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Coal-Induced Enhancement of Ethanol and Biomass Production

J. Kevin Polman,* Catherine Rae, and Karen M. Delezene-Briggs

Idaho National Engineering Laboratory, Lockheed Idaho Technologies Company, Idaho Falls, ID 83415-2203

ABSTRACT

Inclusion of coal in *Saccharomyces cerevisiae* cultures enhanced ethanol and biomass production (from dextrose) similarly to yeast extract. Coal-induced enhancement was slower in comparison to yeast extract. Ethanol/biomass ratios were twice as high for coal-induced enhancement. Humic acid also enhanced ethanol and biomass production. Coal supplied nitrogen, phosphorus, magnesium, and zinc to cells. Coal facilitated the utilization of cobalt, and reduced the toxicity of aluminum and manganese. Coal-induced enhancement may be a chelation effect; ethylenediaminetetraacetic acid mimicked coal enhancement. Coal also enhanced ethanol and biomass production by *Zygosaccharomyces rouxii*.

Index Entries: Ethanol; coal; chelator; *Saccharomyces*; *Zygosaccharomyces*.

INTRODUCTION

Scientific and engineering investigations, which are directed at improving the efficiency with which ethanol is fermentatively produced, are important because of the huge commercial market represented by ethanol. In 1992, 2.4 billion gallons of ethanol were produced in the United States. Some of the major producers of ethanol are Midwest Grain Products, Archer Daniels Midland, Cargill, Chief Ethanol Fuels, Union Carbide, Quantum Chemical, and Eastman Kodak. The current US presidential administration has supported an increase in the use of ethanol as an oxygenate in reformulated gasoline. This presents a considerable opportunity for the ethanol production industry, which has a projected market growth of at least 10%/yr *(1)*. Citizen Action, a US lobby group, has pressed the administration to encourage this use of ethanol *(2)*. Canada has been involved in a major effort to market gasoline–ethanol blends *(3)*. Problems that occur in using ethanol as a gasoline additive include increased emissions of nitroxides and volatile organic carbon *(4)*.

Raw materials that have been used as fermentable feedstocks for the production of ethanol include sugar cane and sugar beet juices, high-test and blackstrap molasses, sweet sorghum, fruit juices, whey, cereal grains, roots, tubers, cacti, cellu-

*Author to whom all correspondence and reprint requests should be addressed.

losic raw materials, and sulfite waste liquor (*5*). The microorganisms used for conversion include *Saccharomyces cerevisiae, Saccharomyces ellypsoideus, Saccharomyces carlsbergensis, Saccharomyces fragilis, Schizosaccharomyces pombe, Torula cremoris, Candida pseudotropicalis, Clostridium thermosaccharolyticum, Thermoanaerobacter ethanolicus, Pachysolen tannophilus,* and *Zymomonas mobilis* (*5*). Of these, *S. cerevisiae* is probably the most commonly utilized and studied.

Using this organism, theoretical ethanol yield from dextrose is 0.511, actual yield under optimal conditions is 0.485, and observed industrial yield using complex substrates is 0.46 (*5*). A well-defined, high-productivity/yield growth medium was devised for ethanol-producing yeast, which contains dextrose, nitrogen, sulfur, potassium, chlorine, phosphorus, magnesium, calcium, boron, zinc, aluminum, copper, manganese, iodine, iron, cobalt, pyridoxine, pantothenate, thiamine, inositol, and biotin (*5*). A cultivation problem that is universal to industrial biotechnological fermentations is toxicity arising from the components that are used to formulate the growth medium (*6*). These components have been found in carbonaceous feedstocks as well as the feed water, and may be organic or inorganic in nature (*6,7*). Detoxification of growth medium can be accomplished by ion exchange, calcium hydroxide/sodium sulfite treatment (which results in a separable calcium sulfate sludge that contains inhibitors), molecular sieves, or steam stripping at a cost that can be as high as 22% of the overall production cost (*7*).

