Article

Anaerobic digestion of kitchen waste generated from Atomic Energy Research Establishment (AERE) cafeteria for lactic acid production

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Abstract: Due to accelerated economic growth and increased food production, per capita rate of waste generation is also increasing in Bangladesh. Being the ninth most populous and twelfth most densely populated country in the world, Bangladesh will face serious crisis in both food scarcity as well as food loss if food wastage problem is not addressed. At household level, 5.5 percent food is wasted on daily basis. Due to its large volume, the disposal of food waste will be a major problem. Production of Organic acid from kitchen waste via anaerobic digestion can eliminate both waste pollution problem and high cost production of organic acid. Such organic acid can be used in food and beverages, cosmetics, and detergent industries. The present study was undertaken to convert kitchen waste generated from cafeteria of Atomic Energy Research Establishment (AERE), Savar, Dhaka into lactic acid using natural microflora. The number of indigenous microflora in kitchen waste were found to be 1.25×10^7 cfu/mL and pH range of 5.0-6.0. The ratio of rice, meat and vegetables in the kitchen waste was found to be 3:1:1. Kitchen waste was found to contain approximately 19.03% protein, 3.2% fat and 1.5% ash. Anaerobic digestion was carried out in shake flasks at various initial pH (5.0, 6.0 and 7.0) and different temperature (30℃, 37℃ and 45℃) for 96 hours. Highest lactic acid from Kitchen waste was produced (24.00 g/L) at 24 h at initially adjusted pH-7.0. An attempt to recover Lactic acid from fermented broth was conducted using rotary evaporation at 100℃ and at (60-65) cm. Hg vac. The results indicated that, the volume of food waste can be greatly reduced and can be converted into value-added products such as lactic acid via anaerobic fermentation.

1. Introduction

According to the world report 'State of Food Security and Nutrition in 2018' by UN Food and Agriculture Organization (FAO), the approximate worth of food wastage in developing countries including Bangladesh is around 310 billion dollars. According to FAO, food waste refers to food that is good in quality and fit for human consumption but that does not get consumed because it is discarded - either before or after it spoils. The report also states that 1.3 billion tons of food is wasted globally (FAO, 2011).

For the past few years, food and food waste has been an emerging global problem. It will be a matter of grave concern also in Bangladesh due to accelerated economic growth and increased food production. Bangladesh is

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

89

the ninth most populous and twelfth most densely populated country in the world. Consequently, Bangladesh is likely to face serious crisis in both food scarcity as well as food loss, if food wastage problem is not addressed.

In Bangladesh, within 15 years (1991-2005), per capita waste generation rate in urban areas have been increased from 0.31 to 0.41. Projected value of per capita waste generation rate in urban areas in 2025 will be close to 0.60. Since, waste generation increases exponentially with GDP and population, food waste will be a challenging issue, indeed (Waste Concern, 2014).

At present, one of the most common practices of food waste disposal is dumping in landfill. However, food, food waste and environment are correspondingly related. Food waste has an environmental impact when disposal of food waste creates emission of greenhouse gas (Omar *et al*., 2009).

On the other hand, the high percentage of organic waste, both food waste from domestic sources and other organics from non-domestic sources, means that great potential lies in the changing practices and patterns of municipal solid waste management and resource recovery in the urban areas of Bangladesh. As the country continues its journey towards middle income nation, integrated resource recovery strategies such as recycling this organic waste into organic fertilizer through aerobic composting or into biogas through anaerobic digestion and then to electricity can be well adopted.

Organic waste that consist of uneaten food and waste generated during food preparation from residences, restaurants and cafeteria are known as kitchen waste. Such food waste contains high level of organic contents such as starch, sugars, protein, cellulose which are readily biodegradable (Wang *et al*., 2005). As food waste especially kitchen waste has high organic compound with high moisture contents; anaerobic digestion (AD) is the best treatment method than incineration, landfill, and composting (Zinatizadeh *et al*., 2006). Anaerobic digestion which involves hydrolysis, acidogenesis, acetogenesis and methanogenesis are a complex process (Bo *et al*., 2007). It is also considered as means of volume reduction, waste stabilization and biogas recovery process (Wang *et al*., 2008).

