

2005 Project Abstract

For the Period Ending June 30, 2008

PROJECT TITLE: *Bio-conversion of Potato Waste Into Marketable Biopolymers*

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FUNDING SOURCE: LCCMR and CEESS-BSU

LEGAL CITATION: ML 2005, First Special Session, Chp.1, Art. 2, Sec. 11, Subd. 9(f).

Appropriation Language: \$175,000 the first year and \$175,000 the second year are from the trust fund to the commissioner of natural resources for an agreement with Bemidji State University to evaluate the bioconversion of potato waste into plant-based plastics. This appropriation is available until June 30, 2008, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

APPROPRIATION AMOUNT: LCCMR Appropriation \$350,000.00

Overall Project Outcome and Results

Potato processing plants generate large quantities of potato waste that pollutes air, water, and soil; no solution to the problem has yet been found. Biopolymers are renewable and biodegradable materials that could replace petroleum based plastics, which are the polluting, environmentally unfriendly materials. We studied production of two biopolymers (i.e. xanthan and polylactic acid) on potato waste. Xanthan has many applications in the chemical, food, oil, pharmaceutical, and other industries. Its global market was estimated at \$300-400 million and is expected to grow at an annual rate of 4 to 10%. Polylactic acid is also an attractive raw material. Its market is smaller than that for xanthan; however, polylactic acid has a large growth potential.

The objectives of the project were to study: 1) Lactic acid fermentation of potato waste and subsequent polymerization of the lactic acid into polylactic acid (PLA), 2) Xanthan (XA) fermentation of potato waste, and 3) To compare economic feasibility of PLA and XA production from the potato waste. The major results were: 1) Lactic acid average yield was 60% (i.e. kg lactic acid / kg potato waste starch), 2) Xanthan average yield was 24.90 % (i.e. kg xanthan/ kg dry potato waste), 3) Both lactic acid and xanthan fermentations can be more profitable on potato waste than on current production media, and 4) PLA polymerization from the lactic acid fermented on potato waste would be less profitable than conventional processes.

Fermentation of potato waste into xanthan or lactic acid could generate net social benefits regardless of whether these processes are commercially viable. The potential for positive externalities emanates from: 1) reducing environmental costs of potato waste disposal, and 2) alleviating the pressure on materials that are both conventional media for fermentation of xanthan and lactic acid and inputs in subsidized markets for food and fuel.

Project Results Use and Dissemination

The project has resulted in a patent application ("Solid or Semi-Solid State Fermentation of Xanthan on Potato or Potato Waste" – Patent Publication No. US-2008-0113414-A1). Additionally, information about project results has been disseminated through multiple conference presentations and posters, news stories in Minnesota media, and multiple manuscripts submitted for publication. Additional information is available in the full Work Program Final Report.

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LCCMR 2005 Work Program Final Report

Date of Report: June 30th, 2008

LCCMR Work Program Final Report

Date of Work Program Approval: June 14, 2005

Project Completion Date: June 30, 2008

I. Project Title: Bio-conversion of Potato Waste Into Marketable Biopolymers

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Location: Center for Environmental, Earth and Space Studies, Bemidji State University, Bemidji, MN 56601

Total Biennial LCMR Project Budget:	LCMR Appropriation:	\$ 350,000.00
	Minus Amount Spent:	\$ 343,315.00
	Equal balance:	\$ 6,685.00

Legal Citation: ML 2005, First Special Session, Chp.1, Art. 2__, Sec. 11__, Subd. 9(f).

Appropriation Language: 2005 Draft Appropriation Language:

9 (f) Bioconversion of Potato Waste into Marketable Biopolymers
\$175,000 the first year and \$175,000 the second year are from the trust fund to the commissioner of natural resources for an agreement with Bemidji State University to evaluate the bioconversion of potato waste into plant-based plastics. This appropriation is available until June 30, 2008, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. and III. FINAL PROJECT SUMMARY

Potato processing plant generates large quantities of potato waste (PW) that pollutes: a) waters, b) air, and c) soils; no solution to the problem has yet been found. Biopolymers are renewable and biodegradable materials that could replace petroleum based plastics, which are the polluting, environmentally unfriendly materials. We studied production of two biopolymers (i.e. xanthan and polylactic acid) on PW. Xanthan has

many applications in the chemical, food, oil, pharmaceutical and other industries; its global market was estimated at \$ 300 – 400 million and is expected to grow at an annual rate of 4 to 10%. Polylactic acid is also an attractive raw material; its market is smaller than that for xanthan; however, polylactic acid has a large growth potential.

The objectives of the project were to study in laboratory: 1) Lactic acid fermentation of PW and subsequent polymerization of the lactic acid into polylactic acid (PLA), 2) Xanthan (XA) fermentation of PW, and 3) To compare economic feasibility of PLA and XA production from the PW.

The major results are: 1) Lactic acid average yield was 60% (i.e. kg lactic acid / kg potato waste starch), 2) Xanthan average yield was 24.90 % (i.e. kg xanthan/ kg dry potato waste), 3) Both lactic acid and xanthan fermentations can be more profitable on PW than on current production media, and 4) PLA polymerization from the lactic acid fermented on PW would be less profitable than conventional processes.

Fermentation of PW into xanthan or lactic acid could generate net social benefits regardless of whether these processes are commercially viable. The potential for positive externalities emanates from: 1) reducing environmental costs of PW disposal, and 2) alleviating the pressure on materials that are both conventional media for fermentation of xanthan and lactic acid and inputs in subsidized markets for food and fuel.

IV OUTLINE OF PROJECT RESULTS:

Result 1: *Production of Polylactic acid from Potato Waste was executed and managed by Dr. Fu-Hsian Chang and his research team.*

Summary Budget Information for Result 1:	LCMR Budget	\$ 165,000.00
	Balance	\$ 186.00

Completion Date: June 30th, 2008

Fermentation Study and Laboratory Survey of Polylactic Acid - Research Tasks 1 to 4 - Result 1.

Laboratory Survey of polylactic acid was initiated upon the approval of this project by the LCCMR in August, 2005. Numerous technological studies reports on lactic acid production have been obtained from Bioresource Technology, Biotechnology and Bioengineering, Journal of Fermentation and Engineering, Polymer, Process Biochemistry, Tetrahedron, European Polymer Journal, and Polymer Engineering and Science.

Five *Lactobacillus* cultures have been purchased from American Type Culture Collection (ATCC) in Manassas, Virginia. They are *Lactobacillus casei* (formerly *Lactobacillus casei* subsp. *casei*), *Lactobacillus* species, *Lactobacillus delbrueckii* subsp. *delbrueckii*, *Lactobacillus plantarum* and *Lactobacillus pentosus*. *Lactobacillus casei* (ATCC 39392) is known to produce lactic acid; *Lactobacillus* sp. is known to produce D-lactic acid; *Lactobacillus delbrueckii* subsp. *delbrueckii* is known capable of degrading molasses and produces D(-) lactic acid from corn sugar and molasses; *Lactobacillus plantarum* is known to metabolize mevalonic acid and produce lactic acid [lactate]; and *Lactobacillus pentosus* is capable of degrading sawdust, ferments pentoses and hexoses and produces lactic acid and acetic acid. All of these *Lactobacillus* cultures are grown on ATCC #416 broth medium (*Lactobacilli* MRS Broth) and maintained on ATCC #416 agar medium (BD 288130). Fermentation study using these five *Lactobacillus* culture were conducted (Figures 1 to 4B - Result 1).

Acid hydrolysis of starch in potato waste and fermentation of starch hydrolysate to lactic acid by three *Lactobacillus* species were tested. Potato waste was collected on different dates from potato processing plants. The collected potato waste contained from 22.72 to 27 % dry matter based on its dry weight.

The estimation of reducing sugar (glucose) in starch hydrolysate was analyzed by enzymatic method (glucose kit from Sigma) and the dinitrosalicylic acid method using glucose as the standard (1). Similar glucose concentrations, 53.85±13.19 g/l and 51.81±15.68 g/l, were obtained for samples when analyzed by enzymatic and the dinitrosalicylic acid method, respectively. There were no significant differences among the glucose concentrations observed by two methods. Dinitrosalicylic acid method is a more economical method. Therefore, glucose assay was performed in other samples using dinitrosalicylic acid method in subsequent analyses.

Acid hydrolysis of starch present in potato waste and fermentation conditions of starch hydrolysate for bioconversion of potato waste to lactic acid was performed. Enzymes and acid can hydrolyze starch present in waste potato. Enzymatic hydrolysis is specific and more efficient hydrolysis method; however, it is an expensive method. Therefore, acid hydrolysis of starch was performed using hydrochloric acid (HCl). Acid hydrolysis of starch was attempted at 0.0, 0.01, 0.1, 0.3, 0.5, 0.75, 1.0, 2.0 and 4.0 N HCl. The overall hydrolysis rate was observed as 57.8 ± 32.5 percentage based on dry matter. Acid hydrolysis with 0.00, 0.1, 2, and 4 N HCl was either ineffective or inconvenient. Therefore, no further hydrolysis was done with 0.00, 0.1, 2, and 4N HCl. Acid hydrolysis by autoclaving with 0.3, 0.5, 0.75 and 1 N HCl acid resulted in starch hydrolysate of about 81.7 ± 12.8 percentage. Both reflux and autoclaving were attempted for starch hydrolysis and found that autoclaving was more convenient and an efficient method. It takes about 1 hr to complete hydrolysis by autoclaving compared to 8 to 12 hrs by refluxing method. No significant difference in reducing sugar was observed between autoclaving and reflux hydrolysis at 1.00 N HCl (Table 1 - Result 1).

Various concentrations of starch such as 12, 33, 400, 440, 476, and 507 g/L starch suspension were used and observed that the lower the starch concentration the higher the concentration of reducing sugar was observed. It was concluded that, based on dry weight, about 82 % starch was hydrolyzed by 0.3 to 1 N HCl during autoclaving for 15 min at 121 °C and 18.5 Pascal pressure. However, for practical purpose about 500 g/L of starch suspension was used and found that approximately 85.6 ± 11.6 percentage of starch was hydrolyzed by autoclaving for 20 min at 121 °C and 18.5 Pascal pressure. Based on reducing sugar (glucose) analysis, 0.75 N HCl was found to be an optimum acid concentration.

Similarly, fermentation conditions of starch hydrolysate to produce lactic acid were tried by using three different *Lactobacillus* species. These bacteria were maintained in *Lactobacillus* MRS broth media from Difco. About one milliliter (mL) of fresh cultures (overnight cultures) were inoculated to 25 mL media prepared using starch hydrolysate and other ingredients. These bacteria were grown at 30 °C in a rotary shaker at 50 rpm in micro-aerobic conditions. One mL of the culture was taken in various intervals for reducing sugar and lactic acid analysis. The cultures were centrifuged at 15000 rpm for 10 min to separate bacteria. Supernatants containing residual glucose and lactic acid were diluted to proper concentration. Reducing sugars were assayed by the dinitrosalicylic acid method using glucose as the standards. The analysis of lactic acid was carried out using colorimetric method developed by Taylor (2) and using lithium lactate as standards. Preliminary data indicates that all three species (*Lactobacillus casei*, *L. plantarum*, and *L. pentosus*) ferment starch hydrolysate (17 g/L of dextrose equivalent) to lactic acid in 24 hours with 60 % conversion efficiency (Figure 1 and Table 2 - Result 1). Not much lactic acid production was observed after 24 hours when fermentation with 17 g/L of dextrose equivalent in media started. However, it was observed after 24 hours when started with higher concentration of dextrose.

The second set of experiment were performed to see the difference of lactic acid production by fermentation using media with pure dextrose and reducing sugar from starch hydrolysate. The results showed that there was insignificant difference between the pure dextrose and starch hydrolysate in the conversion ratio of sugar to lactic acid. Similarly, there was no significant difference among three *Lactobacillus* specie in both

cases (Figure 2A and 2B - Result 1). In these experiments, the consumption of sugars and production of lactic acid did not stop after 24 hours. This means that fermentation could be continued for longer period if the experiment were to start with higher concentration of sugar in media. However, this result reveals that starch hydrolysate from potato waste can be used to grow various *Lactobacillus* species to produce lactic acid.

Similarly, it was tried to optimize the fermentation conditions using three different species in different glucose concentration to find optimum concentration of glucose. The result did not reveal any difference between three species in different time intervals (Figure 3A and 3B - Result 1). The increasing dextrose concentration did not significantly increase lactic acid production.

The pH was not controlled in above mentioned fermentation experiments that could be the cause of low bioconversion efficiency. Our next experiments would be to optimize the fermentation using buffer solution to control pH. Other *Lactobacillus* species such as *L. delbrueckii* subspecies *delbrueckii* and *L. species* did not grow well in artificial medium. These experiments proved that three *Lactobacillus* species can grow well in the starch hydrolysate from potato waste. Further optimization for fermentation is going on. So far, we have been doing fermentation to optimize the condition for batch fermentation. We have recently ordered Electrodialyzer for Lactic acid purification. Once Electrolyzer is received, continuous fermentation will be applied to get high yield and higher productivity.

Table 1 – Result 1: Acid Hydrolysis of Starch in Waste Potato.

% Glucose/DW					
Overall	Sample Batch 1 Potato Waste Autoclave Average	Sample Batch 1 Potato Waste Reflux Average	Sample Batch 2 Potato Waste Autoclave Average		
HCl acid				Average	St.Deviation
0.3 N	70.5	1.86	76.6	49.65333333	41.50246418
0.5	78.4	90.3	85	84.56666667	5.961822987
0.75	107.2	43.6	85.6	78.8	32.34068645
1	58.9	60.9	91.1	70.3	18.04106427
Ave	78.75	49.165	84.575	70.83	
StDev	20.58810336	36.96183031	5.983519031		27.57080604
% Glucose/DW					
Overall	Sample Batch 1 Potato Waste Autoclave Average	Sample Batch 1 Potato Waste Autoclave Average			
HCl acid			Average	Standard Deviation	
0.3 N	70.5	76.6	73.55	4.313351365	
0.5	78.4	85	81.7	4.666904756	
0.75	107.2	85.6	96.4	15.27350647	
1	58.9	91.1	75	22.76883835	
Ave	78.75	84.575	81.6625		
StDev	20.58810336	5.983519031		12.75340505	

Table 2 – Result 1: Consumption of glucose and production of lactic acid by *Lactobacillus* species at initial 17 g/L of glucose concentration from potato waste.

Time (hrs)	Glucose Analysis (g/L)				Lactic acid Analysis (g/L)			
	Control	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. pentosus</i>	Control	<i>L. casei</i>	<i>L. plantarum</i>	<i>L. pentosus</i>
0	17.44	16.83	16.26	16.83	2.25	1.87	1.91	1.71
8	16.39	14.42	8.18	10.74	3.27	7.46	9.84	11.92
24	16.45	0.72	0.85	0.85	3.3	18.6	22.94	18.307
32	16.05	0.95	0.95	0.93	3.87	19.46	21.89	22.59
48	16.93	1.76	1.76	1.79	2.15	13.35	14.98	14.02
72	13.72	0.88	1.235	0.93	2.7	17.43	20.9	21.43

Table 3A Result 1: Glucose consumption: Glucose analysis (g/mL) to compare between media containing pure dextrose (D) and starch hydrolysate (SH) for control and three *Lactobacillus* species.

Time (hrs)	Glucose							
	Control D	<i>L. casei</i> D	<i>L. plantarum</i> D	<i>L. pentosus</i> D	Control SH	<i>L. casei</i> SH	<i>L. plantarum</i> SH	<i>L. pentosus</i> SH
0	56.25	56.25	56.25	56.25	89.76	89.76	89.76	89.76
24	55.07	24.22	18.63	25.81	79.81	50.29	33.61	48.6
72	55.55	16.46	10.18	13.81	81	34.62	30.01	34.93

Table 3B – Result 1: Lactic Acid Production: Lactic acid analysis (g/mL) to compare between media containing pure dextrose (D) and starch hydrolysate (SH) for control and three *Lactobacillus* species.

Time (hrs)	Lactic Acid							
	Control D	<i>L. casei</i> D	<i>L. plantarum</i> D	<i>L. pentosus</i> D	Control SH	<i>L. casei</i> SH	<i>L. plantarum</i> SH	<i>L. pentosus</i> SH
0	3.95	3.77	4.57	4.39	9.49	8.63	7.93	8.54
24	1.12	14.42	22.05	19.41	7.09	17.38	26.62	23.42
72	1.28	26.69	29.9	35.02	7.14	29.99	34.02	37.45

Table 4 – Result 1: Glucose consumption and lactic acid production at different glucose concentration by three Lactobacillus species.

A: Starting with 35 g/L

Time (hrs)	Glucose Analysis (35)				Lactic acid Analysis			
	Control	L. casei	L. plantarum	L. pentosus	Control	L. casei	L. plantarum	L. pentosus
0	34.92	35.8	36.24	36.24	4.3	4.7	4.02	
24	28.14	4.1	1.2	4.8	7.1	21.42	37.86	27.05
48	34.2	6.3	5.1	6.4	4.85	29.3	19.9	28.1
72	28.48	6.8	7.3	7.4	7.4	30.6	28.1	21.2
96	36.7	6.13	6.2	6.4	4.65	37.85	35.75	34.1

B: Starting with 70 g/L

Time (hrs)	Glucose analysis (70)				Lactic Acid Analysis			
	Control	L. Casei	L. plantarum	L. pentosus	Control	L. casei	L. plantarum	L. pentosus
0	78.53	76.39	82.01	72.59	6.7	8.7	9.1	
24	73.3	52.3	37.7	46.9	9	20.85	31.05	24.4
48	71.92	39.2	31.11	41.09	8.85	27.15	29.6	32.82
72	71.57	42.6	32.85	39.45	11.8	31.7	26.6	31.2
96	72.47	40.1	38.6	43.7	7.75	32.7	40.9	36.01

C: Starting with 105 g/L

Time (hrs)	Glucose analysis				Lactic acid analysis			
	Control	L. casei	L. plantarum	L. pentosus	Control	L. casei	L. plantarum	L. pentosus
0	125.93	135.11	125.93	125.93	10.4	9.7		
24	109.4	100.3	87.6	96.6	13.8	16.3	24.96	17.15
48	102.68	80.3	75.06	79.64	11.7	25.8	26.65	33.6
72	109.44	82.9	77.6	84.26	14.8	24.3	31	24.7
96	110.5	83.96	84.2	87.73	13.85	39.2	34.65	41.35

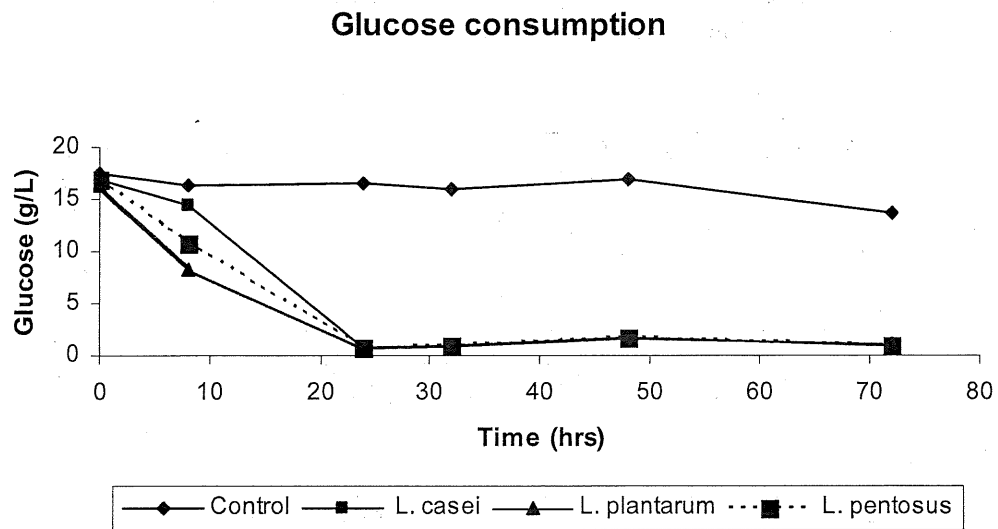


Figure 1A – Result 1. Consumption of glucose (starch hydrolysate) by *Lactobacillus* starting at 17 g/L.

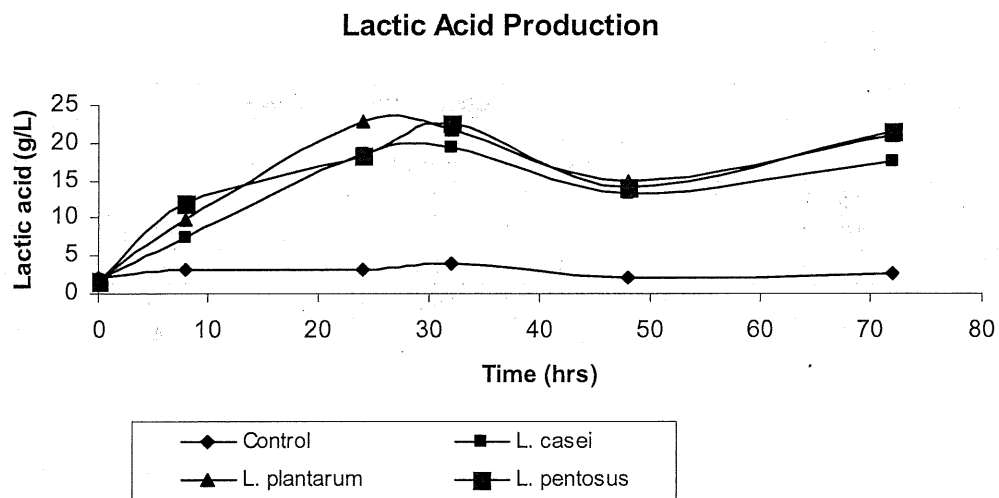


Figure 1B – Result 1. Production of lactic acid from starch hydrolysate by *Lactobacillus* starting at 17 g/L of dextrose equivalent.

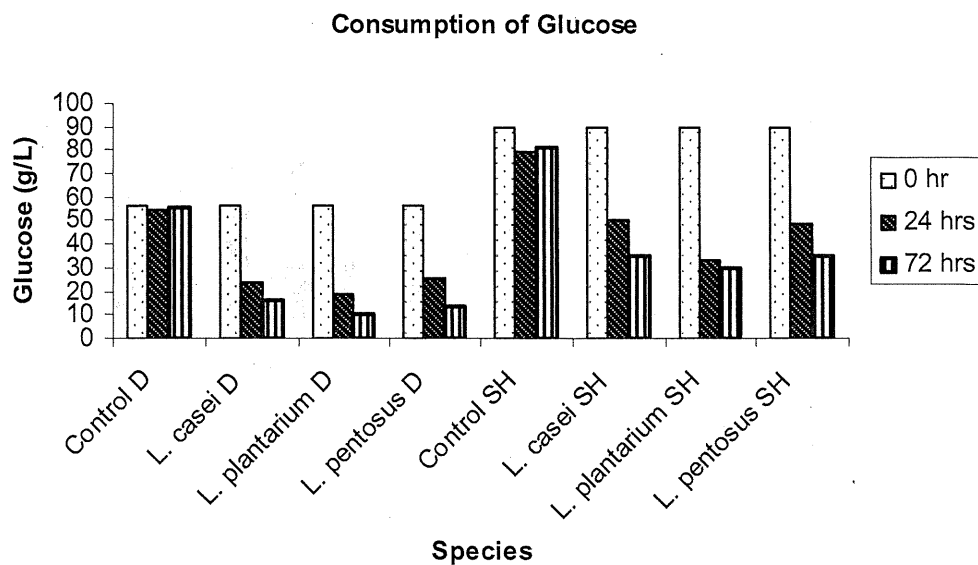


Figure 2A – Result 1: Consumption of glucose (pure dextrose, D and starch hydrolysate, SH) by various *Lactobacillus* species with media containing pure dextrose (D) and starch hydrolysate (SH).

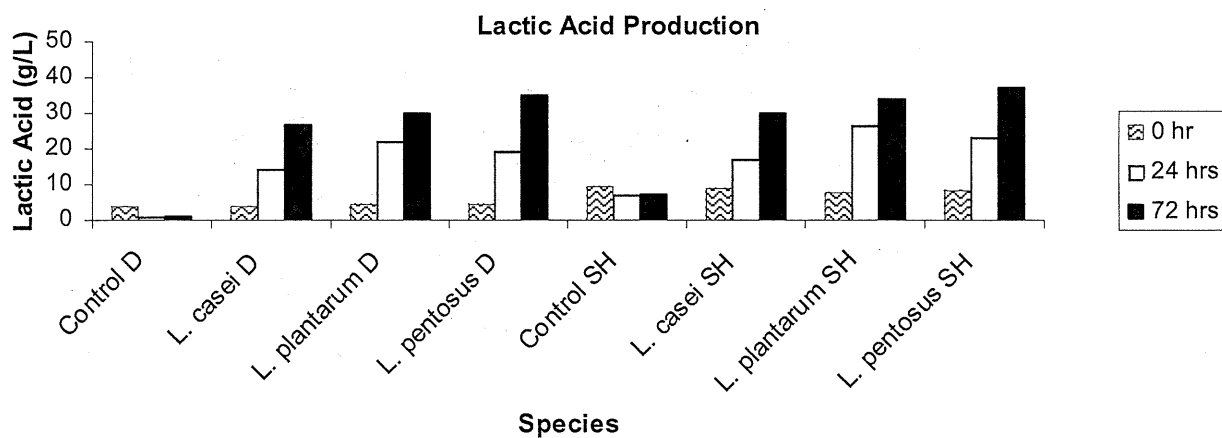


Figure 2B – Result 1: Production of lactic acid by various *Lactobacillus* species with different media containing pure dextrose (D) and starch hydrolysate (SH).

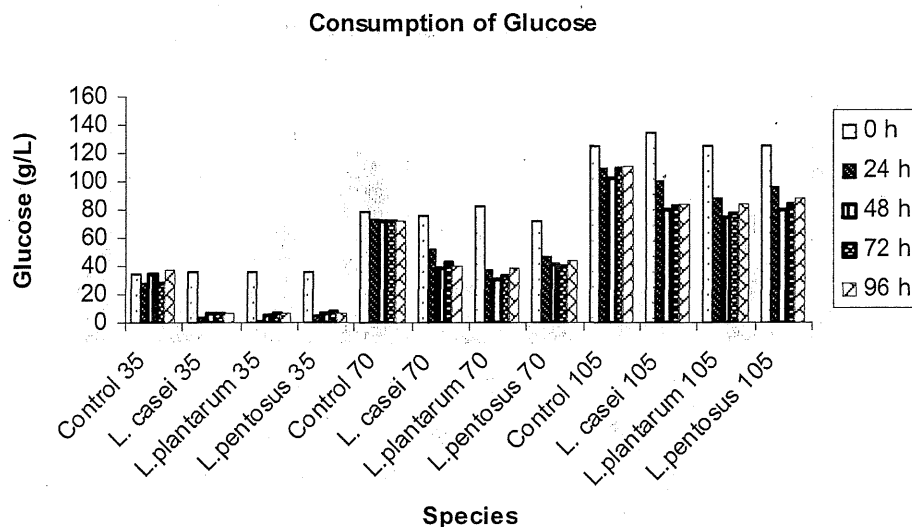


Figure 3A – Result 1: Consumption of glucose (starch hydrolysate) by various *Lactobacillus* species at different sugar level in media.

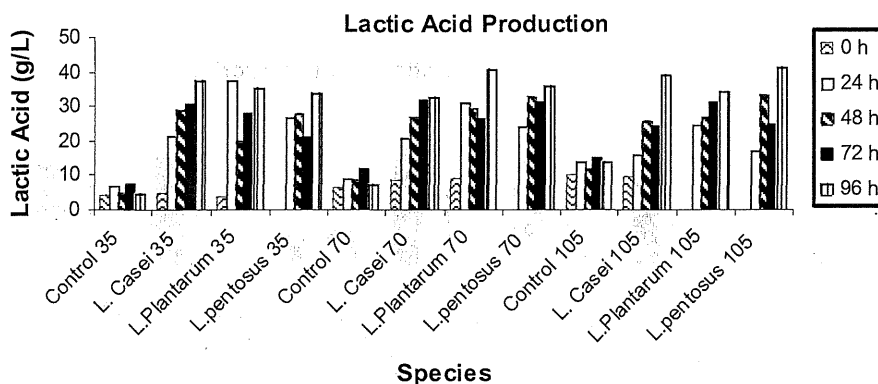


Figure 3B – Result 1: Production of lactic acid from starch hydrolysate by various *Lactobacillus* species at different sugar level in media.

Direct fermentation of starch (5%) into lactic acid was tried by using *L. plantarum* (Tables 6A and 6B and Figures 5A and 5B - Result 1) and *L. amylovorus*, respectively. *L. plantarum* did not ferment starch into lactic acid efficiently. Therefore, *L. amylovorus* was used for direct fermentation of starch for further experiments. After several experiments for optimizing fermentation, *Lactobacillus amylovorus* was found to yield the most lactic acid out of the other species of bacteria namely: *L. casei*, *L. plantarum*, *L. pentosus* and *L. amylovorus*. The starch present in waste potato was converted to lactic

acid by direct fermentation of starch using *Lactobacillus amylovorous*. About 90 % of starch present in potato waste was converted to lactic acid. About 56 g/L of lactic acid was obtained at 37 °C by direct fermentation of 6 % of starch by *Lactobacillus amylovorous*.

Potato waste was collected on different dates from potato processing plants. The collected potato waste contained about 22.72 to 27 % dry matter based on its dry weight. The bacteria, *L. casei*, *L. plantarum*, *L. pentosus* and *L. amylovorous*, were maintained in *Lactobacillus* MRS Broth media from Difco. About one milliliter (mL) of fresh cultures (overnight cultures) were inoculated to 25 mL media prepared using starch hydrolysate/starch and other ingredients. These bacteria were grown at 30 °C or 37 °C in a rotary shaker at 100 rpm in micro-aerobic conditions. One mL of the culture was taken in various intervals for reducing sugar and lactic acid analysis. The cultures were centrifuged at 15000 rpm for 10 min to separate bacteria. Supernatants containing residual glucose and lactic acid were diluted to proper concentration. Reducing sugar was assayed by the dinitrosalicylic acid method using glucose as the standards (1). The analysis of lactic acid was carried out using colorimetric method developed by Taylor (2) and using lithium lactate as standards.

Lactic acid analysis indicates that all three species (*Lactobacillus casei*, *L. plantarum*, and *L. pentosus*) ferment starch hydrolysate (31 g/L and 23 g/L of dextrose equivalent) to lactic acid in 120 hours with 50 % conversion efficiency when neutralized with potassium hydroxide and sodium hydroxides, respectively (data not shown). There was no significant difference when starch hydrolysates were neutralized either by sodium hydroxide or potassium hydroxides after acid hydrolysis of starch present in potato waste. About 50 % of glucose was converted to lactic acid.

The second set of experiments were performed to see the effects of pH on lactic acid production by fermentation of starch hydrolysate. The pH was controlled by adding either sodium hydroxide or phosphate buffer (mixture of KH_2PO_4 and K_2HPO_4). Surprisingly, the results showed that there was insignificant difference between the fermentation of starch hydrolysate into lactic acid with pH control and without pH control. When lactic acid was analyzed at 96 hrs, the average conversion of starch hydrolysates to lactic acid were 28.3 and 28.0 % with pH control at or above 6.5 by the addition of sodium hydroxide and without pH control, respectively. Similarly, when lactic acid was analyzed at 96 hrs, the average conversion of starch hydrolysates to lactic acid were 27 and 29 % with pH control at or above 6.5 by the addition of phosphate buffer and without pH control, respectively (Tables 5A and 5B and Figure 4A and 4B - Result1).

We tried direct fermentation of starch by *L. amylovorous* at 30 °C and 37°C and did not find significant difference in lactic acid production. Surprisingly, there was insignificant difference in lactic acid production with pH control by adding phosphate buffer and without pH control (Table 7 and Figure 6 - Result 1). Various concentrations of starch such as 3, 4, 5 and 6 % were tried for direct fermentation without pH control and it was concluded that the optimum concentration of starch was 6 % starch (Table 8 and Figure 7 - Result 1) for lactic acid production. The experiments proved that lactic acid could be produced from potato waste as the cheap starting material by above-mentioned bacteria. Pure lactic acid is the starting material for producing biodegradable plastics.

