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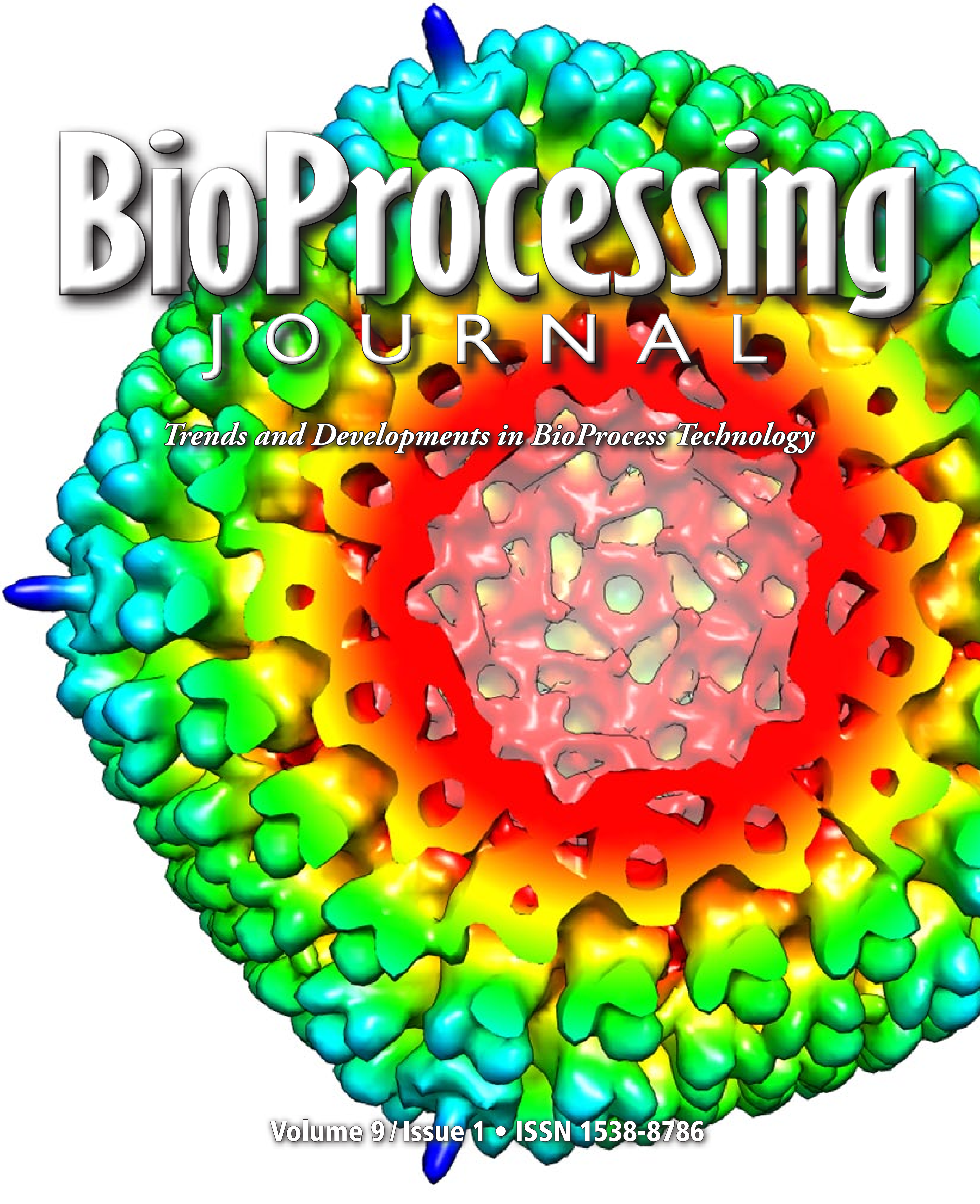

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The Effects of Temperature Variation In the Bioethanol Production Process