In anaerobic digestion, the organic acid production was influenced by pH and temperature (Zhang *et al*., 2008). Organic acids such as lactic acid was found to be the key product of kitchen waste fermentation (Bo *et al*., 2007). Such lactic acid has been used in the food and beverage industries, pharmaceutical industries, cosmetics, and detergents. Now a days, the demand for lactic acid has been increasing in industries as it can be utilized as a raw material for polylactic acid, a polymer used as medical disposal and environmental ecofriendly bioplastics, which can replace synthetic plastic derived from petroleum feedstocks (Narayanan *et al*., 2004).

Extraction of organic acid especially lactic acid from the fermentation broth has been demonstrated by several methods such as electrodialysis (Huang *et al*., 2007), membrane filtration (Kang and Chang, 2005), solvent extraction (Harington and Hossain, 2008), precipitation, adsorption on resins (Cao *et al*., 2002) as well as crystallization. Physical separations such as filtration and evaporation are widely used as organic acids recovery strategies in industries (Mumtaz *et al*., 2008). However, those methods face a few problems like the usage of chemicals, production of large volume of wastes, high product loss and high-power consumption. Freezing and thawing is a conventional method but yet is still being applied nowadays.

Production of organic acid from kitchen waste may help to mitigate waste pollution problem and mediate cost-effective production of lactic acid (Wang *et al*., 2003). Kitchen waste generally contain few compounds that inhibit bacteria (Wang *et al*., 2003b). There will be an advantage if the kitchen waste with high fraction of organic content can be utilized as a high value of carbon resource. Lactic acid could be stably accumulated during kitchen waste fermentation by controlling some fermentation parameters such as temperature, pH etc. (Sakai and Wang, 2002).

With this aim, the present study was undertaken to investigate the effect of different pH and different temperature on anaerobic digestion of kitchen waste collected from Atomic Energy Research Establishment (AERE) cafeteria. The study also aimed at isolating bacteria from kitchen waste and also recovering lactic acid from fermented kitchen waste after anaerobic digestion by evaporation.

2. Materials and Methods
2.1. Sample collection
Leftover food (kitchen waste) as shown in Figure 1 was collected from central cafeteria of Atomic Energy Research Establishment, Ganakbari, Savar during lunch time. Sample was brought to laboratory immediately after collection and kept in refrigerator before processing. Each time, the gross ratios of rice, meat and vegetable in food waste samples were recorded.

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

90

Figure 1. Kitchen waste collected from central cafeteria of AERE.

2.2. Sample processing
After collection of samples, kitchen waste was ground using heavy duty waring blender. Water was added in order to adjust the ratio of kitchen waste to water to be 1:1(Wang *et al*., 2005). pH of the slurry produced was measured and slurry was kept in refrigerator for further analyses viz. proximate composition (moisture content, crude protein, ash and fat content) and microbial load etc. Finally, anaerobic digestion of food slurry was carried out as described in section 2.11.

2.3. Proximate analysis
Proximate analyses of the kitchen waste were performed in order to quantify the proportion of carbohydrate, lipids content, crude protein, fat, fiber, and moisture content of collected food waste samples (Wang *et al*., 2005). Determination of moisture was based on standard method (Adegunwa *et al*., 2011). Kjeldhal's method as given in Chow (1980) was followed for crude protein analysis. Ash content was determined as described by Carpenter (1960). Fat content was determined as described by Folch (1957). And carbohydrate was determined as described by Dubois (1956). We also performed total solid (TS) and total suspended solid (TSS) content in samples.

2.4. Microbial analysis of food sample
A serial dilution was performed with blended kitchen waste and spread plate technique was used to count microbial load. Streak plate method was used to isolate and purify the isolates. The purified isolates were transferred to nutrient agar slant in small glass vials with sterile loop, incubated at 37˚C for 24-48 hours. After incubation the vials were stored at 4˚C and were revived every month. Colony morphology, microscopic examination of bacteria by gram staining and biochemical test such including catalase, oxidase, MR, VP, Indole etc. were carried out as per the method given by Cappuccino and Sherman (1992).