Table 5A - Result 1: Lactic acid analysis (g/L) of fermentation of starch hydrolysates by *L. casei*, *L. plantarum* and *L. pentosus* with or without pH control by sodium hydroxide (NaOH)s

Time (hrs)	Control	<i>L. casei</i>	<i>L. casei</i> (pH)	<i>L. plantarum</i>	<i>L. plantarum</i> (pH)	<i>L. pentosus</i>	<i>L. pentosus</i> (pH)
0	17.39	12.69	8.74	15.64	14.83	11.61	12.06
24	0.41	-4.21	5.43	6.12	8.1	8.39	1.75
48	8.29	21.85	23.96	30.89	24.65	21.78	22.83
96	4.13	33.8	28.13	38.85	24.32	27.67	26.63

Table 5B - Result 1: Lactic acid analysis (g/L) of fermentation of starch hydrolysates by *L. casei*, *L. plantarum* and *L. pentosus* with or without pH control (phosphate buffer)

Time (hrs)	Control	<i>L. casei</i>	<i>L. casei</i> (pH)	<i>L. plantarum</i>	<i>L. plantarum</i> (pH)	<i>L. pentosus</i>	<i>L. pentosus</i> (pH)
0	7.8	3.73	1.81	2.04	5.63	11.07	6.6
24	14.75	13.1	6.6	21.4	18.9	17.14	14.5
48	0.41	11.4	8.9	28.2	25.2	14.8	27.6
96	12.2	38.4	48.2	48.9	50.5	52.5	24.3

Table 6A - Result 1: Glucose analysis (g/L) of fermentation of starch and starch hydrolysates by *L. plantarum*

Time (hrs)	0.00	24.00	120.00
Contol-Starch	1.33	5.57	8.34
Contol-Starch hydrolysate	87.25	67.75	60.99
<i>L. plantarum</i> (Starch)	1.33	5.57	8.71
<i>L. plantarum</i> (Starch hydrolysate)	87.25	26.97	20.50

Table 6B - Result 1: Lactic acid analysis (g/L) of fermentation of starch and starch hydrolysates by *L. plantarum*

Time (hrs)	0.00	24.00	120.00
Contol-Starch	4.29	6.19	8.78
Contol-Starch Hydrolysate	4.75	7.57	7.59
<i>L. plantarum</i> (Starch)	4.29	5.81	12.28
<i>L. plantarum</i> (Starch hydrolysate)	4.75	25.78	30.98

Table 7 - Result 1: Lactic acid analysis (g/L) of fermentation of starch (4 %) by *L. amylovorous* (with and without pH control by phosphate buffer)

Time (hrs)	Control	<i>L. amylovorous</i>	pH	<i>L. amylovorous</i> -pH
0	6.09	2.21	4.52	10.4
24	0.23	9.26	5.13	9.2
48	2.2	23.24	0.12	29.39
96	5.07	29.92	7.58	29.4
120	7.61	38.34	5.22	34.63
144	7.83	41.91	5.65	38.23
168	5.21	45.22	4.07	37.85

Table 8 - Result 1: Lactic acid analysis (g/L) of fermentation of starch by *L. amylovorous*

Time (hrs)	Starch (3%)	Starch (4%)	Starch (5%)	Starch (6%)
0	0.96	5.9	10.4	13.9
24	35.29	14.78	32.93	38.49
48	25.49	27.01	36.5	42.16
96	22.52	17.97	20.43	26.12
120	38.23	39.57	43.73	56.14

The objective of the Result 1 was to convert potato waste into biodegradable plastics. There are various processes involved in getting plastics from potato waste, such as conversion of starch present in potato waste into glucose or reducing sugar by enzymes or by acid hydrolysis, bioconversion (fermentation) of sugar by *Lactobacillus* bacteria into lactic acid, purification of lactic acid, and polymerization of lactic acid into biodegradable plastics.

Direct fermentation of starch (5%) into lactic acid was tried by using *L. plantarum* and *L. amylovorous*, respectively. *L. plantarum* did not ferment starch into lactic acid at all. Therefore, *L. amylovorous* was used for direct fermentation of starch for further experiments. The starch present in waste potato was converted to lactic acid by direct fermentation of starch using *Lactobacillus amylovorous*. About 90 % of starch present in potato waste was converted to lactic acid. About 56 g/L of lactic acid was obtained at 37 °C by direct fermentation of 6 % of starch by *Lactobacillus amylovorous*. We also tried direct fermentation of starch by *L. amylovorous* at 30 °C and 37°C and did not found significant difference in lactic acid production. Various concentrations of starch such as 3, 4, 5 and 6, 10 and 12 % were tried for direct fermentation and it was concluded that the optimum concentration of starch was 6 % starch. Surprisingly, there was not significant difference in lactic acid production with pH control and without pH control.

We have optimized fermentation condition for batch fermentation to produce lactic acid. We tried direct fermentation of starch present in potato waste by *L. amylovorous*. The fermentation was carried out at 30°C and 6% starch was used. We attempted to use 12 % starch but did not yield better results. The fermentation optimizations were performed to reduce the cost of lactic acid production. The bacteria in fermented broth

were recycled and our experiments showed that there was no significant difference in lactic acid production between fresh bacteria (maintained in MRS media) or recycled bacteria (Table 9 and Figure 8 - Result 1). Similarly, we attempted to use less amount of ingredients such as beef extract (25%) in potato waste fermentation and we obtained comparable results. We tried to reuse the ingredients from previous experiments to save ingredients costs. Lactic acid analysis confirmed that the ingredients can be recycled to produce lactic acid without significant loss of lactic acid production (Table 10 and Figure 9). Fermentation is almost completed in 48 hrs. Potato waste is very economical way of producing lactic acid for synthesizing biodegradable poly (lactic acid).

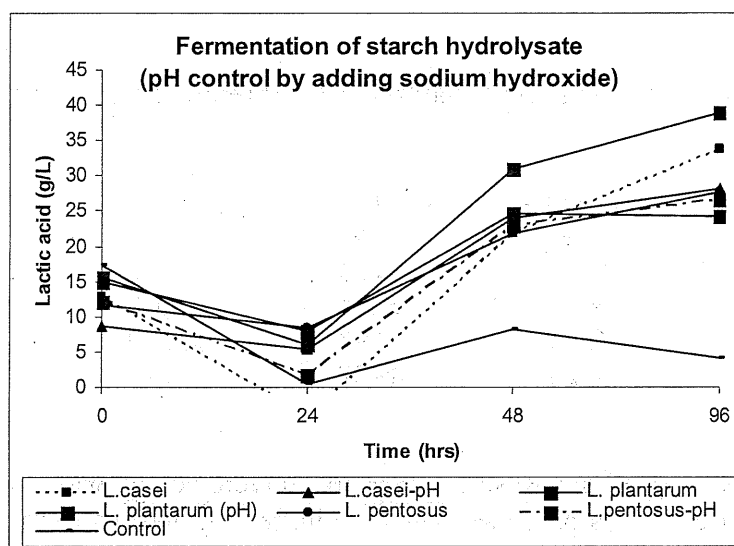


Figure 4A - Result 1: Lactic acid analysis (g/L) of fermentation of starch hydrolysates by *L. casei*, *L. plantarum* and *L. pentosus* with or without pH control by addinsg sodium hydroxide

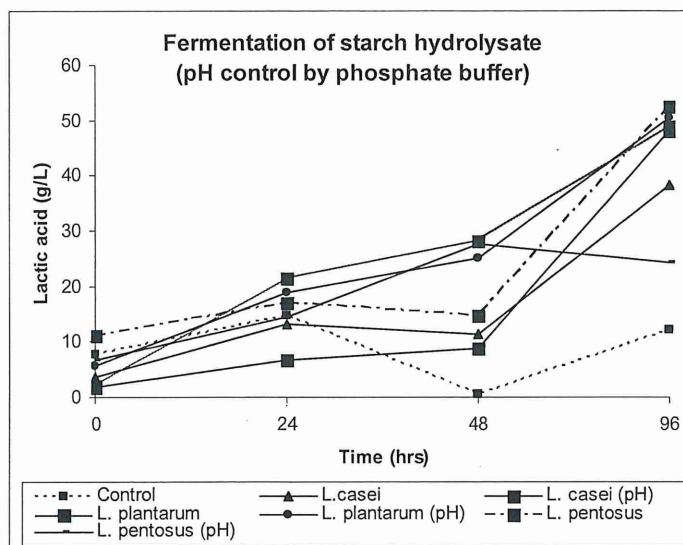


Figure 4B - Result 1: Lactic acid analysis (g/L) of fermentation of starch hydrolysates by *L. casei*, *L. plantarum* and *L. pentosus* with or without pH control by adding phosphate buffer

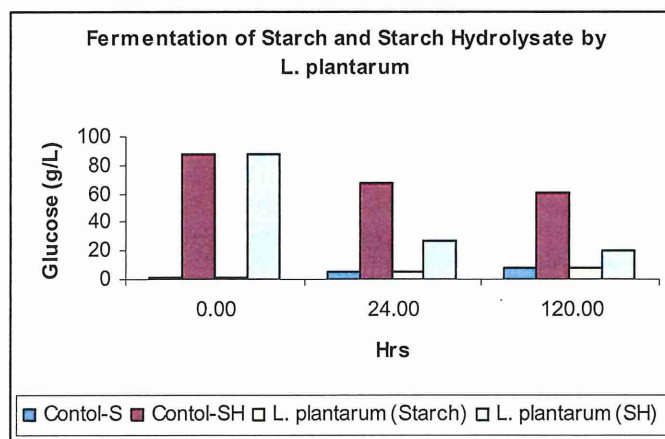


Figure 5A - Result 1: Glucose analysis (g/L) of fermentation of starch and starch hydrolysates by *L. plantarum*

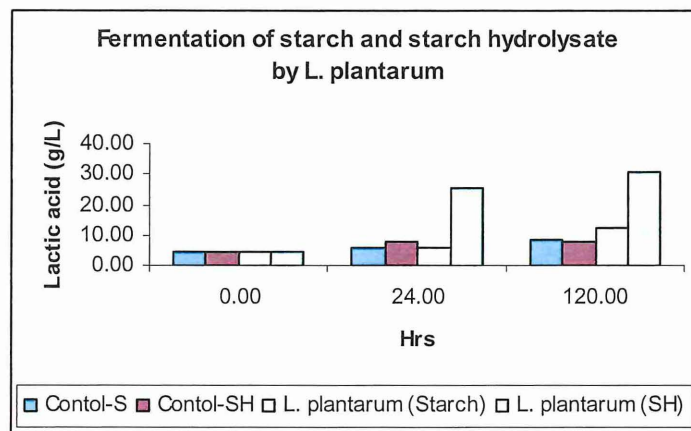


Figure 5B - Result 1: Lactic acid analysis (g/L) of fermentation of starch and starch hydrolysates by *L. plantarum*

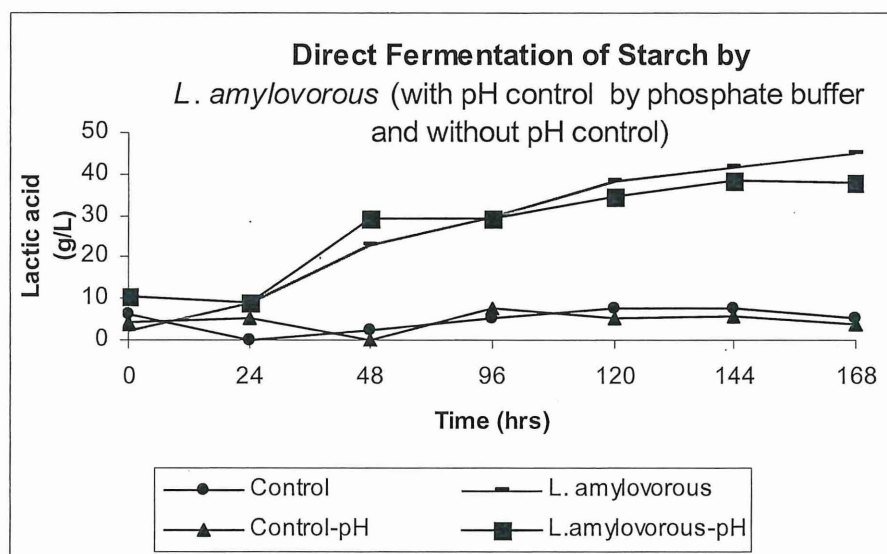


Figure 6 - Result 1: Lactic acid analysis (g/L) of fermentation of starch (4 %) by *L. amylovorous* (with and without pH control by adding phosphate buffer)

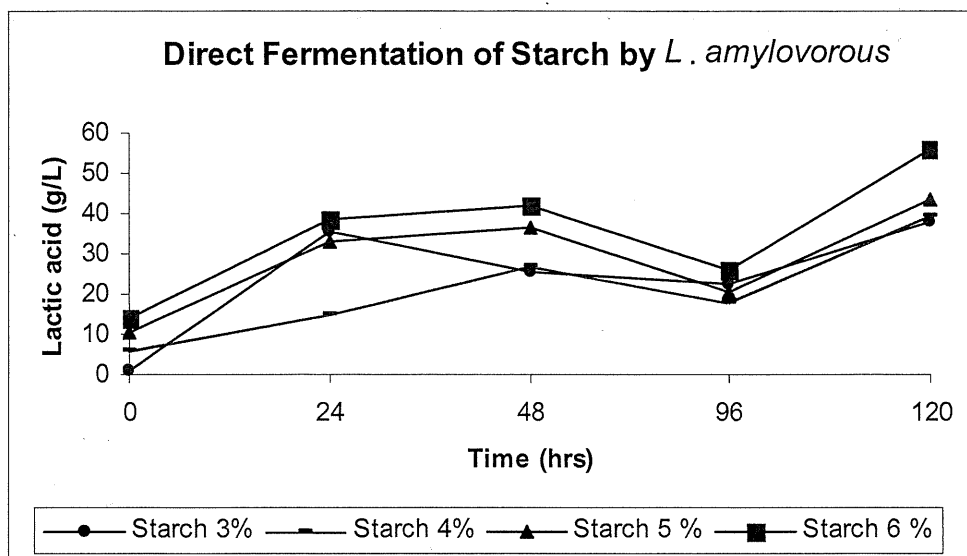


Figure 7 - Result 1: Lactic acid analysis (g/L) of Direct Fermentation of starch by *L. amylovorous*

Table 9 - Result 1: Lactic acid analysis (g/L) produced by *Lactobacillus amylovorous* either maintained in MRS media or recycled from potato waste

Lactic acid analysis (g/L) MRS and rejuvenated
bacteria (RAM)

Time (h)	MRS	RAM
0	1.61	0
24	37.79	45.54
48	37.24	47
72	39.13	35.1

Table 10 - Result 1: Lactic acid analysis produced by *Lactobacillus amylovorus* either fresh media or recycled media with or with out additional ingredients:

Lactic acid Analysis (g/L) (48 h) of recycled Nutrients		
Bacteria	Average	StDev
AM	41.68667	13.11904
FBAM	45.17	9.427041
FBAM	40.20333	3.266991
2FB	46.72667	12.59359

AM: Bacteria from 67 % strength media of previous experiments;

FBAM: Bacteria from media with recycled nutrients (FB) into media with 67% strength ingredients;

FB: Bacteria from 67% strength media into new media with recycled nutrients (FB);

2FB: Bacteria from previous fermentation broth with recycled nutrients (FB) into new media with recycled nutrients and 25% strength (2.5 g/L) fresh ingredients (FB).

Figure 8 - Result 1: Lactic acid analysis produced by *Lactobacillus amylovorus* either maintained in MRS media or recycled from potato waste

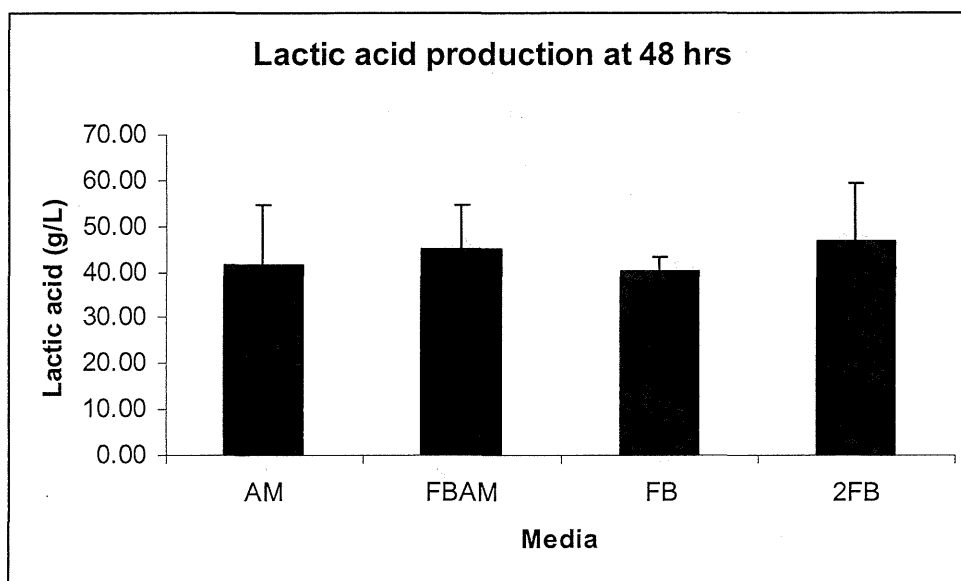


Figure 9 - Result 1: Lactic acid analysis at 48 hrs produced by *Lactobacillus amylovorus* maintained in MRS media or recycled bacteria in potato waste with regular media or recycled media

AM: Bacteria from 67 % strength media of previous experiments;

FBAM: Bacteria from media with recycled nutrients (FB) into media with 67% strength ingredients;

FB: Bacteria from 67% strength media into new media with recycled nutrients (FB);

2FB: Bacteria from previous fermentation broth with recycled nutrients (FB) into new media with recycled nutrients and 25% strength (2.5 g/L) fresh ingredients (FB)

Lactic Acid Purification and Polymerization - Research Tasks 5 and 6 - Result 1

We have recovered, purified and concentrated lactic acid in the form of sodium lactate for standard lactic acid and lactic acid produced by direct fermentation of starch in potato waste by electrodialysis. Electrodialysis (ED) is an electromembrane process in which ions are transported through ion permeable membrane from one solution to another under the influence of a voltage between two end electrodes (3). The diluates (dilute solution, ~ 13 g/L) and concentrates (higher concentration) were prepared either from lactic acid or fermentation broth by neutralizing with sodium hydroxide to pH 5.5. In conventional electrodialysis, lactate in the concentrate is transferred through anion exchange membranes. The standard sodium lactate solution was spiked with 6 g/L glucose and subjected to electrodialysis at 21 V for 30 min. The samples were collected every five minutes for thirty minutes.

Lactic acid analysis showed that the lactic acid concentration increased in diluate but decreased in concentrates from 0 min to 30 min. There was no glucose in diluate (**Table 11** and **Figure 10**). The lactic acid concentration from fermentation broth was about 36 g/L and were neutralized to pH 5.5 by adding sodium hydroxide solution. The lactic acid in fermentation broth was transferred from concentrate to diluate and recovered up to 40 g/L by electrodialysis (**Table 12** and **Figure 11**). After optimizing fermentation conditions and electrodialysis conditions, continuous fermentation will be applied to produce maximum lactic acid. Therefore, the next improvement will be the continuous fermentation using electrodialysis unit to get maximum lactic acid production.

Table 11 - Result 1: Glucose and Lactic acid analysis (g/L) of electro dialysis of standard lactic acid solution spiked with 6 (g/L) glucose

Glucose Analysis (g/L)			Lactic Acid Analysis (g/L)		
Samples	Average	StDev	Samples	Average	StDev
D0	0.654201	0.04076	D0	14.03	2.96
D5	0.644648	0.027251	D5	26.81	1.55
D10	0.654201	0.04076	D10	33.38	3.02
D15	0.654201	0.04076	D15	40.99	2.18
D25	0.663156	0.028095	D25	39.74	3.79
D30	0.6441	0.001146	D30	44.46	2.66
C0	5.963589	0.44973	C0	25.29	4.09
C5	5.360828	0.834739	C5	22.11	3.04
C10	5.998769	0.04557	C10	11.36	5.43
C15	6.124071	0.222774	C15	4.39	4.65
C25	5.402035	0.237257	C25	0.34	2.05
C30	6.298784	0.099346	C30	-0.51	2.22

D= Diluate, C= Concentrate, 0...30 Time (minutes)

Table 12 - Result 1: Lactic acid analysis (g/L) of Electro dialysis of Fermentation broth

Time (min)	Diluate	Concentrate
0	18.267233	37.2051983
5	27.14944	28.7762438
10	36.03127	21.8060931
15	38.055307	12.4735473
20	40.760145	10.5627688
30	41.70862	3.0753247

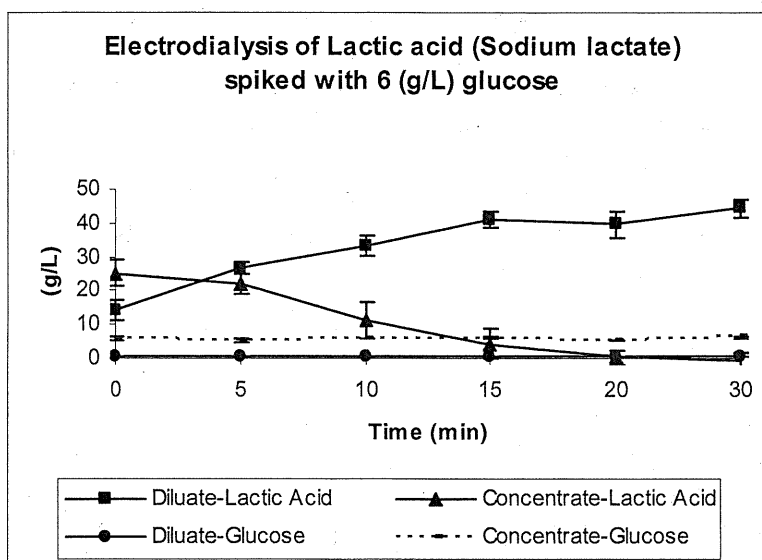


Figure 10 - Result 1: Glucose and Lactic acid analysis (g/L) of electro dialysis of standard lactic acid solution spiked with 6 (g/L) glucose

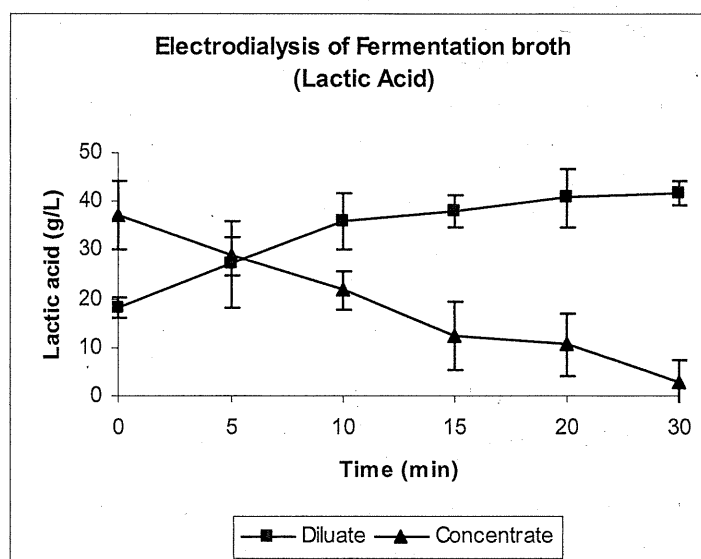


Figure 11 - Result 1: Lactic acid analysis (g/L) of Electro dialysis of fermentation broth

Electrodialyzer has been used for lactic acid purification. We purified and concentrated lactic acid in the form of sodium lactate for standard lactic acid and lactic acid produced by direct fermentation of starch in potato waste by conventional electro dialysis. Lactic acid (in the form of sodium lactate) produced by the direct fermentation was concentrated by conventional electro dialysis. The lactate solutions

obtained from fermentation were passed through activated carbon to reduce color of the broth. Then the solution was passed through chelating resin (Purolite S940) to remove multi-valent cations. Removal of cations is essential before electrodialysis to lengthen the lifetime of cartridge used in electrodialysis. The diluant was prepared from lactic acid and neutralized with sodium hydroxide at pH 5.5. The lactic acid from fermentations were diluted to 26 g/l and concentrated to 40 g/L by conventional electrodialysis. We have added the fresh fermentation broth to the running of conventional electrodialysis to achieve higher sodium lactate concentration. Sodium lactate obtained from conventional electrodialysis was converted to lactic acid and sodium hydroxide by bipolar electrodialysis at 18 V. Different voltages were tried and bipolar electrodialysis at 18 V was found to be optimum condition. The concentration of lactic acid obtained by this method was about 0.723 M (65 g/L). The lactic acid solution was concentrated to 95 to 100 % for polymerization process in a rotavapor under vacuum. Sodium hydroxide obtained in this way can be used to control the pH of fermentation and/or for other uses. The percentage recovery of sodium lactate and lactic acid was about 90 % and 87 % for conventional and bipolar, respectively. The experiments proved that lactic acid could be produced economically from potato waste and purified for preparing biodegradable poly(lactic acid) from potato waste as the cheap starting material by above mentioned bacteria and purification steps. Lactic acid is the starting material for producing biodegradable plastics.

We conducted polymerization reactions in two steps. The first step is to prepare low molecular weight pre-polymer by condensation of lactic acid and second step is chain linking reaction of pre-polymer with chain extenders such as 1,6- hexamethylene diisocyanate(HMDI) and 2,2'-bis(2-oxazoline) (BOX) to get high molecular weight polymer. The pre-polymers were condensation polymerized in 250 mL round-bottom three-necked flasks with water-cooling condenser. The reaction temperature was controlled by a knob in a oil-bath. The appropriate capillary inlet for nitrogen and vacuum were fitted in side tubes. We tried polymerization condensation under various reaction conditions such as in presence of nitrogen, under vacuum, without catalyst and with catalyst [tin(II) octoate]. Polymerization reaction at 200 °C under vacuum in presence of nitrogen for 24 hours was optimum condition. In this condition, pre-polymers were obtained with low acid value and higher molecular weight. Low acid value is essential for chain extension polymerization. We used 1,4- butane diol with lactic acid monomer to reduce acid value. Based on molecular weight, pre-polymers prepared from lactic acid from potato waste are similar to pre-polymers from commercial lactic acid. We were able to prepare pre-polymer with molecular weights 2000 and 2600 g/mol from lactic acid produced potato waste without using any catalyst and with catalyst, respectively. The detailed information about monomers, catalyst, reagents and reaction conditions are given in **Table 14**. The pre-polymers with such molecular weight prepared without catalyst can be used in medical applications

The linking polymerization of the pre-polymers were done in three-necked round bottom flasks fitted with stirrer to mix BOX and HMDI as chain extenders. The polymerizations were carried out at mixing speed of 60 rpm and 150 °C for defined time.

Generally, for linking reaction, 25 g of the dried pre-polymer powder was charged into the flask. BOX and HMDI were added to the flask based on acid value and hydroxyl value from titration when temperature reached about 150 °C. The molecular

weight of polymer was increased to 12000 when HMDI was reacted with pre-polymer in the ratio of 1.2:1 of isocyanate /hydroxyl (NCO/OH). Surprisingly, molecular weight of polymer was obtained only 5000 when HMDI was added to the reaction flask and reacted for 90 min after BOX was reacted with pre-polymers for 20 minutes. The chain linking polymerization of pre-polymer from potato waste with BOX and HMDI yield very desired products (**Figure 13**). The molecular weight was increased from 2500 to 12000 in 30 min and started to decrease from 30 to 60 min. We need longer period and better resources to produce commercial plastics from potato waste

The polymers were characterized by molecular weight. The NMR in our university is down. Therefore, molecular weight of polymer was determined by an alternative method, viscosimetry. We have done cost-benefit analysis of producing poly(lactic acid). The costs to produce poly(lactic acid) is \$145.49/kg which is based on laboratory scale which include operational cost (chemicals, power and water) excluding labor and start-up costs (glassware, instrumentation and their installments). The details of the analysis are given in Result 3 of the project.

Table 13 - Result 1: Electrodialysis (Bipolar) of fermentation broth

Time (min)	Current (A)	Conductivity (Sample)	Conductivity (Base)	Lactic acid (M)	Sodium Hydroxide (M)
0	0	38.6	0	0.01	0.02
10	1.6	33.7	0	0.01	0.1
20	1.73	26.7	28.5	0.12	0.2
30	1.72	19.7	62.8	0.26	0.28
40	1.55	11.1	89.4	0.42	0.38
50	1.37	6.7	104.5	0.58	0.44
55	1.46	7.3	107.6	0.68	0.5
60	1.72	8.2	109.9	0.723	0.48

Table 14 - Result 1: Polymerization at various conditions

POLYMERIZATION	
Polymers	Lactic acid Source
Poly-1**	D, L (Evaporation, 4h)
Poly-2**	D, L -Lactic acid
Poly-3**	Lactic acid solution (134 g/125mL, 449 g/L Lactic acid) from EDBP (Distillation 3.5 hrs)
Poly-4**	Lactic acid solution (109.3 g/100 mL) from EDBP/ (447g/L Lactic acid Distillation of 15 hr)
Poly-51**	L-Lactic acid/1,4 butanediol (4%mole)/Tin octanoate (0.01% mole)
Poly-52**	L-Lactic acid/Butanediol/tin octanoate (continuation of Poly-51)
Poly-6**	L-Lactic acid
Poly-7**	Lactic acid solution -Potato waste Broth
Poly-8**	Lactic acid solution (46.5 g LA)(112.8 g/104 mL) from EDBP (447g/L Lactic acid Distillation of 15 hr)
Poly-9*	L-Lactic acid (Distillation at 100 to140 ⁰ C under Vacuum for one hr)/Tin octanoate (0.01% mole)
Poly-10*	L-Lactic acid (Distillation at 100 to140 ⁰ C under Vacuum for one hr) (Synthesis of Lactide)
Poly-11**	L-lactic acid (Distillation at 100 ⁰ C at 15"Hg for 4 h)/1,4 butanediol (4% mole)/Tin octanoate(0.01%mole)
Poly-12**	L-lactic acid/Adipic acid (0.1 % Mole) /Tin octanoate (0.1% Mole)
Poly-13**	Lactic acid solution (53.27g/140mL)/ EDBP/potato waste)
Poly-14**	Lactic acid (60 g/180 mL) solution from EDBP/potato waste
Poly-15**	L-lactic acid / Butanediol (4%mole)/Tin octanoate (0.01)
Poly-16	L-lactic acid/1,4 butanediol (2%mole)/Tin octanoate (0.01% mole)
Poly-17	L-lactic acid/1,4 butanediol (2%mole)/Tin octanoate (0.01% mole)
Poly-18	L-lactic acid/1,4 butanediol (4%mole)/Tin octanoate (0.01% mole)
Poly-19	Lactic acid solution from EDBP/potato waste)/1,4 butanediol (4%mole)/ Tin octanoate (0.1% mole)
Poly-20	Pre-polymer-18/HMDI
Poly-21	Pre-polymer-18/BOX/HMDI
Poly-22	Pre-polymer-19/BOX/HMDI (Simultaneous addition of BOX/HMDI)
	* No Nitrogen
	** No Vacuum

Continued on the next page

Table 14 - Result 1 (Continued)

Polymers	Time (h)	Heating Temp (°C)	Post-heating at 140 °C (h)	Yield (%)	Acid Value	Hydroxyl value	Mol Wt (Vis)
Poly-1**	6	150 to 200	3	49	246.5	nd	2078
Poly-2**	6.5	151 to 200	3.5	47	179.6	nd	nd
Poly-3**	7.5	125 to 200	3	20	275.4	nd	1059
Poly-4**	9	125 to 200	4	22	192	nd	458
Poly-51**	8.5	125 to 200	4	80	267	nd	418
Poly-52**	3	125 to 200	4		225	nd	915
Poly-6**	8.5	125 to 225	4	55	158.5	nd	1312.3
Poly-7**	8.5	125 to 200	NO PRODUCT	nd	nd	nd	nd
Poly-8**	8.5	126 to 200	4 hr at 18" Hg	37	120	nd	1415
Poly-9*	8	150 (Vacuumn)	4		672	nd	1319
Poly-10*	14	100 to 200 (18" Hg)	1 hr at 18" Hg		220	nd	939
Poly-11**	8.5	125 to 200	3 hr (10 min at 18" Hg)	77	314	nd	657
Poly-12**	8.5	125 to 200	4 hr N2 and 1 hr at 29" Hg	79	457	nd	639
Poly-13**	13	125 to 225	3 hr at 29" Hg	72	291	nd	1197
Poly-14**	13	125 to 225	3 hr at 29" Hg	72	188	nd	1325
Poly-15**	10	125 to 225	3 hr at 29" Hg	70	264	nd	1161
Poly-16	9	125 to 200/15 to 29" Hg 125 to 200°C/15 to 29"			31	nd	2111
Poly-17	18	Hg 125 to 200°C/15 to 29"			23	nd	3895
Poly-18	24	Hg			8	91.33	1740
Poly-19	20	125 to 205	4 hr at 29" Hg	28	42	50	1325
Poly-20	Half 30 min/80	150	-	-	nd		27624
Poly-21	min	150	-		nd		6651
Poly-22	90 min	150			nd		12801

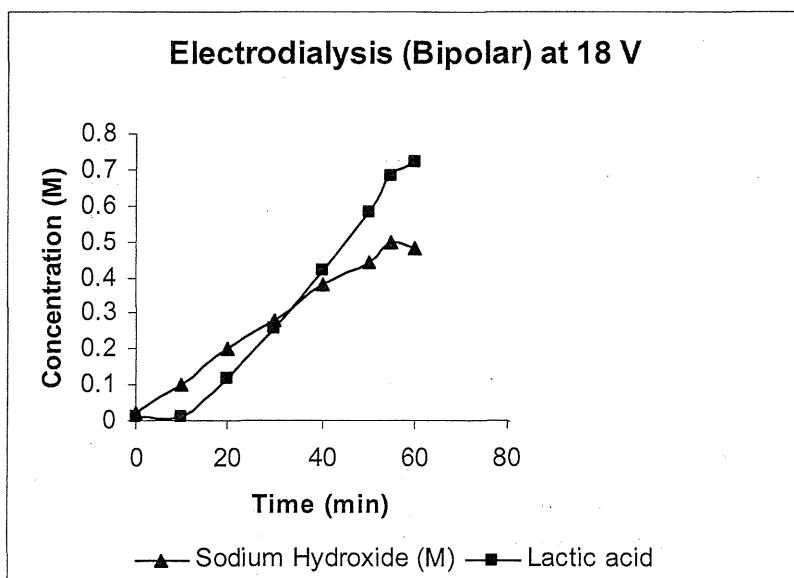


Figure 12 - Result 1: Sodium hydroxide and Lactic acid analysis (M) of Electrodesialysis-Bipolar of Fermentation Broth

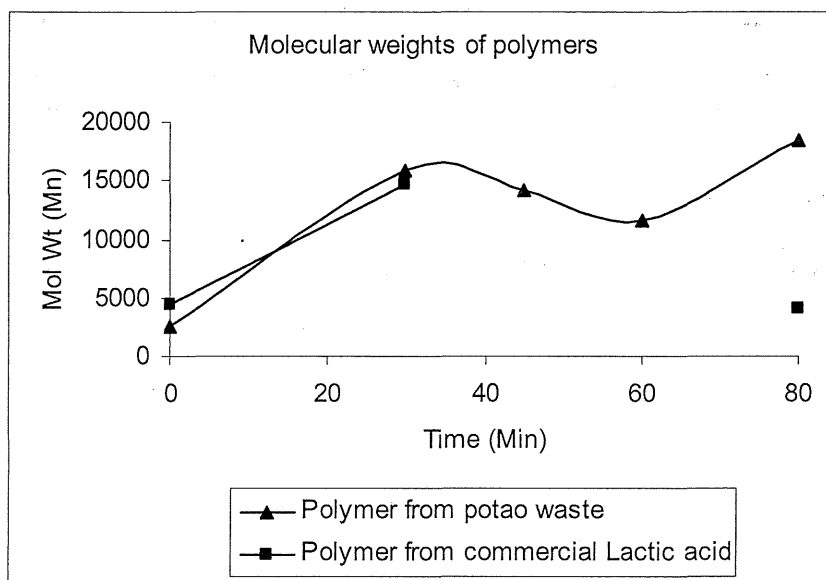
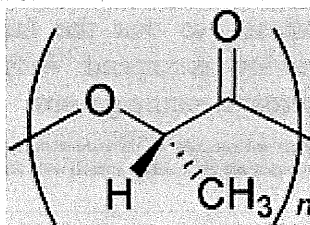


Figure 13 - Result 1: Molecular weights of polymers prepared from potato waste and commercial lactic acid

Synthesis of Polylactic Acid Using Chain Extending Process - Result 1

Poly lactic acid (PLA) is a polymer with a future. Best known for its ability to biodegrade, the polymer can be processed to replace less environmentally friendly hydrocarbon-based polymers, such as polystyrene or polyethylene. Since it does biodegrade, and can be processed to have such a wide variety of properties. Nowadays, Biodegradable polymers are more and more widely used in many industries such as packaging, paper coating, fibers, films, and other disposable articles, as well as in biomedical applications, Poly lactic acid (PLA) is one of the most important and the most popularly used biodegradable polymers in the world, so the study on its producing process become an important research in this field (Stephen J Duffy, 2000).