The study that is described herein was directed at determining the effects of coal on ethanol production. We have observed that the inclusion of coal in microbial growth media often enhances growth, although it is rarely used as a primary carbon source (refs. *8–10*, and several unpublished experiments from this laboratory). Other investigators have observed that compounds leached from coal can either inhibit or enhance growth, depending on the microbial species (*11*). The chemical structure of coal is complex and highly variable; a variety of organic and inorganic moieties are present (*12,13*). Because coal contains several elements, it can provide cells with a variety of nutrients. The chemical complexity of coal also renders different functions to coal molecules, such as the ability to chelate metals (*13*). As we will demonstrate in this article, such functions play a role in how coal affects microbial metabolism.

METHODS

Soluble coal, solid coal, humic acid, and yeast extract were used as metabolic enhancers in initial ethanol and biomass production experiments using *S. cerevisiae* ATCC 36026 and *Zygosaccharomyces rouxii* ATCC 28253. The basal medium used in these experiments contained (g/100 mL): dextrose (1), NH_4Cl (0.02), NaCl (0.0001), K_2HPO_4 (0.001), $MgSO_4 \cdot 7H_2O$ (0.001), $CaCl_2 \cdot 2H_2O$ (0.0001), sodium EDTA (0.01), $FeSO_4 \cdot 7H_2O$ (0.0035), and 5 mL/L of medium of a trace elements solution containing 0.1 g/100 mL of each of the following: $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$, $CuSO_4 \cdot 5H_2O$, $Na_2B_4O_7 \cdot 10H_2O$, $CoCl_2 \cdot 6H_2O$, and $Na_2MoO_4 \cdot 2H_2O$ (*9*). Enhancers that were added included soluble North Dakota Beulah Zap lignite (*14*), soluble Mississippi Wilcox lignite, granular (–100 mesh) Beulah Zap, soluble humic acid (Aldrich Chemicals), and yeast extract (Difco). All experiments were performed using 25 mL of medium in 50-mL culture flasks (200 rpm, 30°C, aerobic). Soluble coal was prepared by dissolving 2 g of lignite in 1 L of 0.1*N* NaOH overnight, neutralizing the mixture

with HCl, and then filtering to remove undissolved coal. Ethanol was measured by gas chromatography (Stabilwax column, Restek, 30 m, 0.25 mm id, FID, 0.1-mL injection). Biomass was determined indirectly by measuring turbidity at A_{660} (absorbance owing to dark coal solutions or particulate coal was considered by determining time zero values for cultures containing these components and using these values to correct and thus standardize measurements). Dextrose was measured by the classic phenol/sulfuric acid assay *(15)*.

Other experiments were designed to determine the mechanism of coal-induced enhancement of biomass production by *S. cerevisiae*. Two growth media were used: 25X optimized media (OM) and 1X OM. 25X OM contained (g/100 mL): dextrose (2), $(NH_4)_2SO_4$ (0.35), KCl (0.06), H_3PO_4 (0.04), $MgSO_4 \cdot 7H_2O$ (0.006), $CaCl_2 \cdot 2H2O$ (0.002), H_3BO_3 (0.0005), $ZnSO_4 \cdot 7H_2O$ (0.0003), $Al_2(SO_4)_3 \cdot 17H_2O$ (0.00015), $CuSO_4 \cdot 5H_2O$ (0.00013), $MnSO_4 \cdot H_2O$ (0.00013), KI (0.00005), $FeSO_4 \cdot 7H_2O$ (0.00005), 0.1% $CoCl_2 \cdot 6H_2O$ (0.00003), pyridoxine (0.0003), pantothenate (0.0002), thiamine (0.00013), inositol (0.00013), and biotin (0.0000005) *(5)*. 1X OM was 25X OM diluted 25 times (except the dextrose, which was still present at 2 g/100 mL). 25X OM was used in experiments that examined (1) replacement of ammonium, dextrose, magnesium, and zinc by coal and (2) detoxification of manganese and aluminum. 1X OM was used in experiments that examined:

1. Replacement of phosphate and cobalt by coal;
2. Effects of EDTA; and
3. Effects of coal on culture pH.

RESULTS AND DISCUSSION

Soluble Beulah Zap lignite and yeast extract enhanced ethanol levels produced by *S. cerevisiae* to approximately the same extent (Fig. 1A,B; Table 1). However, enhancement by coal required a significantly longer period of time to reach maximum level. In addition, both Beulah Zap lignite and yeast extract enhanced biomass production. The level of biomass production caused by yeast extract was much higher than that observed for coal (Fig. 1C,D; Table 1). This resulted in ethanol:biomass ratios that were approximately two times higher for coal compared to yeast extract, despite the fact that product:substrate ratios for both coal and yeast extract were the same (Table 1). In the case of coal, perhaps the dextrose was funneled into the production of another metabolite rather than biomass. Nonetheless, it is an unusual observation that warrants further investigation.