2.5. Anaerobic digestion of food waste
Anaerobic digestion process of kitchen waste is shown in Figure 2. 100 ml of blended kitchen waste was placed separately into 250 ml of shake flasks without any supplement. The shake flask was marked as A1, A2, B1, B2, C1, and C2 with varied initial pH of 5.5, 6.5, and 7.5, respectively. Fermentation was carried out for several days at different temperature. An aliquot of 1 ml sample was withdrawn from each flask for analyses every 24 hours interval.
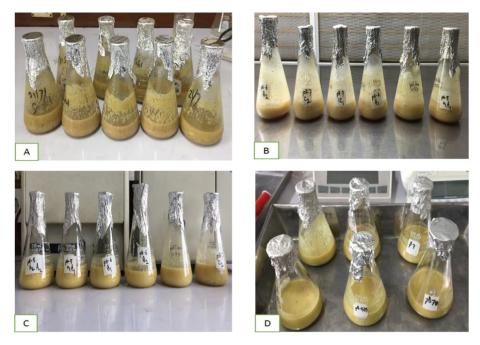
Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

91

Figure 2. (a-d). Photograph showing anaerobic digestion process of kitchen waste.

2.6. Recovery of organic acid from fermentation broth

The recovery process of organic acids from fermented kitchen waste was performed by freezing and thawing method (Phang *et al*., 2001). The freezing process was carried out using a deep freezer at -30°C for overnight. The thawing process was conducted in room temperature on a cloth mesh for 2-3 h. The filtrate was then centrifuged (8000 g for 20 min) and filtered (0.8 μm, cellulose acetate filter paper) by using a vacuum pump filter. Evaporation was carried out by using a rotary evaporator (Mumtaz *et al*., 2008) until the desired concentration of the organic acids was achieved. Lactic acid concentration was determined for the final concentrate during evaporation.

2.7. Spectrophotometric determination of lactic acid
2.7.1. Construction of standard curve of lactic acid

At first, 1.2 ml/L of Lactic acid was placed in 10ml volumetric flask and made the stock solution. Then various concentration of Lactic acid (2.5-10.5) ml/L were made from the stock solution. 50μL of lactic acid from various concentration were added to 2 ml of 0.2% solution of iron (III) chloride (Appendix) and stirred. Optical density (OD) was taken for different concentration of lactic acid at 390 nm against 0.2% solution of iron (III) chloride. A standard curve was prepared by plotting the values of known concentration in y axis as shown in Figure 3.
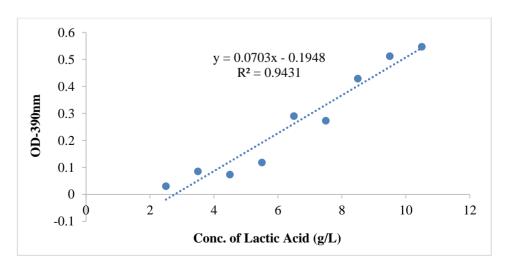

$$y = 0.0703x - 0.1948$$
$$R^2 = 0.9431$$

Figure 3. Standard curve for the estimation of lactic acid.

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

92

2.7.2. Iron (iii) chloride (0.2%) solution preparation

Iron (iii) chloride was placed in 100 ml volumetric flask. Diluted to the mark with water and stirred to complete dissolution of salt.

A solution of lactic acid (50 µl) of a corresponding concentration was added to 2 ml of 0.2% solution of iron (iii) chloride.

The absorbance of the obtained colored solution was measured at 390 nm against 2 ml 0.2% solution of iron (iii) chloride.

2.7.3. Method of the spectrophotometric determination of Lactic acid

A test solution (50 µl) containing Lactic acid was added to 2 ml of a 0.2% solution of iron (iii) chloride. Absorbance was measured at 390 nm against 0.2% iron (iii) chloride.

2.7.4. Determination of lactic acid in food sample

Cells/solid portion were separated from cultural liquid by centrifugation. Lactic acid in the sample was determined by the spectrophotometric method.