Lactic acid is the simplest hydroxy acid with an asymmetric carbon atom and it exists in two optically active configurations.



The L-isomer is an intermediate of carbohydrate metabolism in humans and other mammals whereas both the D- and L-enantiomers are produced in bacterial systems. Pure L- and D-lactic acid can be obtained by fermentation or culture techniques.

The hydroxyl and a carboxyl group in lactic acid enables it to be converted directly into polyester via a polycondensation reaction. However, the conventional condensation polymerization of lactic acid does not increase the molecular weight sufficiently unless organic solvents are used for azeotropic distillation of condensation water and the polymerization time is very long. Conventional polycondensation of lactic acid yields a brittle glassy polymer, which is unusable for most applications. (Jukka Tuomenie, 2002).

In this research paper, a modified method is used, an alternative way to achieve high molecular weight polyesters is to treat condensation polymers with chain extenders.

Methodology

This polymerization method consist of two main steps: Polycondensation to prepare prepolymer and chain extending process. The intermediate lactide, a cyclic lactic acid dimer, is formed in the first step when the condensation product water is removed by evaporation during oligomerization. L-Lactic acid, D-lactic acid or mixtures thereof can be polymerized to corresponding low-molecular-weight poly(lactic acid) oligomer. Many catalyst systems have been evaluated for the polymerization of lactide. Tin compounds, especially tin(II) bis-2-ethylhexanoic acid (tin octoate), are preferred for the bulk polymerization of lactide due to their solubility in molten lactide, high catalytic activity, and low rate of racemization of the polymer. The lactic acid prepolymer can be obtained by condensation polymerization in a rotation evaporator. L-Lactic acid (88% in water.) was purified by distillation under vacuum. Sn octoate (0.01 mol%, used as received.) was used as a catalyst. The flask was purged with nitrogen and placed in an oil bath. The

reaction mixture was polymerized at 200 centigrade for 24 h, with a continuous nitrogen stream fed under the surface of the melt at a reduced pressure of 20 minibar.

The linking polymerization of the prepolymer was carried out in a melt mixer with use of HMDI and BOX as chain extenders. The polymerizations were carried out at 150 centigrade at a mixing speed of 60 rpm and for a predetermined time. generally speaking, 50g of the dried prepolymer powder was charged into the preheated melt mixer chamber. After 1 min the prepolymer was completely molten, and then HMDI was added. Simultaneously, BOX was added. However, different OH/HMDI and COOH/BOX ratios were used to control the dosage of HMDI and BOX. The hydroxyl number and acid value is used to complete that. (Jukka Tuominen, Janne Kylma, 2003)

Results and discussion: Two different prepolymers for linking reaction study were prepared by polycondensation of L-lactic acid. The typical diisocyanate linking reaction for formation of polyurethane requires prepolymer with only hydroxyl ending groups. I used 1,4-butanediol in the synthesis, so that the lactic acid chains were hydroxyl terminated and the Acid value was low as required. Without butanediol we obtained PLA chains with both carboxyl and hydroxyl ending groups.

Fig. 1. Development of the weight average molecular weight and molecular weight distribution (dashed line) for prepolymer E2% (open symbols) with OH/NCO ratios of 1:1 (□), 1:1.1 (○) and 1:1.2 (△), and for prepolymer E0% (solid symbols) with OH/NCO ratios of 1:1.1 (•) and 1:2 (◆).

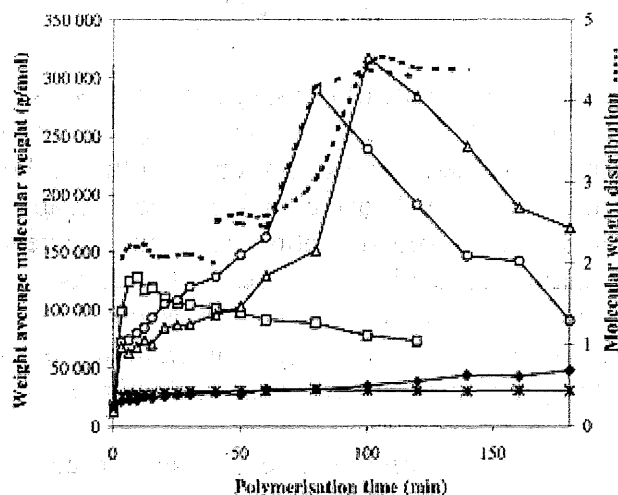


Figure 14 - Result 1

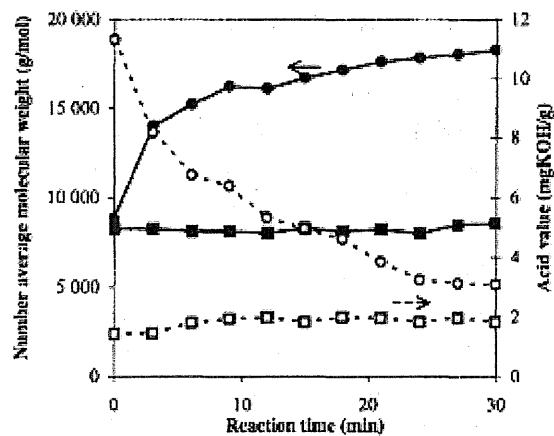


Fig. 2. Effect of 2,2'-bis(2-oxazoline) (BOX) in COOH/BOX ratio 1:1.1 on molecular weight (—) and acid (---) value of E0% (○) and E2% (□) prepolymers.

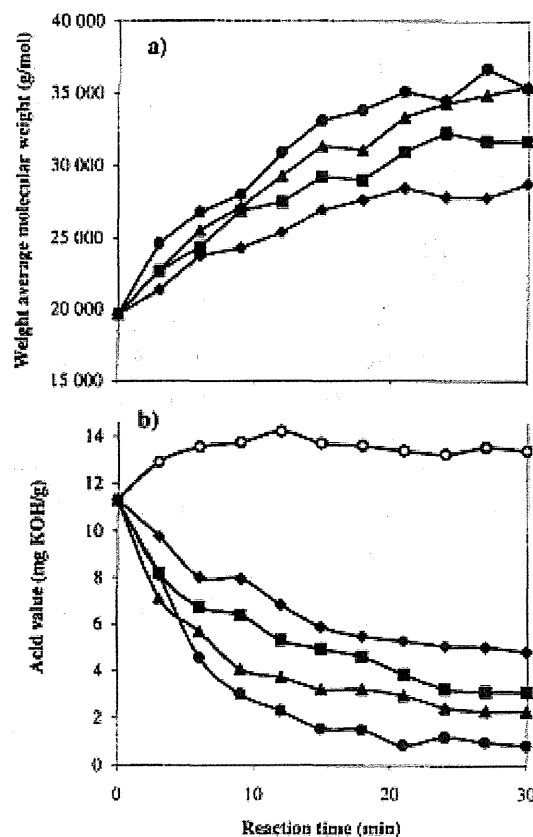


Fig. 3. Effect of COOH/BOX ratio on (a) weight average molecular weight and (b) acid value of prepolymer E0% plain polymer without BOX (○), COOH/BOX ratio of 1:1 (◆), 1:1.1 (■), 1:1.4 (▲) and 1:2 (●).

Figures 15 and 16 - Result 1

Biodegradation of Polylactic Acid - Research Task 8 - Result 1

Recently, the production amounts of biodegradable plastics sold in the market are gradually increasing. Especially, polylactic acid (PLA) has received the most attention because its raw material, L-lactic acid can be efficiently produced by fermentation from renewable resources such as starchy materials and sugars. Moreover, it has good properties such as high melting point (175°C), high degree of transparency, and ease of fabrication. PLA can be synthesized either by condensation polymerization of lactic acid or by ring opening polymerization of lactide. Currently, PLA is primarily used for medical applications such as drug delivery devices, absorbable sutures, and as a material for medical implants and other related applications. So producing PLA is not only due to the prevention of environment pollution, but also due to the human health for a society. The degradation of PLA has been studied several years ago, but understanding on this subject is still inadequate. This paper provides a short overview on the biodegradability and biodegradation of PLA.

In general, polymer degradation takes place through the scission of the main chains or side chains of polymers. Different degradation mechanisms whether chemical or biological can be involved in the degradation of biodegradable polyesters. A combination of these mechanisms can also happen at some stage of degradation.

Types of Degradation of PLA:

1. Microbial Degradation of PLA

Polymers are degraded in the soil by the action of a wide variety of microorganisms. But research shows that PLA-degrading microorganisms are not widely distributed in the natural environment and thus, PLA is less susceptible to microbial attack in the natural environment than other microbial and synthetic aliphatic polyesters. The biodegradability of PLA depends on the environment to which it is exposed. Soil burial tests show that the degradation of PLA in soil is slow and that it takes a long time for degradation to start. No degradation was observed on PLA sheets after 6 weeks in soil. In the soil, PLA can be degraded in a composting environment where it is hydrolyzed into smaller molecules after 45-60 days at 50-60°C. These smaller molecules are then degraded into CO₂ and H₂O by microorganisms in the compost (Figure 1).

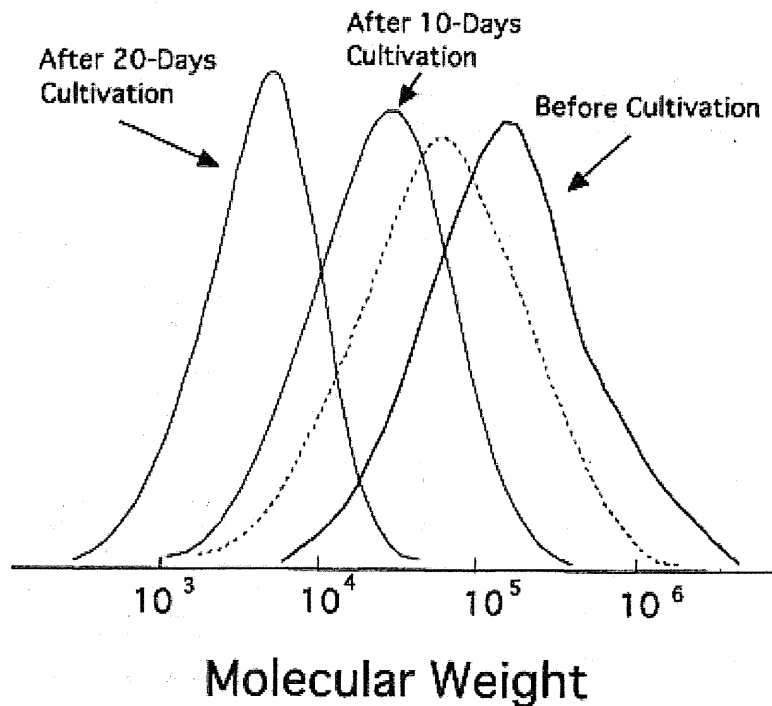


Figure 17 - Result 1. GPC curve of PLA before and after 10- and 20-days cultivation of strain 41 at 60°C. Control after 20-days cultivation without the strain is also shown (dotted line)

In spite, under normal condition, the degradation of PLA is very slowly, but recently scientist find some special microbes can degrade PLA very quickly. For example, an *actinomycete Amycolatopsis* strain which isolated from the soil can degrade 60% of the 100 mg film after 14 days. The table 1 shows us some microbes which can degrade PLA very quickly. Some research also report that several microorganisms exhibited a high level of L-PLA degradation activity when natural substrates such as silk fibroin and gelatin were added in the liquid culture medium. For instance, the L-PLA degrading activity of *Amycolatopsis strain HT-41* was increased significantly upon addition of silk fibroin as an inducer into the culture medium. Generally, the degradation process of PLA takes place inside the cells; hence, the inducers should have lower molecular weight so that they could easily penetrate into the cell. These inducers stimulate the production of enzymes capable of degrading L-PLA.

Table 15 - Result 1. PLA-degrading microorganism, their enzyme and substrate specificities and detection methods used for degradation.

Strain	Type of enzyme	Substrate specificity	Detection method for PLA degradation	Reference
<i>Amycolatopsis</i> sp. strain HT 32	Protease	L-PLA	Film-weight loss; monomer production (lactic acid)	Pranamuda et al. (1997)
<i>Amycolatopsis</i> sp. strain 3118	Protease	L-PLA	Film-weight loss; monomer production (lactic acid)	Ikura and Kudo (1999)
<i>Amycolatopsis</i> sp. strain KT-s-9	Protease	Silk fibroin, L-PLA	Clear-zone method	Tokiwa et al. (1999)
<i>Amycolatopsis</i> sp. strain 41	Protease	L-PLA, silk powder, casein, Suc-(Ala) ₃ -pNA	Film-weight loss; monomer production (lactic acid)	Pranamuda et al. (2001)
<i>Amycolatopsis</i> sp. strain K104-1	Protease	L-PLA, casein, fibrin	Turbidity method	Nakamura et al. (2001)
<i>Lentzea waywayandensis</i> (formerly <i>Saccharothrix waywayandensis</i>)	Protease	L-PLA	Film-weight loss; monomer production (lactic acid)	Jararat et al. (2003a)
<i>Kibdelosporangium aridum</i>	Protease	L-PLA	Film-weight loss; monomer production (lactic acid)	Jararat et al. (2003b)
<i>Tritirachium album</i> ATCC 22563	Protease	L-PLA, silk fibroin, elastin	Film-weight loss; monomer production (lactic acid)	Jararat and Tokiwa (2001)
<i>Brevibacillus</i> (formerly <i>Bacillus brevis</i>)*	Protease	L-PLA	Change in molecular weight and viscosity	Tomita et al. (1999)
<i>Bacillus stearothermophilus</i> *	Protease	D-PLA	Change in molecular weight and viscosity	Tomita et al. (2003)
<i>Geobacillus thermocatenulatus</i> *	Protease	L-PLA	Change in molecular weight and viscosity	Tomita et al. (2004)
<i>Bacillus subtilis</i> strain PL 21*	Lipase (Esterase)	L-PLA, pNP-fatty acid esters	Change in molecular weight	Sakai et al. (2001)
<i>Paenibacillus amylolyticus</i> strain TB-13	Lipase	DL-PLA, PBS, PBSA, PES, PCL, triolein, tributyrin	Turbidity method	Shigeno et al. (2003)
<i>Cryptococcus</i> sp. strain S-2	Lipase (Curtinase)	L-PLA, PBS, PCL, PHB	Turbidity method	Masaki et al. (2005)

*Thermophilic PLA-degrading microorganisms

2. Enzymatic Degradation of PLA

Enzymes play a significant role in the degradation of polymers, although they are not solely responsible for the hydrolysis of polymers. The enzymatic degradation of aliphatic polyesters by hydrolysis is a two-step process. The first step is adsorption of the enzyme on the surface of the substrate through surface-binding domain and the second step is hydrolysis of the ester bond. The major types of enzyme which can degrade the PLA are: (1) Proteases, such as trypsin, elastase, subtilisin, and proteinase K. (2) Lipase. Researchers often get enzyme which can degrade the PLA from purification. Pranamuda et al. (2001) purified a L-PLA depolymerase from *Amycolatopsis* sp. The purified enzyme has a molecular weight of 43 kDa. The optimum pH and temperature for L-PLA depolymerase activity were 6.0 and 37-45°C. Another PLA-degrading enzyme which was able to degrade high molecular weight L-PLA was also purified from *Amycolatopsis*. The maximum activity of the enzyme was observed at pH 9.5 and 55-65°C. So if using the enzymatic method to degrade the PLA, pH and Temp is the major factor which can significantly influence the degrade rate.

Factors Influence the Process of Degradation

There are several important factors that affect the biodegradability of polymers. These are: (1) factors associated with the **first-order structure** such as chemical structure, molecular weight and molecular weight distribution; High molecular weight polyesters are degraded at a slower rate than those with low molecular weights; (2) factors associated with the **higher order structure** such as melting temperature, crystallinity, crystal structure and modulus of elasticity; the higher melting point the lower the degradability tends to be; (3) factors related to **surface conditions** such as surface area, hydrophilic, and hydrophobic properties.

1. The Influence of Particle Size on the Degradation of PLA

In order to stimulate the process of degradation of PLA, we often grind the PLA into powder. Different size powder will have different degradation rate. Here is an experiment: after grinding, the PLA power was separated using sieves of 30 mesh (500 μm), 60 mesh (250 μm) and 120 mesh (125 μm). Under 58°C, 60 days, use MODA to investigate the degradation rate. The result shows us that the difference in the biodegradation speed for the PLA powders with different size distributions was observed. In the case of PLA with a size distribution between 0 and 125 μm , the biodegradation speed was two times faster than that between 125 and 250 μm . Over 100% degradation was observed in this sample. This may be due to the more active respiration of the microorganisms in the compost by the well metabolized carbon source. Degradation from the PLA to a metabolite by microorganisms occurs on the surface of the particles. Smaller particles will be degraded faster by the microorganisms in compost. However, PLA particles with a size over specific level will be degraded at the same speed. In this way, fine PLA powders under 100 μm were activated for the biodegradation of PLA. (Figure 18 - Result 1)

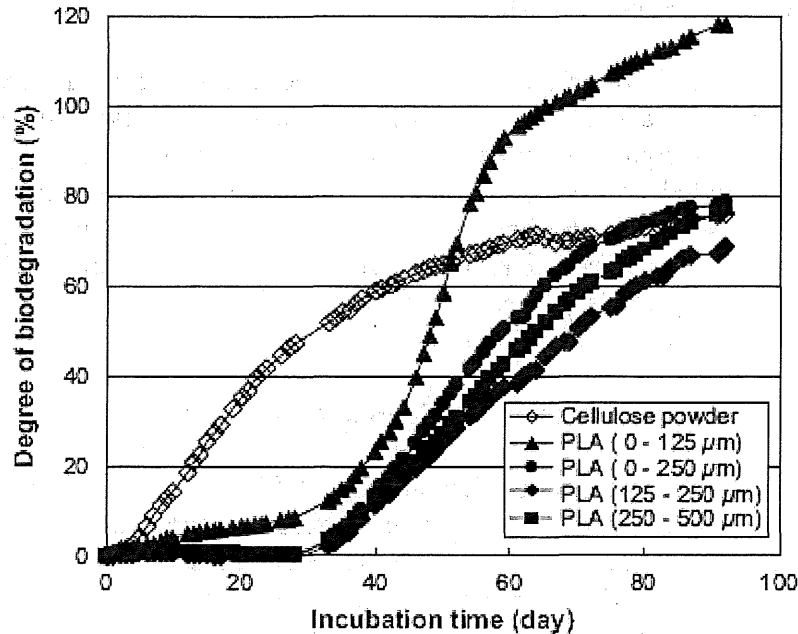


Figure 18- Result 1 Biodegradation test of PLA with different size in controlled compost at 58' C

2. The influence of Incubation Time, Temperature and Inducers on Degradation of PLA

Figure 19 - Result 1 shows effect of incubation time and temp on the biodegradation of PLA. The samples were measured the biodegrade rate in the controlled compost at 58°C and 70°C after no preincubation, normal preincubation with polycaprolactone (PCL). No PLA biodegradation was observed in the case without preincubation. Using the normal preincubation step without PCL described in Section 2. PLA was degraded to 80% in 50 days after the induction periods at 58°C. In the cases at 70°C and using the preincubation step with PCL, the PLA was degraded faster with shorter induction periods. As we mention former, the inducers will significantly stimulate the process of biodegradation, PCL has the same action. The biodegradation of PLA was promoted by a higher reaction temperature and using an activation step with PCL.

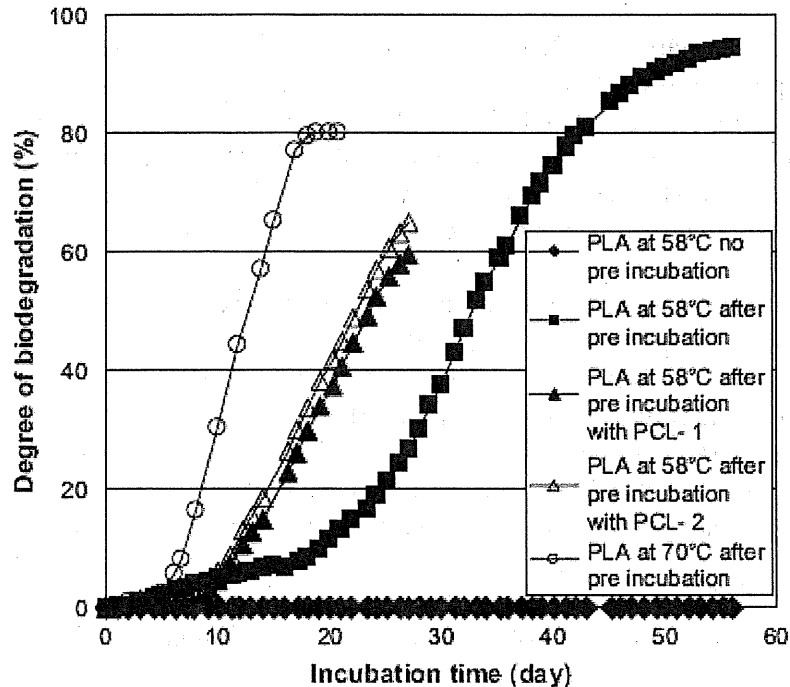


Figure 19 - Result 1. Biodegradation test of PLA in controlled compost at 58°C and 70°C with and without activation step

Remark - Result 1

Although the production of PLA is in its early stage compared with conventional plastics, the potential applications of PLA as biodegradable plastics are promising. As the use of PLA is expected to increase in the future, the information obtained from this review will be very useful in the evaluation of the degradability of PLA.

Note on Request of Budget Amendment on May 5, 2007 - Result 1

In the original proposal "LCMR 05-09f" a sum of \$2,000 was budgeted toward Outside MN (professional meeting) and additional \$3,000 was budgeted toward in-MN sampling expenses under "travel Expenses" of Result I. Also, under the "equipment/Tool" the PI of result I requested a total of \$40,000 to be used toward equipment purchasing expenses.

Due to the fact that we did not need to make as many trips to Park Rapids as we originally planned to collect potato waste for our R & D work, we projected to have \$2,700 remained in our in-state travel budget.

The PI of Result 1 has presented one research paper entitled "Bioconversion and Optimization of Potato Waste to Lactic Acid" at the American Chemical Society Annual Meeting in Chicago, IL on March 26-29, 2007. This Out-of-State Trip to professional meeting has spent \$976.96.

The second research paper entitled "Bioconversion of Potato Waste into Polylactic Acid" has been accepted by the ASA-CSSA-SSSA International Conference committee and it will be presented on November 4-8, 2007, in New Orleans, Louisiana. The PI would like to request that the LCCMR approves and allows the PI to use the remaining, out-of-state travel money \$1,023.04 to cover the registration and plane ticket cost to New Orleans to present the second research paper produced from the result I of this project.

Another budget amendment request is to move the balance of "Equipment/Tool" budget to "Chemicals and Supplies" budget. The PI has purchased the required equipments with a savings of \$940.00 on Detector of HPLC/SEC and \$2,432.00 on electrodialyzer plus all the accessories. It is requested that the LCCMR allows the PI to transfer this remaining equipment money $\$940.000 + \$2,432.00 = \$3,372.00$ plus the in-state travel projected remaining budget of \$2,700.00 (a total combined \$6,072.00) to "Chemicals and Supplies" budget item so we can purchase needed chemicals and supplies to complete this project.

The attachment A shows "proposed budget" amount, "amount spent" and "balance" of result I and the whole project of Potato waste Bioconversion project for Bemidji State University as of May 5, 2007. Your approval of our request would be extremely helpful and very much appreciated.

Request of Budget Amendment of Result I was approved by the LCCMR on May 17, 2007

Remark on Budget Balance - Result 1

Sampling expenses were \$ 186.00 less than originally planed.

References - Result 1

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Result 2: *Production of Xanthan from Potato Waste was executed and managed by Dr. Dragoljub Bilanovic and his research team. Dr Steven Spigarell was co-investigator on "Result 2".*

Summary Budget Information for Result 2: LCMR Budget \$ 168,000.00
Balance \$ 4,722.00

Completion Date: June 30th, 2008

Introduction - Result 2

Commercial xanthan fermentations are usually conducted in batch-mode for up to five days. Glucose, sucrose, and hydrolyzed-starches are common carbon sources (CS). Xanthan yield is affected by concentration of carbon source (CS) and carbon to nitrogen ratio (Moraine and Rogovin 1971, 1973; Souw and Demain 1979, 1980). Various alternative carbon sources were tested on xanthan fermentation - for example: apple pomace and spent malt grains (Stredansky and Conti 1999), citrus waste (Bilanovic 1990, Bilanovic et al. 1994), potato waste (Bilanovic et al. 2006, Bilanovic and Welle 2007), olive-mill wastewater (Lopez and Ramos-Cormenzana 1996), sugar beet pulp (Yoo and Harcum 1999), and whey (Nitschke et al. 2001).

Literature (i.e. References listed under Results 1, 2 and 3) and our experiments show that the ranges of carbon to nitrogen (C/N), carbon to phosphorous (C/P) and carbon to magnesium (C/Mg) of potato waste overlap broadly with C/N, C/P and C/Mg of standard xanthan fermentation media (Table 1). This implies that addition of both nutrients and micronutrients to the potato waste should be studied if potato waste is to be used in xanthan and lactic acid and, probably, many other fermentations instead of sugars, and similar expensive carbon sources. Relatively high water content of standard media for xanthan production indicates that xanthan fermentation of potato waste will be more economical if conducted in solid and semi-solid mode (Bilanovic et al. 2006, Bilanovic and Welle 2007).

The objective of the Result 2 was to study and develop solid state or semi-solid fermentations of xanthan on potato waste. We found that xanthan fermented on standard glucose medium and xanthan fermented on the potato waste compare favorably, with respect to yield and rheological characteristics. It is our understanding that xanthan fermented on the potato waste could be used as emulsifier, stabilizer, thickener, friction and water mobility reducer in oil, paint, plastic, agriculture and other industries excluding of food and pharmaceutical industries. Use of xanthan fermented on potato waste in food and pharmaceutical industry will, probably, be subject of FDA approval although it should be added that xanthan fermented on certain potato waste streams (i.e. peeled and steam-treated waste) might be used in those industries after a minimal FDA involvement.

Data generated during execution of Result 2 represent a good basis for construction of pilot-plant for scale-up studies (i.e. not part of this project) that should be

conducted prior to industrial implementation of solid state or semi-solid xanthan fermentation of potato waste (Bilanovic et al 2006).

Operational difficulties experienced during submerged fermentations like reduced oxygen and heat transfer, and problematic mixing are minimized in solid-state fermentation; this is an additional outcome of the Result 2.

Another additional outcome of Result 2 is a reduction, up to a complete elimination, of solid waste generated by the potato processing plants. It should also be noted that xanthan fermentation on potato waste is not expected to generate any new waste. This will in turn help minimize pollution of waters and soils in potato growing/processing regions.

Table 1 - Result 2. Carbon, Nitrogen, Phosphorus and Magnesium Content of Potato Waste and Standard Media for Xanthan Fermentation.

Nutrients, Ratios, and H ₂ O Content	Standard Medium for Xanthan Fermentation (g/L, g/kg, kg/ton)		Potato Waste Composition (g/L, g/kg, kg/ton)	
	Minimum	Maximum	Minimum	Maximum
C	8.00	40.00	60.00	74.80
N	0.030	0.250	1.260	2.330
P	0.220	0.700	0.450	0.630
Mg	0.025	0.500	0.220	0.290
C/N	32.00	1,333.00	25.75	59.36
C/P	11.43	181.82	95.24	166.20
C/Mg	16.00	1600.00	206.90	340.00
H ₂ O	892.00	978.60	720.00	925.00

Assumptions: 1) Yeast extract and peptone contain on average 10% N, 2) Carbon sources like glucose, starch and molasses contain on average 40% C, 3) P provided as KH₂PO₄, 4) Mg provided as MgCl₂.

Outcomes - Result 2

Throughout execution of Result 2 the "Response Surface Methodology (RSM)" was used to plan the experiments and to randomize the order in which the experiments were conducted (Design-Expert 6.1 and 7.1 software); for illustration please see Table 2-Results 2 which lists experiments, conducted with *Xanthomonas campestris* NRRL B 1013, in which the effects of 1) enzymatic pretreatment, 2) mechanical pretreatment, 3) moisture control, and 4) pH control on production of xanthan on potato waste were studied. The experiments listed in Table 2-Result 2 were conducted in duplicate with *Xanthomonas campestris* NRRL B 1013 and with strains NRRL B 1003 and NRRL B 1459.

RSM was also used to analyze the effects of variables on xanthan fermentation of potato waste. The results were analyzed using analysis of variance (ANOVA), and the statistical significance of the variables on xanthan fermentation was determined by means

of a F-test; details of statistical procedures can be found elsewhere (Anderson and Whitcomb 2000).

Table 2 - Result 2. Example of Experimental Schedule - *Xanthomonas Campestris* NRRL B 1013

Experiment No/ Medium	Pre-Treatment (Pt) or Control (C)			
	Mechanical (Pt)	pH (C)	Moisture (C)	Enzymatic (Pt)
1/SM	0	1	0	0
2/SM	0	1	0	0
3/PW	1	0	1	0
4/PW	2	0	1	0
5/PW	2	0	0	0
6/PW	2	1	0	1
7/SM	0	1	0	0
8/SM	0	1	0	0
9/PW	0	0	1	0
10/PW	0	1	1	1
11/PW	2	1	1	1
12/PW	0	1	0	0
13/SM	0	1	0	0
14/SM	0	1	0	0
15/PW	1	0	1	1
16/PW	3	0	0	1
17/PW	3	0	0	0
18/PW	0	1	0	1
19/SM	0	1	0	0
20/SM	0	1	0	0
21/PW	0	0	0	0
22/PW	1	1	1	0
23/PW	2	0	1	1
24/PW	3	1	1	0
25/SM	0	1	0	0
26/SM	0	1	0	0
27/PW	3	1	0	0
28/PW	3	1	0	1
29/PW	1	1	0	0
30/PW	1	1	1	1
31/SM	0	0	0	0
32/SM	0	0	0	0
33/PW	3	0	1	1
34/PW	1	0	0	1
35/PW	0	0	0	1
36/PW	2	1	1	0

SM = Standard Medium for xathan production. PW = Potato Waste medium, Mechanical pre-treatment: 0 = No treatment, 1 = cook in microwave for 3 minutes, 2 = grind in blender for 3 minutes, 3 = grind and cook. Enzymatic pre-treatment: 0 = No treatment, 1 = PW treated with bacterial α amylase prior to sterilization. pH Control: 0 = No control, 1 = medium pH adjusted to 7. Moisture: 0 = No water added, 1 = 50 g H₂O added per 100 g potato waste.

