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Determination of amounts of some vitamin B groups in domestic wastewater treatment plants

Fatih Selimoğlu[1], Erdal Öbek[2*], Fikret Karataş[3], E.Işıl Arslan[1] and Şule Yüksel Tatar[1]

[1]Department of Environmental Engineering, Faculty of Engineering, Firat University, Elazig 23119, Turkey
[2]Department of Bioengineering, Faculty of Engineering, Firat University, Elazig 23119, Turkey email:
[3]Department of Chemstry, Faculty of Science, Firat University, Elazig 23119, Turkey
[*]eobek@firat.edu.tr

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Abstract

Some living species need vitamins required for matter modifications which are realized particularly through biochemical reactions, while some need a few of them or do not need any of them. The living organisms obtain those matters, which are essential for surviving, from their existing environments. Therefore, the quantity of vitamins in those environments should be identified. In this sense, the quantity of vitamins in wastewater treatment plants which are the environments of microorganisms also should be determined. In this study, the levels of B_1 (Thiamin), B_2 (Riboflavin), B_3 (Niacin), B_6 (Pyridoxine), B_{11} (Folic Acid) and B_{12} (Cobalamin) among vitamin B groups which can be dissolved in water were determined in water and treatment sludge of Malatya and Gaziantep wastewater treatment plants (Turkey). The levels of B_1, B_2, B_3, B_6, B_{11} and B_{12} in the samples obtained from Malatya and Gaziantep wastewater treatment plants were found out to be significantly different. The results are statistically significant with respect to $p<0.05$ and $p<0.01$. Such differences emerged depending on the characteristics of purified water and microbial characteristics. The determined vitamin levels were found out to be satisfactory for the vitamin requirements of heterotrophic microorganisms in both biological wastewater treatment and soil.

Keywords Wastewater; Treatment; Vitamin B; Water; Sewage Sludge

1. Introduction

The basis of the biological treatment plants bottoms to ensure the nutrient need for energy satisfiying, making the cell components, growth, reproduction and living. Biological cells need six macronutrients for metabolic processes which contain the synthesis of nucleic acids, proteins, fats and carbonhydrates. These are carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus. In addition to these six macronutrients, cells need trace elements as micronutrients (mangenese, zinc, cobalt, molybdenum, nickel, copper, vanadium, boron, iron and iodine) and vitamins (K, B_1, B_2, B_6, B_{12}, biotin, niacin and pantothenic acid) [1].

It was reported that the influent of the activated sludge process must contain all the specific micronutrients to activate the cellular enzymes [2,3].

Vitamin B complex is used in microbiological cycles during biological treatment. It was reported that the "vitamin B complex" is a group of vitamins originally thought to be a single substance, but it is actually made up of Thiamin, riboflavin, niacin, pantothenic acid, biotin, pyroxidine, folic acid, lipoic acid, inositol, and vitamin B_{12}. These substances have different effects on microorganisms and should be considered separately [3]. Todar [4] was reported that some microorganisms need all of these while some do not need any one. Todar [4] was also reported that the needed vitamins must be added from the outside to the environment which the microorganisms exist. In the garland of Anderl [5], vitamin deficiency was reported in 65% of 71 biological wastewater treatment plants in USA. The vitamin B_{12}, vitamin B_2, pantothenic acid, tiamine and folic acid deficiencies were seen in the 4, 4, 7, 10 and 21 of the plants, respectively. Addition of the deficient vitamins increased bacterium growth. Clark and Stephenson [6] were reported that vitamin B complex accelerated biomass formation.

The determination of most of the water soluble vitamins at the same time is difficult and many different methods are used. The most used method for determination of group B vitamins is inverse phase high performance liquid chromatography (HPLC) method that uses C18 column. The other chromatography systems are used when the concentrations of B_1, B_6 and B_{12} are same. But these systems are not used when B_1 and B_6 concentrations in the complex are higher two or three order than B_{12} concentration [7].