Soluble Mississippi Wilcox lignite also enhanced biomass and ethanol production by *S. cerevisiae* (Fig. 2), suggesting that the enhancement effect may be general with respect to coal type. In the cases of both coal types used here, the amount of soluble coal required to bring about enhancement was very low. Granular Beulah Zap lignite was also capable of enhancing ethanol and biomass production by *S. cerevisiae* implying that the solubilization step may not be necessary; this observation might be cost effective with respect to potential industrial processes (Fig. 3A,B). Humic acid enhanced ethanol and biomass production, as well (Fig. 3C,D), which is not surprising since soluble lignite coal has a humic chemical nature *(16,17)*. Overall, the data indicated that humic acid and granular Beulah Zap lignite provided higher ethanol and biomass levels than soluble Mississippi Wilcox and Beulah Zap lignites (Figs. 1–3).

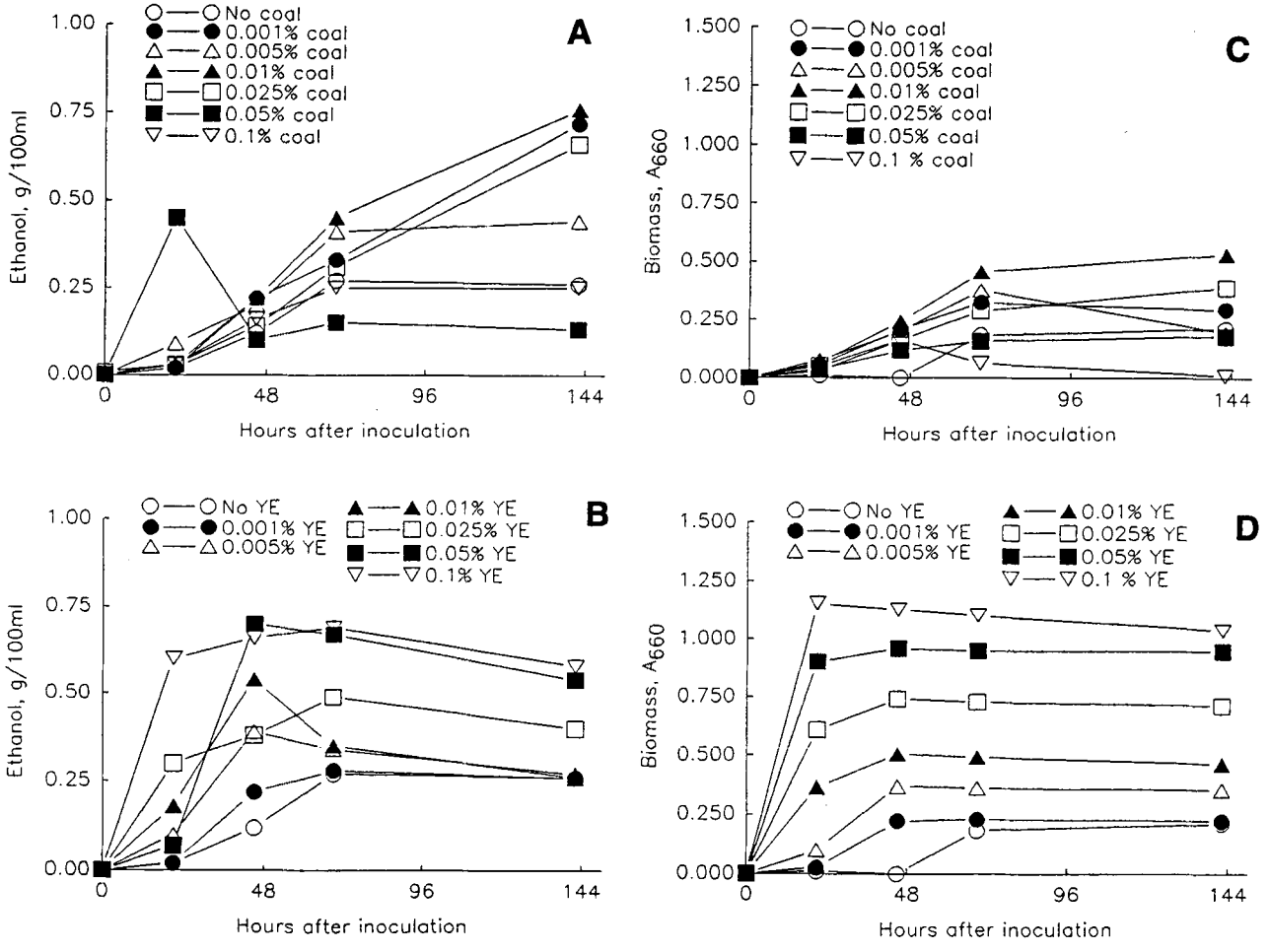

Fig. 1. Enhancement of ethanol and biomass production by *S. cerevisiae* in the presence of soluble Beulah Zap coal and yeast extract.

Table 1
Biomass Production, Ethanol Production, and Dextrose Utilization by *S. cerevisiae* in the Presence of Coal and Yeast Extract

Culture medium[a]	0.001C	0.01C	0.05YE
Time after inoculation (d)	3	6	2
B, biomass (A_{660})	0.144	0.370	0.926
E, ethanol (g/100 mL)	0.32	0.79	0.86
D1, dextrose provided (g/100 mL)	2.384	1.995	2.190
D2, dextrose remaining (g/100 mL)	1.344	0.033	0.046
D1-D2, dextrose utilized (g/100 mL)	1.04	1.962	2.144
E/(D1-D2), product yield (g/g)	0.31	0.40	0.40
E/B, product-to-biomass ratio (g/A_{660})	2.222	2.135	0.929

[a]Media contain dextrose and basal salts, plus 0.001% (0.001C) or 0.01% (0.01C) alkali-soluble Beulah Zap lignite, or 0.05% yeast extract (0.05YE).