Strain Comparison - Research Task 1 - Result 2

Xanthomonas campestris strains NRRL B-1003, NRRL B-1013 and NRRL B-1459, were obtained from the National Center for Agricultural Utilization Research, United States Department of Agriculture Peoria, IL. Maintenance and propagation of the strains and preparation of inoculum for fermentation were conducted on standard media and according to procedures described previously (Bilanovic 1990 and Bilanovic et al 1994).

Xanthan fermentation experiments in which strains were compared on xanthan fermentation were conducted on: a) standard production medium, b) solid medium prepared from potato waste and c) semi-solid medium prepared from potato waste (Table 2-Result 2). Potato waste medium was prepared following procedures described by Bilanovic (1990), Bilanovic et al (1994) and Stredansky et al (1999).

Results of experiments listed in Table 2-Result 2 are summarized in Table 3-Result 2; the model predicted values versus experimental data are given in Figures 1 to 3a.

Table 3 - Result 2: Crude Xanthan Fermented by Xantomonas Strains Tested

	NRRL B 1003		NRRL B 1013		NRRL B 1459	
	Xt	Y%	Xt	Y%	Xt	Y%
Mean	3.21	32.15	1.76	15.04	2.94	31.2
St. deviation	1.07	11.76	0.43	7.48	0.77	16.55
No. of experiments	48	48	48	48	48	48

Xt = Crude Xanthan (total g) isolated after 96 hours fermentation; Y% = Xanthan Yield percent after 96 hours fermentation (g Crude Xanthan/ g Dry Weight Potato Waste at t=0); *Xantomonas campestris* strains: NRRL B 1003, NRRL B 1013 and NRRL B 1459.

Xanthomonas campestris strains NRRL B-1003 and NRRL B-1459 were used in fermentations intended on optimization of xanthan fermentation of potato waste after analysis of data given in Tables 3 to 7 - Result 2 and Figures 1 to 3a - Result 2.

Mechanical Pre-treatment - Research Task 2 - Result 2

Experiments were conducted to decide should solid potato waste be treated mechanically prior to fermentation (Table 2 - Result 2)

NRRL B 1003 strain ferments on average 20.6% less xanthan on mechanically treated potato waste when compared to not-treated potato waste (Table 4 - Result 2). NRRL B 1013 strain ferments on average 27.62% more xanthan on mechanically treated potato waste when compared to not-treated potato waste (Table 4 - Result 2). NRRL B 1459 strain ferments on average 36.09% less xanthan on mechanically treated potato waste when compared to not-treated potato waste (Table 4 - Result 2); predicted versus experimental data are given in Figures 1 to 3a.

Table 4 - Result 2. Mechanical Pre-Treatment of Solid Potato Waste and Xanthan Production

	NRRL B 1003		NRRL B 1013		NRRL B 1459	
	Xt NM	Xt M	Xt NM	Xt M	Xt NM	Xt M
Mean	3.84	3.05	1.81	2.31	4.35	2.78
St. deviation	1.37	1.04	1.65	0.42	1.31	1.23
No. of experiments	12	12	12	10	12	12

Xt NM = Crude Xanthan (total g) isolated after 96 hours fermentation on potato waste that was not treated mechanically; Xt M = Crude Xanthan (total g isolated) with Mechanical Treatment after 96 hours fermentation; *Xantomonas campestris* strains: NRRL B 1003, NRRL B 1013 and NRRL B 1459

Research Task 9 - pH control

Control of the fermentation medium pH is important in almost all industrial fermentations. We used K_2HPO_4 to control pH in solid and semi-solid xanthan fermentations and in fermentations on standard medium (Table 2-Result 2). NRRL B 1003 strain ferments almost equal amount of xanthan with and without pH control (Table 5 - Result 2). NRRL B 1013 strain ferments 46.27% more xanthan on medium with than without pH control (Table 5 - Result 2). NRRL B 1459 strain ferments 16.54% more xanthan on medium with than without pH control (Table 5 - Result 2); predicted versus experimental data are given in Figures 1 to 3a.

Table 5 - Result 2. pH Control in Solid and Semi-solid Xanthan Fermentation.

	NRRL B 1003		NRRL B 1013		NRRL B 1459	
	Xt NpH	Xt pH	Xt NpH	Xt pH	Xt NpH	Xt pH
Mean	3.23	3.19	1.34	1.96	2.72	3.17
St. deviation	1.34	1.48	0.69	1.70	1.30	1.56
No. of experiments	24	24	24	20	24	24

Xt NpH = Crude Xanthan (total g) isolated after 96 hours fermentation without pH control; Xt M = Crude Xanthan (total g) isolated after 96 hours fermentation with pH control; *Xantomonas campestris* strains: NRRL B 1003, NRRL B 1013 and NRRL B 1459

Research Task 3 - Enzymatic Pre-Treatment

Experiments were conducted to decide should enzymatic pre-treatment of solid potato waste be conducted to increase xanthan yield (Table 2 - Result 2). Potato waste (50 g) was treated with α amylase (0.25 mL) for 12 hours at 25°C prior to sterilization and solid or semi-solid fermentation; we used E 3.2.1.1. type XII A bacterial α amylase from Sigma: 21 mg protein per mL, 786 units/mg protein.

Table 6 - Result 2. The effect of α Amylase Pre-treatment of Potato Waste on Xanthan Fermentation.

	NRRL B 1003		NRRL B 1013		NRRL B 1459	
	Xt NET	Xt ET	Xt NET	Xt ET	Xt NET	Xt ET
Mean	3.35	3.07	1.24	2.08	2.98	2.91
St. deviation	1.31	1.50	0.55	1.90	1.39	1.51
No. of experiments	24	24	24	22	24	24

Xt NET = Crude Xanthan (total g isolated) without Enzymatic Treatment after 96 hours fermentation; Xt ET = Crude Xanthan (total g isolated) with Enzymatic Treatment after 96 hours fermentation; *Xantomonas campestris* strains: NRRL B 1003, NRRL B 1013 and NRRL B 1459

NRRL B 1003 strain ferments almost equal amount of xanthan with and without enzymatic pre-treatment (Table 6 - Result 2). NRRL B 1013 strain ferments on average 40.40% less xanthan on medium with than without enzymatic treatment (Table 6 - Result 2). NRRL B 1459 strain ferments almost equal amount of xanthan with and without enzymatic pretreatment (Table 6 - Result 2); predicted versus experimental data are given in Figures 1 to 3a.

Research Task 8 - Substrate Moisture Control

To study solid and semi-solid xanthan fermentations we conducted experiments in which xanthan was fermented on:

- solid potato waste without water addition = solid state fermentation,
- solid potato waste to which water was added in an amount 50 mL H₂O per 100 g potato waste = semi-solid fermentation (Table 2 - Result 2).

Table 7 - Result 2. Moisture Control - Solid and Semi-Solid Xanthan Fermentation

	NRRL B 1003		NRRL B 1013		NRRL B 1459	
	Xt NC	Xt MC	Xt NC	Xt MC	Xt NC	Xt MC
Mean	3.63	2.78	2.38	0.95	2.72	3.17
St. deviation	1.37	1.32	1.69	0.55	1.30	1.56
No. of experiments	24	24	22	22	24	24

Xt NC = Crude Xanthan (total g) isolated after 96 hours fermentation without Moisture Control; Xt MC = Crude Xanthan (total g) isolated after 96 hours fermentation with Moisture Control; *Xantomonas campestris* strains: NRRL B 1003, NRRL B 1013 and NRRL B 1459

NRRL B 1003 strain ferments 23.42% less xanthan in semi-solid than in solid state xanthan fermentation (Table 7 - Result 2). NRRL B 1013 strain ferments on average 60.00% less xanthan in semi-solid than in solid state fermentation (Table 7 - Result 2). NRRL B 1459 strain ferments 16.54% more xanthan in semi-solid than in solid-state xanthan fermentation of potato waste (Table 7 - Result 2); predicted versus experimental data are given in Figures 1 to 3a.

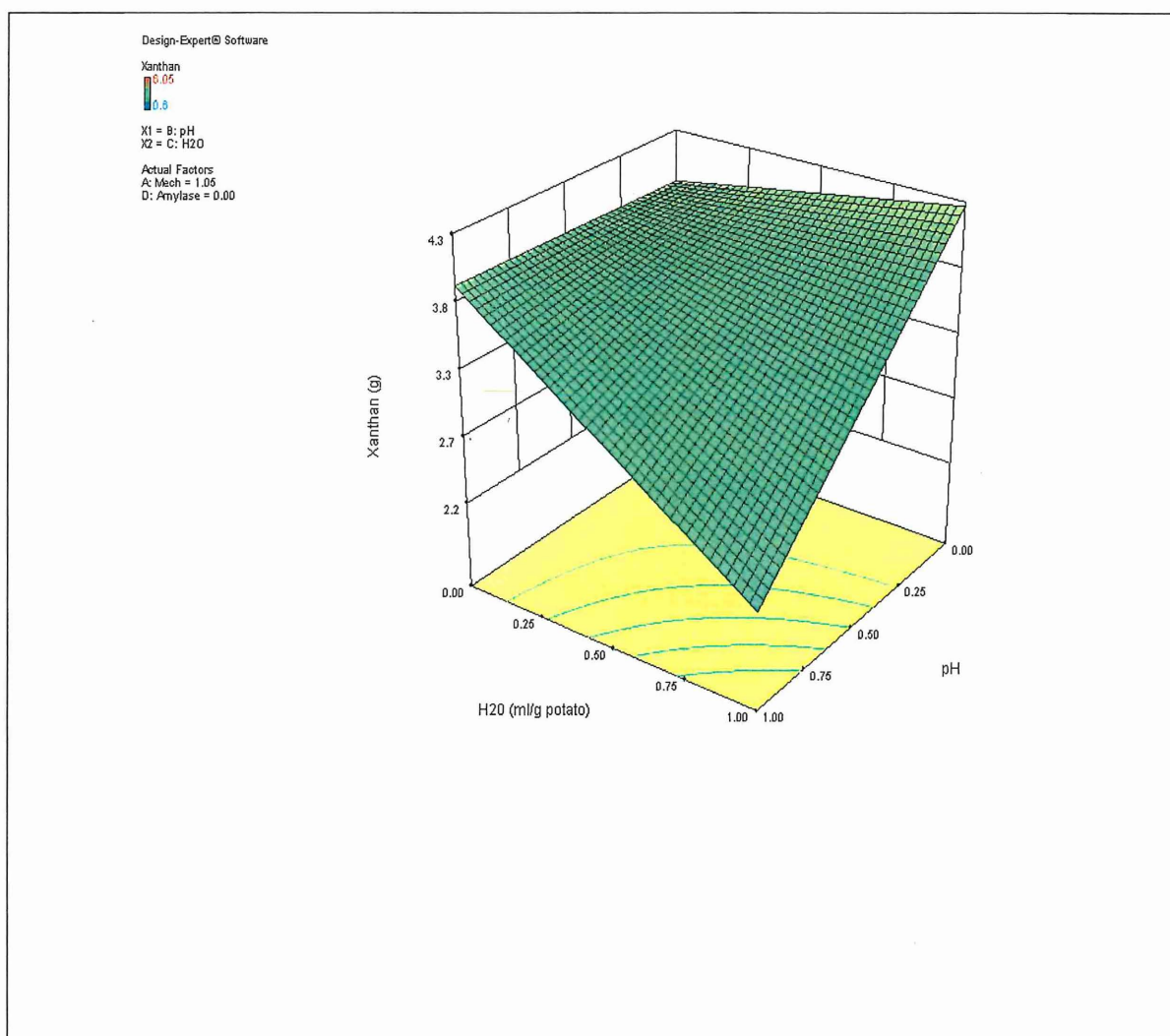


Figure 1 - Result 2. *Xanthomonas campestris* NRRL B 1003 xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control. Remarks: pH 0 stands for absence of pH control; pH 1 stands for pH control with phosphate buffer. H₂O = 0 stands for no water added; H₂O =1 stands for addition of 50 mL H₂O per 100 g potato waste.

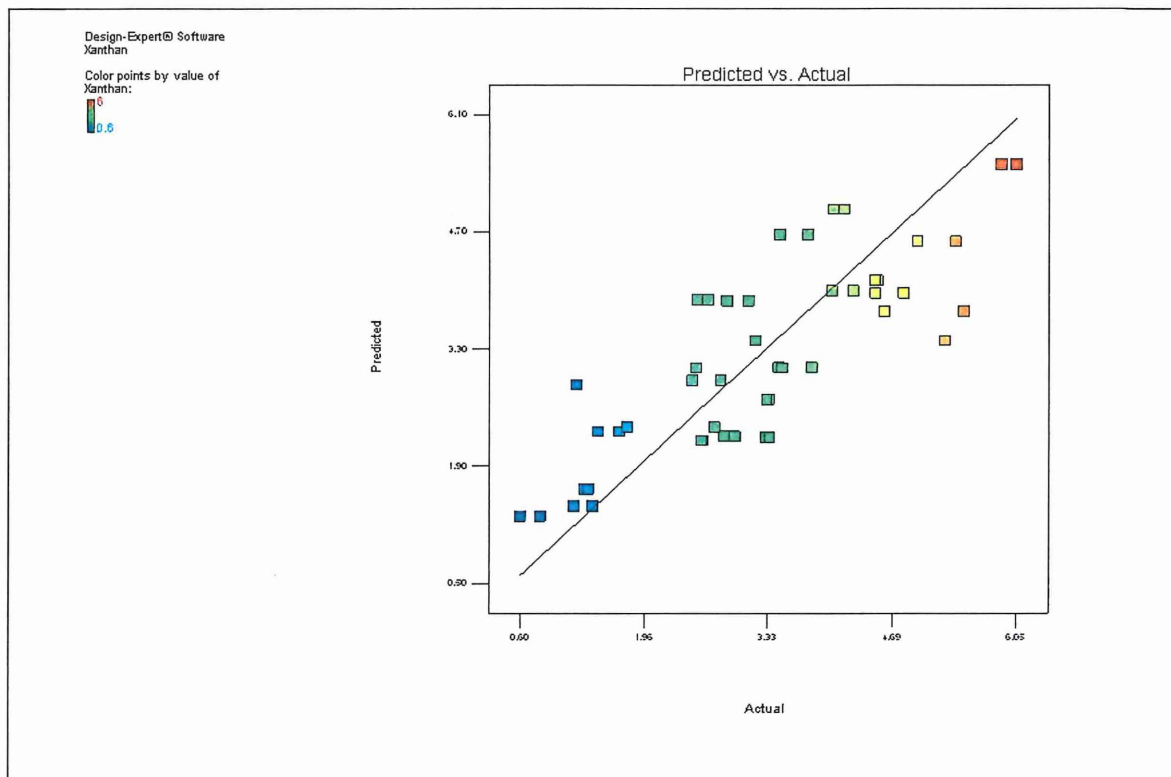


Figure 1a - Result 2. Predicted versus actual xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control - *Xanthomonas campestris* NRRL B 1003; Mean 3.21 g, Std.Dev.=1.07, $R^2=0.6503$.

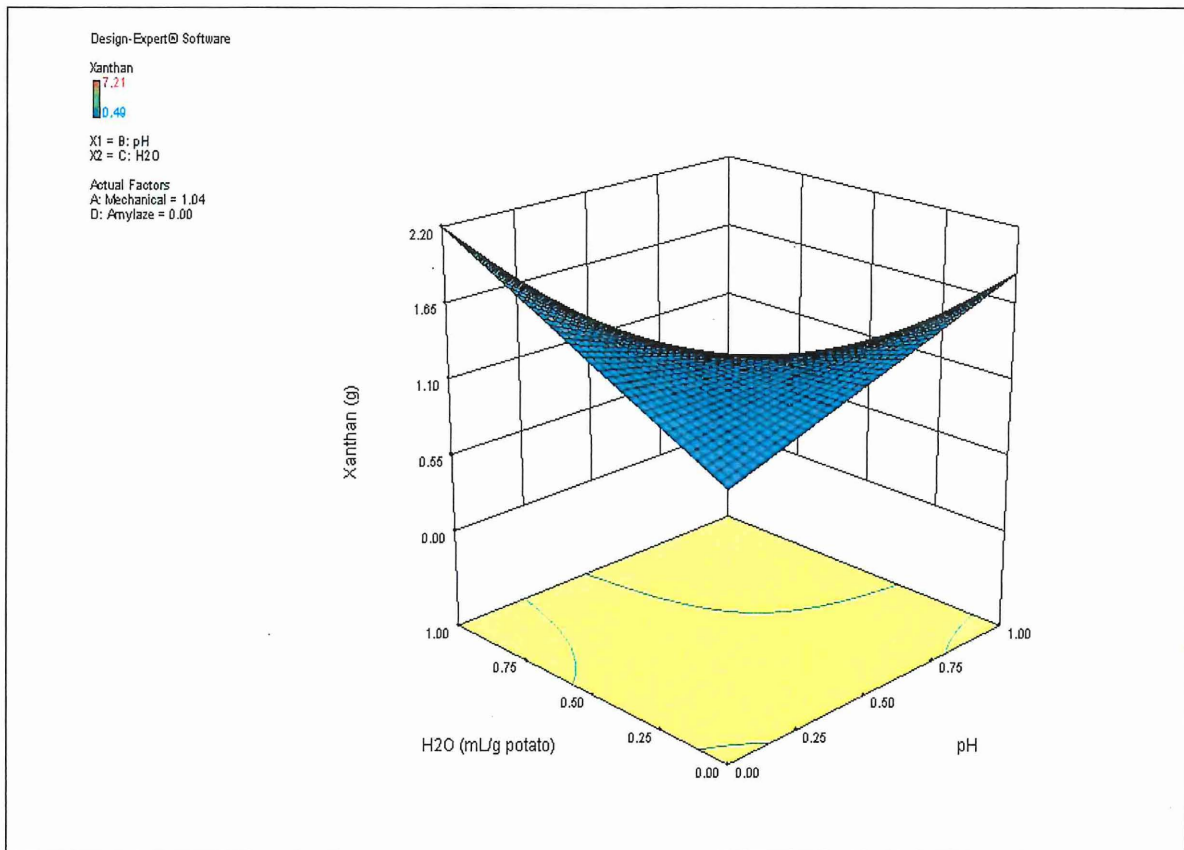


Figure 2 - Result 2. *Xanthomonas campestris* NRRL B 1013 xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control. Remarks: pH 0 stands for absence of pH control; pH 1 stands for pH control with phosphate buffer. H₂O = 0 stands for no water added; H₂O =1 stands for addition of 50 mL H₂O per 100 g potato waste.

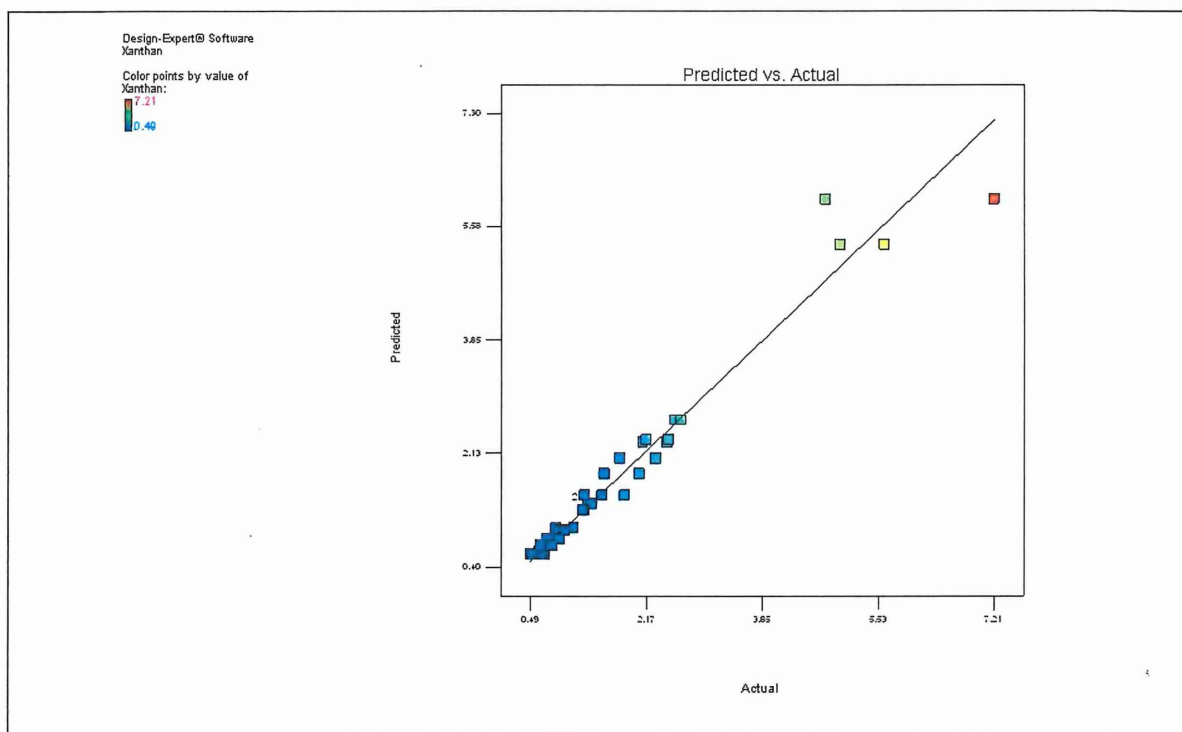


Figure 2a - Result 2. Predicted versus actual xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control. *Xanthomonas campestris* NRRL B 1013; Mean 1.76 g, Std.Dev.=0.43, $R^2=0.9548$.

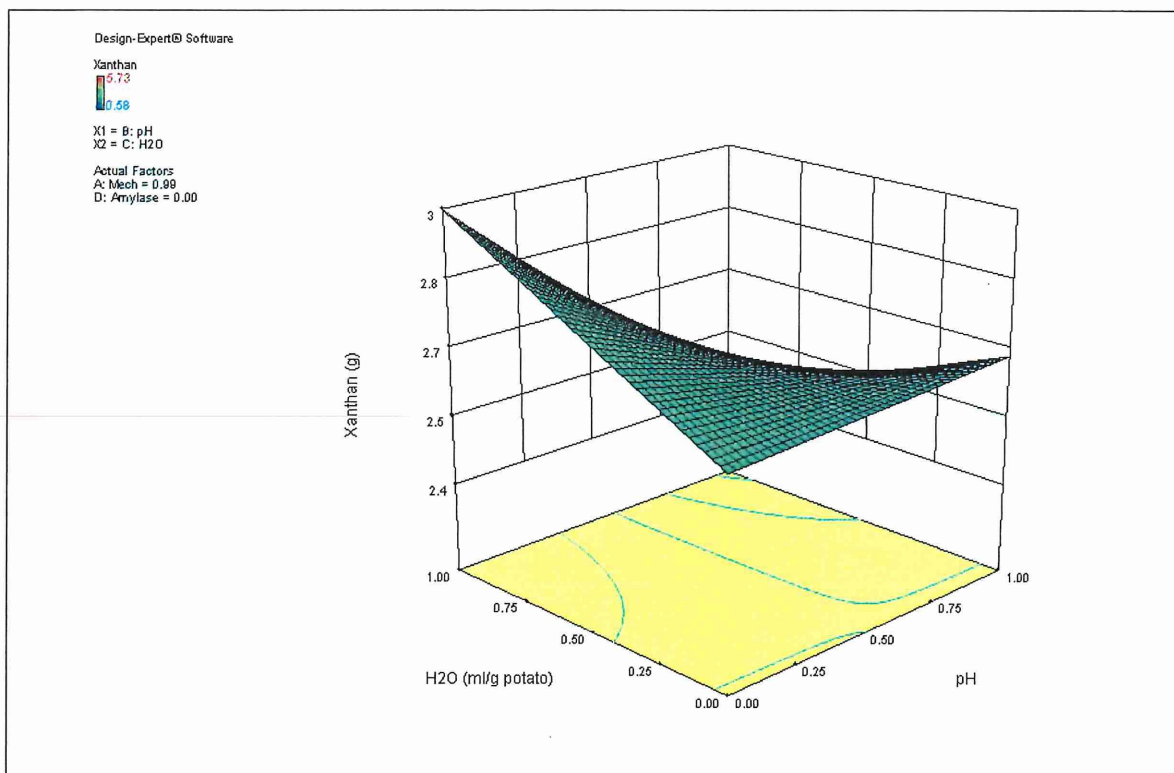


Figure 3 - Result 2. *Xanthomonas campestris* NRRL B 1459 xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control. Remarks: pH 0 stands for absence of pH control; pH 1 stands for pH control with phosphate buffer. H₂O = 0 stands for no water added; H₂O = 1 stands for addition of 50 mL H₂O per 100 g potato waste.

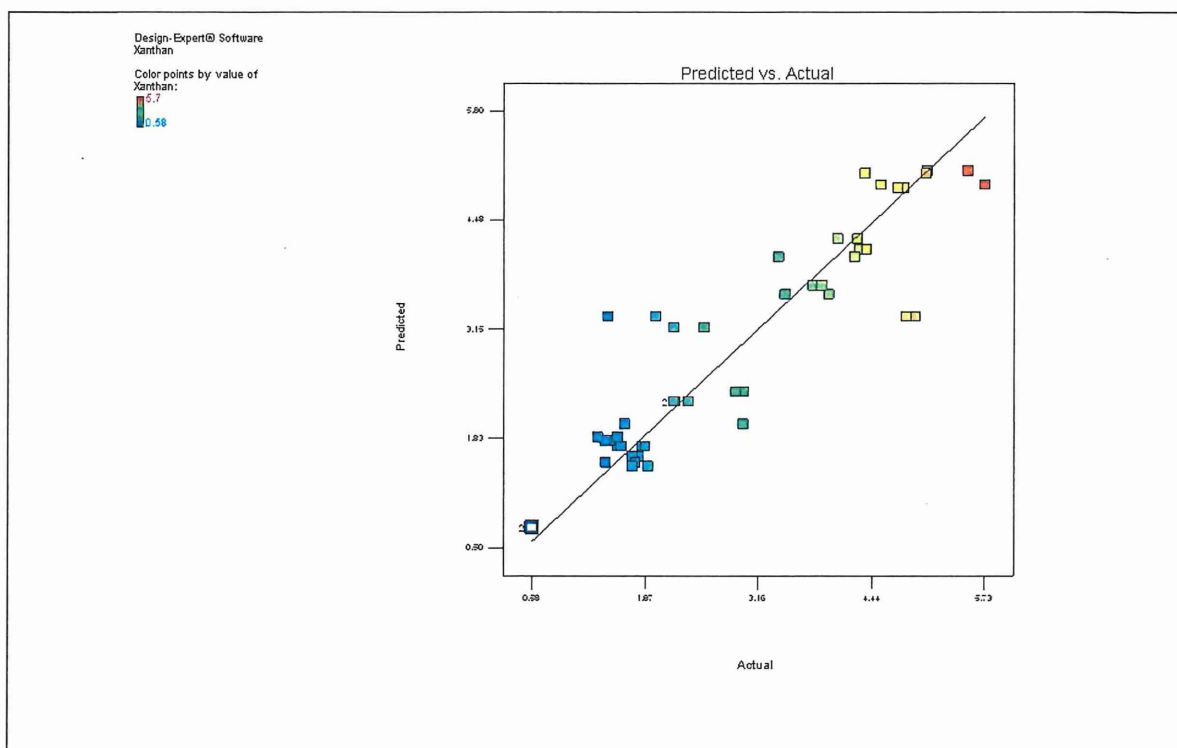


Figure 3a - Result 2. Predicted versus actual xanthan yield in solid and semisolid xanthan fermentation with and without: mechanical and enzymatic pre-treatments and pH control - *Xanthomonas campestris* NRRL B 1459; Mean 2.94 g, Std.Dev.=0.77, $R^2=0.8286$.

Fermentation Kinetics, Medium Optimization, and Xanthan Characterization - Research Tasks: 4, 5, 6, 7, and 10 - Result 2

Fermentation Kinetics - Result 2

Xanthomonas campestris strains NRRL B 1003 and B 1459 were used to study kinetics of xanthan fermentation of solid potato waste. Total solids were used at almost equal rate by both strains of *Xanthomonas* (i.e. B 1459 and B 1003). Maximal amount of xanthan was fermented after six days of fermentation using B 1459 strain while it took nine days of fermentation with strain B 1003 to reach maximal concentration of xanthan (Figure 4 – Result 2).

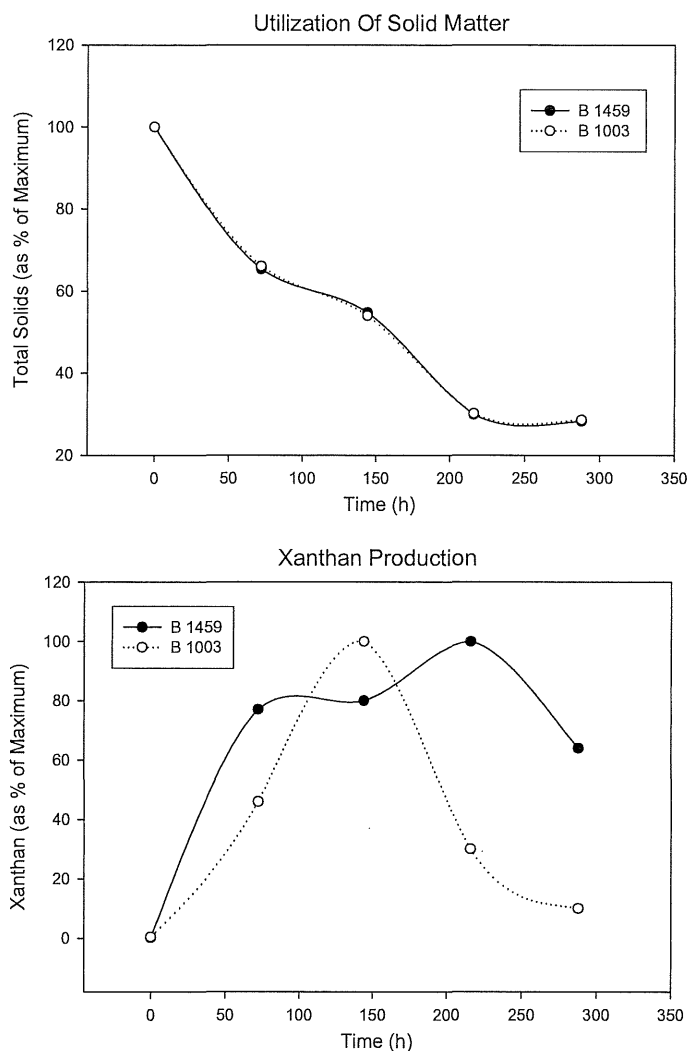


Figure 4 - Result 2: Xanthan production and utilization of Total solids

Medium Optimization Experiments - Research Tasks: 4, 5, 6, and 7

Nutritional studies intended on optimization of concentration of macro and micro nutrients in semi-solid and solid xanthan fermentations of the potato waste were conducted with *Xantomonas campestris* NRRL B 1003 (Table 8 - Result 2) and with *Xantomonas campestris* NRRL B 1459 (Table 9 - Result 2). Response Surface Methodology (Design-Expert 7.0 software) was also used to plan the experiments and to randomize the order in which experiments were conducted (Tables 8 and 9 - Result 2). Macro and micro nutrients were added to potato waste prior to sterilization as: Na_2HPO_4 , NaNO_3 , Na_2SO_4 , CaCl_2 , $\text{Fe}_2(\text{SO}_4)_3$ and MgCl_2 . The results were analyzed using analysis of variance (ANOVA), and the statistical significance of the variable(s) on xanthan fermentation was determined by means of a F-test (Anderson and Whitcomb 2000).

Table 8 - Result 2. RSM Design for Optimization of Nutrient Content of Potato Waste Medium - *Xanthomonas Campestris* NRRL B 1003.

