oxidation, β-Hydroxybutyric acid, Acetone

1. Introduction

Titanium dioxide (TiO2), as a semiconductor photocatalyst has been intensively investigated since Fujishima and Honda studied the photocatalytic splitting of water on TiO2 electrodes in 1972.[1] Since then, many research efforts have been performed in understanding the fundamental processes and in enhancing the photocatalytic efficiency of TiO2. Titanium dioxide is when exposed to UV light, electrons in the uppermost valence band will jump to the conduction band and create conduction band electrons and valence band holes. In most instances, the valence band holes and conduction band electrons simply recombine liberating heat or light, a process known as recombination. Recombination is responsible for the low quantum yields.[2-4] Photoelectron trapping has long been regarded as an effective mechanism to reduce the charge recombination on semiconductor photocatalysts. Many successful efforts have made to enhance the catalytic efficiency of TiO2 by doping with metals (like Ag) and various non metal doping agents like N, P, S.[5-7]

The presence of pharmaceuticals, personal care products (PPCPs) and other hazards in surface water is an emerging environmental issue and provides a new challenge to drinking water, wastewater, and water reuse treatment system.[8-11] These discharged sulfa pharmaceuticals could persist in environmental waters for a long time and accumulate in various organisms of the food chain[12], which may adversely affect the environmental ecosystems[13] and human health.[14] Although these kinds of sulfa pharmaceuticals have been used for several decades, it is surprised that there are only few studies to investigate their environmental and health effect. TiO_2 also, due to its non-toxicity, long term photo stability, and high effectiveness, has been widely utilized in mineralizing toxic and non-biodegradable environmental contaminants as well as the novel organic synthesis. As a food additive[15-17], TiO2 (titanium dioxide) is approved for use in various countries like EU, USA ,Australia and New Zealand; it is listed by INS number 270 or E number E-270.

The oxidized and polymeric derivatives of β-hydroxybutyric acid occur widely in nature. In humans, D-β-hydroxybutyrate can be synthesized in the liver via the metabolism of fatty acids e.g. β-hydroxy β-methylbutyrate, and ketogenic amino acids through a series of reactions that metabolize these compounds into acetoacetate, which is the first ketone body that is produced in the fasting state. The biosynthesis of D-β-hydroxybutyrate from acetoacetate is catalyzed by the β-hydroxybutyrate dehydrogenase enzyme. Butyrate can also be metabolized into D-β-hydroxybutyrate via a second metabolic pathway that does not involve acetoacetate as a metabolic intermediate.[18]

The concentration of β-hydroxybutyrate in human blood plasma, as with other ketone bodies, increases through ketosis.[19] This elevated β-hydroxybutyrate level is naturally expected, as β-hydroxybutyrate is formed from acetoacetate. The compound can be used as an energy source by the brain when blood glucose is low.[20] Diabetic patients can have their ketone levels tested via urine or blood to indicate diabetic ketoacidosis. In alcoholic ketoacidosis, this ketone body is produced in greatest concentration. Ketogenesis occurs if oxaloacetate in the liver cells is depleted, a circumstance created by reduced carbohydrate intake (through diet or starvation), prolonged, excessive alcohol consumption, and/or insulin deficiency. Like the other ketone bodies (acetoacetate and acetone), levels of 3-hydroxybutyrate in blood and urine are raised in ketosis. In humans, 3-hydroxybutyrate is synthesized in the liver from acetyl-CoA, and can be used as an energy source by the brain when blood glucose is low.

β-Hydroxybutyric acid is able to cross the blood-brain-barrier in to the central nervous system.[21] Levels of β-hydroxybutyric acid increase in the liver, heart, muscle, brain and other tissues with exercise, calorie restriction, fasting, and ketogenic diets.[22]
β-Hydroxybutyric acid is the precursor to polyesters, which are biodegradable plastics. This polymer,poly(3-hydroxybutyrate), is also naturally produced by by the bacteria Alcaligenes eutrophus.[23] β-Hydroxybutyrate can be extracted from poly(3-hydroxybutyrate) by acid hydrolysis.[24,25] β-Hydroxy-butyric acid was determined by Edson's [1935] modification of Van Slyke's method. Five substances, lactic, acetic, succinic, ,B-hydroxybutyric acids and CO_2 have now been identified as products of the anaerobic metabolism of pyruvic acid.

Hence β-hydroxybutyric acid has a great biological and pharmaceutical importance as discussed above and it is one of the members of the series of hydroxy acids undertaken in this study. In addition; although many remarkable organic methodologies were developed in the last century, but toxic properties of many reagents and solvents were not known. It is therefore planed to investigate photo- oxidation of β-hydroxybutyric acid by semiconductors.