3. Results and Discussion

3.1. Sample processing

Leftover food (kitchen waste) was mostly collected from central cafeteria of Atomic Energy Research Establishment, Ganakbari, Savar during lunch time. It has been observed that, the composition of kitchen waste varied during each sampling. However, gross ratios of rice, meat and vegetables in the kitchen waste were determined as 3:1:1. For further analysis and anaerobic fermentation, the kitchen waste was added with water in 1:1 ratio and then grounded using a blender (Wang *et al*., 2005) as shown in Figure 4.

Figure 4 (a-b). Processing of kitchen waste collected from central cafeteria of AERE.

3.2. Proximate analysis

Moisture content, carbohydrate, crude protein, fat, fiber and ash were analyzed for kitchen wastes (Wang *et al*., 2005). Kitchen waste was found to contain 19.03% protein, 3.2% fat and 1.5% ash. The content of carbohydrate and fiber were not determined. The moisture content of kitchen was found to be in the range of 60-70%. The total solid (TS) and Total suspended solid (TSS) observed were 300 g/L and 83.86 g/L in kitchen waste.

Whilst the composition in terms of carbohydrates, lipids and proteins gives an overall idea of the composition, together with the solids content, a more extensive characterization in terms of carbon content or nitrogen is also necessary to finally differentiate them. Since kitchen waste is generated by the consumers, its composition depends strongly on different eating and cooking habits (Zhang *et al*., 2014). Thus, composition and characterization of food waste largely depends on its origin, geographical location, social behaviours, habits, and quality of ingredients etc. (Iacovidou *et al*., 2012). The results obtained in this study is relatively higher compared to Hafid *et al*. (2010) where the total solid (TS) and total suspended solid (TSS) content of food waste were 118 g/L and 84 g/L, respectively.

Total viable count after serial dilution in nutrient agar media was found to be 1.25×10^7 cfu/ml. This result is considered slightly lower than that of Hafid *et al*. (2010) which is 1×10^9 cfu/ml. However, no growth in

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

93

MacConkey agar was observed in this study which indicated that the collected food waste was devoid of any pathogenic microbes.

3.3. Microbial analysis of food waste samples

Discrete bacterial colonies appeared on dilution plates were streaked onto nutrient agar for the purpose of isolation and purification of isolates from food waste samples collected from AERE cafeteria. A total of 3 bacterial colonies were recovered on the basis of morphological differences and were purified. The size, form, pigmentation, margin, and elevation of the colonies were recorded for each isolate. Gram staining of all three isolates revealed that all the isolates were gram-positive, and coccus (Figure 5).
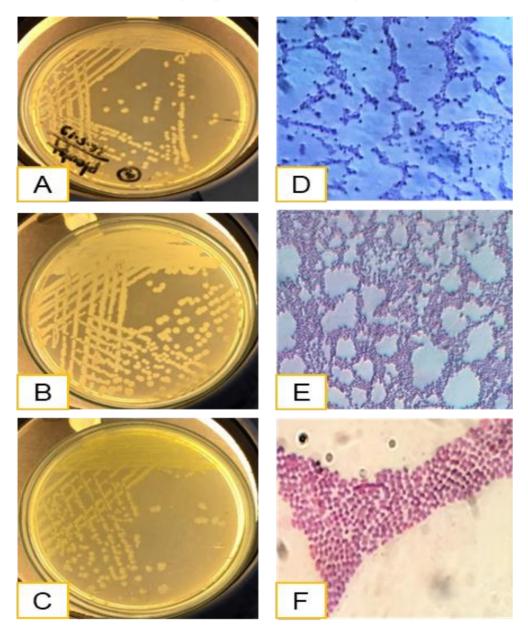

Figure 5. Photograph showing colonies of lactic acid producing isolate no.C1 (b), P1 (c) and Y1 (d), respectively and their morphology under light microscope.