In literature, there is not a study about determination of amounts of vitamin B_1, B_2, B_3, B_6, B_{11} and B_{12} in the biological wastewater treatment plants in Turkey. In this study, HPLC method was used to determine the amounts of these vitamins which the activated sludge microorganisms need in the treatment sludges and wastewaters of Malatya Wastewater Treatment Plant (MWTP) and Gaziantep Wastewater Treatment Plant (GWTP) (Turkey). The treatment sludges of these plants are used by farmers.

2. Materials and method

2.1. Materials

In our study, waters and treatment sludges of MWTP and GWTP which designed for treatment of domestic wastewaters were used as materials. The biomass those obtained aerobically and anaerobically had 22% dry solids for MWTP and GWTP, respectively.

Samples which taken from both of the plants were put individually into black plastic sample containers those have lids. Sample containers were covered with aluminium foil and protected from light to prevent the vitamin loss. Then these sample containers were put into the thermos with ice and immediately analyzed in the laboratory.

2.2. Method

The determinations of vitamin B_1, B_2, B_3, B_6, B_{11} and B_{12} were done according to the methods of Wongyai [8] and Amidzic et al. [7] by modification as given below.

Stock solutions of vitamin B_1, B_2, B_3, B_6, B_{11} and B_{12} and diluted solutions from these stocks with different concentrations were prepared to determine the peak of the standards of the vitamins and consequently the retention time.

1.0 g of sludge sample and 2.0 ml of water sample was taken for each analysis. 0.5 ml of 0.5 M $HClO_4$ was added to the taken samples and then the samples were vortexed for 3 minutes. The samples were vortexed again after addition of 2.5 ml of deionized water and then soluted after allowing in the ultrasonic water bath for 5 minutes. Then the samples were centrifuged at 4000 rpm for 8-10 minutes and the curd and filtrate was seperated. 20 µl from the top of the filtrate of the centrifuged samples was injected to HPLC. 250 ml of A solution as mobile phase was prepared by solving sodium salt of the 5 mM heptano sulfonic acid in the methanol. 750 ml of B liquid solution of 0.1 % triethylamine was prepared in the same manner. The A and B solutions prepared were mixed in a proportion of 25:75 (v/v) and pH was adjusted to 2.8 with phosphoric acid. The flow rate of the mobile phase was adjusted to 0.7 ml/min. The wavelengths for the determination of vitamin B_1, B_2, B_3 in C18DB column (15 cm length, inner diameter 4.6 cm, particle size 5 µm) were determined. The mixture prepared from the studied vitamin standards was seperated by HPLC. The detection of the compounds seperated by HPLC was done at 260, 290 and 500 nm wavelengths. Thiamin, riboflavin, nicotinamide gave absorbance at 260 nm wavelength while pyridoxine and folic acid gave absorbance at 290 nm and cobalamin gave absorbance at 500 nm wavelength.

All analyses were done in triplicate. Arithmetic mean and standard deviation of the analyses were determined. Then the evaluations were done at significance levels of $p<0.05$ and $p<0.01$. The result of experiment series of a vitamin was calculated by taking the mean of all values those obtained from these experiment series.

3. Result and discussion

The vitamin amounts determined were given in Table 1. The amounts of vitamin B_1, B_2, B_3, B_6, B_{11} and B_{12} differed from plant to plant. Also the amounts were different in the waters and the digested sludges of the plants. The water and the treatment sludge digested aerobically of the MWTP had higher amounts of vitamin than the ones of the GWTP. There was $p>0.05$ difference between amounts of vitamin B_{11} in plant water while the difference between the other amounts of vitamin (B_1, B_2, B_3, B_6, and B_{12}) was statistically important at $p<0.01$ level (Table 1). The difference between the amounts of vitamin B_1, B_2, B_3, B_6, B_{11} and B_{12} in the treatment sludges used for agricultural purposes was important at $p<0.01$ level (Table 1).

Table 1. B_1, B_2, B_3, B_6, B_{11} ve B_{12} vitamin levels in the MWTP and GWTP samples.