2. Experimental

The organic compounds i.e. β-hydroxybutyric acid, Silica gel- G, Resublimed Iodine (sm), ninhydrin, titanium oxide, tungsten oxide, iron oxide, zinc oxide, cadmium sulphide, stannic oxide, copper oxide, some other semiconductors and other analytical chemicals are purchased by SD Fine or E Merck chemical suppliers.

UV chamber with UV tube 30 W (Philips), spectrophotometer (Systronic), spectrometer (Systronic), tungsten filament lamps 2 x 200 W (Philips) for visible light, 450 W Hg-arc lamp, water shell to filter out IR radiations and to avoid any thermal reaction, necessary glass wares, thin layer chromatography and paper chromatography kits for to determine the progress of reaction, conductivity meter (Systronic) to determine the optimum yields of photoproducts, magnetic stirrer, pH meter (Eutech pH 510), spectrophotometer (Systronic) and I.R. spectrometer (Perkin- Elmer Grating-377) was used.

The β-hydroxybutyric acid solutions are prepared in water and acetonitrile solvent as the required concentrations as mentioned in the Tables**.** The required concentration of semiconductor or mixed semiconductors has been added to the reaction mixture for heterogeneous photocatalytic reactions. Variations were made to obtain the optimum yield of photoproducts in the given practical conditions.

The progress of reaction was monitored by running thin layer chromatography at different time intervals, where silica gel-G was used as an adsorbent. For colorless spot detection a slide spot detector; UV chamber (Chino's) was used. At the end of reaction or the process the photoproducts has been isolated as its salts and by preparing appropriate derivatives were identified by spectrophotometer, IR-spectrometer, NMR-spectrometer. The optimum yield of obtained 2, 4-DNP [with 0.50gm and 84 ml HCl in 500 ml aqueous solution] was measured by using spectrophotometers and conductivity meter. Various probable variations like the role of different semiconductors, mixed semiconductors, visible and UV-light etc., was studied. Some sets of experiments are also made in controlled conditions such as in absence of UV or visible light, semiconductors and stirring etc.

3. Results and Discussion

Acetone was the photoproduct in the TiO2-sensitised photooxidation of dilute solution in water of β-hydroxybutyric acid. Following variations are studied in this part of thesis:-

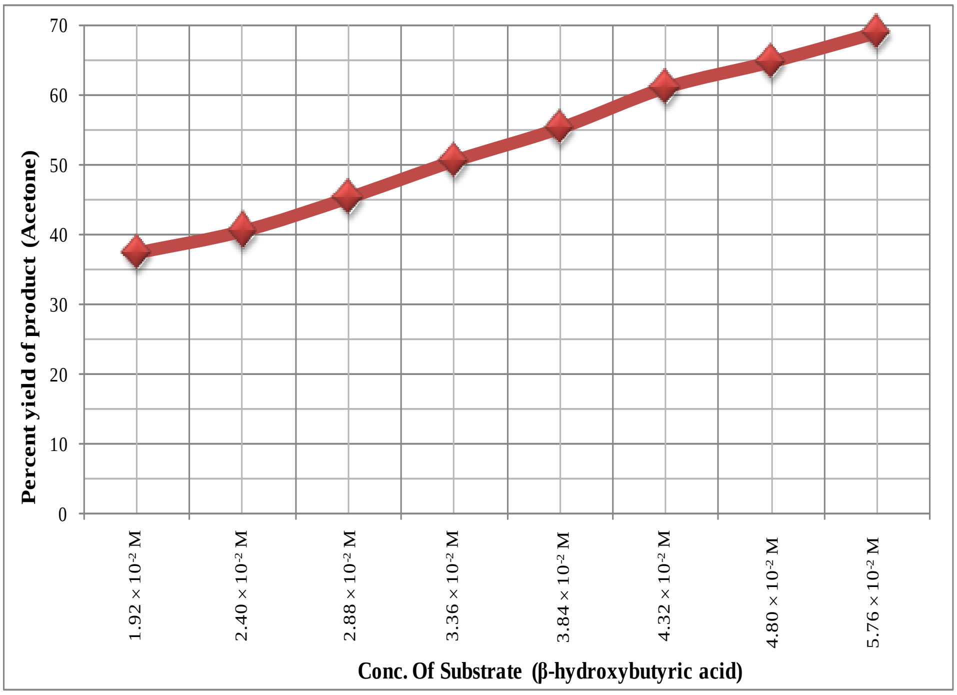
(1) The effect of substrate

The effect of amount of substrate on the oxidation of β-hydroxybutyric acid was studied at different concentrations varying from 1.92×10^{-2} M to 5.76×10^{-2} M (2 gm to 6 gms per litre) at fixed amount TiO2 (1.25×10^{-2} M, i.e. 1 gm/Lt). The total volume of reaction mixture is 50 ml and the results are reported in the following Table 5.21 and shown in Plot 5.21.