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Fermentation utilizes organic substrates and organic electron acceptors to produce reduced organic substances as end-products.[1] Bioethanol has a number of advantages over conventional fuels because it is produced from renewable resources. Ethanol is a high octane fuel that can replace lead as an octane enhancer in petrol by helping to oxygenate the fuel mixture so it burns more completely and reduces polluting emissions. The burning of ethanol more closely represents a natural carbon dioxide cycle since the released CO_2 is recycled back into plants where the carbon dioxide becomes the carbon source for new sugars, starches and cellulose.[2] In addition, the toxicity of exhaust emissions from bioethanol is lower than that of other petroleum sources such as coal, oil, or gasoline.[3]

Bioethanol is produced by using bacteria along with the action of yeast on substrates such as carbohydrates, starch, and cellulose. And the bacteria *Zymomonas mobilis* is currently the most widely used microorganism for ethanol production.[4] *Zymomonas mobilis* is a unique bacterium offering a number of advantages over current ethanol producing microorganisms.[5]

First generation bioethanol was made from sugars and a starch-based feedstock like corn, sugar beets, sugar cane, and barley. But second generation bioethanol is being produced from feedstock containing cellulosic biomass such as stalks, leaves, and husks from corn plants, wood chips, and sawdust. And such lignocellulosic biomass contains complex carbohydrates that need micro-organisms capable of fermenting sugars not fermentable by brewer's yeast.

The greatest success has been with genetically engineered gram-negative bacteria such as *Escherichia coli, Klebsiella oxytoca*, and *Zymomonas mobilis*.[6] Swings and Deley[7] explained that *Zymomonas mobilis* is a fermentative, gram negative, and ethanologenic bacteria that ferments only glucose, fructose, and sucrose. Previous studies were performed to measure the effect of temperature on cell growth for ethanol production by *Zymomonas mobilis*.[8]

This study evaluates bioethanol production levels when different bacteria strains are used at different temperature levels with barley, oats, maize, and sugar beets as the carbohydrate source. The bacteria used in the experiments were isolated from rotten fruit, and while they were not positively identified, they were all rod shaped, gram positive and acid-fast negative. Further work is underway to characterize the bacteria, but for the purposes of this study, they will be referred to as strains A, B, X and Y.

Materials and Methods

Ethanol production was observed from each sample using Jones reagent[9] [$K_2Cr_2O_7 + H_2SO_4$]. According to Barwick *et al.*[10] in the presence of sulfuric acid, ethanol is oxidized to acetic acid with an excess of potassium dichromate that gives off a blue-green color and indicates that the carbon source was converted to bioethanol.

To quantify the amount of bioethanol produced, we developed a standard curve with pure ethanol and a series of dilutions (0.5 mL, 1.0 mL, 1.5 mL, 2.0 mL, 2.5 mL, 3.0 mL, 3.5 mL, and 4 mL) of ethanol in distilled water to a total volume of 4 mL (Table 1). Then after oxidizing each dilution with Jones reagent, optical density (OD) was measured with spectrophotometry at 600 nm[11] and the resulting data are shown in Figure 1. Then the amount of ethanol was read from the standard curve.

TABLE 1. Standard curve of ethanol.

Serial #	Ethanol (mL)	H_2SO_4 (mL)	$K_2Cr_2O_7$ (mL)	Distilled H_2O (mL)	Optical Density (nm)
1	0.5	0.5	1.0	3.5	0.176
2	1.0	0.5	1.0	3.0	0.196
3	1.5	0.5	1.0	2.5	0.201
4	2.0	0.5	1.0	2.0	0.207
5	2.5	0.5	1.0	1.5	0.212
6	3.0	0.5	1.0	1.0	0.215
7	3.5	0.5	1.0	0.5	0.227
8	4.0	0.5	1.0	0.0	0.241

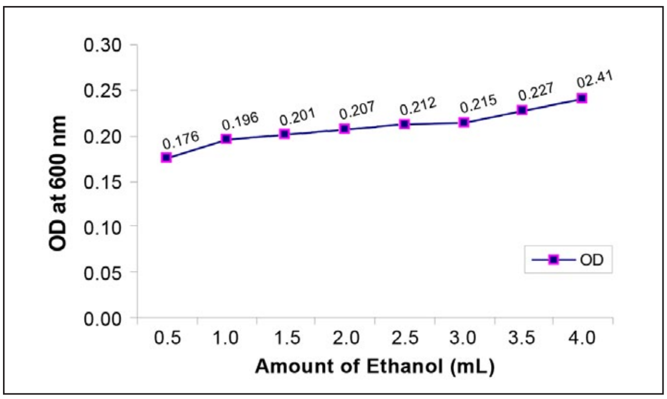

FIGURE 1. The standard curve of ethanol.