3.4. Biochemical characteristics of isolates

Four biochemical tests viz, oxidase, catalase, Methyl Red, Voges Proskauer were performed in this study. All isolates were oxidase negative and catalase positive. For methyl red test, isolate P1 and R1, showed positive reaction and Y1 showed negative reaction. In case of Voges-Proskauer, all showed negative reaction.

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

94

3.5. Anaerobic digestion of food waste

Initially, pH was adjusted to pH 5.0, pH 6.0 and pH 7.0 and were referred to as A, B and C, respectively. Fermentation was continued till 120 hour and lactic acid and pH were measured every 24 hours throughout the fermentation process.

3.5.1. Production of lactic acid from food waste at 30°C

Figure 6 shows the fermentation profile of lactic acid at 30°C with the initially adjusted pH-5.0, pH-6.0 and pH-7.0. In this study, the highest production of lactic acid was achieved at 24h (18.00 g/L) at initially adjusted pH-7. And the lowest production of lactic acid was achieved at 72h (8.6 g/L) at initially adjusted pH-7.0.
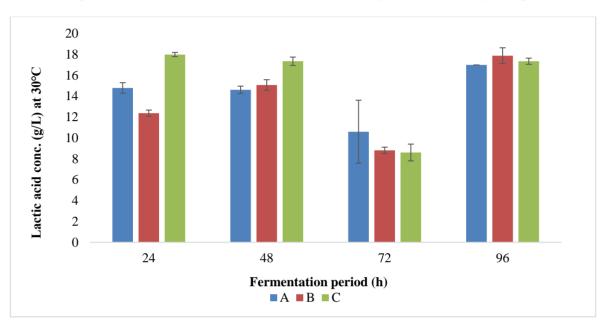

Figure 6. Lactic acid production during fermentation of kitchen waste at 30°C with initially adjusted pH-5.0(A), pH-6.0(B) and pH-7.0 (C).

Table 1 shows pH variation during anaerobic digestion of food waste at 30°C. Rapid reduction in pH was witnessed after 24 h of fermentation regardless of initial pH. After 24 h, pH value was decreasing gradually from 4.1 to 3.8 and after 72 hours, pH was maintained in the range of 3.6 to 3.8.

Table 1. pH profile of anaerobic digestion of food waste at 30°C.

Incubation period (h)	Fermentation at different initial pH		
	A	B	C
0	5.0 ± 0.00	6.0 ± 0.00	7.0 ± 0.00
24	3.9 ± 0.03	4.0 ± 0.00	4.1 ± 0.01
48	3.7 ± 0.00	3.8 ± 0.01	3.9 ± 0.01
72	3.6 ± 0.00	3.7 ± 0.00	3.8 ± 0.00
96	3.6 ± 0.01	3.6 ± 0.00	3.8 ± 0.00

3.5.2. Production of lactic acid at 37°C under static condition

Figure 7 shows the production of lactic acid at 37°C with initially adjusted pH-5.0, pH-6.0, and pH-7.0. Highest lactic acid (19.59 g/L) was produced at initially adjusted pH-7.0 at 24 h. The lowest concentration of lactic acid (4.02 g/L) was found at 72 h at initially adjusted pH-6.0.
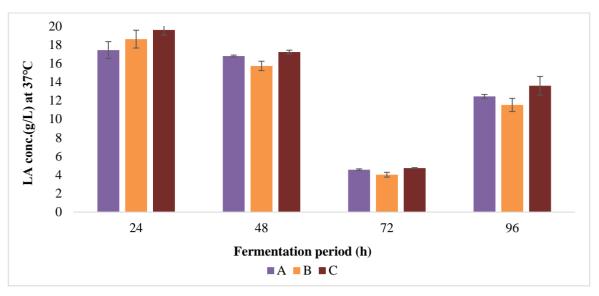
Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

95

Figure 7. Lactic acid production during fermentation of kitchen waste at 37°C with initially adjusted pH-5.0, pH-6.0, pH-7.0.

At 37°C, sharp decline in pH was observed for all treatments within 24 hours (Table 2). pH was steady till 48 hour and was gradually increased after 72 hours (Table 2).

Table 2. pH profile of anaerobic digestion of food waste at 37°C.