1) Solvent : Water
2) TiO_2 : 1.26×10^{-2} M (1.00 g/L)
3) Irradiation time : 120 min
4) Visible light : 2 × 200 W Tungsten lamps

Table 5.21

S. No.	Conc. Of Substrate (β-hydroxybutyric acid)	Percent yield of product (Acetone)
1	1.92×10^{-2} M	37.4%
2	2.40×10^{-2} M	40.5%
3	2.88×10^{-2} M	45.3%
4	3.36×10^{-2} M	50.6%
5	3.84×10^{-2} M	55.3%
6	4.32×10^{-2} M	61.1%
7	4.80×10^{-2} M	64.8%
8	5.76×10^{-2} M	69.0%



Plot 5.21: The effect of substrate

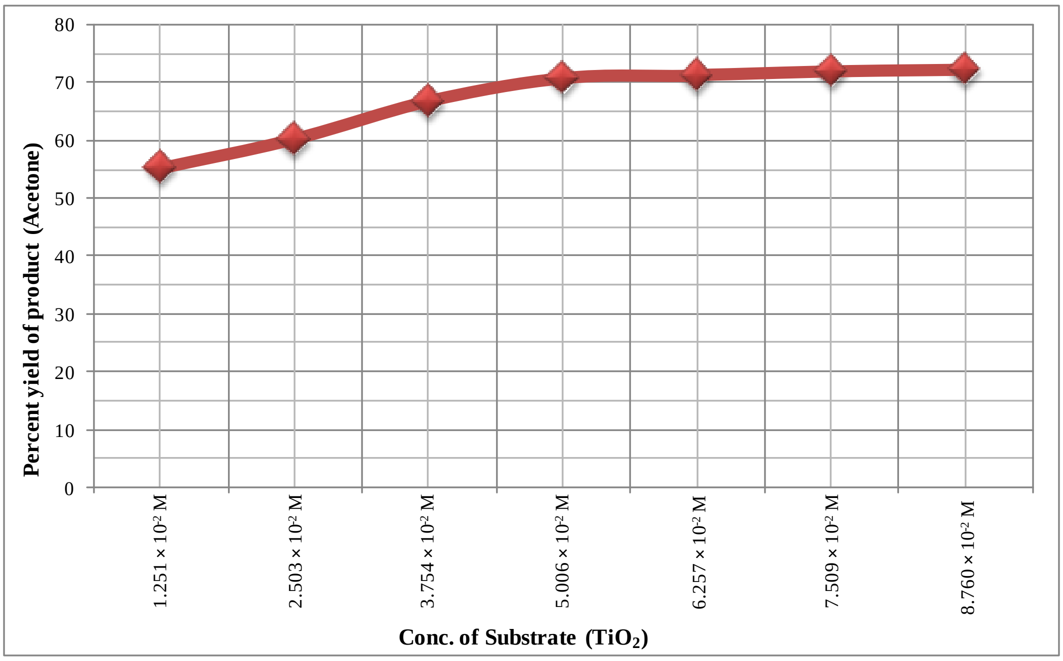
(2) The effect of photocatalyst

Keeping all other factors identical the effect of amount of TiO_2 has also been observed. The total volume of reaction mixture is 50 mL and the results are reported in the following Table 5.22 and shown in Plot 5.22

1) Solvent : Water
2) β-hydroxybutyric acid : 3.84 x 10^{-2} M (4.00 g/L)
3) Irradiation time : 120 min
4) Visible light : 2 × 200 W Tungsten lamps

Table 5.22

S. No.	Conc. Of Substrate (TiO_2)	Percent yield of product (Acetone)
1	1.251 × 10^{-2} M	55.3%
2	2.503 × 10^{-2} M	60.2%
3	3.754 × 10^{-2} M	66.7%
4	5.006 × 10^{-2} M	70.8%
5	6.257 × 10^{-2} M	71.2%
6	7.509 × 10^{-2} M	72.0%
7	8.760 × 10^{-2} M	72.3%



Plot 5.22: The effect of light

(3) The effect of type of radiations

The effect of type of radiations on photocatalytic reaction was studied in visible light and ultraviolet light keeping all other factors identical. The total volume of reaction mixture is 50 mL and the results are reported in the following Table 5.23 and shown in Plot 5.23.