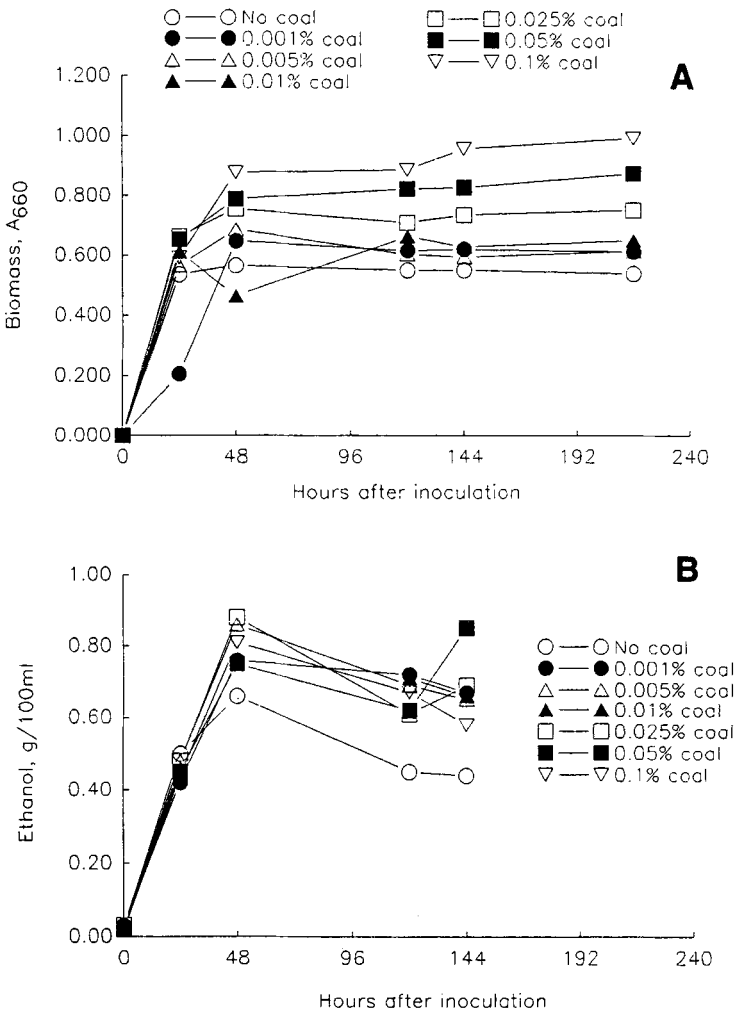

Fig. 2. Enhancement of ethanol and biomass production by *S. cerevisiae* in the presence of soluble Mississippi Wilcox coal.