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Collection of Samples

Rotten fruit was collected and kept for 2–3 days. Then the juice was poured onto nutrient agar medium (NAM) and cultured for 24 hours at 37°C. A number of bacteria colonies were isolated and each was grown in NAM using the streak plate method. Then from the seven different strains of bacteria cultured, we tested the fermentation capability of each and found that four of the seven demonstrated fermentation activity.

Selection of Bioethanol-Producing Bacteria By Process of Fermentation

As fermentation substrates, we chose four grains (maize, barley and oats) as well as sugar beets, which we submersed in water overnight. Then we crushed each substrate with a mortar and pestle, added distilled water, and autoclaved the resulting material. Following that, we inoculated each of the sterilized samples with each of the four bacterial that demonstrated fermentation activity and incubated the samples on a shaker platform for 24 hours at 37°C. To determine the amount of ethanol produced, we added 1 mL of 2% $K_2Cr_2O_7$ and 5 mL H_2SO_4 to 3 mL of each sample.

Results and Discussion

After the initial incubation period of 24 hours at 37°C, the amount of ethanol produced by each sample was determined through OD measurement and the standard curve described previously. Different bacteria resulted in the highest ethanol production in the different substrates. For barley and maize, bacteria Y produced the most ethanol. Then for the oats, bacteria B worked the best, and for the sugar beets, bacteria A produced the most ethanol. And while all four of the bacteria were able to utilize carbon from starchy substrates, the most ethanol produced in these experiments was from barley. Then taking each substrate with the bacteria that worked the best, samples were incubated at a different temperature for an additional 24 hours.

The amount of ethanol produced in each sample was determined through OD measurement and the standard curve described previously. The temperatures used were 30°C, 40°C, 50°C, and 60°C (Table 2 and Figure 2). Ethanol production was best at 40°C, and decreased with temperatures above and below this level (Table 3).

TABLE 2. Amounts of bioethanol produced by combinations of the four selected bacteria and substrates at 37°C.

Bacteria	Barley	Oats	Maize	Sugar Beets
A	3.00 mL	1.50 mL	0.54 mL	1.14 mL
B	3.32 mL	2.02 mL	0.46 mL	0.57 mL
X	1.50 mL	0.83 mL	0.84 mL	0.47 mL
Y	3.40 mL	1.26 mL	2.08 mL	0.39 mL

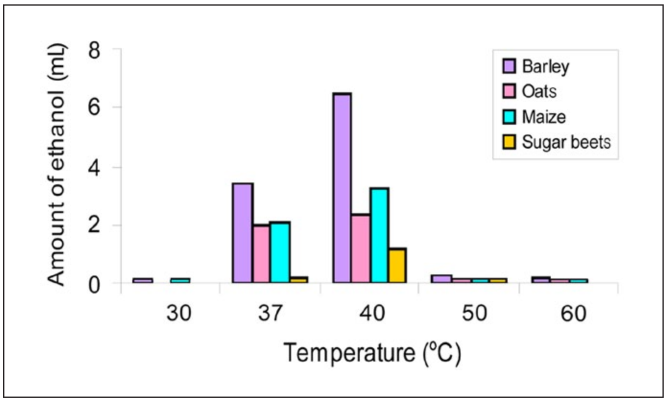

FIGURE 2. Comparing the effects of different temperature ranges on ethanol production.

TABLE 3. Amount of bioethanol produced at different temperatures.

SUBSTRATE	30°C	37°C	40°C	50°C	60°C
Barley	0.07 mL	3.40 mL	6.50 mL	0.23 mL	0.14 mL
Oats	0.02 mL	2.02 mL	2.37 mL	0.12 mL	0.10 mL
Maize	0.05 mL	2.08 mL	3.27 mL	0.09 mL	0.07 mL
Sugar Beets	0.02 mL	1.14 mL	1.18 mL	0.05 mL	0.02 mL

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