1) Solvent : Water
2) TiO_2 : 1.26×10^{-2} M (1.00 g/L)
3) Irradiation time : 180 min
4) Visible light : 2 × 200 W Tungsten lamps
5) UV Light : UV Chamber 30 W (Philips Tube)

Table 5.23

S. No.	Conc. Of Substrate (β-Hydroxy- butyric acid)	Percent yield of product (In visible light)	Percent yield of product (In UV light)
1	1.92×10^{-2} M	37.4%	48.3%
2	2.40×10^{-2} M	40.5%	56.7%
3	2.88×10^{-2} M	45.3%	64.5%
4	3.36×10^{-2} M	50.6%	71.2%
5	3.84×10^{-2} M	55.3%	78.1%
6	4.32×10^{-2} M	61.1%	84.3%
7	4.80×10^{-2} M	64.8%	88%
8	5.76×10^{-2} M	69.0%	94.1%

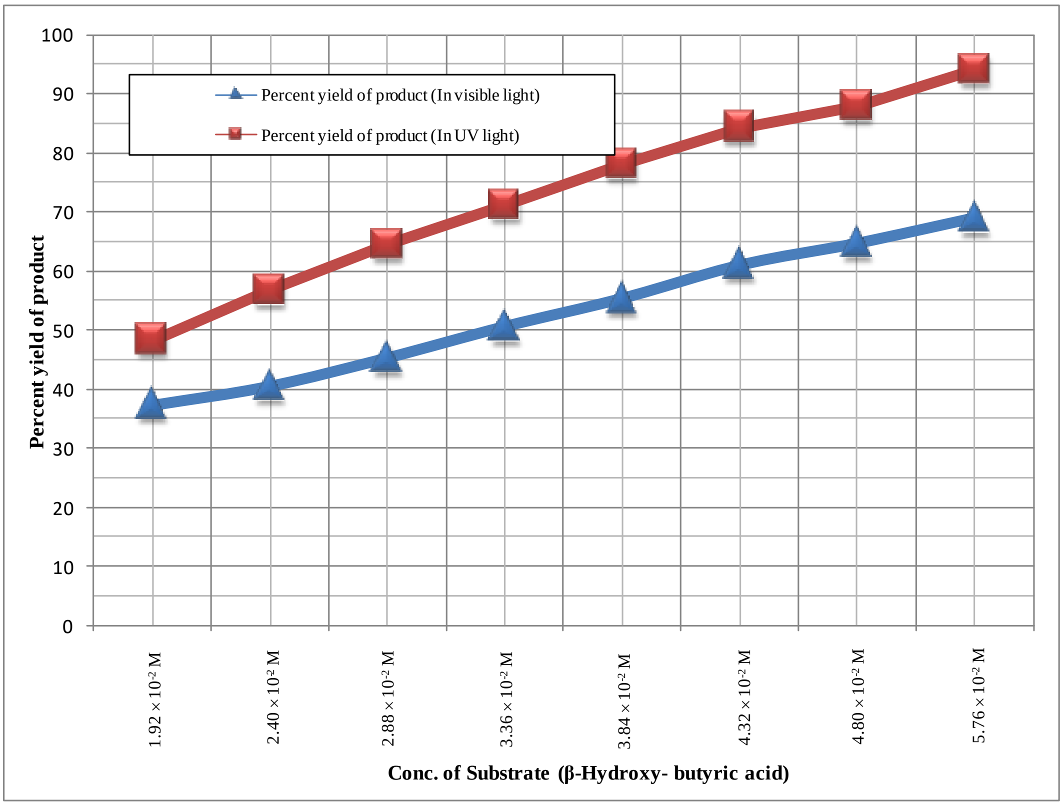


Figure 5.23: Percent yield of product (Acetone)