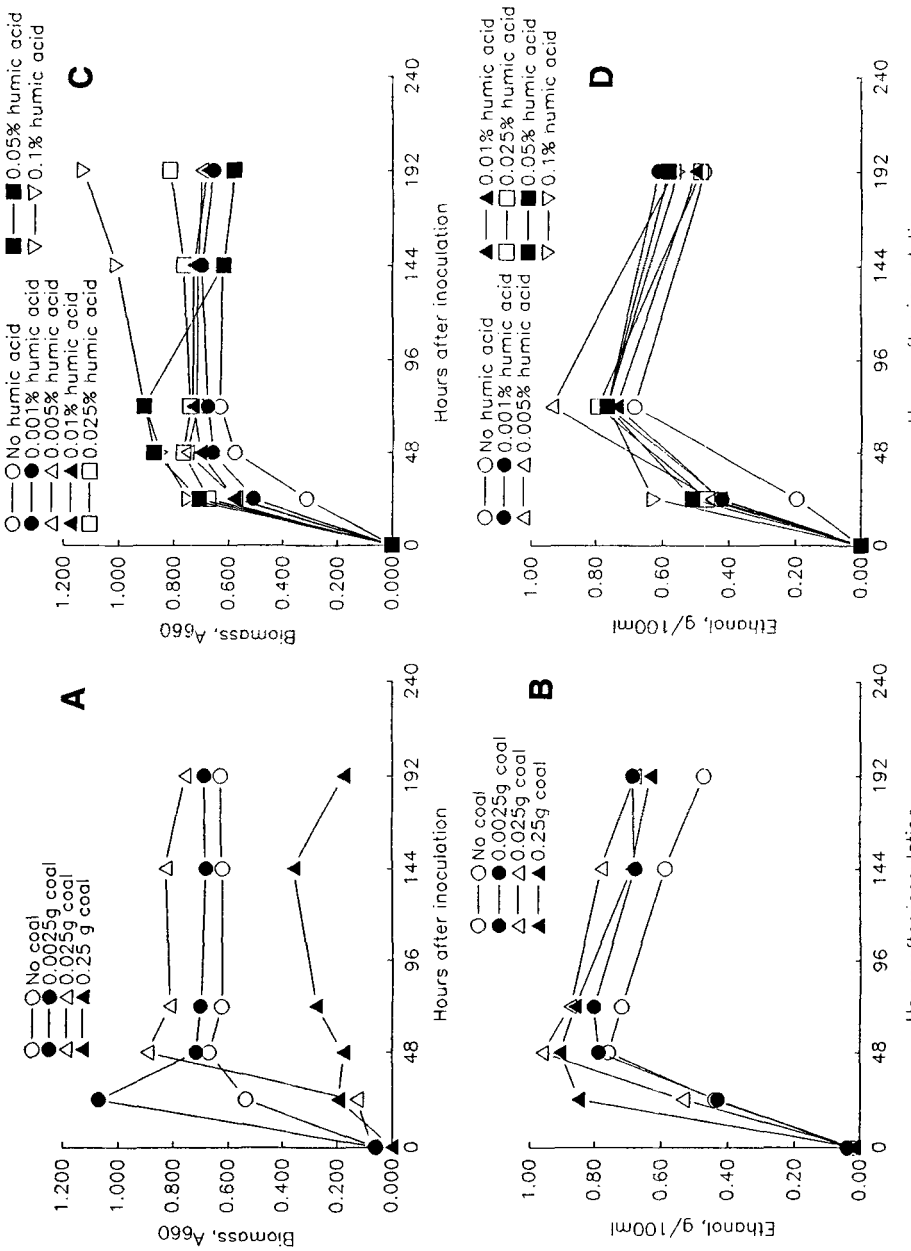

Fig. 3. Enhancement of ethanol and biomass production by *S. cerevisiae* in the presence of granular Beulah Zap coal and humic acid.