Run	Concentration [g/L]						XT	X/DWo
	[PO ₄]	[NO ₃]	[SO ₄]	[Ca]	[Fe]	[Mg]		
1	4.33	1.22	0.00	0.08	0.00	0.36	1.878	0.27
2	4.33	1.22	0.00	0.08	0.00	0.36	1.806	0.227
3	0.00	1.22	0.13	0.08	0.02	0.00	1.645	0.217
4	0.00	1.22	0.13	0.08	0.02	0.00	1.347	0.176
5	0.08	0.00	0.00	0.00	0.00	0.00	5.793	0.816
6	0.08	0.00	0.00	0.00	0.00	0.00	5.57	0.806
7	4.33	1.22	0.00	0.00	0.02	0.04	5.452	0.855
8	4.33	1.22	0.00	0.00	0.02	0.04	5.153	0.646
9	0.00	1.22	0.13	0.08	0.00	0.04	1.138	0.154
10	0.00	1.22	0.13	0.08	0.00	0.04	1.336	0.154
11	4.33	1.22	0.00	0.00	0.00	0.00	1.868	0.173
12	4.33	1.22	0.00	0.00	0.00	0.00	2.15	0.22
13	0.00	0.00	0.00	0.00	0.02	0.00	4.941	0.751
14	0.00	0.00	0.00	0.00	0.02	0.00	4.396	0.628
15	0.00	1.22	0.13	0.00	0.00	0.00	1.562	0.159
16	0.00	1.22	0.13	0.00	0.00	0.00	1.641	0.174
17	4.33	0.00	0.13	0.00	0.00	0.04	1.613	0.175
18	4.33	0.00	0.13	0.00	0.00	0.04	1.687	0.174
19	4.33	0.00	0.13	0.08	0.00	0.00	1.518	0.208
20	4.33	0.00	0.13	0.08	0.00	0.00	1.239	0.176
21	4.33	1.22	0.00	0.00	0.02	0.04	1.637	0.193
22	4.33	1.22	0.00	0.00	0.02	0.04	1.559	0.175
23	0.00	0.00	0.00	0.00	0.00	0.04	2.988	0.514
24	0.00	0.00	0.00	0.00	0.00	0.04	3.14	0.52
25	4.33	1.22	0.00	0.08	0.02	0.00	1.476	0.18
26	4.33	1.22	0.00	0.08	0.02	0.00	1.309	0.146
27	4.33	0.00	0.13	0.08	0.02	0.04	1.022	0.155
28	4.33	0.00	0.13	0.08	0.02	0.04	1.131	0.177
29	4.33	0.00	0.13	0.00	0.02	0.00	1.159	0.17
30	4.33	0.00	0.13	0.00	0.02	0.00	1.331	0.141
31	0.00	0.00	0.00	0.08	0.02	0.04	3.276	0.546
32	0.00	0.00	0.00	0.08	0.02	0.04	3.085	0.474

Crude xanthan (XT) and xanthan per gram of dry potato waste (XDWo) are averages of two determinations. Symbols: [PO₄] = Na₂HPO₄; [NO₃] = NaNO₃, [SO₄] = Na₂SO₄, [Ca] = CaCl₂, [Fe] = Fe₂(SO₄)₃, [Mg] = MgCl₂, XT = Xanthan Total xanthan (i.e. crude xanthan grams after 96 hours fermentation), XDWo = crude xanthan grams per gram potato waste(dry weight at t=0).

Table 9 - Result 2. RSM Design for Optimization of Nutrient Content of Potato Waste Medium - *Xanthomonas Campestris* NRRL B 1459.

Run	Concentration [g/L]						XT	X/DWo
	[PO ₄]	[NO ₃]	[SO ₄]	[Ca]	[Fe]	[Mg]		
1	4.33	1.22	0.13	0.08	0.00	0.00	1.516	0.193
2	4.33	1.22	0.13	0.08	0.00	0.00	1.451	0.175
3	4.33	1.22	0.13	0.00	0.00	0.04	3.982	0.873
4	4.33	1.22	0.13	0.00	0.00	0.04	3.401	0.727
5	4.33	1.22	0.13	0.08	0.02	0.04	4.203	0.589
6	4.33	1.22	0.13	0.08	0.02	0.04	4.099	0.363
7	0.00	0.00	0.13	0.08	0.00	0.04	3.969	0.443
8	0.00	0.00	0.13	0.08	0.00	0.04	4.109	0.532
9	0.00	1.22	0.00	0.08	0.02	0.04	2.761	0.382
10	0.00	1.22	0.00	0.08	0.02	0.04	3.372	0.501
11	4.33	0.00	0.00	0.00	0.00	0.00	4.57	0.956
12	4.33	0.00	0.00	0.00	0.00	0.00	3.462	0.454
13	4.33	0.00	0.00	0.08	0.00	0.04	3.615	0.432
14	4.33	0.00	0.00	0.08	0.00	0.04	3.543	0.426
15	0.00	0.00	0.13	0.08	0.02	0.00	2.789	0.332
16	0.00	0.00	0.13	0.08	0.02	0.00	2.586	0.286
17	0.00	0.00	0.13	0.00	0.02	0.04	2.422	0.266
18	0.00	0.00	0.13	0.00	0.02	0.04	3.096	0.329
19	4.33	0.00	0.00	0.08	0.02	0.00	3.202	0.539
20	4.33	0.00	0.00	0.08	0.02	0.00	2.292	0.357
21	0.00	0.00	0.13	0.00	0.00	0.00	3.285	0.644
22	0.00	0.00	0.13	0.00	0.00	0.00	3.709	0.552
23	0.00	1.22	0.00	0.00	0.00	0.04	2.32	0.247
24	0.00	1.22	0.00	0.00	0.00	0.04	2.718	0.297
25	4.33	1.22	0.13	0.00	0.02	0.00	3.326	0.515
26	4.33	1.22	0.13	0.00	0.02	0.00	3.332	0.489
27	0.00	1.22	0.00	0.08	0.00	0.00	2.307	0.361
28	0.00	1.22	0.00	0.08	0.00	0.00	1.898	0.281
29	0.00	1.22	0.00	0.00	0.02	0.00	2.528	0.434
30	0.00	1.22	0.00	0.00	0.02	0.00	2.909	0.358
31	4.33	0.00	0.00	0.00	0.02	0.04	2.89	0.407
32	4.33	0.00	0.00	0.00	0.02	0.04	2.854	0.516

Crude xanthan (XT) and xanthan per gram of dry potato waste (XDWo) are averages of two determinations. Symbols: [PO₄] = Na₂HPO₄; [NO₃] = NaNO₃, [SO₄] = Na₂SO₄, [Ca] = CaCl₂, [Fe] = Fe₂(SO₄)₃, [Mg] = MgCl₂, XT = Xanthan Total xanthan (i.e. crude xanthan grams after 96 hours fermentation), XDWo = crude xanthan grams per gram potato waste(dry weight at t=0).

Model Equations for Optimization of Nutrient Content in Solid or Semi-Solid Fermentation of Xanthan of Potato Waste

Data in Tables 8 and 9 - Result 2 were used to derive the following model equations that describe the effects of addition of Na_2HPO_4 , NaNO_3 , Na_2SO_4 , CaCl_2 , $\text{Fe}_2(\text{SO}_4)_3$, and MgCl_2 to potato waste on xanthan fermentation:

Xanthomonas campestris NRRL B 1003 - Total Crude Xanthan in Terms of Coded Factors

$$\sqrt{TX96-1003} = 1.39 - 0.16A + 0.43B - 7.294C - 0.12D + 0.058E - 0.06F - 7.43AB - 0.026AD + 0.062BE + 0.57BF + 0.56CF$$

Where:

$$\sqrt{TX96-1003} = \text{Total Xanthan Isolated after 96 hours fermentation (g total)}^{0.5}$$

A = Na_2HPO_4

B = NaNO_3

C = Na_2SO_4

D = CaCl_2

E = $\text{Fe}_2(\text{SO}_4)_3$

F = MgCl_2

Table 10 - Result 2. Model Statistics for Total Xanthan Isolated after 96 hours Fermentation. *Xanthomonas campestris* NRRL B 1003.

	Xanthan (g total) ^{0.5}				
	S ²	df	M ²	F	P>F
Model	4.57	11	0.42	5.73	0.0004
A	0.55	1	0.55	7.55	0.0124
B	0.069	1	0.069	0.95	0.3410
C	0.069	1	0.069	0.95	0.3425
D	0.29	1	0.29	4.05	0.0577
E	0.069	1	0.069	0.96	0.3396
F	0.00127	1	0.00127	0.018	0.8960
AB	0.074	1	0.074	1.02	0.3244
AD	0.016	1	0.016	0.22	0.6478
BE	0.085	1	0.085	1.17	0.2922
BF	0.098	1	0.098	1.35	0.2588
CF	0.046	1	0.046	0.64	0.4333
Pure Error	1.12	17	0.066		
CT	6.02	31			
R ² = 0.7590 Adjusted R ² = 0.6265					
Coefficient of variation % = 18.10 Mean = 1.49 St.Dev. = 0.27					
CT=Sum of squares total corrected for the mean, df=degrees of freedom, F= F value, M ² = Mean Square, P>F = probability value, and S ² =Sum of Squares.					

The Model F-value of 5.73 implies the model is significant. There is only a 0.04% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.1000 indicate that A and D are significant model terms (Table 10 Result 2). Experimental and model predicted amounts of xanthan fermented by *Xantomonas campestris* NRRL B 1003 are given in Figure 5 - Result 2.

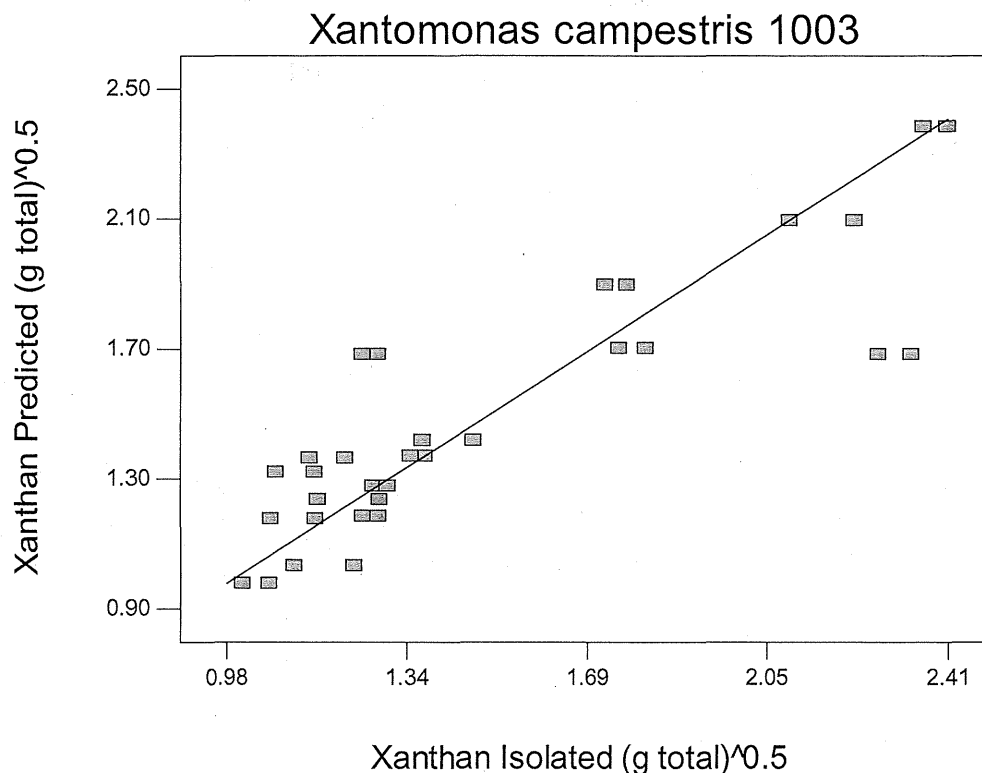


Figure 5 - Result 2. *Xantomonas campestris* NRRL B 1003 fermentation of potato waste. For modeling purposes xanthan isolated and xanthan predicted values are expressed as square root of the total xanthan isolated or predicted. Xanthan isolated is average of two measurements at each data point.

Xanthomonas campestris 1459 - Total Crude Xanthan in Terms of Coded Factors

$$\sqrt{TX96-1459} = 2.44 + 0.29A + 0.53B + 0.54C - 0.035D - 0.0025E + 0.78F - 0.045AD + 0.025AE + 0.28AF - 0.031BD + 0.14BE + 0.66BF + 0.56CF$$

Where:

$$\sqrt{TX96-1459} = \text{Total Xanthan Isolated after 96 hours fermentation (g total)}^{0.5}$$

A = Na_2HPO_4

B = NaNO_3

C = Na_2SO_4

D = CaCl_2

E = $\text{Fe}_2(\text{SO}_4)_3$

F = MgCl_2

Table 11 - Result 2. Model Statistics for Total Xanthan Isolated after 96 hours Fermentation. *Xanthomonas campestris* NRRL B 1459

	Xanthan (g total) ^{0.5}				
	S ²	df	M ²	F	P>F
Model	1.43	13	0.11	11.30	< 0.0001
A	0.034	1	0.034	3.46	0.0794
B	0.11	1	0.11	11.43	0.0033
C	0.11	1	0.11	11.58	0.0032
D	0.038	1	0.038	3.94	0.0625
E	0.000197	1	0.000197	0.020	0.8883
F	0.19	1	0.19	20.00	0.0003
AB	0.000	0			
AC	0.000	0			
AD	0.066	1	0.066	6.74	0.0182
AE	0.020	1	0.020	2.04	0.1702
AF	0.026	1	0.026	2.62	0.1226
BD	0.031	1	0.031	3.17	0.0920
BE	0.61	1	0.61	62.86	< 0.0001
BF	0.14	1	0.14	14.38	0.0013
CF	0.10	1	0.10	10.39	0.0047
Pure Error	0.16	16	0.00988		
CT	1.61	31			
<p>R² = 0.8909 Adjusted R² = 0.8121 Coefficient of variation % = 5.76 Mean = 1.74 St.Dev. = 0.099</p> <p>CT=Sum of squares total corrected for the mean, df=degrees of freedom, F= F value, M² = Mean Square, P>F = probability value, and S² =Sum of Squares.</p>					

The Model F-value of 11.30 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.1000 indicate that the following model terms are significant: A, B, C, D, F, AD, BD, BE, BF, CF (Table 11 - Result 2). Experimental and model predicted amounts of xanthan fermented by *Xantomonas campestris* NRRL B 1459 are given in Figure 6 - Result 2.

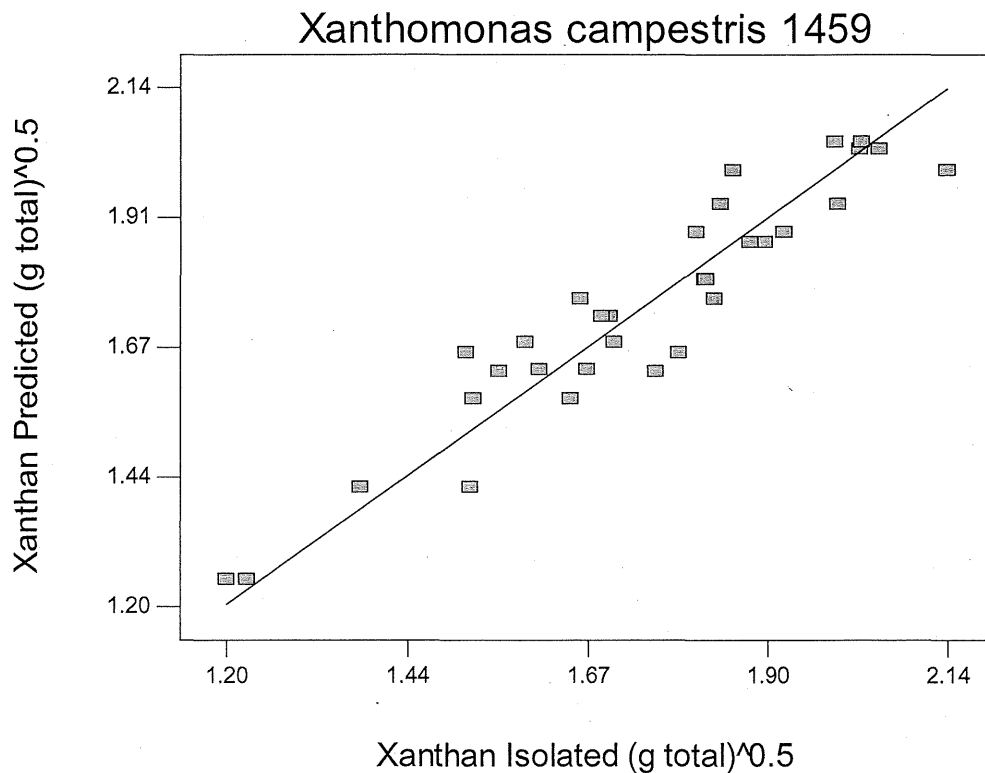


Figure 6 - Result 2. *Xanthomonas campestris* NRRL B 1459 fermentation of potato waste. For modeling purposes xanthan isolated and xanthan predicted values are expressed as square root of total xanthan isolated or predicted. Xanthan isolated is average of two measurements at each data point.

***Xanthomonas campestris* NRRL B 1003 - Xanthan Yield in Terms of Coded Factors**

$$\sqrt{XPWDW - 1003} = 0.56 - 0.074A - 0.066B - 0.11C - 0.038D + 0.015E + 0.023F$$

Where:

$\sqrt{XPWDW - 1003}$ = Xanthan yield gram per gram potato waste added (i.e potato waste dry weight PWDW) after 96 hours of fermentation (g X/ g DWol)^{0.5}

A=Na₂HPO₄

B=NaNO₃

C=Na₂SO₄

D=CaCl₂

E=Fe₂(SO₄)₃

F=MgC₁₂

Table 12 - Result 2. Model Statistics for Total Xanthan Isolated after 96 hours Fermentation. *Xanthomonas campestris* NRRL B 1003

	Xanthan Yield (g X/ g PWDW) ^{0.5}				
	S ²	df	M ²	F	P>F
Model	0.82	6	0.14	9.29	< 0.0001
A	0.15	1	0.15	10.19	0.0038
B	0.13	1	0.13	8.63	0.0070
C	0.30	1	0.30	20.00	0.0001
D	0.032	1	0.032	2.14	0.1561
E	0.00558	1	0.00558	0.38	0.5443
F	0.00235	1	0.00235	0.16	0.6935
Pure Error	0.21	17	0.012		
CT	1.19	31			

R² = 0.6904

Adjusted R² = 0.6161

Coefficient of variation % = 22.18 Mean = 0.55

St.Dev. = 0.12

CT=Sum of squares total corrected for the mean, df=degrees of freedom, F= F value, M² = Mean Square, P>F = probability value, and S²=Sum of Squares.

The Model F-value of 9.29 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.1000 indicate that A,B and C are significant model terms (Table 12 - Result 2). Experimental and model predicted xanthan yield are given in Figure 7 - Result 2. (i.e. *Xantomonas campestris* NRRL B 1003 fermentation of potato waste).

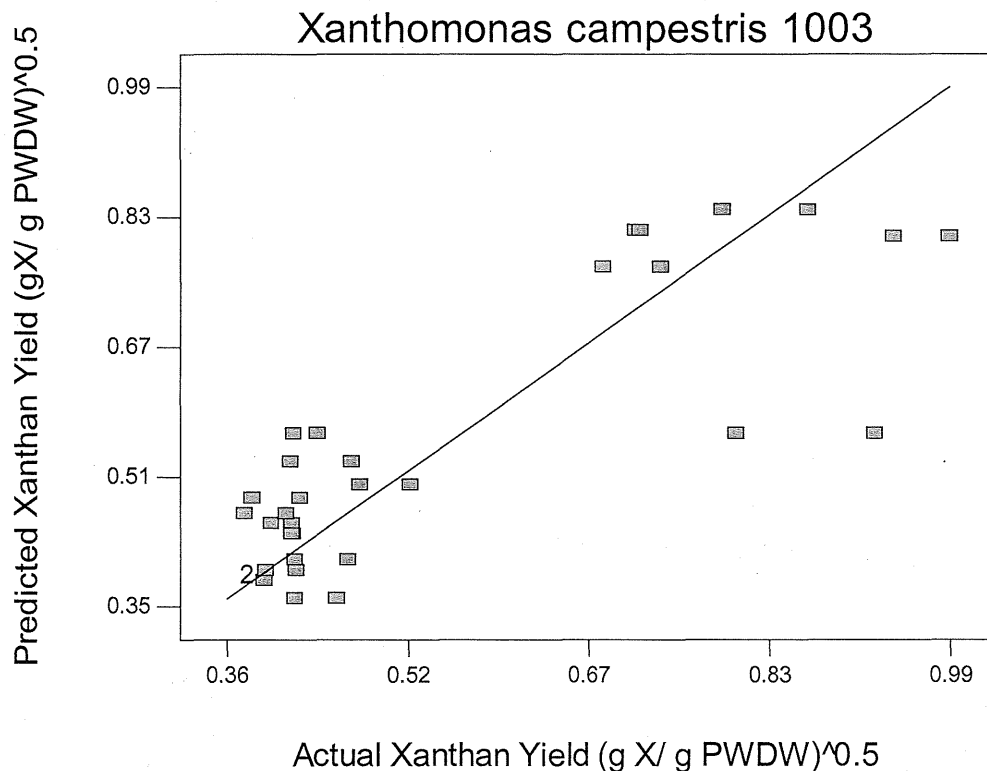


Figure 7 - Result 2. Xanthan Yield - *Xantomonas campestris* NRRL B 1003. X = Xanthan, PWDW = Potato Waste Dry Weight at the beginning of experiment. For modeling purposes xanthan actual yield and xanthan predicted yield after 96 hours are given as $(g\ X/ g\ PWDW)^{0.5}$. Each data point is average of two measurements. Number "2" on the Figure 7 - Result 2 indicate overlap of two experimental points.

***Xanthomonas campestris* NRRL B 1459 - Xanthan Yield in Terms of Coded Factors**

$$\sqrt{XPWDW - 1459} = 0.76 + 0.25A + 0.36B + 0.30C - 0.041D - 0.014E + 0.12F - 0.043AD + 0.0062AE + 0.24AF - 0.010BD + 0.049BE + 0.42BF - 0.028CD - 0.023CE + 0.33CF$$

Where:

$\sqrt{XPWDW - 1459}$ = Xanthan yield gram per gram potato waste added (i.e potato waste dry weight PWDW) after 96 hours of fermentation $(g\ X/ g\ PWDW)^{0.5}$

A=Na₂HPO₄

B=NaNO₃

C=Na₂SO₄

D=CaCl₂

E=Fe₂(SO₄)₃

F=MgC₁₂

Table 13 - Result 2. Model Statistics for Total Xanthan Isolated after 96 hours Fermentation. *Xanthomonas campestris* NRRL B 1459

	Xanthan Yield (g X/ g PWDW) ^{0.5}				
	S ²	df	M ²	F	P>F
Model	0.41	15	0.027	4.78	0.0017
A	0.024	1	0.024	4.25	0.0558
B	0.051	1	0.051	8.87	0.0089
C	0.035	1	0.035	6.10	0.0251
D	0.054	1	0.054	9.39	0.0074
E	0.00666	1	0.00666	1.16	0.2976
F	0.00434	1	0.00434	0.76	0.3976
AB	0.000	0			
AC	0.000	0			
AD	0.059	1	0.059	10.22	0.0056
AE	0.00124	1	0.00124	0.22	0.6488
AF	0.018	1	0.018	3.13	0.0961
BC	0.000	0			
BD	0.00326	1	0.00326	0.57	0.4620
BE	0.077	1	0.077	13.34	0.0021
BF	0.057	1	0.057	9.94	0.0062
CD	0.025	1	0.025	4.38	0.0528
CE	0.017	1	0.017	2.94	0.1059
CF	0.034	1	0.034	5.96	0.0266
DE	0.000	0			
DF	0.000	0			
EF	0.000	0			
Pure Error	0.092	16	0.00575		
CT	0.50	31			

$R^2 = 0.8177$
Adjusted $R^2 = 0.6467$
Coefficient of variation % = 11.56 Mean = 0.66 St.Dev. = 0.076

CT=Sum of squares total corrected for the mean, df=degrees of freedom, F= F value, M² = Mean Square, P>F = probability value, and S²=Sum of Squares.

The Model F-value of 4.78 implies the model is significant. There is only a 0.17% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.1000 indicate that the following model terms are significant: A, B, C, D, AD, Af, BE, BF, CD, and CF (Table 13 - Result 2). Experimental and model predicted xanthan yield are given in Figure 8 - Result 2 (i.e. *Xantomonas campestris* NRRL B 1459 fermentation of potato waste).

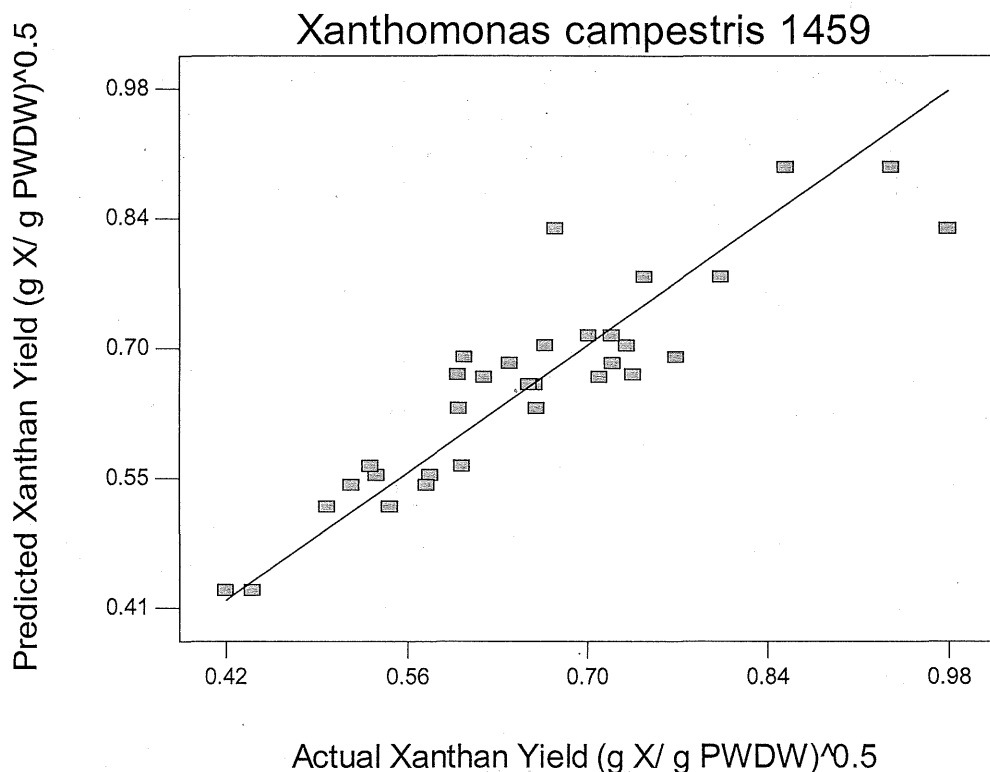


Figure 8 - Result 2. Xanthan Yield - *Xanthomonas campestris* NRRL B 1459. X = Xanthan, PWDW = Potato Waste Dry Weight at beginning of experiment. For modeling purposes xanthan actual yield and xanthan predicted yield after 96 hours are given as (g X/ g PWDW)^{0.5}. Each data point is average of two measurements.

Xanthan Characterization and Comparison with Commercial Xanthan - Research Task 10

Xanthan fermented by *Xanthomonas campestris* strains has cellulosic backbone to which thiosaccharide side chains are attached to alternate backbone residues. Acetyl and pyruvate content of the side chains varies from strain to strain and also with fermentation conditions (Cadmus et al 1978, Cadmus and Knutson 1983, Tait et al. 1986, and elsewhere). Viscosity of xanthan solution is function of its: mean molecular mass, pyruvate and acetyl content.

Crude xanthan was determined: a) gravimetrically (Bilanovic 1994), b) spectrophotometrically using alcian blue procedure adapted from Aruda Fatibello et al (2004) and Powell et al (1982), and c) from viscosity measurements (Flores-Candia and Deckwer 1999)

Spectrophotometric determination of acetyl and pyruvate content of xanthan fermented on potato waste were conducted. Following HCl hydrolysis of xanthan its acetyl content was determined at 480 nm and its pyruvate content at 320 nm.

Results on pyruvate and acetate content, molecular weight and viscosity of potato waste fermented xanthan and commercial xanthan are given in Tables 14 - 17 - Result 2. Pyruvate and acetate content of xanthan fermented on potato waste are within range of literature reported values (Flores Candia and Deckwer 1999, and elsewhere). Molecular weight of xanthan (Table 15 - Result 2) and viscosity of partly purified xanthan solutions (Tables 16 and 17 - Result 2) fermented on potato waste are also comparable to commercially available xanthan.

Table 14 - Result 2. Acetyl and Purynate Content of Xanthan Fermented on Potato Waste.

<i>Xantomonas campestris</i> strain	NRRL B1003		NRRL B 1459	
A= Acetyl; P = Pyruvate	A (wt %)	P (wt %)	A (wt %)	P (wt %)
Average	4.49	3.08	2.42	2.74
St.Deviation	0.70	1.06	0.92	1.13
Minimum	3.00	1.41	1.45	1.05
Maximum	5.74	6.15	5.74	4.44
No of observations	32	32	32	32

Table 15 - Result 2 Molecular Weight Comparison

	Xanthan		
	Commercial	NRRL B 1003	NRRL B 1459
Average	4589173	3236302	3364717
St. Deviation	390079	796775	904985
Minimum		1920591	1608466
Maximum		4480012	4906271
No of observations	5	18	18

Table 16 - Result 2. Viscosity of Commercial and NRRL B 1459 Fermented Xanthan

	NRRL B 1459 Xanthan	Commercial Xanthan
Mean	26.875	25.191
Variance	117.148	113.761
Observations	32	32
Pooled Variance	115.454	
Hypothesized Mean Difference	0	
df	62	
t Stat	0.6270	
P(\leq t) one-tail	0.2665	
t Critical one-tail	1.6698	
P(\leq t) two-tail	0.5329	
t Critical two-tail	1.9989	

Table 17 - Result 2 Viscosity of Commercial and NRRL B 1003 Fermented Xanthan

	NRRL B 1003 Xanthan	Commercial Xanthan
Mean	26.096	25.443
Variance	151.233	120.680
Observations	28	28
Pooled Variance	135.957	
Hypothesized Mean Difference	0	
df	54	
t Stat	0.2097	
P(<= t) one-tail	0.4173	
t Critical one-tail	1.6736	
P(<= t) two-tail	0.8347	
t Critical two-tail	2.0049	

Remarks on Patent Application

The outcomes of the projects “Result 2: Production of Xanthan from Potato Waste” were such that a patent application on the subject of xanthan production from potato waste was prepared. Mr. John Velin, Director of the Legislative Commission on Minnesota Resources was informed about the potential patent relevant to outcomes of “Result 2: Production of Xanthan from Potato Waste – of the project “Bio-conversion of Potato Waste into Marketable Biopolymers”; Legal Citation: ML 2005, First Special Session, Chp.1, Art. 2 __, Sec. 11 __, Subd. __9(f) in a letter sent to Mr. Velin on May 09, 2006.

The Client Representation Agreement was signed between Bemidji State University Foundation of Bemidji, Minnesota and Kinney & Lange – A Professional Association of Minneapolis, Minnesota concerning the patent application titled Solid or Semi-Solid State Fermentation of Xanthan on Potato or Potato Waste. The following documents were prepared and mailed to Kinney & Lange on June 29, 2006:

1) Outline of the patent titled: Solid or Semi-Solid State Fermentation of Xanthan on Potato or Potato Waste; this nine page document comprises of: Sixteen Claims, Background of the Invention, Summary of the Invention, Six Examples, and List of References

2) Literature survey; references in this fifteen page document were obtained from the following databases: American Chemical Society Journals at <http://pubs.acs.org/>, Chemical Abstract at <http://stneasy.cas.org>, and ScienceDirect at <http://www.sciencedirect.com/>

Following the “Agreement Between the State of Minnesota and Bemidji State University” concerning the execution of the project and conditions relevant to intellectual property rights as described in “2.4. Ownership of Intellectual Property Rights and Materials (Minn.Stat.116P.10)” the Bemidji State University Foundation was listed as the

patent owner along with the State of Minnesota Environment and Natural Resources Trust Fund on the patent application which Kinney & Lange helped prepare and submit to the US Patent & Trademark Office in November 2006; Dragoljub Bilanovic, Samuel Hunter Malloy and Petra Remeta were listed as inventors on the patent application.

Data generated during execution of research tasks 1 to 10 were in support of our preliminary hypothesis that potato waste is a very good substrate for xanthan fermentation. United States Patent and Trademark Office acknowledged receiving our patent application on December 15, 2006 – a copy of the acknowledgement is provided below. In accordance with the agreement between the State of Minnesota and Bemidji State University relating to this research project, the assignees on the patent application are The Bemidji State University Foundation and The State of Minnesota Environment and Natural Resources Trust Fund.

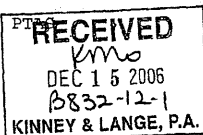


UNITED STATES PATENT AND TRADEMARK OFFICE

UNDER SECRETARY OF COMMERCE FOR INTELLECTUAL PROPERTY AND
DIRECTOR OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECEMBER 08, 2006

LARRIN BERGMAN
KINNEY & LANGE, P.A.
312 SOUTH THIRD STREET
MINNEAPOLIS, MN 55415-1002



UNITED STATES PATENT AND TRADEMARK OFFICE
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RECORDATION DATE: 11/14/2006

REEL/FRAME: 018604/0390

NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:

BILANOVIC, DRAGOLJUB D.