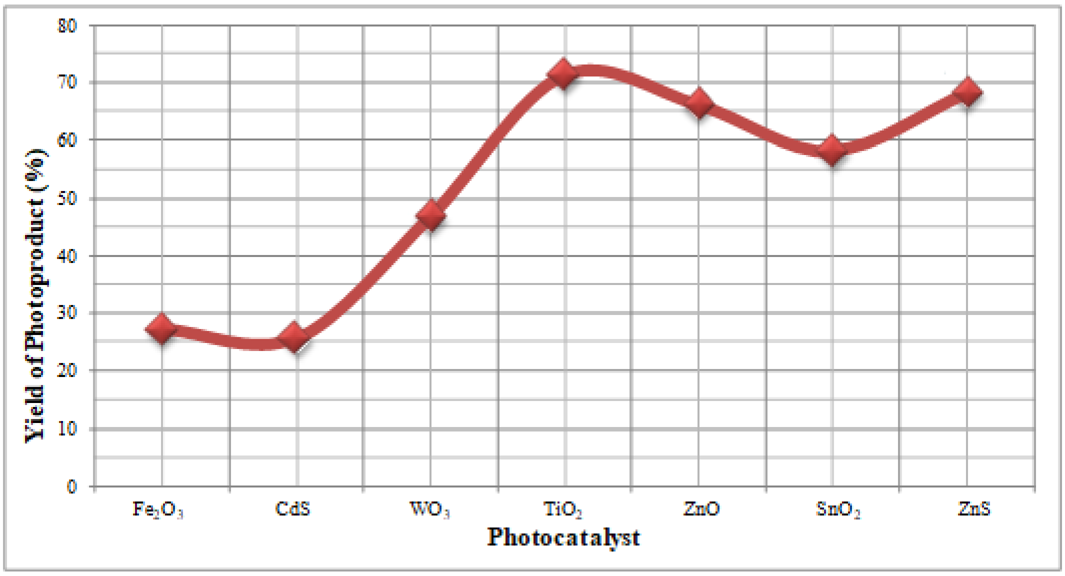
(4) The effect of nature of photocatalyst

The effect of the nature of photocatalyst on photocatalytic reaction was studied by different photocatalysts, which are Ferric oxide, Cadmium sulphide, Tungsten oxide, Titanium oxide, Stannic oxide and Zinc sulphide. The total volume of reaction mixture is 50 ml and the results are reported in the Table 5.24 and shown in Plot 5.24.

1. Solvent : Water
2. β-hydroxybutyric acid: 3.92×10^{-2}M(5.00 gm)
3. Irradiation Time: 180 min.
4. Visible Light: 2 × 200 W Tungsten Lamps.

Table 5.24

S. No.	Photocatalyst	Band gap (eV)	Wavelength (nm)	Yield of Photoproduct
1	Fe_2O_3	2.2	564	27.2%
2	CdS	2.4	516	25.6%
3	WO_3	2.6	477	46.8%
4	TiO_2	3.1	400	71.3%
5	ZnO	3.2	388	66.2%
6	SnO_2	3.5	354	58.1%
7	ZnS	3.6	345	68.2%



Plot 5.24: Percent yield of product

The effect of amount of on the oxidation of β-hydroxybutyric acid was studied by using variable amount of substrate, as reported in Table 5.21 and Plot 5.21. The highest efficiency was observed at optimum concentration. It may be explained on the basis that as the concentration of substrate increases, more substrate molecules are available for photocatalytic reaction and hence an enhancement on the rate was observed with increasing concentration of substrate.

The amount of photocatalyst on oxidation of β-hydroxybutyric acid was investigated employing different concentrations of the TiO_2 as reported in Table 5.22 and Plot 5.22. It was observed that the yield of photo-product increasing with increasing catalyst level up to 5.006×10^{-2} M and beyond this, the yield of photo-product is constant. This observation may be explained on the basis that on the initial stage, even a small addition of photocatalyst will increase the yield of photoproduct as the surface area of photocatalyst increases, but after a certain amount 5.006×10^{-2} M, addition of photocatalyst do not affect the yield of product because of the fact that at this limiting amount, the surface at the bottom of the reaction vessel become completely covered with photocatalyst. Now increase in the amount of photocatalyst will only increase the thickness of the layer at the bottom. Keeping all the factors identical the effect of the nature of photocatalyst on the photo-oxidation of β-hydroxybutyric acid was studied by using visible and UV light as shown in the Table 5.23 and Plot 5.23. As we know that the band gap for the formation of acetone is more suitable for UV light and this property quite resembles the observed data as the table reported.

Titanium dioxide (TiO_2) is the most common photocatalyst and comparably little research has been conducted on zinc oxide, ZnO, which could be a viable alternative for some applications. The effect of other semiconductor particle e.g. Fe_2O_3, CdS, WO_3 (having low band gap than TiO_2 semiconductor) on the TiO_2 catalyst photocatalytic reactions have also been studied. TiO_2 is the most frequently used photo catalyst because of its photo stability and low cost, combined with its biological and chemical inertness and resistant to photo and chemical corrosion. On the other hand, binary metal sulfide semiconductors such as CdS and PbS are regarded as insufficiently stable for catalysis and are toxic. ZnO is also unstable in illuminated aqueous solutions while WO_3 has been investigated as a potential photo catalyst, but it is generally less active catalytically than TiO_2. However, these can be combined with other semiconductors including TiO_2 to achieve greater photo catalytic efficiency or stability. Keeping all the factors identical the effect of the nature of photocatalyst on the photo- oxidation of β-hydroxybutyric acid was studied by using different photocatalysts as shown in the Table 5.24 and Plot 5.24.