Vitamins (ppm)	Wastewater, ml (n=25)		Biosolid, g (n=25)	
	MWTP	GWTP	MWTP	GWTP
Vitamin B1	0.09–0.32	0.13-2.06	14.19±1.79	1.05±0.14
Vitamin B2	0.09–0.65	0.20-2.35	11.16±1.06	0.76±0.06
Vitamin B3	0.17–0.49	0.10-11.10	661.37±17.20	0.56±0.11
Vitamin B6	0.06–0.15	0.11-1.52	23.87±0.47	0.70±0.07
Vitamin B11	0.20–0.51	0.27-0.66	2.60± 0.34	1.48± 0.19
Vitamin B12	0.33–0.52	0.05-5.16	9.23± 1.25	3.61± 0.66

It was reported that the vitamin need for aerobic and anaerobic bacteria was at ppm level for niacin, thiamin, riboflavin, pyridoxine and at ppb level for folic acid [9-11]. In our study these vitamins which needed for bacterial growth were at enough levels in MWTP and GWTP.

Our results could not directly compared with the results reported in the other studies those used mostly different methods.

Lemmer et al. [10] were found thiamin levels of 1-29 ppm in municipal and industrial sludges and 1-6 ppm in digester sludge. Lemmer and Nitschke [9] were found thiamin levels of 1.1-6.6 ppb and 1-6 ppb in dry solids and water phase of municipal plant activated sludge, respectively while 0.9-6.0 ppb and 18 - 386 ppb in the dry solids and water phase of digested material, respectively. Lemmer and Nitschke [9] were also determined the contents of thiamin in the dry solids and the water phase of three activated sludges taken from industrial wastewater treatment plants. Thiamin content in the dry solids of activated sludge from a petrochemical industry was between 5.1 and 29.1 ppb, while the water phase contained 3–14 ppb thiamin. Thiamin content in the dry solids of fruit and paper industry was 2.4 ppb while the water phase contained none. Dry solids of activated sludge from oil industry had 1.9 ppb thiamin while the water phase contained 5 ppb. In our study, thiamin amounts in the aerobically digested sludge and wastewater obtained from MWTP were 14.19±1.79 ppm and 0.09-1.32 ppm, respectively. Thiamin amount in the anaerobically digested sludge obtained from GWTP was under the detection limits while it was 2.06 ppm in the wastewaters of GWTP.

Lemmer et al. [10] were determined that the riboflavin amounts were 18-43 ppm in municipal and industrial sludges and 3-11 ppm in digester sludges. Lemmer and Nitschke [9] were reported the riboflavin content between 17.6–43.2 ppb and 9–25 ppb in the dry solids and water phase of activated sludge from municipal wastewater treatment plants, respectively. They were reported the riboflavin content between 31.5-41 ppb and 1-5 ppb in the dry solids and water phase of petrochemical industry, respectively. The riboflavin contents reported for the dry solids and water phase of fruit and paper industry were between 21.3-24.4 ppb and approximately 26 ppb, respectively while they were 23.4-32.4 ppb and 15 ppb in the dry solids and water phase of oil industry, respectively. In our study, riboflavin amounts in the dry solids of sludgesof MWTP and GWTP were 10.10–12.22

ve 0.76–0.06 ppm, respectively while they were 0.65 and 2.35 ppm in the wastewaters taken from MWTP and GWTP, respectively.

Lemmer et al. [10] were reported that folic acid amounts were 10-13 ppm and 2 ppm in the municipal activated sludge and industrial activated sludge, respectively while they were 0.3-0.6 ppm in the digester sludge. Lemmer and Nitschke [9] were determined that the folic acid amounts were 10-13 ppb and 9-25 ppb in the dry solids of activated sludge and wastewaters taken from municipal wastewater treatment plant, respectively. Dry solids of digested sludges had folic acid level of 0.25-0.6 ppb while water phase did not contain any. They were reported that dry solids of activated sludge taken from industrial wastewater treatment plant had approximately 2 ppb folic acid content. In our study, folic acid amounts in the sludge samples taken from MWTP and GWTP were between 2.26-2.94 and 1.39-1.67 ppm, respectively while they were 0.51 and 0.66 ppm in the wastewaters of MWTP and GWTP. Vitamin amounts differ from plant to plant in the materials used as in the ones of the other studies.