DOC DATE: 11/13/2006

ASSIGNOR:

MALLOY, SAMUEL HUNTER

DOC DATE: 11/13/2006

ASSIGNOR:

REMETA, PETRA

DOC DATE: 11/13/2006

ASSIGNEE:

THE BEMIDJI STATE UNIVERSITY
FOUNDATION
1500 BIRCHMONT DRIVE NORTHEAST,
#17
BEMIDJI, MINNESOTA 56601

018604/0390 PAGE 2

ASSIGNEE:

THE STATE OF MINNESOTA ENVIRONMENT
AND NATURAL RESOURCES TRUST FUND
100 REV. MARTIN LUTHER KING, JR.
BLVD., ROOM 65 STATE OFFICE
BUILDING
A DIVISION OF LEGISLATIVE CITIZEN
COMMISSION ON MINNESOTA
RESOURCES
ST. PAUL, MINNESOTA 55155

SERIAL NUMBER: 11598907

FILING DATE: 11/14/2006

PATENT NUMBER:

ISSUE DATE:

TITLE: SOLID OR SEMI-SOLID STATE FERMENTATION OF XANTHAN ON POTATO OR
POTATO WASTE

MARCUS KIRK, EXAMINER
ASSIGNMENT SERVICES BRANCH
PUBLIC RECORDS DIVISION

11-14-06
**PATENT RECORDATION
COVER SHEET**

11-21-2006



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† No.: B832.12-0001 - kmm

113257 U.S. PTO
11/598907

11406

To the Commissioner for Patents: Mail Stop Assignment Recordation Services - Please record the attached copy thereof.

1. Name of conveying party(ies):

- (1) Dragoljub D. Bilanovic
- (2) Samuel Hunter Malloy
- (3) Petra Remeta

Additional name(s) of conveying party(ies) attached? [] Yes [X] No

2. Name and address of receiving party(ies):

Name: The Bemidji State University Foundation

Street Address: 1500 Birchmont Drive Northeast, #17

City: Bemidji, State: MN ZIP 56601

-and-

Name: The State of Minnesota Environment and Natural
Resources Trust Fund

Internal Address: A division of Legislative Citizen Commission
on Minnesota Resources

Street Address: 100 Rev. Martin Luther King, Jr. Blvd., Room
65 State Office Building

City: St. Paul, State: MN ZIP 55155

Additional name(s) & address(es) attached? [] Yes [X] No

3. Nature of Conveyance:

[X] Assignment [] Merger [] Security Agreement
[] Change of Name [] Other _____

Execution Date: November 13, 2006

4A. Application No.(s)

If this document is being filed together with a new application, the
execution date(s) of the application is: November 13, 2006

Additional numbers attached? [] Yes [X] No

4B. Patent No.(s)

Additional numbers attached? [] Yes [X] No

5. Name and address of party to whom correspondence concerning
document should be mailed:

Name: Larrin Bergman

Street Address: Kinney & Lange, P.A.

312 South Third Street

City: Minneapolis State: MN ZIP 55415-1002

6. Total number of applications and patents
involved: [1]

7. Total fee (37 C.F.R. 3.41):\$ 40.00

The Commissioner is hereby authorized to charge any additional fee
required under 37 C.F.R. 1.16 and 1.17 and credit any over payments to
Deposit Account No. 11-0982, Deposit Account Name: Kinney & Lange,
P.A. A duplicate copy of this communication is enclosed.

DO NOT USE THIS SPACE

9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original
document.

Larrin Bergman

11/20/2006 DBYRNE

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11-14-06
Date

Total number of pages including cover sheet, attachments and document: [5]

Kinney & Lange professionals suggested in the summer of 2007 to Dr. Bilanovic to file international patent application to protect the patent application titled "Solid or Semi-Solid State Fermentation of Xanthan on Potato or Potato Waste" that was filed with the U.S. Patent and Trade Mark Office on 11/14.2006. Due date for the international patent application was November 2007. Dr. Bilanovic did never receive a permission from the BSU administration to proceed with international patent application. This was, possibly, a consequence of the absence of timely communication between BSU and MnSCU administrations concerning partial funding of the international patent application. As a consequence, and to Dr. Bilanovic's regret, the international patent application was not filed. This unfortunate development could have negative impact on marketability of the patent if it would be approved by the United States Patent and Trademark Office.

Additional Activities Relevant to Result 2

Regular monthly survey of research literature was conducted through the execution of Result 2 using the following databases: a) Academic Search Primer, b) Agricola, c) Biological Abstract, d) Chemical Abstract, e) Pollution Abstract and f) ScienceDirect.

Remarks on Budget Balance - Result 2

Result 2 shows a positive budget balance of \$ 4,722.00.

The initial plan was to hire 1.9 graduate students as research assistants for Result 2 for two research years. The first graduate student was hired in September of 2005 instead in July of 2005. The second graduate student was hired in January 2006, instead in July 01 of 2005. The third graduate student was recipient of "Muskie Fellowship" and was hired at reduced rate without tuition expenses. As a result \$ 4,073 was not used under category "Personnel Expenses".

Sampling expenses were \$ 280 less than originally planned.

Fringe was \$ 315.00 less than originally planed.

Chemicals, glassware and plasticware were \$54.00 less than originally planned.

Note on Match Dollars - Result 2.

The following letter was sent to Mr. John Velin on May 6, 2006; the letter includes Table: Encountered and Expected Expenses Relevant to Potential Patent on "Result 2: Production of Xanthan from Potato Waste – of the project "Bio-conversion of Potato Waste into Marketable Biopolymers" that list estimated Match Dollars.

To: Mr. John Velin, Director
Legislative Commission on Minnesota Resources
Room 65, State Office Building
100 Rev. Dr. Martin Luther King Jr. Blvd.
St. Paul, Minnesota 55155

Subject: Note on intended patent application relevant to outcomes of "Result 2: Production of Xanthan from Potato Waste – of the project "Bio-conversion of Potato Waste into Marketable Biopolymers"; Legal Citation: ML 2005, First Special Session, Chp.1, Art. 2__, Sec. 11__, Subd. 9(f) .

Date: May 09, 2006

Dear Mr. Velin,

Last year we were granted LCMR funding towards execution of research project titled "Bio-conversion of Potato Waste into Marketable Biopolymers"; Legal Citation: ML 2005, First Special Session, Chp.1, Art. 2__, Sec. 11__, Subd. 9(f) .

After talking to Ms. Thornton and following "Agreement Between the State of Minnesota and Bemidji State University" concerning the execution of the project and conditions relevant to intellectual property rights as described in "2.4. Ownership of Intellectual Property Rights and Materials (Minn.Stat.116P.10)" this is to inform you that the outcomes of the projects "Result 2: Production of Xanthan from Potato Waste" which is being executed and managed by Dr. Dragoljub Bilanovic and his research team are such that we intend to file patent application on the subject in very near future.

As stipulated in the agreement within Patent Application Bemidji State University Foundation and the State of Minnesota Environment and Natural Resource Trust Fund will be listed jointly as owners of potential patent, Dr. Dragoljub Bilanovic will be listed as primary patent inventor; Mr. Samuel Malloy and Ms. Petra Remeta will be listed as secondary patent inventors.

In addition to the above, and following instructions kindly provided Ms. Thornton of LCMR, a summary of expenses encountered up to date and expected in future is summarized in the following table

Table: Encountered and Expected Expenses Relevant to Potential Patent on “Result 2: Production of Xanthan from Potato Waste – of the project “Bio-conversion of Potato Waste into Marketable Biopolymers”

Item	Funded By:		
	LCMR	BSU	Total \$ (Row)
Particle sizer	32,500.00	0.00	32,500.00
Incubated shaker	5,870.00	0.00	5,870.00
Microscope Leica	2,000.00	27,759.46	29,759.46
D.Bilanovic wages & fringe (130 work days: Saturdays, Sundays and holidays and 2 – 3 hours per day after 4:30 p.m.)	9,845	48,730.00 (in kind)	58,575.00
Student wages	11,565.00	0.00	11,565.00
Chemicals	6,897.00	1,500.00	8,397.00
Patent application	2,000.00	5,500.00	7,500.00
Patent maintenance	0.00	5,000.00	5,000.00
Sampling and travel	450.00	0.00	450.00
Total	71,127.00	88,489.46	159,616.46
Percent	44.56	55.54	100

Kind Regards

Dr. Dragoljub Bilanovic

CC. Rob Bollinger
Executive Director,
Bemidji State University Foundation

References - Result 2.

Anderson, J.M., and Whitcomb, J.P., 2000. *DOE simplified – Practical tools for effective experimentation*. Productivity, Inc, Portland, Oregon. ISBM1-56327-225-3, 1-236.

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Result 3: *Assessment of Economic Feasibility of Result 1 and Result 2 - was executed and managed by Drs. Patrick Welle and Dragoljub Bilanovic in cooperation with Drs Fu-Hsian Chang and Steven Spigarell and graduate and undergraduate students.*

Summary Budget Information for Result 3: LCMR Budget \$ 17,000.00
Balance \$ 1,777.00

Completion Date: June 30th, 2008

Summary Result 3:

Results 1 and 2 experimental data and literature data were used to execute Result 3. The economic analysis generating Result 3 compares laboratory production of polylactic acid and xanthan on potato waste to estimate the profit impacts of these processes. Internal cost implications were assessed to understand the commercial viability of these processes. To broaden this economic perspective, benefit-cost analysis was conducted which factors in the social economic effects of managing this waste in less environmentally costly manner.

Outcomes Result 3:

Result 3; analyzes the economic potential of bio-conversion processes described in under Results 1 and 2. The economic approach employed here is two-fold:

1. estimate the potential for commercial-scale viability of the bio-conversion processes conducted in the laboratories, and
2. assess the potential for social net benefits of producing xanthan and polylactic acid (PLA) using potato waste in light of the negative cost of utilizing potato waste (alleviating a disposal burden) as a substitute for scarce materials used as inputs conventional processes, such as sugar and corn.

The laboratory data (please see Results 1 and 2) are informative in that the materials costs and input conversion efficiencies allow comparisons to conventional methods used in producing xanthan and PLA. While it would be ideal to have detailed information about all the cost components from the conventional methods, much of this is not available due to its proprietary nature. However, the bio-physical aspects of the conversion of materials are known and can be compared with some precision. The conversion efficiencies are favorable using potato waste in xanthan production while the conversion to PLA seems less favorable than conventional conversion using current materials.

Prospects for Commercial Viability: General Principles

The basic method for assessing commercial viability in this research has been to compare results obtained in the laboratory to outcomes from conventional production methods. In the case of xanthan fermentation (Result 2), the research was designed to isolate materials in order to compare the conversion efficiencies of potato waste into xanthan to the efficiencies achieved under conventional methods with inputs currently used. This approach obviates the need for seeking proprietary information on Total Production Costs (TCP). In the case of xanthan production, material conversion is equal

or better than conventional methods. The conclusion is that these newly developed solid and semi-solid fermentation have potential to be more profitable than conventional submerged fermentation. The information yielded on PLA (Result 1) is not as specific in comparing conversion efficiencies but instead tracks production costs in more detail. These costs exceed production costs reported in the literature and are greater than revenue figures gleaned from market reports so would yield losses (negative profits) due to costs exceeding revenue.

Key variables in determining commercial viability, i.e. profit potential

1. Conversion efficiencies of materials (i.e. carbon sources like: sugar, molasses, potato waste, etc.)
2. Costs of material inputs: potato waste free or negative cost as disposal burden
3. Future trends in relative prices: sugar, corn and other inputs increasing in value due to competing uses
4. Portion of total production costs comprised of materials costs
5. Scaling-up of laboratory fermentations to pilot plant scale and to commercial scale production
6. Applicability of cost-saving principle from Industrial Ecology: residual from one process is utilized as productive input for another process and locational choices pursue these advantages.

The results from the laboratory fermentations reported in the previous sections of this report (please see Results 1 and 2) indicate the relative conversion efficiencies and cost comparisons between processes using potato waste versus conventional processes and inputs. Key findings are highlighted below for xanthan and PLA production.

Items 2 and 3 are closely related. The assumption for item 2 is that, even though some potato waste is distributed as livestock feed and therefore generates positive value, shipping costs are so high that most is too expensive to ship as livestock feed so it becomes a disposal burden. Item 3 focuses on the potential changes in the long run and the likelihood that the advantage of using potato waste as an input will increase as sugar/corn and other conventional materials become more scarce. It must be stressed that the potato inputs are obtained from the waste stream after the primary food outputs have been processed from the potatoes. In the case of corn as an input, not only is demand growing for production of ethanol but this use precludes its use as food for humans and/or livestock. Use of corn as an input for xanthan or PLA production precludes these other highly-valued uses. In contrast, using potato waste does not displace other production processes as it remains after primary food processing. In the case of xanthan production from potato waste (being xanthan is used as food ingredient), this could be defined as secondary food production from these same inputs.

Items 4 and 5 are variables that serve as caveats on the ability to predict commercial-scale viability from laboratory findings. Item 4 qualifies the results in that proprietary information on total production costs is not available so that the context is lacking for determining the relative importance of materials costs as a percentage of TCP. In the case of xanthan, lower materials costs using potato waste as the raw material could be significant in reducing costs and increasing profits. In the case of PLA, the conversion efficiencies are inferior so any cost savings would have to originate in the relative cost of potato waste as an input being even less than the relative inferiority of conversion efficiencies. For example, if conversion efficiencies are only half what they are for

conventional inputs, cost savings could still be realized if costs of potato waste as an input is even less than half that of conventional inputs. As is reported below in the section containing specifics on PLA and previously in the report, many operating costs (energy, labor, etc.) also are higher given the poor conversion efficiencies of potato waste into PLA.

For item 5, the conversion efficiencies for xanthan production yield cost savings in materials even without considering the lower cost of a kg of potato waste as an input compared to a kg of conventional inputs. The laboratory findings suggest that other operating costs are at most equal to, and likely less than, the operating costs for producing xanthan with conventional materials. Therefore the savings are likely to not only occur for material inputs but other operating costs as well.

Item 6 emphasizes a growing awareness of the benefits of applying the principles of ecology to industrial production. Traditionally, by-products or residuals from a production process have often been regarded as merely a disposal challenge. Industrial ecology recognizes the Laws of Conservation of Matter and Energy in searching for ways to utilize the potential in matter and energy to be multiple times. Eco-Industrial Parks are designed to locate multiple processes so that the residuals from one manufacturing process can be used as an input in another. Locating a facility converting potato waste to xanthan or PLA adjacent to or near a potato processing facility would be an application of these principles with great economic potential.

Prospects for Net Social Value: General Principles

It is possible that bio-conversion of potato waste into xanthan or PLA could generate net social benefits regardless of whether these processes are commercially viable. The potential for positive externalities emanates from two sources:

1. reducing environmental costs of potato waste disposal and
2. alleviating the pressure on inputs that are conventional media for bio-conversion to xanthan and PLA but are also inputs in subsidized markets for food and fuel.

1. Disposal Costs: There will be social and environmental benefits from using potato waste being it has disposal costs. Again, while some potato waste is distributed as livestock feed, most is too expensive to ship as livestock feed so becomes a disposal burden.

Whether the benefits of finding an outlet for a disposal burden are sufficient to yield positive net benefits depends on their relative importance compared to the private components of costs and benefits (profits.) If revenues exceed costs, as is estimated for xanthan production, then the alleviation of a disposal burden (that can translate into environmental costs) only adds to the social net benefits when these social cost reductions are factored in. For PLA, even though the conversion does not seem to be commercially viable on a private for profit basis, net benefits could be enjoyed depending on the relative magnitude of commercial losses and decreases in social costs.

Using Potato Waste as an Input for Xanthan and/or PLA production exemplifies a basic principle from Industrial Ecology: there can be both commercial and social gains from using residuals from one process as an input for another productive process.

2. Benefits of Reducing Pressures on other Subsidized Inputs: Benefit Cost Analysis from a long-term perspective needs to consider the importance of potato waste as substitute input for conventional inputs that have higher opportunity costs. Corn and other raw materials used as conventional inputs for xanthan and/or PLA are scarce being they have high valued competing uses. Corn, for example, is not only in high demand as a food source but also as an input for PLA and xanthan production. Furthermore, considering the increasing demand for corn as an input for bio-fuels strengthens the long-term potential for potato waste to yield net benefits as a substitute raw material for PLA and xanthan production. If these reductions in social costs combined with the social benefit of taking pressure off of corn and other inputs with competing uses, are sufficient the social gain could outweigh commercial losses. Put another way, if these social gains were to be captured in a subsidy for PLA production, they could be great enough to make PLA production from potato waste profitable when it would not be in the absence of a subsidy.

Prospects for Commercial Viability: Results for Xanthan

The specifics on cost savings from xanthan production using potato waste are based on the data shown in Table 1 - Result 2 and Figures 1 and 2 - Result 3.

	Xanthan		Lactic Acid	
	Minimum	Maximum	Minimum	Maximum
Carbon source (kg/m ³)	20.00	100.00	30.00	170.0
Carbon source (\$/kg)	0.06	0.35	0.06	0.35
Nitrogen source (kg/m ³)	0.300	2.500	1.000	21.00
Nitrogen source (\$/kg)	0.21	1.50	0.21	1.50
Phosphorus source (kg/m ³)	1.000	3.500	0.150	5.000
Phosphorus source (\$/kg)	0.10	0.42	0.10	0.42
Magnesium source (kg/m ³)	0.100	2.000	0.200	0.750
Magnesium source (\$/kg)	2.30	2.50	2.30	2.50
Yield % (Kg/Kg Carbon source)	40	70	21	50
References: Chemical Marketing Reporter, Biocompare Database, Fine Chem. Trading Ltd, .Milling and Baking News, New York Board of Trade, USDA Agricultural marketing Services/ Grain and Feed Market News, USDA-NASS, List of References, and results of this study.				

Table 1 - Result 3. Reported Nutrient Requirements, Costs and Yields of Lactic Acid and Xanthan Fermented on Standard Media. Carbon sources: glucose, lactose, starch and molasses; Nitrogen sources: (NH₄)₂SO₄, peptone and yeast extract; Phosphorus sources: KH₂PO₄, K₂HPO₄, Na₂HPO₄; Mg sources: MgCl₂ and MgSO₄.

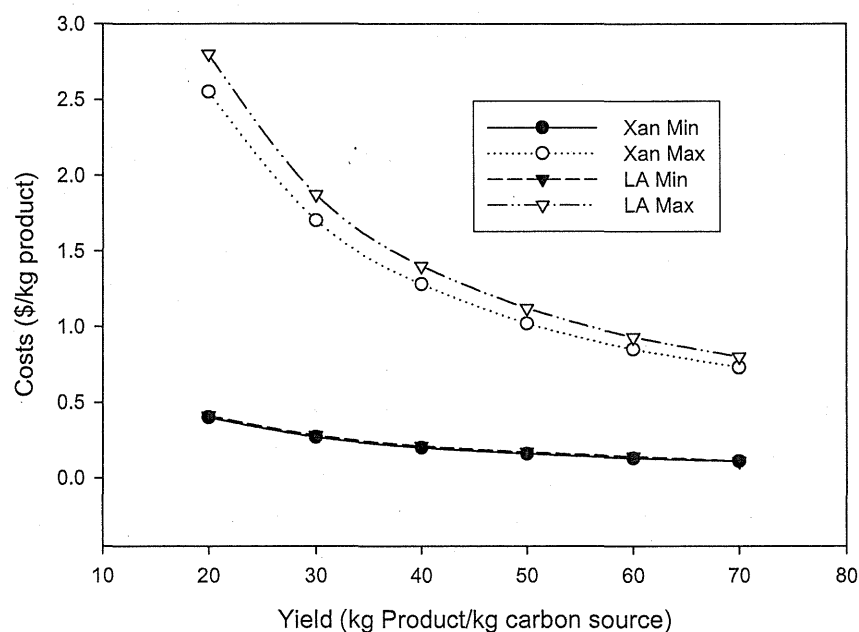


Figure 1 - Result 3. Estimated Minimal and Maximal Media Costs of Lactic Acid and Xanthan Fermented on Standard Media.

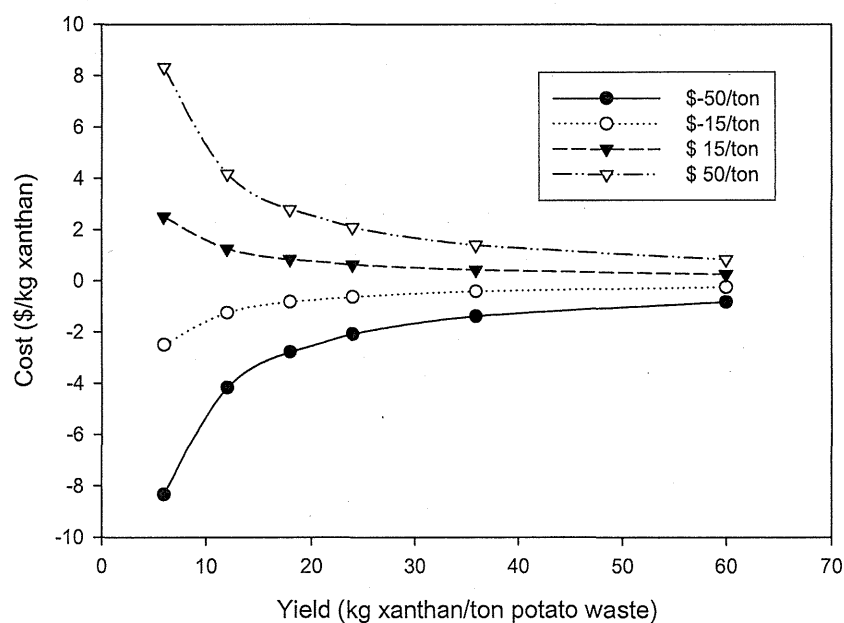


Figure 2 - Result 3. Estimated Media Costs of Lactic Acid and Xanthan Fermentation on Potato Waste at the Waste Cost in Range -\$50.00 to \$50.00/ton.

Based on the findings of the research team working on Result 2, the cost savings for material inputs are determined to be in range 5 to 15% of TCP. This evidence suggests that xanthan production can be more profitable using potato waste as the carbon

sources rather than inputs currently used. The cost savings should be even greater assuming potato waste can be obtained at lower cost than these other inputs. Good xanthan yields in the solid and semi-solid fermentations employed in this research provide the key evidence of potential for increased profits.

Table A in Appendix A - Result 3 attempts to put these cost savings in context by showing the relative magnitudes of cost components for chemical production processes based on the literature across various products. These cost estimates are taken from a classic source on economics of chemical engineering, "Plant Design and Economics for Chemical Engineers" by Peters and Timmerhaus. Raw materials are shown to range from 10-50% of total production cost across chemical production processes. Using the median of this range provides a context for how significant a 20% savings in material costs could be in total commercial costs and profitability. For example, saving 20% on a cost component that comprises 30% of total production costs would yield a cost reduction of 6%. If materials cost comprise only 10% of total production costs, than use of potato waste would mean a 2% cost savings, but at 50% the cost savings from use of potato waste would lead to a 10% reduction in total production cost.

The proprietary nature of cost, revenue and profit figures make it difficult to paint a complete picture of the commercial viability of xanthan production using potato waste as the media. However, the revenue side can be understood given available data on the price of xanthan in the marketplace. The price of xanthan per pound over the last couple of years has been in the \$5 range for food grade xanthan, and \$4.50 per pound for industrial grade xanthan. While profit margins are not available a 5% to 10% decrease in production costs estimated based on these laboratory findings could yield a considerable increase in profit margins.

Further information on chemical prices can be found on the web at the homepage of ICIS, and the Chemical Business Americas webpage, www.icispricing.com. While this site provides price listings for numerous chemicals, a current price for xanthan is not provided.

Prospects for Net Social Value: Results for Xanthan

The section above on potential net benefits discusses conditions under which bio-conversion of potato waste could yield positive net benefits to society. Given that the social effects are conceptualized as positive externalities, this potential is obviously stronger when the process is expected to generate profit on commercial basis as well. Reducing social/environmental costs by alleviating a disposal burden and taking pressure off other resources that are becoming increasingly scarce improves the results in terms of economic efficiency.

In the absence of market failures or market distortions, the conclusion that xanthan production from potato waste is profitable in the private market would also imply that xanthan production increases net benefits to society from the standpoint of benefit cost analysis. The positive externalities of improving potato waste disposal and

the existing distortions in the markets for conventional bio-conversion media (especially corn, given the subsidies in related markets for food and ethanol production) suggest that net social value could be increased substantially. This conceptual case is strong but it would be difficult to place empirical estimates on these net benefits. It is beyond the scope of this study to place a magnitude on the external benefits from avoiding disposal costs and reducing pressures on corn as an input for food and fuel production

Prospects for Commercial Viability: Results for Poly Lactic Acid

The information on costs for producing PLA using potato waste is provided in Tables 2 and 3 - Result 3. Based on the findings of the research team led by Dr. Chang, the costs of producing PLA using potato waste appear to be substantially higher than with conventional media. The evidence generated in the laboratory suggests that PLA production using potato waste as the raw material would be less profitable than production with inputs currently used. In fact, the evidence suggests PLA production using potato waste would cause a commercial loss, even with the potato assigned zero input cost.

In particular, the summary cost figures in Table 2 - Result 3 show that even the partial accounting for production costs fall well short of revenue per kg. The price of commercial lactic acid from Sigma-Aldrich (which is 85% pure) is \$ 80.59 per kg compared to our costs of \$ 92.88. Similarly, the price of commercial Poly (DL-Lactide) from Sigma-Aldrich (which is pure) is \$ 20,000 per kg compared to our raw poly(lactic acid) of \$ 145.49. These costs (\$145.49/kg) are laboratory scale costs which include operational cost (chemicals, power and water) excluding labor and start-up costs (glasswares, instrumentation and their installments). In other words, costs exceed revenue even without assigning costs to labor and other cost components. The cost information found in the literature for LA and PLA production and the ranges provided in Peters and Timmerhaus that omitted costs would be substantial perhaps ranging as high as 50% of total production cost. This would imply that the costs recorded in the laboratory might need to be doubled to accurately reflect production costs at a commercial scale.

A noteworthy result from Table 3 - Result 3 is that the total media costs are 28% of total cost, without assigning any cost to the potato waste as a raw material. If conventional media were used instead of potato waste at zero cost, the media cost would be above the median rate reported in the book by Peters and Timmerhaus. This corroborates the conclusion that the bioconversion of potato waste into PLA is a less efficient use of media than is currently occurring using conventional media.

Table 2 - Result 3. Summary of Costs Involved in Bioconversion of Potato Waste to Poly-lactic Acid

Descriptions	\$ (Lactic acid/kg)	\$
Production of Lactic acid (Fermentation)(90 % conversion)	55.69	
Purification of Lactic acid	31.11	
Concentration of Lactic acid (Evaporation)	4.16	
Lactic acid Analysis (24 samples)(colorimetric)	1.92	
Total Lactic acid production cost	92.88	
Lactic acid (70 % conversion of Lactic acid)		132.68
Polymerization		9.74
Polymerization characterization (triplicate)		3.07
Total cost to synthesize polymers per kg (Raw polymer)		145.49

Note: The price of commercial lactic acid from Sigma-Aldrich (which is 85% pure) is \$ 80.59 per kg compared to our costs of \$ 92.88. Similarly, the price of commercial Poly(DL-Lactide) from Sigma-Aldrich (which is pure) is \$ 20,000 per kg compared to our raw poly(lactic acid) of \$ 145.49.

The above-mentioned costs (\$145.49/kg) are laboratory scale costs which include operational cost (chemicals, power and water) excluding labor and start-up costs (glasswares, instrumentation and their installments).

Table 3 - Result 3. Detailed Costs Involved in Bioconversion of Potato Waste to Poly-lactic Acid

Fermentation Media	MRS Media Composition (g/L)	\$ / Kg	\$/kg PLA
Lactobacillus amylovorus (Bacteria)			
Potato waste	20.00		
Proteose peptone No. 3	10.00	6.587	
Beef extract	10.00	8.517	
Yeast Extract	5.00	1.035	
Tween 80	1.00	0.349	
Ammonium citrate	2.00	3.500	
Sodium acetate	5.00	4.086	
Magnesium sulfate	0.10	0.053	
Manganese sulfate	0.05	0.032	
Dipotassium phosphate	2.00	2.003	
Total Media Cost			26.163

Continued on the Following Page

Power (Autoclave and Boiling) (1000 watt for 35 min)	0.5 hrs	0.795
Heating (Electricity)	48 hrs	27.545
Centrifuge (Electricity)	1.5 hrs	0.424
Water	40 Gallons	0.758

Total Fermentation Cost **55.686**

Purification of Lactic acid (100%):

Decolorization		17.642
Charcoal (g)	185.20	4.208
Power (Rejuvenation of charcoal) (5.5 KW for 3 hours)	16500.00	2.747
Chemicals (Sodium hydroxide)(463 g)	463 (g)	10.418
Water	48.7 Gallons	0.270

Removal of multivalent ions		16.949
Resin (use 100 times, \$ 52.51 kg) (g)	185.20	9.723
Chemicals (Hydrochloric acid) (\$8.9875/L)		3.680
Chemicals (Sodium hydroxide)		3.330
Water		0.216

Electrodialysis (Conventional)		3.000
Power		0.556
Chemicals (Sodium sulfate)		2.037
Water		0.407

Electrodialysis (Bipolar)		6.479
Power		0.662
Chemicals (Sodium sulfate)		2.037
Chemicals (Sodium hydroxide)		0.417
Recovered (Sodium hydroxide)		10.00
Water		0.405

Total purification of lactic acid **31.112**

Evaporation (78%):	Duration (hrs)	
Vacuum pump	1.000	
Heater (Electricity)	1.000	
Rotavapor	1.000	
Water	1.000	
Evaporation Cost (KG of Lactic acid)		4.162

Lactic acid Analysis (24 samples)(Colorimetric)	\$0.08/samples	1.920
Total Lactic Acid Production Cost (1 KG)	92.880	132.685

Continued on the Following Page

Polymerization (70%) conversion:	Duration (hrs)	
Heater (Electricity)	25.000	1.929
Pump	25.000	0.400
Water	25.000	0.943
Nitrogen Gas	25.000	0.402
Tin (II) hexanoate (\$14.31/100g)(0.01 mole%)(Fisher)	0.540g	0.110
1, 4 Butanediol (S36.5/L) or Succinic anhydride (\$30.50/kg)(1 %mole)(Sigma)	12.5 ml	0.651
1,6 hexamethylene diisocyanate (\$185.70/kg) (2% of prepolymer)(Fisher)	20 g	5.306
Polymerization cost	9.741	9.741

Polymerization characterization

(Acid value, molecular weight, melting point, and FTIR)

Triplicate analysis	3.073	3.073
Total Cost to get 1 KG of PLA	105.693	145.499
DL-Lactic Acid (85% pure)(Fisher Scientific)(IL)		80.590
Poly (DL-Lactide)(Sigma)(Pure) \$100/5G		20000.000

Prospects for Net Social Value: Results for Poly Lactic Acid

The differential appears to be so large as to cast doubt on the profitability of commercial production of PLA using potato waste as the medium, even if this was subsidized to account for the positive externality of reducing the disposal costs of potato waste. However it is plausible in the long run, that the positive externalities of reducing pressure on corn and other conventional media used to produce PLA could be sufficiently large to warrant production with potato waste. Again it is beyond the scope of this study to assign empirical estimates to these social and environmental benefits, but it could generate social net benefits and if subsidies were commensurate with high external benefits, it could become commercially profitable due to large external financial support, along the lines of current subsidies to ethanol production.

The interconnections go further in that much of the impetus for PLA production using biological media comes from the desire to meet the demand for plastic without relying on petroleum to produce these synthetics. If trends continue it is plausible that PLA production using potato waste could become viable as an "indirect" substitute for petroleum. If bio-fuels grow as projected, the preferred use of corn and other conventional media could be for energy so that potato waste serves as the input for PLA production instead of either corn or oil. Just as bio-fuels subsidies are being justified to take the pressure off of conventional energy sources, so to PLA production using potato waste could be justified to take the pressure off conventional raw materials for plastics.

The costs (\$) involved in synthesis of poly (lactic acid) are categorized as follows (per kg). The cost of bacteria is not included because the bacteria can be reused from the previous fermentation batch. Raw material (potato waste) is assumed free. Fermentation is performed at 60 g/L of dry weight of potato waste (i.e. 240 g/L raw potato waste) at 30 or 37°C. About 54 g/L of lactic acid is produced in 48 hours from the potato waste. Ingredients for fermentation media is recycled from the previous runs and only 25% of fresh ingredients is replenished to reduce the costs of the process.

Result 3 - LITERATURE REVIEW

Abstracts of Selected Sources on Xanthan

These abstracts are provided verbatim as provided at the websites cited below.

“Industrial Uses of Agricultural Materials.” Economic Research Service, U.S. Department of Agriculture. (1997): 7 Nov. 2006
<http://search.usda.gov/search?q=cache:nqVZE_D1uWEJ:http://usda.mannlib.cornell.edu/reports/erssor/specialty/ius-bb/ius7f.asc+economic+benefits+xanthan&access=p&output=xml_no_dtd&ie=UTF-8&client=usda&num=10&site=usda&oe=UTF-8&proxystylesheet=OC>.

“The three major U.S. producers are Archer Daniels Midland Company (ADM); Cargill, Inc.; and Haarmann and Reimer Corporation (H&R), a subsidiary of Bayer. Currently, most citric acid in the United States is produced via submerged (deep-tank) fermentation of corn-derived glucose or dextrose. Other carbohydrate sources, such as potato, sweet potato, and wheat starch, may be utilized, but on a smaller scale due to their higher cost relative to cornstarch. H&R, the only major producer not vertically integrated back to feedstocks, is looking to sell its citric acid plant. ADM, on the other hand, recently announced plans to build a new bioproducts plant in Cedar Rapids, Iowa, that will produce citric acid, lactic acid, lysine, xanthan gum, and glycerine. In recent years, vertical integration and large facilities, which take advantage of economies of scale, have become very important to the profitable operation of fermentation facilities.”