It is now well established that the photocatalytic oxidation of several organic compounds by optically excited semiconductor oxides is thermodynamically allowed in presence of oxygen at room temperature. On the basis of analytical, chemical and spectral data the product was characterized acetone.

After completion of photocatalytic reaction the photoproduct acetone was characterized by usual qualitative tests treatment with (1) Semicarbeazide; m.pt-189^{0}C (2) Hydroxylamine hydrochloride, (3) 2, 4-Dinitrophenyl phenyl hydrazones m.pt-126^{0}C shows that photoproduct is acetone. The confirmatory test for acetone and calculation for percentage yield is carried out as following method. In this method the 2, 4-dinitrophenylhydrazone is separated out and percent yield can easily be calculated by measuring the optical density of the its collided suspension.

Separation of 2, 4-DNP and Yield Calculations

In the case of poor yield of photoproduct the visible amount of 2,4 -DNP precipitate may or may not appear, according to the amount of hydrazone substances present. The whole is now shaken with 20 ml of ethyl acetate in a glass stopper 50 ml separating funnel. After separation, the aqueous layer, which is nearly colorless, is extracted with further 10 ml of ethyl acetate; as soon as the aqueous layer is colorless, as usually is the case after two extractions, it is discarded. The united ethyl acetate extracts now contain all the unchanged 2, 4-dinitrophenyl hydrazine together with the hydrazones which have been formed. The liquid is also acid, owing to the extraction of certain amount of hydrochloric acid; these must be neutralized by shaking with solid calcium carbonate (1, 2). The solution is decanted into a glass evaporating basin, washing the calcium carbonate with further ethyl acetate until it is colorless. The washing is added to the main bulk of fluid. The substances in solution must now be transferred to toluene (3).This is accomplished by evaporating the contents of the dish on a water bath to 1-2 ml and then, after removal from the bath, adding about 20 ml of toluene. The slightly cloudy orange- yellow solution is again transferred to the separating funnel and is thoroughly shaken with 5 ml of cold 25%sodium carbonate solution (4). If acetone was originally present as its hydrazone dissolves in the aqueous layer, coloring it brown. This extraction is repeated with fresh Na_2CO_3 solution until the latter remains colorless; two or three repetitions usually sufficient. The united sodium carbonate layers are now acidified by adding concentrated HCl drop by drop. The 2, 4-DNP of acetone is precipitated and orange suspension results. This extracted in a separating funnel with successive 10 ml portions of ethyl acetate until the aqueous layer is colorless (5). It is now usually to employ more than 20 ml of ethyl acetate to achieve this. The ethyl acetate solution now contains all the acetone hydrazone which was present, and is, evaporated to dryness in a glass basin on a water bath. The orange residue is dissolved when cool in 5% alcoholic KOH (6), giving a deep red solution which is made up in a graduated vessel with further alcoholic KOH to a volume such that the color is suitable for reading in colorimeter. In practice it is found that the total volume at this stage should be about 50 ml for every mg of acetone that was originally present in the reaction mixture.[39, 40]

1) If neutralization be omitted, the subsequent heating of the solution leads to the formation of small quantities of compounds other than the acetone derivative which are extracted by the sodium carbonate, coloring it brown, and which are afterwards precipitated upon acidification, giving a reddish color in the final treatment with alcoholic potash. When the neutralization is properly carried out, the sodium carbonate always remains colorless in blank determinations, i.e. when no acetone is present.
2) It was that first the practice of neutralize the acid solution before extracting with ethyl acetate, but if this be done it is found that extraction has to be repeated a much larger number of times before the aqueous layer is colorless.
3) The solubility of acetone derivative in ethyl acetate is so great that it is not completely removed by sodium carbonate solution. Moreover, shaking the ethyl acetate solution with sodium carbonate solution to lead to a small amount of saponification, with the result that separation into layers is imperfect. The substitution of toluene for the ethyl acetate overcome both of these difficulties, but it cannot be used for extraction at the outset because the hydrazine and hydrazones are not sufficiently soluble in to render the process efficient.
4) The toluene extract and the sodium carbonate solution must both be at room temperature. Warm Na_2CO_3 react with other 2, 4-DNPs besides that of acetone.
5) A good deal of care must be taken at this stage that the effervescence produced by the librated CO_2 does not leads to loss by splashing.

4. Mechanism

On the basis of results and discussion the following tentative mechanistic part has discussed for photocatalytic oxidation of β-hydroxybutyric acid, with collaborating the results already reported for other studied compounds.