Incubation period (h)	Fermentation at different initial pH		
	A	B	C
0	5.0 ± 0.00	6.0 ± 0.00	7.0 ± 0.00
24	3.8 ± 0.00	3.8 ± 0.01	4.1 ± 0.01
48	3.8 ± 0.00	3.9 ± 0.01	4.1 ± 0.00
72	4.0 ± 0.04	4.2 ± 0.00	4.2 ± 0.01
96	4.3 ± 0.04	4.5 ± 0.03	4.5 ± 0.01

3.5.3. Production of lactic acid at 45°C under static condition

Figure 8 shows the production of lactic acid at 45°C with initially adjusted pH-5.0, pH-6.0, and pH-7.0. Highest lactic acid from Kitchen waste was produced (24.00 g/L) at 24h at initially adjusted pH-7.0. And the lowest concentration of lactic acid (10.17 g/L) was found at 72h at initially adjusted pH-6.0.
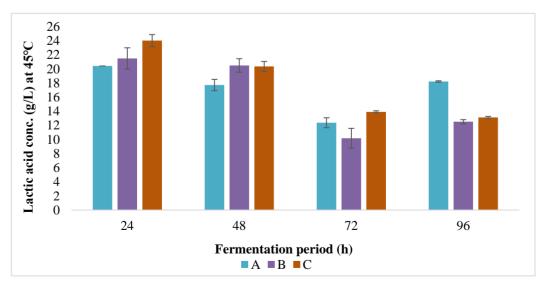

Figure 8. Lactic acid production during fermentation of kitchen waste at 45°C with initially adjusted pH-5.0, pH-6.0, pH-7.0.

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

96

As shown in Table 3, anaerobic digestion of kitchen waste carried out at 45°C resulted into decrease of pH value to 3.6-3.8 which was kept unchanged throughout the fermentation. pH was steady from 24 hour till 96 hours of anaerobic fermentation.

Table 3. pH profile of anaerobic digestion of food waste at 45°C.

Incubation period (h)	Fermentation at different initial pH		
	A	B	C
0	5.0 ± 0.00	6.0 ± 0.00	7.0 ± 0.00
24	3.6 ± 0.00	3.7 ± 0.00	3.8 ± 0.01
48	3.7 ± 0.01	3.7 ± 0.01	3.8 ± 0.01
72	3.6 ± 0.01	3.6 ± 0.01	3.7 ± 0.00
96	3.6 ± 0.01	3.6 ± 0.02	3.8 ± 0.01

Out of three temperatures, anaerobic digestion of kitchen waste with highest yield was observed at 45°C which is thermophilic condition. pH initially adjusted to 7.0 changed to 3.8 after the fermentation at 45°C and the acid produced was 24.0 g/L which is highest from all treatment.

In this study, the concentration of lactic acid was about 24 g/L. The results obtained was relatively lower compared to Hafid *et al*. (2010). They demonstrated 37g/L lactic acid from the fermentation of kitchen waste. This difference could be due to the difference in process parameters. The most important factors which affect the production of lactic acid in the anaerobic digestion process are substrate characteristics, pH, and temperature. Kitchen waste, by nature, contains a lot of active indigenous microbes where it exerts significant roles in converting large molecules into simpler molecules (Wang *et al*., 2005). Kitchen waste also contains high levels of organic compounds, nutrients, carbohydrates, fat and protein. The carbohydrate is broken down to low molecular weight sugars such as maltose, glucose, galactose and fructose which can be easily taken up by indigenous acid producing bacteria (Zhang *et al*., 2008). At 45°C, the rate of lactic acid production was high and accumulation of lactic acid resulted in the reduction of pH value in fermentation broth.

3.6. Recovery of lactic acid

Figure 9 shows the unit operations involved in recovery of lactic acid by combination of freeze-thawing, filtration and evaporation techniques. The recovery process includes physical separation that is freezing and thawing, centrifugation and filtration while the rotary evaporation step was employed to concentrate the recovered organic acids (Lactic acid) by eliminating excess water. The centrifugation and filtration process do not have any effect on the concentration of the lactic acids. Water was evaporated at 100°C and at vacuum pressure of (60-65) cm.Hg.