Lachke, Anil, “Xanthan - A Versatile Gum.” Resonance (2004): 1-9. 6 Nov. 2006
<<http://www.ias.ac.in/resonance/Oct2004/pdf/Oct2004p25-33.pdf>>.

“Many types of natural gums can be acquired from a variety of plant sources. The collection of the gums is expensive and calls for skilled laborers. The backbone of xanthan is made up of D-glucose units. The production of xanthan is a complicated process that involves a multienzyme system. The industrial production of xanthan uses inexpensive substrates and nutrients. A special relationship between xanthan gum and plant polysaccharides exists as well.”

R. A. Moraine, P. Rogovin. “Kinetics of the Xanthan Fermentation.” Biotechnology and Bioengineering. 15.2 (2004): 225 - 237. 9 Nov. 2006
<<http://www3.interscience.wiley.com/cgi-bin/abstract/107618418/ABSTRACT>>.

“Xanthan gum, a heteropolysaccharide with unusual and useful properties, is now

produced commercially by fermentation with *Xanthomonas campestris* NRRL B-1459 in a medium containing glucose, minerals, and a complex nitrogen source - distillers' dried solubles (DDS). Understanding the kinetics of the fermentation should contribute to process improvements and increase the market potential for the gum. Earlier studies showed that although DDS determined initial growth rate, growth was stopped by some mechanism other than substrate exhaustion, probably an effect related to product formation. Product formation did not require active growth, but its rate increased with cell concentration. Specific product formation rate declined at high viscosities. Varying glucose concentration from 0.5 to 5.0% and dissolved O₂ tension between 20 and 90% air saturated had no effect on the rates, but pH had to be maintained near 7 and temperature near 28°C to permit continued product formation. Xanthan yield could be explained by the energy required for growth and polymerization, that energy coming from dissimilation of the part of the carbohydrate substrate not converted to polymer.”

Stredansky M.; Conti E. “Xanthan production by solid state fermentation.” Process Biochemistry. 34.6 (1999): 581-587. 11 Nov. 2006
<<http://www.ingentaconnect.com/content/els/00329592/1999/00000034/00000006/art00131>>.

“*Xanthomonas campestris* strains were cultivated on a variety of solid substrates in order to evaluate their ability to produce the exopolysaccharide xanthan. The solid substrates used were agro-industry wastes or by-products, including spent malt grains, apple pomace, grape pomace, and citrus peels, which can be obtained at a very low cost. With most substrates, xanthan yields were comparable to those obtained from conventional submerged cultivation. The basic characteristics of the process were studied on solid substrates prepared by impregnating the inert support (spent malt grains) with a solution of nutrients. Fermentations were performed on the laboratory scale either under static conditions or in rotating cultures. The production conditions were optimized so as to achieve polymer yields ranging from 32.9 to 57.1 g l⁻¹, as referred to the liquid volume impregnating the various substrates. The products were analysed by NMR spectroscopy, revealing a composition consistent with that of commercial xanthan.”

Thompson, Steve, “Greener for bio-tech co-ops?” Rural Coopertatives. 71.2 (2004): 7 Nov. 2006. <<http://www.rurdev.usda.gov/rbs/pub/mar04/greener.htm>>.

“The manufacture of new products that are bio-based may replace or supplement products that are made with conventional, non-renewable materials. This could provide new opportunities for farmer co-ops to add value to their crops. Many useful substances can be extracted from these, including xanthan gums, lignins, and complex sugars and proteins. In order for this to be successful, there needs to be an improved demand for bio-based products.”

“Use of Agricultural Wastes for Xanthan Production by *Xanthomonas campestris*.” Journal of Industrial Microbiology and Biotechnology. 21.4-5 (1998): 242-246. 9 Nov. 2006. <<http://www.springerlink.com/content/adry2br2wacag1dh/>>.

“Four different acid-hydrolyzed wastes, from melon, watermelon, cucumber and tomato were compared for xanthan production. Growth of *Xanthomonas campestris*, xanthan biosynthesis, kinetics and chemical composition were investigated. Both growth and

xanthan production were dependent on the acid hydrolysate concentrations and available nitrogen. Melon acid hydrolyzed waste was the best substrate for xanthan production. Exopolysaccharide obtained throughout this study was compared to commercial xanthan, showing a very similar chemical composition. Acid hydrolyzed wastes are proposed as a new carbon source for xanthan production."

"Xanthan Gum Production from Waste Sugar Beet Pulp." Bioresource Technology. 70.1 (1999): 105-109. 9 Nov. 2006
<<http://www.ingentaconnect.com/content/els/09608524/1999/00000070/00000001/art00013>>.

"The feasibility of using waste sugar beet pulp (WSBP) as a supplemental substrate for xanthan gum production from *Xanthomonas campestris* was investigated. For the range of incubation periods and contact times investigated (1 to 5 days), there were no differences in the mean WSBP degradation. The mean WSBP degradation was significantly greater for incubation temperatures of 28°C as compared to incubation temperatures of 32°C. WSBP degradation was insensitive to the contact temperatures evaluated. These results indicate that optimal cell growth might optimize WSBP degradation. Xanthan gum production from the WSBP supplemented cultures was significantly greater than the unsupplemented production medium. Based on a preliminary analysis, the use of WSBP for xanthan gum production has the potential to be a cost-effective supplemental substrate to produce non-food grade xanthan gum."

"Xanthan Production - Effect of Agitation." Bioprocess and Biosystems Engineering. 15.1 (2004): 35-37. 9 Nov. 2006
<<http://www.springerlink.com/content/q4u3w40122625736/>>.

"Glucose fermentation by *Xanthomonas campestris* to xanthan was investigated in a stirred tank fermenter for the effect of agitation on cell growth and product synthesis. In the range investigated for speed between 300 to 500 rpm, these factors registered a rise with increasing degrees of turbulence, the experiments being carried out over a span of two days."

Krause, Carey. "Demand for xanthan on the rise, leading prices out of the doldrums. (Markets: Pharmaceuticals & Fine Chemicals).(Brief Article)(Statistical Data Included)." Chemical Market Reporter. (August 2002).

< http://www.findarticles.com/p/articles/mi_hb4250/is_200208/ai_n13216062>.

"The market for xanthan gum is expected to tighten as oil field sales begin to rebound. Although pricing remains depressed, healthy demand, combined with tight supply, could help producers capture price improvements in coming months. Manufacturers are beginning to see an uptick in the market. Aiding the rebound is the food industry, which outperforms all other market segments for xanthan. Xanthan gum prices are estimated at \$3.20 to \$3.70 per pound, according to Dennis Seisun of IMR International, a San Diego-based hydrocolloid consultancy."

"*Xanthan Demand on the Rise, but Price Is Still an Issue. (Markets: Pharmaceuticals & Fine Chemicals).(Brief Article).*" Chemical Market Reporter. (December 2001).
<<http://www.highbeam.com/doc/1G1-81007462.html>>.

"After a two year price slump in the xanthan gum market, producers are optimistic that improvements could be on the horizon. A surge in consumption in the oil industry is helping market recovery, while demand for food-grade applications appears to be rebounding. Virtually across the board, producers are investing in or considering capacity expansions to stay competitive. Yet even with the recent uptick in demand, prices remain depressed."

Mirasol, Feliza. "Xanthan Gum Market Eases As Producers Add Capacity." Chemical Market Reporter. (November 1999).

<<http://www.highbeam.com/doc/1G1-57578526.html>>.

"Debottleneckings and expansions by major producers have boosted supplies of xanthan gum, ending tightness that plagued the market from late 1997 through most of 1998. As a result, prices have dropped. Suppliers quote a price of \$5 per pound for food-grade xanthan, though prices may fluctuate, depending on quality and intended end use. Industrial Market Research (IMR) analyst Dennis Seisun estimates that prices of food-grade xanthan range from \$4.50 to \$5 per pound."

Lerner, Matthew. "Xanthan market upturn brings higher prices and more supply." Chemical Market Reporter. (October 1997).

<<http://www.highbeam.com/doc/1G1-19917343.html>>.

"After suffering downward pressure for years, prices for xanthan are rebounding and producers are expanding to meet growing demand from a variety of sectors. Xanthan is a semi-synthetic hydrocolloid, derived from fermentation, which has applications in food and industrial sectors. Market leader NutraSweet Kelco Company, a unit of Monsanto Company, is increasing prices by 10 percent effective November 1 and says it will expand production. The world's three other xanthan suppliers--Jungbunzlauer, Rhone-Poulenc and SBI--are expected to join in the price increase."

"Xanthan gum. (Markets: Chemical Commodities).(prices, market information)(Brief Article)(Statistical Data Included)." Chemical Market Reporter. (August 2002).

<<http://www.highbeam.com/doc/1G1-90892614.html>>.

"Prices for xanthan gum range from \$3.20 to \$3.70 per pound. Specialty grades are closer to \$3.70 or above, but the bulk of the market is regular grade. The average represents a further drop from last year's already depressed price level of \$3.50 to \$4.00 per pound on food grade product. The market for xanthan gum is expected to tighten as oil field sales begin to rebound. Although pricing remains depressed, healthy demand, combined with tight supply, could help producers capture price improvements in coming months."

"Xanthan gum has great market potential. (Market Report)." China Chemical Reporter. (October 2002). <<http://www.highbeam.com/doc/1G1-93027896.html>>.

"Xanthan gum is a biological gum with unique properties. It is compatible with various foods, pharmaceuticals and cosmetics. The FAO and the WHO of the United Nations approved xanthan gum as a food additive in 1983 and the production and development of xanthan gum has therefore been stimulated and promoted."

Abstracts of Selected Sources on Polylactic Acid

These abstracts are provided verbatim as provided at the websites cited below.

Drumright, R.E., P. R. Gruber, D. E. Henton. "Polylactic Acid Technology." Advanced Materials. 12.23 (2000): 1841-1846. 10 Nov. 2006

<<http://www3.interscience.wiley.com/cgi-bin/abstract/76502855/ABSTRACT?CRETRY=1&SRETRY=0>>.

"Polylactic acid is proving to be a viable alternative to petrochemical-based plastics for many applications. It is produced from renewable resources and is biodegradable, decomposing to give H₂O, CO₂, and humus, the black material in soil. In addition, it has unique physical properties that make it useful in diverse applications including paper coating, fibers, films, and packaging."

Garlotta, D.V., Donane, W., Shogren, R.L., Lawton Jr, J.W., Willett, J.L. "Mechanical and Thermal Properties of Starch-filled Poly (D,L-Lactic Acid)/ Poly (Hydorxy Ester Ether) Biodegradable Blends." Journal of Applied Polymer Science." 88 (2003): 1775-1786. 9 Nov. 2006

<http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=129365>.

"Cornstarch has been blended with poly (lactic acid) in order to improve its biodegradability and reduce the cost of the final product. Introduction of cornstarch into poly (lactic acid) leads to products with reduced, and usually unsatisfactory, mechanical properties and processability. A new plastic, PHEE was introduced into starch/poly (lactic acid) blends in order to improve their mechanical properties and processability. PHEE was found to enhance the mechanical properties and processability of starch/poly (lactic acid) blends to satisfactory levels. By introducing PHEE into starch/poly (lactic acid) blends, one can maximize the starch content of these blends, increasing the biodegradability and decreasing the cost of these blends without sacrificing their mechanical properties and processability."

Lunt, J. "Large-scale production, properties and commercial applications of polylactic acid polymers." Polymer Degradation and Stability. 59.1 (1998): 145-152. 11 Nov. 2006
<<http://www.ingentaconnect.com/content/els/01413910/1998/00000059/00000001/art00148>>.

"Polylactic acids (PLA) are not new polymers. However, recent developments in the capability to manufacture the monomer economically from renewable feedstocks have placed these materials at the forefront of the emerging biodegradable plastics industry. Increasing realisation of the intrinsic properties of these polymers, coupled with a knowledge of how such properties can be manipulated to achieve compatibility with thermoplastics processing, manufacturing, and end use requirements has fueled technological and commercial interest in PLA products. This paper discusses the various technologies being used to produce polylactic acids. In addition, attention is drawn to how monomer stereochemistry can be controlled to impart targeted utility in the final polymers. Specific applications are described to illustrate further the range of properties that can be developed by utilising both the basic monomer/polymer chemistries in

combination with post-modification techniques. Finally, the biodegradation mechanism of polylactic acids will be discussed and contrasted with other biodegradable polymers.”

Rathin Datta, Shih-Perng Tsai, Patrick Bonsignore, Seung-Hyeon Moon, James R. Frank. “Technological and economic potential of poly(lactic acid) and lactic acid derivatives.” FEMS Microbiology Reviews. 16 (1995): 221. 10 Nov. 2006 <<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6976.1995.tb00168.x>>.

“In the past, efficient and economical technologies for the recovery and purification of lactic acid from crude fermentation broths and the conversion of lactic acid to the chemical or polymer intermediates had been the key technology impediments and main process cost centers. The development and deployment of novel separations technologies, such as electrodialysis (ED) with bipolar membranes, extractive distillations integrated with fermentation, and chemical conversion, can enable low-cost production with continuous processes in large-scale operations. The use of bipolar ED can virtually eliminate the salt or gypsum waste produced in the current lactic acid processes. Thus, the emerging technologies can use environmentally sound processes to produce environmentally useful products from lactic acid. The process economics of some of these processes and products can also be quite attractive. In this paper, the recent technical advances in lactic and polyactic acid processes are discussed. The economic potential and manufacturing cost estimates of several products and process options are presented. The technical accomplishments at Argonne National Laboratory (ANL) and the future directions of this program at ANL are discussed.”

“Assessing Economic Implications of New Technology: The Case of Cornstarch-Based Biodegradable Plastics.” Keith H. Coble, Ching-Cheng Chang, Bruce A. McCarl, Bobby R. Eddleman. Review of Agricultural Economics Vol. 14, No. 1 (Jan., 1992), pp. 33-43 <<http://links.jstor.org/sici?sici=10587195%28199201%2914%3A1%3C33%3AAEIONT%3E2.0.CO%3B2-F&size=LARGE>>.

“A nonlinear mathematical programming model of the agricultural sector is utilized to examine the potential economic impacts of producing cornstarch-based biodegradable plastics. The model simulates market conditions in terms of prices, quantities, farm program expenditures, and resource usage while also generating social welfare measures. Six alternative biodegradable plastic production and technology scenarios are examined. The results indicate that biodegradable plastics production, even under optimistic scenarios, does not have major impacts on the agricultural sector. Producers gain welfare benefits, largely through corn and sorghum production. Biodegradable plastics production causes increased farm program expenditures as well. A net welfare cost to the agricultural sector would need to be met by consumer and environmental gains due to biodegradable plastics use.”

Corinne Gangloff “Degradable Plastic Demand to Reach 500 Million Pounds in 2010.” Market Wire. November 2006.

<http://www.findarticles.com/p/articles/mi_pwwi/is_200611/ai_n16822344>.

“Demand for degradable plastic in the U.S. is forecast to expand nearly 17 percent yearly to 500 million pounds in 2010, valued at \$610 million. Average prices will continue to decline as a result of higher capacity and greater production efficiencies, as well as price

mixes reflecting fastest growth for the lowest priced resins. Myriad opportunities are anticipated based on a more competitive pricing structure, high feedstock costs for petroleum-based polymers, and growing environmental, governmental and consumer initiatives for greater use of sustainable resources. Degradable plastic applications are also being expanded by enhanced performance properties brought about by more sophisticated polymerization and blending techniques. These and other trends are presented in "Degradable Plastics," a new study from The Freedonia Group, Inc., a Cleveland-based industry market research firm."

"Compost-Ready Packaging: Waste Management Strategies and Surging Oil Prices Are Helping Bioplastics Manufacturers to Increase Their Market Presence." Chemistry and Industry. (October 2006). <<http://www.highbeam.com/doc/1Y1-100253857.html>>.

"Entrepreneurs often see opportunity in adversity. But the bioplastics industry has been waiting patiently in the wings for market forces to align in its favour for two decades. Dwindling fossil fuel reserves, limited landfill capacity and consumers with a thirst for sustainability without compromising on lifestyle mean that biopolymers--renewable polymers--are now edging into the marketplace. According to the industry association European Bioplastics, consumption in Europe is still dominated by petrochemical-based plastics."

"New from the Freedonia Group: US Degradable Plastics Demand to Reach 370 Million Pounds in 2008. " Business Wire. (December 2004).

<<http://www.highbeam.com/doc/1G1-126122008.html>>.

"Degradable plastic demand in the US is projected to grow 13.7 percent annually to 370 million pounds in 2008, valued at \$490 million. Gains will be stimulated by widening applications brought about by a more competitive pricing structure, improved performance characteristics, the continued development of degradability standards, and consumer education as to the benefits of these materials. Prospects will continue to improve as markets emerge and the prices and properties of degradable plastics begin to approximate those of conventional polymers."

Summaries of Selected Articles on PLA

Wee, Y., Kim, J., and Ryu, H. "Biotechnological Production of Lactic Acid and Its Recent Applications." Food Technology and Biotechnology. 44 (2) 163-172. (2006)
This is a well integrated summary of major findings in the field of LA (lactic acid). It is a good beginning for those at the stage of designing their biotechnological LA production. The article provides informative tables regarding:

1. Microorganisms used for recent investigations of the biotechnological production of LA
2. Reports in the literature about recent investigations on the biotechnological production of LA from cheap raw materials and
3. Reports in the literature about recent investigations on the biotechnological production of LA by different fermentation approaches.

The paper presents information from economic forecasts. Particularly it states the following:

- the currently worldwide consumption of LA is estimated to be 130,000-150,000 metric tons per year (ref: 11, 95)
- LA consumption in chemical applications including PLA polymer and new “green” solvents is expected to expand 19%/year (Ref: 96)
- the commercial prices of food grade LA range between 1.38 USD/kg (for 50% purity) and 1.54 USD/kg (for 88% purity) (ref: 11, 95)
- Technical grade LA with 88% purity has been priced as much as 1.59 USD/kg (ref: 96)

On an industrial scale the predictions are as follows:

- the manufacturing cost of LA monomer will be less than 0.8 USD/kg
- The selling price of PLA should decrease by half from its present price of 2.2 USD/kg.

It also contains a summary of the cost analysis conducted by Datta et.al (see below) which calculates the base manufacturing cost of lactic acid as 0.55 USD/kg (this study was sensitive to various factors such as plant size, raw material cost and capital investment) (ref: 4)

Rathin Datta, Shih-Peng Tsai, Patrick Bonsignore, Seung-Hyeon Moon, James R. Frank. “Technological and economic potential of poly(lactic acid) and lactic acid derivatives.” FEMS Microbiology Reviews. 16 (1995): 221. 10 Nov. 2006 <<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6976.1995.tb00168.x>>. (Abstract provided above.) This paper presents current and future perspectives on LA and PLA. Fields in which these two products play an important role are discussed in terms of current capacities and predictions of future potential. For example, prior to 1991, the annual US consumption of LA was established at 18,500 metric tonnes, with domestic production of approximately 8,600 tonnes, by Sterling chemical and the rest imported from EU and Brasil. US food and food related industries account for 85% of LA consumption. Worldwide production volume of LA by 1990 had grown to approximately 40,000 ton/year with two major producers, CCA Biochem b.v. from the Netherlands and its subsidiaries in Brasil and Spain which resulted in the global production in 1991 to 400,000 tonnes/year.

Table 1 in this paper contains information about potential products that are derived from LA, their use, volumes and selling prices in the States. Some of the products listed are:

- degradable plastics (0.4 -0.6 \$/lb)
- green chemicals/ solvents (0.5 \$/lb)
- plant growth regulators (1 \$/lb)

Several useful tables on economic analysis tables that are presented below.

Table 4. summarizes manufacturing cost of LA from carbohydrates.

Capacity	10 ⁸ lb LA (>99% pure, 85% d.s.)/year
Year	1992
Location	Midwest
Process	ED membrane based

Capital (-15% +30%)		
Direct fixed, \$10 ⁶		35.3
Total Invest, \$10 ⁶		40.9
Cost per lb LA, cents		
Raw material		7.2
Chemicals, supplies		3.4
Variable utilities		5.5
Total var. cost		16.1
Labor and maintenance		2.4
Plant overhead		1.0
Insurance and taxes		0.5
Total fixed direct cost		4.0
Cash cost		20.1
Depreciation (12.5%)		5.1
Manufacturing cost (cash + dep)		25.2

Table 5 summarized parameters for economics (for 1989 – 1991)

Process parameters		
Fermentation yield		0.95 lb Lactate/lb starch
Lactate recovery in desalting ED		94%
Current efficiency in desalting ED		90%
Membrane life of desalting ED		1 year
Lactate recovery in water splitting ED		99%
Current efficiency in water splitting ED		80%
LA recovery in ion exchange		99%
Resin life		2 year
LA recovery in evaporation		100%
Utility cost		
Electricity		5 cents/kWh
Steam		400 cents/10 ³ lb
Process water		150 cents/ 10 ³ gal
Cooling water		10 cents/ 10 ³ gal
Miscellaneous		
Labor with benefits		\$25/hr
Supervision with benefits		\$35/hr
Depreciation (8yr. straight line)		12.5%

Table 6. Target manufacturing costs of lactic derivatives

Product	Process	Target manufacturing cost (cent/lb)
Lactic acid	ED membrane based	25.2
Methyl lactate	Membrane based	29.1
Propylene glycol	John Brown (Dave Mckee)	44.7

Finally this paper presents sensitivity analysis (fig 2) of LA manufacturing costs to various variables such as plant size, carbohydrate price, and percent change in capital investment. This analysis shows that in large scale plants producing LA as an intermediate for other products, the manufacturing cost can be in the range of \$0.25 – 0.30/lb.

Bailly, M. "Production of organic acids by bipolar electrodialysis (EDBM): realization and perspectives." Desalination. 144 (2002) 157-162.

This paper is a short introduction to EDBM technology in organic acids recovery. It provides a summary of costs of a plant that produces lactic acid from sodium lactate in France at a capacity of 5,000 tons/year (100 wt%). According to the author, there are currently around 1,300 sq. meters of bipolar membrane in operation in the USA, EU and Japan together, involved in recovery and production of organic acids.

The economics of electrodialytic steps of concentration and conversion of 5,000 tons/year (100 wt%) of lactic acid presented in this paper are as follows (provided in EU currency):

Concentration / purification of Sodium Lactate (Conventional ED):	
Investment cost	1,900,00 Euro
Operating costs:	
Electrical consumption	0.55 kWh/kg of produced acid
Membrane replacement	0.065 Euro / kg of produced acid
Electrode replacement	0.009 Euro / kg of produced acid
Conversion of sodium lactate to lactic acid (Bipolar ED):	
Investment cost	2,300,000 euro
Operating cost:	
Electrical consumption	1.18 kWh / kg of produced acid
Membranes replacement	0.061 euro / kg of produced acid
Electrodes replacement	0.001 euro / kg of produced acid

Operating costs of the downstream process for the production of LA (including clarification and purification) by EDBM are 0.47 euro / kg of LA produced compared to its selling price of 1.4 – 1.8 Euro / kg. These costs include:

- Consumables replacement (membranes, electrodes, resins)
- Energy
- Chemicals and water
- Depreciation
- Savings related to the base production by EDBM.

To convert euros into dollar currency author of this article suggests to use 1 EURO = 0.90 USD conversion rate.

Summary of the Nature Works Website

Nature Works on its website (<http://www.natureworkslc.com/>) presents description of its products that are made from organic waste and degradable under normal environmental conditions. The information presented on this site is mostly related to the promotion of environmentally friendly polymers produced by Cargill.

OUR VALUES AND VIEWS (<http://www.natureworkslc.com/Our-Values-And-Views.aspx>): contains information about life cycle assessment of products produced by Natureworks. It explains “green manufacturing” and “environmental stewardship”.

PRODUCTS AND APPLICATIONS (<http://www.natureworkslc.com/Product-And-Applications.aspx>): gives an overview where polymers produced by Natureworks are successfully being used. Subheading Ingeo fibers describes a new polymer that is the world’s first man-made fiber derived from 100% annually renewable resources – not oil. Under Ingeo fibers there is a set of downloadable materials that can be used by those who are to design polymer production and can be readily accessed from <http://www.natureworkslc.com/product-and-applications/natureworks-polymer/technical-resources/natureworks-polymer-technical-data-sheets.aspx>.

There is statistical information under CASE STUDIES >> GLOBAL CONSUMER MARKET RESEARCH. This contains information on people’s perception of polymer fibers.

INGREDIENT BRANDING (<http://www.natureworkslc.com/Ingredient-Branding.aspx>): focuses on new products produced by Natureworks Ingeo fiber. This page also contains documents for companies that work with ingredient-branding or licensing agreements and suggests reference to these documents which will help provide direction on how to effectively communicate their co-branded status.

NEWS AND EVENTS (<http://www.natureworkslc.com/News-And-Events.aspx>): contains articles and newsletters that describe advantages of polymers.

PARTNER RESOURCES LOGIN (<http://branding.natureworkslc.com/>): contains guidelines for producers on how to proceed with branding of independent products with similar characteristics.

Appendix - Result 3

Table A - Result 3: Total Production Costs (TPC) from Peters and Timmerhaus

1. Manufacturing Cost = Direct Production Cost + Fixed Charges + Plant Overhead Costs		
	Category	Range
A. Direct Production Costs (DPC)		~60% of TPC
A.1	Raw materials	10-50% of TPC
A.2	Operating labor (OL)	10-20% of TPC
A.3	Direct supervisory and clerical labor	10-25% of OL
A.4	Utilities	10-20% of TPC
A.5	Maintenance and repair (MR)	2-10% of FC
A.6	Operating supplies	10-20% of MR or 0.5-1% of FC
A.7	Laboratory charges	10-20% of OL
A.8	Patents and royalties	0-6% of TPC
B. Fixed Charges (FC)		10-20% of TPC
B.1	Depreciation (depends on life period, salvage value, and method of calculation): • Machinery and equipment • Buildings	~10% of FC 2-3% of FC
B.2	Local taxes	1-4% of FC
B.3	Insurance	0.4-1% of FC
B.4	Rent (of value of rented land and buildings)	8-10%
C. Plant Overhead Costs (POC) (50-70% of OL, supervision and maintenance)		2-5% of TPC
D. General Expenses = Administrative Costs + Distribution and Selling Costs + Research and Development Costs		
D.1	Administrative Costs	~15% of OL Or 2-5% of TPC
D.2	Distribution and selling costs (includes costs of sales offices, salesmen, shipping and advertising)	2-20% of TPC
D.3	Research and development costs (2 – 5% of every sales dollar)	~5% of TPC
D.4	Financing (interest)	0-7% of TPC
E. Total Production Costs = Manufacturing Costs + General Expenses		
F. Gross Earning Costs = Total Income – TPC (amount of gross earnings cost depends on amount of gross earnings for entire company and income tax regulations; a general range of gross -earnings cost is 30-60% if gross earnings).		

Adapted from: Max S. Peters, Klaus D. Timmerhaus, "Plant Design and Economics for Chemical Engineers McGraw Hill, 1968. p.141

Remark on Budget Balance - Result 3

Result 3 shows a positive budget balance of \$ 1,777.00

Request for filing of international patent application was not approved and as a consequence \$1,683.00 was not used in category "Project final report and other publications" Please see "Amendment "Result 3" - Concerning International Patent Filing Expenses - September 30, 2007" given below.

Fringe was \$ 87.00 less than originally planned.

Professional meeting expenses were \$7.00 less than planned.

Amendment "Result 3" - Concerning International Patent Filing Expenses – September 30, 2007

In the original project budget a sum of \$2,000.00 was allocated to budget item "Project final report and other publications" under "Result 3" – budget line 23. Current balance under this item is \$1,790.00.

It is proposed here that the sum of \$1,790.00 (i.e. the current balance under "Project final report and other publications" for "Result 3") be used towards covering international patent filing costs (i.e. line 24 – Result 3) in the file "Attachment A Budget Amendment September 2007".

Note: Amendment "Result 3" concerning "International Patent Filing Expenses" of September 30, 2007 was not approved.

V. TOTAL LCMR PROJECT BUDGET

All Results: Personnel: \$ 225,000.00

All Results: Equipment: \$ 85,000.00

All Results: Development: \$0.00

All Results: Acquisition: \$ 25,000.00

All Results: Other \$ 15,000.00

TOTAL LCMR PROJECT BUDGET: \$350,000

Explanation of Capital Expenditures Greater Than \$ 3,500:

Equipment "Result 1": HP Gas Chromatograph with Integrator and Auto Sampler (\$22,000 - BSU owned equipment) will be used to analyze purified lactic acid and other organic acids. Multiangle Light Scattering Detector (SEC/MALS) will be purchased (\$10,000) and used for polylactic acid using a solvent with relatively low refractive index as the mobile phase. The use of a MALS detector with an HPLC/SEC system will eliminate the need for polymer calibration standards since the absolute molar mass of the polylactic acid is measured directly. Bipolar Electrodialyzer with Fermentation System will be purchased (\$30,000) and used for production of lactic acid. Bipolar

electrodialysis with bipolar membrane and water splitting provide an attractive compliment to organic acid fermentation. This process enables the separation, purification and concentration of salts and converting salts into acid and base without producing high concentrations of salts in the effluents

Equipment Amendment "Result 2" – Approved April 20, 2006

In the original proposal "Bioconversion of potato waste into marketable biopolymers - LCMR05-09f" \$ 45,000 was budgeted towards acquisition of: a) Brookhaven 90Plus (Result 2 budget - line 18), and b) Refrigerated incubator shaker (Result 2 budget - line 19) following prices given in vendor's quotes. The instruments were acquired at discounted price resulting in savings of \$6,630.

It is proposed here to use \$ 2,000 of the saved \$ 6,630 towards cost sharing in acquisition of research grade microscope "Leica" quoted by vendor at \$ 29,759.46. The Center for Environmental, Earth and Space Studies will provide the difference \$27,749.49.

It should be noted that the microscope was not budgeted in initial proposal because of its cost. If the revised budget would be approved "Result 2" would benefit from the proposed microscope which would be used during the second research year: i) to acquire images of xanthan producing bacterium *Xantomonas campestris* at various fermentation times, and ii) to study emulsions containing xanthan following procedures adapted from Vanapalli A.S., Palanuwech, J., and J.N. Copland 2002. The use of the microscope would justify allocation of \$2,000 towards cost sharing with Center for Environmental, Earth, and Space Studies (Result 2 Revised Budget 04/18/2006 line 20).

Equipment "Result 2": Laser Particle counter "Spectrex 2000 PC" (\$15,000 - BSU owned equipment) will be used to trace distribution of particles sizes in processed potato waste (range 5.0 to 100 μm . Malvern "Zetasizer N" (\$ 37,000), or equivalent instrument like Wyatt Technology "MiniDawn", light scattering instrument will be purchased to measure: i) distribution of particle sizes in range 0.6 nm to 6 μm , and ii) molecular mass of xanthan during its fermentation of potato processing waste. Refrigerated incubator shaker (\$ 8,000) is required for precise control of temperature during xanthan fermentation since the temperature strongly affects the process.

The equipment listed under "Result 1" and "Result 2" above will continue to be used at BSU in biopolymer and environmental research; however the equipment will be put out for sale in consultation with LCMR provided LCMR and the Trust Fund would require that. It should be added that the major goal of the proposed research is to answer the question whether fermentations of xanthan and poly-lactic-acid on potato waste are scientifically and technological sound. Once this basic question will be answered we will approach LCMR for continuation of the project in the coming biennium cycles. If continuation of the project would be granted by LCMR the equipment requested here will continue to be used through its useful life in the product(s) development, scale-up, and pilot plant studies to be executed during continuation of the project.

Acquisitions: Twenty five thousand dollars (\$ 25,000.00) will be directed towards acquisition of: chemicals, filters, chemical kits, glass-ware, plastic-ware, and microbiological media and other consumables needed for the execution of Result 1 and Result 2. No expenses of this kind will be associated with Result 3.

Other (\$ 15,000): **a) Travel:** During the course of the project: a) Dr Bilanovic intends to deliver two to three presentations at meetings of American Chemical Society, b) Dr. Chang plans another two presentations at annual meetings of American Society for Microbiology c) Drs. Bilanovic, Chang, Spigarelli and Welle also intend to deliver a joint presentation on forthcoming meetings of professional societies in Minnesota or USA. Eight thousand dollars (\$ 8,000.00) will be directed towards in-state and out-of-state travel to scientific meetings. **b) Sampling:** Five thousand dollars (\$ 5,000.00) will be directed towards sampling and sampling related expenses like: a) acquisition of sampling container, b) mileage expenses, and c) meal allowance. **c) Printing:** Two thousand dollars (\$ 2,000.00) will be allocated to printing of reports and preparation of other publications and posters relevant to the project

VI: OTHER FUNDS AND PARTNERS

A. Project Partners:

Four scientists of significant national and international research and teaching experience of the Center for Environmental, Earth and Space Studies (CEESE) – Bemidji State University (BSU), namely: Dr. Dragoljub D. Bilanovic, Dr. Fu-Hsian Chang, Dr. Steven A. Spigarelli and Dr. Patrick Welle will participate in the project.

Project manager Dr. Bilanovic, holds DSc and MSc in Environmental Engineering/Sciences and BS in chemistry (engineering), participated as principal investigator, co-investigator and scientist in more than 20 research and consulting projects, has five publications relevant to xanthan fermentation, more than 70 other publications including three patents. Dr. Bilanovic will act as an administrative manager of the project and as principal investigator on "Result 2"; \$ 38,300 will be allocated to Dr. Bilanovic (i.e. Wages and fringe - please see Attachment A – Budget Detail).