With respect to a semiconductor oxide such as TiO_2, photocatalytic reactions are initiated by the absorption of illumination with energy equal to or greater than the band gap of the semiconductor. When the suspension of titanium oxide irradiated with visible light electron will be promoted from valence band to conduction band leaving a positive hole in the valence band:

$$TiO_2 + h\nu \rightarrow (h - e) \text{ Excitation } \ldots(1)$$

$$(h - e) \rightarrow h^+ + e^- \text{ Separation } \ldots(2)$$

It was explained before, that the surface of TiO_2 with high surface area retains subsets of hydroxyls, where the net surface density is 4-5 hydroxyl per nm. In addition, suspension of TiO_2 in solution of β-hydroxybutyric acid gives a surface hydroxide ion as locations for primary photo-oxidation processes. Photo holes are trapped by surface hydroxyl groups, whereas electrons are trapped by adsorbed oxygen:

$$h^+ + OH^-_{(s)} \rightarrow OH^\bullet \ldots(3)$$

$$e^- + O_2 \text{ (abs)} \rightarrow O_2^{\bullet -} \text{ (abs)} \ldots(4)$$

The formed $OH^\bullet$ radicals are reacted with adsorbed on the surface, is reacted with the β-hydroxybutyric acid to form β-hydroxybutyric acid radical and water molecule:

$$CH_3CH(OH)CH_2COOH + OH^\bullet \rightarrow CH_3\text{-}C^\bullet(OH)\text{-}CH_2COOH + H_2O \ldots(5)$$

The formed radicals are reacted with adsorbed on the surface, is reacted with the formed water to regenerate hydroxyl group on the surface of the catalyst:

$$O_2^{\bullet -} \text{ (abs)} + H_2O \rightarrow OH^-_{(s)} + OH_2^\bullet \ldots(6)$$

β-Ketobutyric acid formed according the following steps:

$$CH_3CH^\bullet(OH)CH_2COOH + OH_2^\bullet \rightarrow CH_3\text{-}C(O)\text{-}CH_2COOH + H_2O_2 \ldots(7)$$

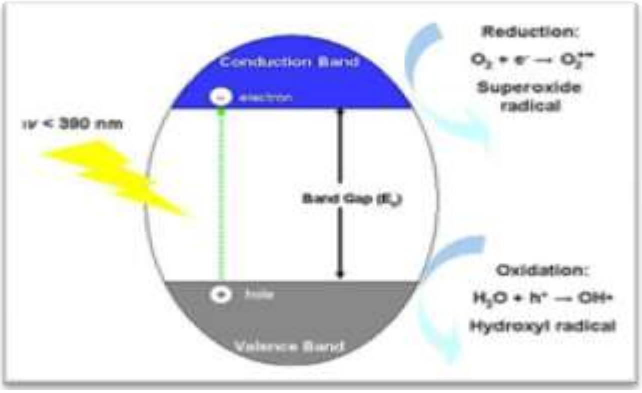
$$CH_3CH^\bullet(OH)CH_2COOH + OH^\bullet \rightarrow CH_3\text{-}C(O)\text{-}CH_2COOH + H_2O \ldots(8)$$

$$2\ CH_3CH^\bullet(OH)CH_2COOH \rightarrow CH_3\text{-}C(O)\text{-}CH_2COOH + CH_3CH(OH)CH_2COOH \ldots(9)$$

The elementary photoproduct β-ketobutyric acid may does not have stability in these practical circumstances and undergoes to release CO_2 and the final photoproduct acetone is produced which was well identified by the simple chemical as well as spectral tests. The evolution of CO_2 was also checked during the reaction under controlled conditions.

$$CH_3\text{-}C(O)\text{-}CH_2COOH \rightarrow CH_3\text{-}C(O)\text{-}CH_3 + CO_2 \ldots(10)$$

The above mechanism is summarized in the following figure:



References

[1] Fujishima, A.; Honda, K. *Nature* **1972**, *238*, 37.
[2] Muruganandham, M.; Swaminathan, M. *Dyes Pigm.* **2006**, *68*,133.
[3] Tan, L. K.; Kumar, M. K.; An, W. W.; Gao, H. *ACS Appl. Mater.Interfaces* **2010**, *2*, 498.
[4] Fei, H. L.; Liu, Y. P.; Li, Y. P.; Sun, P. C.; Yuan, Z. Z.; Li, B. H.; Ding, D. T.; Chen, T. H. *Microporous Mesoporous Mater.* **2007**, *102*, 318.
[5] L. Pan, J. Tang, and F. W. Wang, "Facile synthesis of nanoscaled *a*-Fe2O3, CuO and CuO/Fe2O3 hybrid oxides and their electrocatalytic and photocatalytic properties", *Cent. Eur. J. Chem*., Vol. [**11**], 5, (2013), 763.
[6] S. M. Lam, J. C. Sin, A. Z. Abdullah, and A. R. Mohamed, "ZnO nanorods surface-decorated by WO3 nanoparticles for photocatalytic degradation of endocrine disruptors under a compact fluorescent lamp", *Ceram. Int., Vol.* [**39**], 3, (2013), 2343.
[7] M. Ahmad, E. Ahmed, Y. W. Zhang, N. R. Khalid, J. F. Xu, M. Ullah, and Z. L. Hong, "Preparation of highly efficient Al-doped ZnO photocatalyst by combustion synthesis", *Curr.*
[8] *Appl Phys.*, Vol. [**13**], 4, (2013), 697.
[9] K. Ikehata, N.J. Naghashkar, M.G. Ei-Din, Ozone Sci. Eng. 28 (2006) 353–414.
[10] W.H. Song, W.J. Cooper, S.P. Mezyk, J. Greaves, B.M. Peake, Environ. Sci. Technol. 42 (2008) 1256–1261.
[11] T.C. An, H. Yang, G.Y. Li, W.H. Song, H.Y. Luo, W.J. Cooper, J. Phys. Chem. A 114 (2010) 2569.
[12] T.C. An, H. Yang, G.Y. Li, W.H. Song, W.J. Cooper, X.P. Nie, Appl. Catal. B: Environ.
[13] 94 (2010) 288.
[14] B. Halling-Sorensen, S.N. Nielsen, P.F. Lanzky, F. Ingerslev, H.C.H. Lutzhoft, S.E.
[15] Jorgensen, Chemosphere 36 (1998) 357–394.
[16] R.A. Brain, D.J. Johnson, S.M. Richards, M.L. Hanson, H. Sanderson, M.W. Lam, C.
[17] Young, S.A. Mabury, P.K. Sibley, K.R. Solomon, Aquat. Toxicol. 70 (2004) 23–40.
[18] D.A. Ribeiro, P.C.M. Pereira, J.M. Machado, S.B. Silva, A.W.P. Pessoa, D.M.F. Salvadori,
[19] Mutat. Res. -Gen. Tox. Environ. Mutagen. 559 (2004) 169–176.
[20] UK Food Standards Agency: Current EU approved additives and their E Numbers. Retrieved 27 Oct 2011.
[21] US Food and Drug Administration: Listing of Food Additives Status Part II. Retrieved Oct 2011.
[22] Australia New Zealand Food Standards Code Standard 1.2.4-Labelling of ingredients. Retrieved Oct 2011.
[23] "Butanoate metabolism-Reference pathway" Kyoto Encyclopedia of Genes and Genomes. *Kanehisa Laboratories.* 1 November 2017. *Retrieved* 1 February2018.
[24] Beta- Hydroxybutyrate.Medscape. *Retrieved* 2017-02-08
[25] O. E. Owen; et al. (1967). "Brain Metabolism during Fasting". *The Journal of Clinical Investigation.* 46 (10): 1589–1595.
[26] Sleiman SF, Henry J, Al-Haddad R, El Hayek L, Abou Haidar E, Stringer T, Ulja D, Karuppagounder SS, Holson EB, Ratan RR, Ninan I, Chao MV (2016). "*Exercise promotes the expression of brain derived neurotropic factor through the action of ketone body β-hydroxybutyrate*"
[27] Gilbert DL, Pyzik PL, Freeman JM (2000). "The ketogenic diet: seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones".Journal of Child Neurology. 15 (3): 787-790.
[28] Yoshiharu Doi; Masao Kunioka; Yoshiyuki Nakamura; Kazuo Soga (1988). "Nuclear magnetic resonance studies on unusual bacterial copolyesters of 3-hydroxybutyrate and 4-hydroxybutyrate". *Macromolecules*. 21 (9): 2722–2727.
[29] Dieter Seebach, Albert K. Beck, Richard Breitschuh, and Kurt Job "Direct Degradation of the Biopolymer Poly[(R)-3-Hydroxybutrric Acid to (R)-3-Hydroxybutanoic Acid and Its Methyl Ester" *Org. Synth.* 1993, 71, 39. (25) Law, John H. (Harvard University, Cambridge, Mass.) and Ralpih A. Splepecky ; Assay of poly-,B-hydroxybutyric acid. *J. Bacteriol*. 82:33-36. 1961.