Neujahr [12] was reported that various factors effect vitamin formation amounts in microbiologically degradaded waste sludges and these could be characterized with fermentation type (aerobic or anaerobic), addition levels those made for degradation of the sludges and pH of the sludges. Neujahr [12] was also reported that temperature and alcali operations have harmful effect on vitamin B_{12} synthesis. In our study, aerobic conditions increased vitamin synthesis while anaerobic conditions did not effect vitamin synthesis. These differences may result from the differences in process parameters as wastewater quality and low food/microorganism rate as reported by Lemmer and Nitschke [9] and Burgess et al. [11].

Vitamins are one of the growth factors and needed as coenzymes and functional groups of certain enzymes. Some bacteria (e.g *E. coli*) do not require any growth factors. They can synthesize essential vitamins, as part of their own intermediary metabolism. Certain other bacteria (e.g. *Lactobacillus*) require vitamins in order to grow. These vitamins must be added in advance to culture media that are used to grow these bacteria. Vitamin B_6, B_2, B_1 and B_{12} are frequently required by certain bacteria as growth factors. The functions of these vitamins in essential enzymatic reactions gives a clue why, if the cell cannot make the vitamin, it must be provided exogenously in order for growth to occur. The functions of vitamin B_1 are decarboxylation of keto acids and transaminase reactions. The functions of vitamin B_2 are oxidoreduction reactions. The functions of vitamin B_6 are transamination, deamination, decarboxylation and racemation of amino acids [4].

Voight et al. [13] were reported that *Eschericia coli* secreted Vitamin B_1 in high loaded sludges. The secreted Vitamin B_1 increased the activity of protozoan species as *Trithigmostoma cucullulus* or *tetrahymena pyriformis*. The organisms which need Vitamin B_3 did not exist in the step which had high load in domestic wastewater treatment plant. However, the organisms as *Staphylcoccus aureus* which need Vitamin B_3 as essential growth factor were exist. Optimum activity of Vitamin B_3 which was needed as a growth material for *Streptobacterium casei* and *Lactobactobacillus arabinosus* satisfied at 1 mg/l. Burgess et al. [11] were reported that the need of vitamin B_1, B_2 and B_6 for bacterium in activated sludge is 0.3-1.2 ppm, 0.5-2.0 ppm and 0.1-10 ppm, in that order. Mohr [14] was reported that folic acid is growth factor for bacterium and fungus and mutagens are low effective at the presence of this acid.

Taking into consideration the need for addition of vitamins needed for the microorganisms which could not synthesis them, it could be said that the need could be overcome by addition of treatment sludges to the soil for fertilization.

4. Conclusions

Vitamins which have catalitic effect in metabolic processes must be enough amount for microbial cycles those are the basis of the biological wastewater treatment process. Vitamins must be added to the environment because some microorganisms could not synthese these materials. In the habitat, determination of the amount of vitamins which required for growth and metabolism of microorganisms helps protection of microorganisms in the process ecology. For these reasons, vitamin amounts in wastewater and treatment sludges were examined. In Turkey, there is not a study which investigates the amounts of vitamins in biological wastewater treatment plants.

The amounts of vitamin B which are required for the growth of microorganisms in biological wastewater treatment plants and soil were at enough levels in treated waters and biosolids of MWTP and GWTP. As a result of the different sludge treatments and urban metabolism, amounts of vitamins in the studied samples were different.

It could be said that treated waters and biosolids those digested aerobically and anaerobically and used for agricultural purposes were rich in vitamin B. The determination of this property of biosolids will provide the good knowledge to the users and aware the researchers and also supply knowledge to the literature.

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