Dr. Fu-Hsian Chang, holds PhD in Environmental Microbiology, MSc in Biology and Biophysics and BS in agricultural chemistry, participated as principal investigator, co-investigator and scientist in more than 20 research and consulting projects, and has more than 70 publications. Dr. Chang will act as a principal investigator and manager of "Result 1"; \$ 15,000 will be allocated to Dr. Chang (i.e. Wages and fringe - please see Attachment A – Budget Detail).

Dr. Welle holds PhD and MSc in Economics, BS in environmental sciences, participated as principal investigator, co-investigator and scientist in more than 25 research and consulting projects, and has more than 70 publications. Dr. Welle will be responsible for "Result 3" and will be assisted by Drs. Bilanovic, Chang, and Spigarelli towards execution of research objectives relevant to Result 3; \$ 9,000 will be allocated to Dr. Welle (i.e. Wages and fringe -please see Attachment A – Budget Detail).

Dr. Spigarelli holds, PhD in Biology, participated as principal investigator, co-investigator and scientist in more than 30 research and consulting projects, and has more

than 80 publications; ; \$ 3,000 will be allocated to Dr. Spigarelli (i.e. Wages and fringe - please see Attachment A – Budget Detail). Dr. F.C. Chang's: doctorate associate will receive \$ 59,000; his graduate students will be allocated 50,000 and his undergraduate students will be allocated \$ 6,000. Dr. Bilanovic's graduate students will receive \$ 58,000 and his undergraduate students will be allocated \$ 6,700

B. Other Funds being Spent during the Project Period: We estimated that the "In Kind Match and Leverage Resources" for the proposed project and time frame are:

BUDGET ITEM	In Kind Match for:			Total (Row)
	Result 1	Result 2	Result 3	
Personnel	\$15,240	\$17,117	\$3,000	\$35,357
Fringe Benefits	\$2,760	\$5,435	\$780	\$8,975
Chemicals and Supplies	\$1,000	\$2,000		\$3,000
Travel in Minnesota	\$500	\$1,000	\$500	\$2,000
Travel outside Minnesota	\$500	\$2,000	\$500	\$3,000
Total (Column)	\$20,000	\$27,552	\$4,780	\$52,332

C: Required Match (if applicable): Not applicable.

D: Past Spending: It was also estimated that CEES-BSU cash and in-kind contributions towards work on biopolymers are approximately \$40,000 for the period January 2001, to July 1, 2005.

E. Time: Laboratory bench-top research and development phase is expected to last for two years (July 1, 2005, to June 30, 2007).

VII DISSEMINATION:

Supplementary Materials, Communications and Outreach Activities

The following is pertinent to use and/or dissemination of the project results:

1. Publications and Presentations

1.1 - Result 2: Patent application titled "Solid or Semi-Solid State Fermentation of Xanthan on Potato or Potato Waste" (application number 11/598,907) was filed with the United States Patent and Trademark Office on 11/14/2006. This nine page document was authored by Dr. Dragoljub D. Bilanovic, Mr. Samuel Hunter Malloy, and Ms. Petra Remeta and comprises of: Sixteen Claims, Background of the Invention, Summary of the Invention, Six Examples, and List of References. This application was published on May 15, 2008 under Patent Publication No. US-2008-0113414-A1. Please also see <http://www.freepatentsonline.com/y2008/0113414.html>.

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Title: Solid or semi-solid state fermentation of xanthan on potato or potato waste

Document Type and Number: United States Patent 20080113414 **Kind Code:** A1

Abstract: The present invention presents a method for the aerobic production of xanthan by bacteria of the genus *Xanthomonas* on a solid or semi-solid substrate. In the exemplary embodiment of the method, a substrate is provided that has a total solids content of about 6.5% or higher. The substrate is sterilized and cooled. Bacteria of the genus *Xanthomonas* are inoculated into the substrate and incubated. After incubation, the bacteria are destroyed, the substrate is either diluted or washed, and the xanthan is isolated.

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Inventors: Bilanovic, Dragoljub D. (Bemidji, MN, US)
Malloy, Samuel Hunter (Bemidji, MN, US)
Remeta, Petra (Bemidji, MN, US)

Application Number: 11/598907

Publication Date: 05/15/2008

Filing Date: 11/14/2006

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Referenced by: [View patents that cite this patent](#)

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Assignee: Bemidji State University Foundation (Bemidji, MN, US)
State of Minnesota Environment and Natural Resources Trust Fund (St. Paul, MN, US)

Primary Class: 435/104

International Classes: C12P19/06

Attorney, Agent or Firm: KINNEY & LANGE, P.A. (THE KINNEY & LANGE BUILDING, 312 SOUTH THIRD STREET, MINNEAPOLIS, MN, 55415-1002, US)

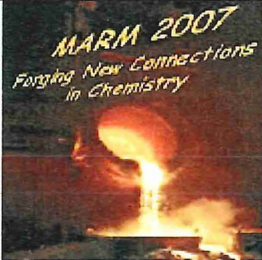
Claims: What is claimed is:

1. A method for the production of xanthan by microbial conversion comprising the steps of: providing a substrate comprising a complex starch medium wherein the total solids content of the substrate is at least about 6.5%; sterilizing the substrate; cooling the substrate; inoculating bacteria of the genus *Xanthomonas* into the substrate; incubating the substrate; heat treating the substrate; diluting or washing the substrate to destroy the bacteria of the genus *Xanthomonas*; and isolating xanthan.

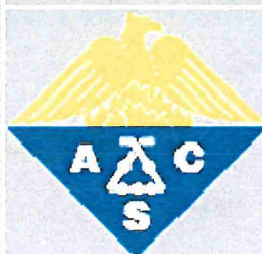
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1.2 - Result 1: Chang, F.H., D.P. Adhikari, E. Ashiamah-Finch, J. Furney, Q. Zhang, Y., Yang, and M. Yan. 2007. "Bioconversion of Potato Waste into Polylactic Acid." Presented at ASA-CSSA-SSSA, 2007 International Annual Meeting, November 4-8, New Orleans, Louisiana.

1.3- Result 2 and 3: Drs Welle and Bilanovic presented a paper titled "Optimization of Solid State Xanthan Fermentation of Potato Waste Using Response Surface Methodology" at the American Chemical Society Mid-Atlantic Regional Meeting an abstract of which is provided the project final report. Collegeville, PA, May 17 -19. 2007. Please see <http://marmacs.org/confex.pl?P42626.HTM> (Accessed 07/25/2008).



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Optimization of solid state xanthan fermentation of potato waste using response surface methodology

Dragoljub D. Bilanovic and Patrick G. Welle. Bemidji State University, Bemidji, MN

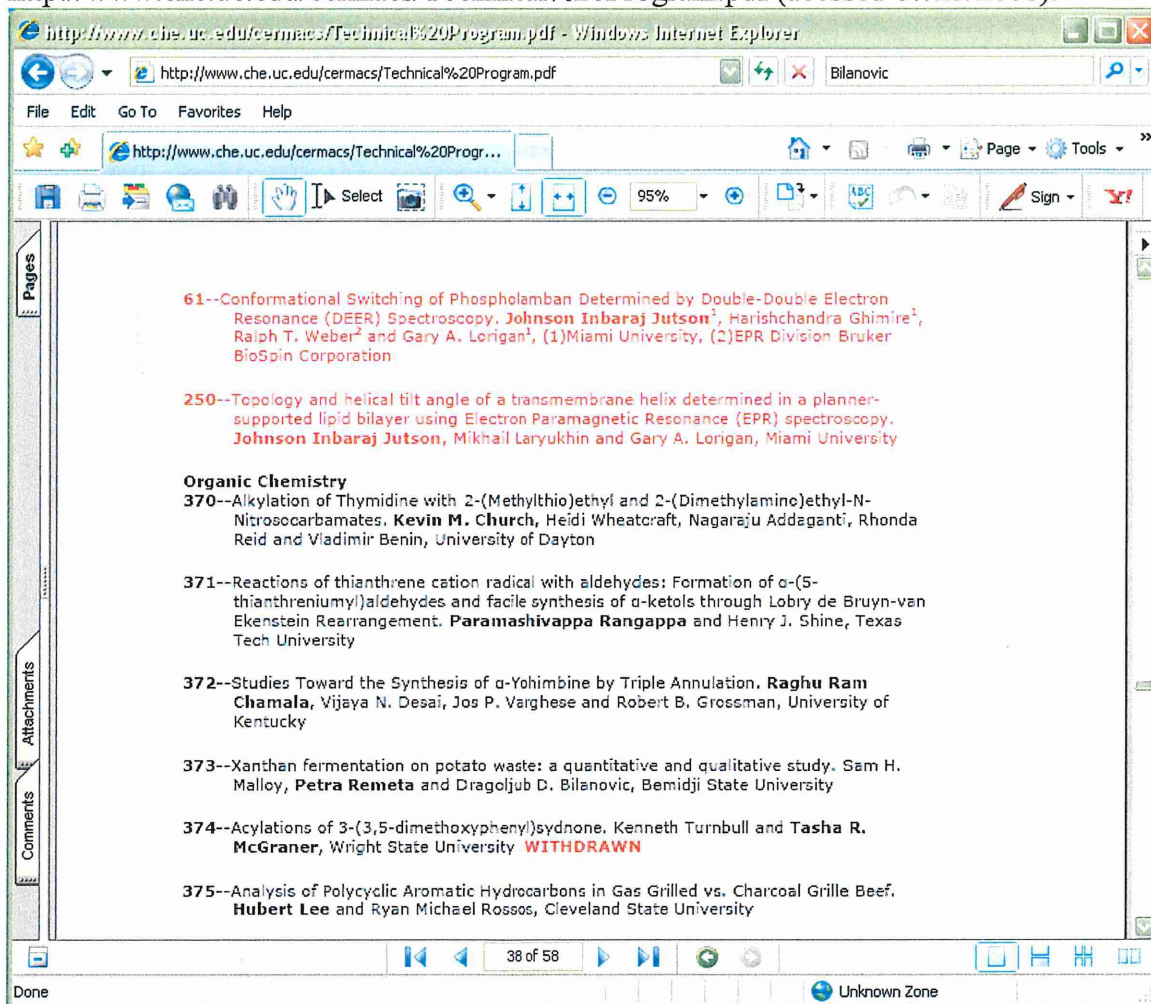
Xanthan is used as stabilizer, thickener, and water mobility reducer in industries as different as food and petroleum. The medium expenses are about one third of its production costs. Utilization of alternative carbon sources, like food industry waste, could reduce xanthan production costs. A potato processing plant generates waste in amounts as high as 25% of its processing capacity.

Xanthan fermentation was conducted on potato waste in solid and semi-solid mode. The individual and interactive effects of: i) enzymatic pre-treatment, ii) mechanical pre-treatment, iii) pH control, and iv) control of moisture content on xanthan production and substrate utilization were analyzed using response surface methodology.

Xanthomons campestris NRRL 1003 strain produces on average sixty percent more xanthan than NRRL 1013 strain which consumed on average 6% more substrate than NRRL 1003 strain. Xanthomons campestris NRRL 1459B strain fermented on average 22% less xanthan than NRRL 1003 strain; these two strains consumed roughly equal amount of potato waste during 96 hours fermentation. All three strains produced more xanthan on solid potato waste medium than on 2% glucose medium. Xanthan fermentation on solid potato waste could compete with xanthan fermentation on standard glucose medium since medium related expenses are at least 20% smaller on the potato waste medium.

[Back to Bioprocess Science/Biomanufacturing Session](#)

1.4 - Result 2: Sam H. Malloy, Petra Remeta, and Dragoljub Bilanovic presented a poster titled "Xanthan Fermentation of Potato Waste: a Quantitative and Qualitative Study" at the Central Regional Meeting of the American Chemical Society, Covington, KY, May 20-23, 2007. Please see page 38 at the <http://www.che.uc.edu/cermacs/Technical%20Program.pdf> (accessed 07/25/2008).



1.5 - Result 1: D.P. Adhikari, E. Ashiamah-Finch, J. Furney, L. Kallestad, A. Frances, and F-H.Chang made presentation " Bioconversion and Optimization of Potato Waste to Lactic Acid" at the American Chemical Society 233rd national Meeting, Chicago, IL, March 25-29, 2007, available at page 96 <http://membership.acs.org/A/AGRO/Picogram/PICOGRAMV72.pdf> (accessed 06/30/2008).

1.6. - Results 1, 2 and 3: Drs Bilanovic, Chang and Welle and Mr. Isobaev made presentation Lactic Acid and Xanthan Fermentations on an Alternative Potato Waste Media at the 2008 World Congress on Industrial Biotechnology and Bioprocessing. April 27-30 in Chicago, IL.

Scientists participating in 2008 World Congress on Industrial Biotechnology and Bioprocessing expressed interest in our work as illustrated by the following email from Dr. Vuyisile Phehane of South Africa.

Subject: World Congress follow-up
Created By: Vuyi@biopad.org.za
Scheduled Date:
Creation Date: 5/12/2008 9:44 AM
From: "Vuyisile Phehane" <Vuyi@biopad.org.za>

Dear Dr Bilanovic,

We previously met at the World Congress on Industrial Biotechnology and Bioprocessing in Chicago. I raised the possibility of in-licensing commercialization-ready technologies in the production of (i) lactic acid and (ii) xanthan using fermentation processes.

Please advise whether such a venture would be possible for set-up in South Africa. I anticipate that issues such as markets and competitors would come into play, as well as other business planning factors. Your advice in this respect is requested please.

The aim would be set-up a local manufacturing facility for domestic value addition, or export into other countries. This approach can only add to alleviating job shortage challenges faced by our country.

I look forward to hearing from you at your convenience.

Sincerely,



VUYISILE PHEHANE, PhD
Portfolio Manager: Industrial and Bioprocessing
BioPAD

Unit 3&4 Enterprise Building
Mark Shuttleworth Street
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Lynnwood
Pretoria, 0001
PO Box 75887
Lynnwood Ridge, 0040

Tel: +27 (0) 12 844 0148
Fax: +27 (0) 12 844 0153
e-mail: vuyi@biopad.org.za
web: www.biopad.org.za

2. Interviews

2.1. Minnesota Public Radio interviewed Drs. Bilanovic, Chang, and Welle titled "Study seeks new uses for potato waste" on January 30, 2006. Mr. Tom Robertson was the reporter. Please see

http://news.minnesota.publicradio.org/features/2005/01/27_robertsont_potatowaste/ (accessed 06/30/2008).

The interview or parts of the interview were also published by the:

AGRIPress - Please see <http://www.agripressworld.com/start/artikel/147830/en> (accessed 06/30/2008)

BSUAlumni - Please see

<http://www.bsualumni.org/horizons/SummerFall06.pdf> (accessed 06/30/2008)

<http://www.bsualumni.org/horizons/SpringSummer07.pdf> (accessed 06/30/2008)

http://news.minnesota.publicradio.org/features/2005/01/27_robertson_potatowaste/

MPR: Study seeks new uses for potato waste - windows internet explorer

http://news.minnesota.publicradio.org/features/2005/01/27_robertson_potatowaste/

MPR: Study seeks new uses for potato waste

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Study seeks new uses for potato waste

by Tom Robertson, Minnesota Public Radio
January 30, 2006

Minnesota is one of the biggest potato producing states in the country. But companies that churn out French fries, Tater Tots and hash browns also produce tons of potato waste each year. Processors have to dispose of that waste somewhere. Sometimes it's thrown onto potato fields to rot. Often, it's sold cheaply to farmers who feed it to cows or pigs.



Environmental scientists at Bemidji State University believe there's a better way. They say fermented potato waste could potentially produce high-value products that are better for the environment.

Bemidji, Minn. — The potato industry generates lots of waste. One-quarter of what goes into a potato processing plant comes out as waste. In Minnesota, that's about 400,000 tons a year.

BSU environmental studies professor Drago Bilanovic enters a small laboratory

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Study seeks new uses for potato waste (story audio)

PHOTOS
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Drago Bilanovic
xanthan
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(5 images)

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RESOURCES
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From potatoes to diapers?[AGRI PRESS BENELUX] - Windows Internet Explorer

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From potatoes to diapers?

Researchers at Bemidji State University are working to find new uses for potato waste from processing plants.

Minnesota is one of the biggest potato producing states in the country. But companies that chum out French fries, Tater Tots and hash browns also produce tons of potato waste each year.

Processors have to dispose of that waste somewhere. Sometimes it's thrown onto potato fields to rot. Often, it's sold cheaply to farmers who feed it to cows or pigs.

Environmental scientists at Bemidji State University believe there's a better way. They say fermented potato waste could potentially produce high-value products that are better for the environment.

The potato industry generates lots of waste. One-quarter of what goes into a potato processing plant comes out as waste. In Minnesota, that's about 400,000 tons a year. BSU environmental studies

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Newsflash

- Barry Callebaut acquires cocoa processing company
- Beet Recall Sinks Topps Meat Co.
- COPA-DOGECA identifies priorities for future European dairy sector
- QSA: Fish counters wage through oceans of data
- Search of biotechnology

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Study seeks new uses for potato waste - Windows Internet Explorer

http://www.hpj.com/archives/2006/feb06/feb27/Studyseeksnewusesforpotatow.cfm

Bilanovic

Study seeks new uses for potato waste

Monday, October 06, 2007 6:41:41 PM

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Study seeks new uses for potato waste

BEMIDJI, Minn. (AP)--The potato industry generates lots of waste--one-quarter of what goes into a potato processing plant comes out as waste, in fact.

In Minnesota, that's about 400,000 tons a year.

In a small laboratory at Bemidji State University, environmental studies professor Drago Bilanovic is doing cutting-edge research on potato waste. In a corner of the lab, Bilanovic shows a visitor a machine that slowly spins about two dozen clear bottles.

"What you see here is a set of bottles in which we have some potato waste and whatnot, and different experimental conditions," said Bilanovic. "So we are trying to figure out how much xanthan we can produce."

Xanthan is something most people haven't heard of. If you're a label reader, you might recognize it as a thickening agent used in salad dressings, yogurt, ice cream and other dairy products.

In its basic form, xanthan is a thin, sticky gel that also has industrial uses. It's used as a stabilizer and thickener in the paint and chemical industry. The

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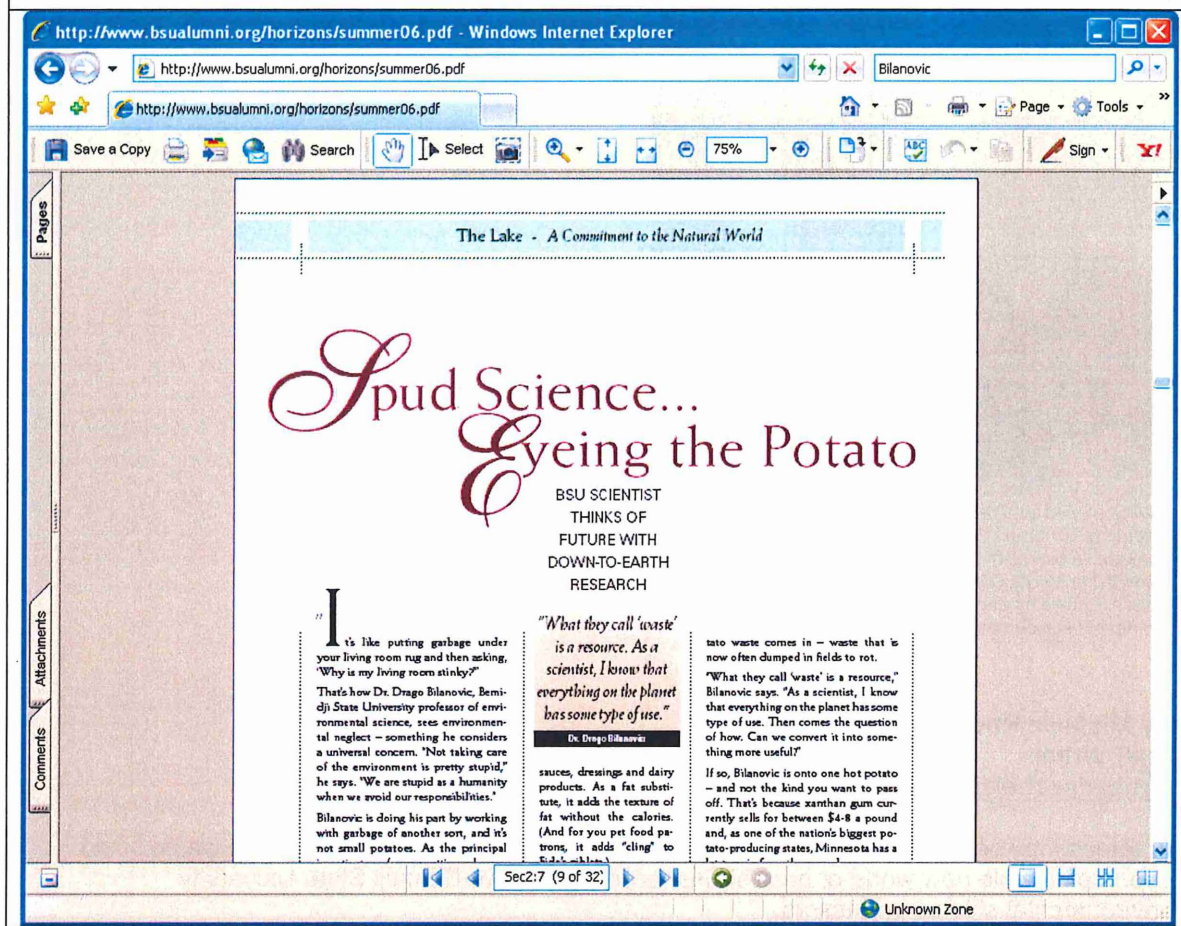
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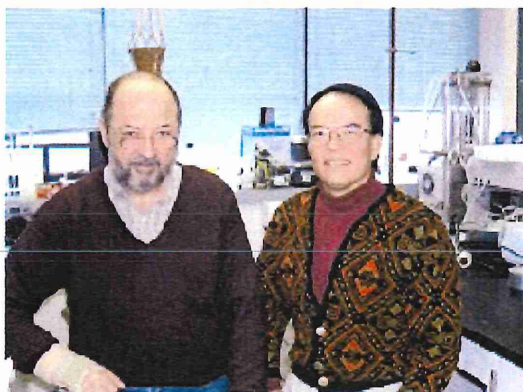
<http://www.bsualumni.org/horizons/summer06.pdf>



2.2. Bemidji Pioneer interviewed Drs. Bilanovic and Chang titled "Two BSU professors lead potato bioconversion research"

Two BSU professors lead potato bioconversion research

Friday, December 16, 2005



Dragoljub Bilanovic and Fu-Hsian Chang, professors of environmental studies at Bemidji State University, are leading research on the conversion of potato waste into marketable biopolymers under a two-year \$350,000 grant awarded to BSU's Center for Environmental, Earth & Space Studies by the Legislative Commission on Minnesota Resources.

By Michelle Ruckdaschel
Staff Writer

mruckdaschel@bemidjipioneer.com

Potatoes may be a staple food in many households around the globe, but potato waste could open up a whole new world of possibilities, according to two Bemidji State University environmental studies professors.

Dragoljub Bilanovic and Fu-Hsian Chang are studying the production of the biopolymers xanthan and polylactic acid through the fermentation of potato waste. Bilanovic is a professor of colloid chemistry and environmental science/engineering. Chang is a professor and director of BSU's Center for Environmental, Earth & Space Studies. He is also an environmental microbiologist and biotechnologist.

Earlier this year, the Legislative Commission on Minnesota Resources awarded the CEES a two-year \$350,000 grant to research the conversion of potato waste into marketable biopolymers, according to the Oct. 14 BSU Insider newsletter.

Biopolymers are renewable, biodegradable materials that could replace petroleum-based plastics, the newsletter states.

"We applied for this grant almost two years ago," Bilanovic said. "We simply can't afford such research if it was not for this grant."

Bilanovic, the project manager, is leading the research on xanthan while Chang is leading the research on polylactic acid. Their colleagues, Stephen Spigarelli and Patrick Welle, professors of

environmental studies, will also work on the project along with up to four graduate students and six undergraduate students, according to the newsletter. And Welle, who is also a professor of economics, will concentrate on a cost-benefit analysis for xanthan and polylactic acid.

The CEESS has a strong interest in promoting environmental technology research that will generate environmentally-friendly products and involve students, Chang noted.

According to the newsletter, the goal of the project is to develop these two valued-added products in parallel for possible implementation into the Minnesota potato industry.

"Hopefully, both products will be attractive and economically feasible," Chang said.

He noted that Minnesota produces about 400,000 tons of potato waste annually.

In Minnesota, potato processors either sell potato waste to cattle farmers as feed at an extremely low price or have to dispose of it, Bilanovic said. He said he believes potato waste has the potential to be converted into something that is more environmentally-friendly and has a higher market value.

Bilanovic said xanthan is already a widely accepted material throughout the world.

"There is a huge market for it," he added.

But, he said, xanthan is typically produced on ordinary sugar and that's expensive. The same bacteria that grow on sugar to produce xanthan can also grow on inexpensive citrus waste, the newsletters states.

According to the newsletter, Bilanovic became the first to convert citrus waste into xanthan as a doctoral student at the Israel Institute of Technology in 1990. The newsletter adds that the global market for xanthan is estimated at \$400 million to \$500 million and is growing at an annual rate of 4 percent or more.

"Xanthan can be used in, and is already used in, almost all fields," Bilanovic said. "It's used in food industry, it's used in pharmaceutical industry, it's used in oil recovery a lot, it's used in paints."

Although xanthan can be produced as either a food-grade or technical-grade material, BSU will focus on the latter, which is used as a stabilizer, emulsifier, thickener and mobility-control and gel-forming agent in the chemical and oil industries, as well as in many other industries, according to the newsletter. The oil industry, for example, uses xanthan to squeeze oil from depleted wells, the newsletter states.

Chang noted that renewable energy is becoming more important as fossil fuels like petroleum continue to diminish.

He said polylactic acid produced by potato waste generated around Minnesota could result in biodegradable plastics that could replace petroleum-based plastics.

"I think that polylactic acid has very good potential," Chang said. "We hypothesized that ... potato waste can be recovered from the waste stream and biologically converted to make valuable polylactic acid plastics."

He said it is also hypothesized that these plastics will compete well with other popular plastics already commercialized for packaging. And, he said, they can be formulated to produce flexible or rigid products and can be mixed with other materials to make different products.

Potential markets for polylactic acid include mulch films, compost bags and sandwich bags, as well as a carrier medium for time-released pesticides and fertilizers. Some other potential uses include industrial and household wipes, diapers and feminine hygiene products.

Chang noted that polylactic acid is a non-volatile odorless product classified by the Food and Drug Administration as Generally Recognized As Safe.

He said biodegradable products are important because garbage made of petroleum-based plastic is not biodegradable and causes environmental pollution.

"As environmental scientists, we have to help solve the environmental pollution problem," he said. "That 400,000 tons of potato waste generated in Minnesota also pose environmental pollution problems."

Both Bilanovic and Chang said they envision the potato waste project continuing after the next two years.

"After the two years of research and development, we hope ... to develop a pilot plant," Chang said.

He added that the hope is to gain partners from industry and the state to create the small scale pilot plant.

But for now, they continue to move forward with research and development.

"We wanted to move forward to carry out all the possible economic analysis to see the market demand and also the cost benefit," Chang said. "We definitely hope that both products will be economically feasible and attractive so that (they) can benefit ... the state economy."

2.3. Bioscience News Note on Potato Research

<http://lists.mnscu.edu/read/messages?id=66400>

Bioscience News, Vol. 6 2006-05-17 13:42:01 <Gail O'Kane>

Faculty outline research programs at Bemidji conference

On April 28, the organization Bemidji Bio held a daylong conference = highlighting the bioscience-related resources that can be found in Bemidji = and the surrounding area. Among the presenters was a panel of system = faculty who described their research.

> Bemidji State environmental studies Professor Drago Bilanovic = described his research into how potato waste can be re-used to make = xanthan, a product used widely in the food industry among other application= s. Professor Fu-Hsian Chang is conducting parallel studies into the = potential use of potato waste to make polylactic acid, the same substance = Cargill's NatureWorks company is extracting from corn to make biodegradeabl=

e plastic and other materials. The potato waste projects are funded by a =
\$350,000 grant from the Legislative Commission on Minnesota Resources.

2.4. Information to MN Lottery

Subject:	"Bio-conversion of Potato Waste into Marketable Biopolymers" project		
Created By:	Marie Hinton		
Scheduled Date:			
Creation Date:	4/8/2008 3:40 PM		
From:	"Marie Hinton" <Marie_H@mnlottery.com>		
Recipient	Action	Date & Time	Comment
To: Dragoljub Bilanovic (DBilanovic@bemidjistate.edu)			

Good afternoon,

I am looking for information about the "Bio-conversion of Potato Waste into Marketable Biopolymers" project. I would like to feature this project in an upcoming issue of the Lottery's newsletter, on our Web site and in a brochure that will be available to the public at the Lottery's booth at the Minnesota State Fair this summer. A portion of the Lottery's proceeds help fund the Environment and Natural Resources Trust Fund (administered by the LCCMR) and the public is very interested in how that money is used. If you wouldn't mind having this project featured, would you please e-mail some information about it to me? I don't need a lot of information, just a summary of the project, how it's going and why it's important. If you happen to have any photos that you think would work well, would you please send those as well?

Thank you,

Marie Hinton

Minnesota State Lottery

888-568-8379, ext. 146

marieh@mnlottery.com

www.mnlottery.com

Good afternoon,

Thank you for your interest in our project. The following links contain information about the project:

http://news.minnesota.publicradio.org/features/2005/01/27_robertsont_potatowaste/

and

<http://www.agripressworld.com/start/artikel/147830/en>

Photos are available at:

<http://info.bemidjistate.edu/News/gallery/events/dragoweb/index.html>

Kind Regards

Dragoljub Bilanovic

CC

susan.thornton@lccmr.leg.mn

VIII REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than January 15, 2006; July 15, 2006; and January 15, 2007. A final work program report and associated products will be submitted by August 15th, 2008

ATTACHMENT A: Budget Detail

Attachment A: Budget Detail for 2005 Projects - Summary and a Budget page for each partner (if applicable)

Proposal Title: Bio-conversion of Potato Waste into Marketable Biopolymers (LCMR05-09f)

Project Manager Name: Dragoljub Bilanovic (D.Sc.)

LCMR Requested Dollars: \$ 350,000.

2005 LCMR Proposal Budget	Result 1 Budget:	Result 1 Original Budget	Result 1 Revised Budget	Amount Spent (6/30/2008)	Balance (6/30/08)	Result 2 Budget:	Result 2 Budget	Result 2 Revised Budget	Amount Spent (6/30/08)	Balance
	Production of Polylactic Acid from Potato Waste.		As ammended on 05/17/07			Production of Xanthan from Potato Waste.		As ammended 4/18/06, 8/25/06 & 3/23/07		
BUDGET ITEM										
PERSONNEL: Staff Expenses, wages, salaries – Be specific on who is paid \$, to do what? Make each person paid a separate line item.	Personnel Expenses Subtotal	98,700	86,333	86,333	0	Personnel Expenses Subtotal	93,700	93,700	89,6	
	Dr. Fu-Hsian Chang: 3 weeks per year	12,700	12,701	12,701	0	Dr. Dragoljub D. Bilanovic: 7.25 weeks per year	29,000	29,000	29,000	
	Dr. Fu-Hsian Chang's Post Doctorate Associate	50,000	38,000	38,000	0	1.9 graduate student per year	58,000	58,000	53,456	
	Graduate student	30,000	24,000	24,000	0	Undergraduate student	6,700	6,700	7,172	
	Undergraduate student	6,000	11,632	11,632	0	N/A	0	0	0	
PERSONNEL: Staff benefits – Be specific; list benefits for each person on a separate line item.	Subtotal Staff Benefits	11,300	23,667	23,667	0	Subtotal Staff Benefits	9,300	9,300	8,9	
	Dr. Fu-Hsian Chang's fringe	2,300	2,667	2,667	0	Dr. D. Bilanovic's fringe	9,300	9,300	8,985	
	Fringe for Post Doctorate Associate	9,000	21,000	21,000	0	N/A	0	0	0	
Equipment / Tools (what equipment? Give a general description and cost)	Electrodialyser with Fermentation System	30,000	27,568	27,568	0	Brookhaven 90Plus (determination of polymer molecular weight)	32,500	32,500	32,	
	MALS DETECTOR for the Gas Chromatograph	10,000	9,060	9,060	0	Refrigerated incubator shaker	5,870	5,870	5,	
						Research Microscope - cost sharing	2,000	2,000	2,	
Printing	N/A	0		0	0	Patent Filing Expenses	0	2,500	2,	
Other Supplies (list specific categories)	Chemicals, glassware and electronics	10,000	16,072	16,072	0	Chemicals, glassware and electronics	19,630	19,630	19,	
Travel expenses in Minnesota	Sampling	3,000	300	114	186	Sampling	2,000	1,000		
Travel outside Minnesota (where?)	Professional meeting	2,000	2,000	2,000	0	Professional meeting	3,000	1,500	1,	
COLUMN TOTAL	\$	165,000	165,000	164,814	186	\$	168,000	168,000	163	