Table 4 shows the volume of filtered sample after freeze-thawing and remaining organic acids (Lactic acid) obtained after evaporation from three experiments. The determination of lactic acid after final evaporation was carried out as described in section 2.13.3-2.13.4. Concentration of lactic acid was generated from the standard curve of lactic acid. For this purpose, concentrated organic acid obtained after evaporation was diluted 100-fold with deionized water. 50 µL of diluted solution was added to 2 mL of 0.2% solution of iron (iii) chloride. The reaction of iron (iii) chloride with lactic acid appeared yellowish-green iron (iii) lactate in the solution (Borshchevskaya *et al*., 2016). The absorbance was measured at 390 nm. The highest lactic acid content was found 183.42g/L.

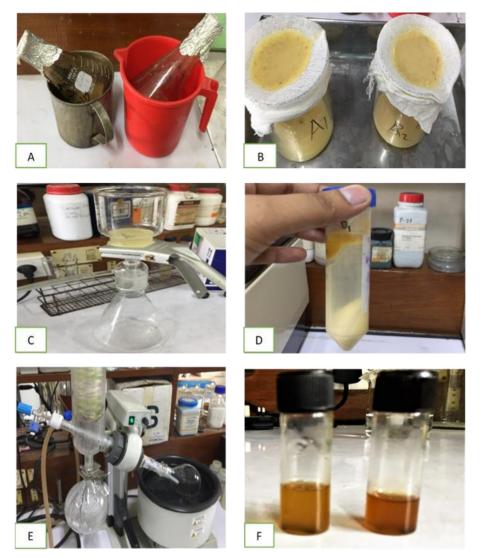Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

97

Figure 9. Photograph showing recovery of organic acid from fermented broth while thawing after freezing (A), vacuum filtration (B, C), centrifugation (D), and rotary evaporation (E) and concentrated lactic acid after evaporation (F).

Table 4. Recovery of Organic acids (Lactic acid) obtained after anaerobic digestion at 37℃ at three initial pH of 5.0, 6.0 and 7.0.

Initial pH	Volume before centrifugation (mL)	Volume after centrifugation (mL)	Volume after rotary evaporation (mL)		Lactic acid concentration (g/L)
			Liquid	Organic acid	
A (5.0)	192	96	86	6	113.79
B (6.0)	190	123	112	9	71.19
C (7.0)	192	124	114	7	183.42

Finally, after evaporation a clear brownish solution containing organic acid (Lactic acid) was successfully recovered.

4. Conclusions

In this study, kitchen waste collected from cafeteria of Atomic Energy Research Establishment (AERE), Savar, Dhaka consisted of 19.03% protein, 3.2% fat and 1.5% ash. The content of carbohydrate and fiber were not determined. The moisture content of kitchen was found to be in the range of 60-70%. The total solid (TS) and Total suspended solid (TSS) observed were 300 g/L and 83.86 g/L in kitchen waste. pH of food samples was found to be in the range of 5.07 to 6.02. Microbial load in kitchen waste was found to be 1.25×10^7 cfu/ml when grown on Nutrient agar plate at 37℃ at pH-6.0. No growth in MacConkey agar was observed in this study which indicated that the collected food waste was devoid of any pathogenic microbes. Anaerobic digestion of

Asian Australas. J. Biosci. Biotechnol. 2020, 5 (3)

98

food waste were carried out to convert wastes into lactic acid using natural microflora at three different temperature and pH. Out of three temperatures, anaerobic digestion of kitchen waste with highest yield was observed at 45°C which is thermophilic condition. pH initially adjusted to 7.0 changed to 3.8 after the fermentation at 45°C and the acid produced was 24.0 g/L which is highest from all treatment. Concentrated organic acid obtained after evaporation was diluted 100-fold with deionized water. The highest lactic acid content was found 183.42 g/L. The overall results indicated that, the volume of food waste can be greatly reduced and can be converted into value-added products such as lactic acid via anaerobic fermentation.

Conflict of interest
None to declare.

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99

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