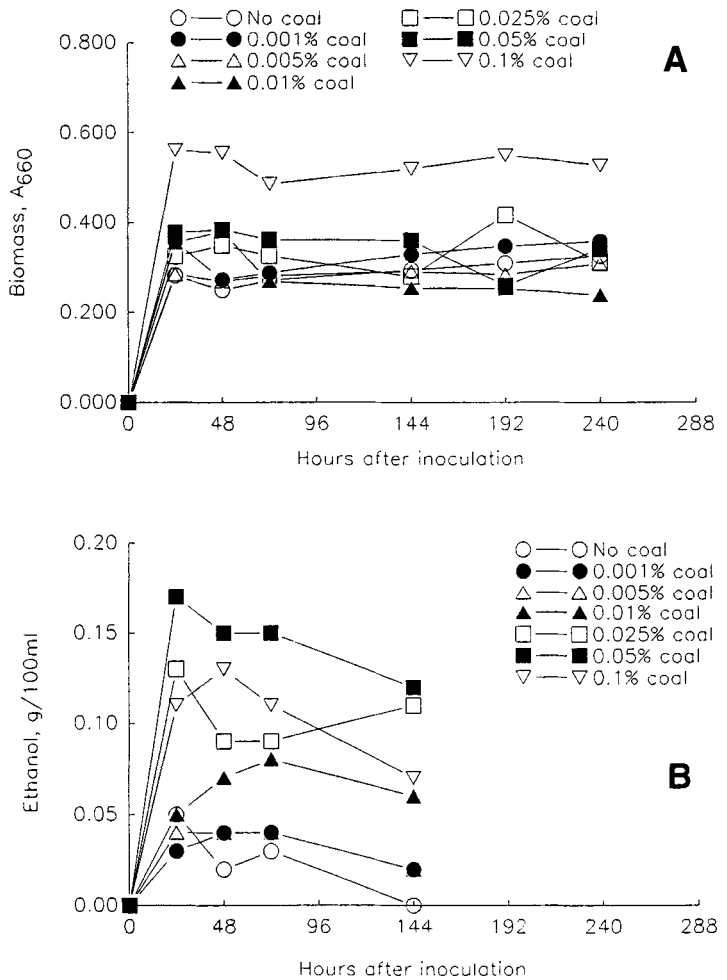

Fig. 4. Enhancement of ethanol and biomass production by *Z. rouxii* in the presence of soluble Beulah Zap coal.

Supporting the hypothesis that coal-induced enhancement of ethanol and biomass production (or metabolic processes in general) may not be limited to one microbial species were the observations of similar enhancements for *Z. rouxii* (Fig. 4). Other results, such as coal-induced enhancement of benzoate degradation by *Pseudomonas chlororaphis*, support this theory *(18)*.

Soluble Beulah Zap lignite did not provide carbon to *S. cerevisiae* (Fig. 5A). This is likely due to the recalcitrant nature of carbon skeletons in coal *(10)*. Coal did provide some nitrogen (Fig. 5B) and phosphate (Fig. 6A), and was able to replace magnesium (Fig. 5C) and zinc (Fig. 7A) deficiencies completely. Coal appeared either to replace cobalt deficiencies or facilitate a faster uptake of trace cobalt (Fig. 6B).

The presence of soluble coal also seemed to alleviate the toxicity imposed by excessive levels of aluminum and manganese (Fig. 7B,C). We hypothesized that this was due to a chelator effect, since coal is known to bind various metals *(13,19)*. This hypothesis was supported by the observation that ethylenediaminetet-

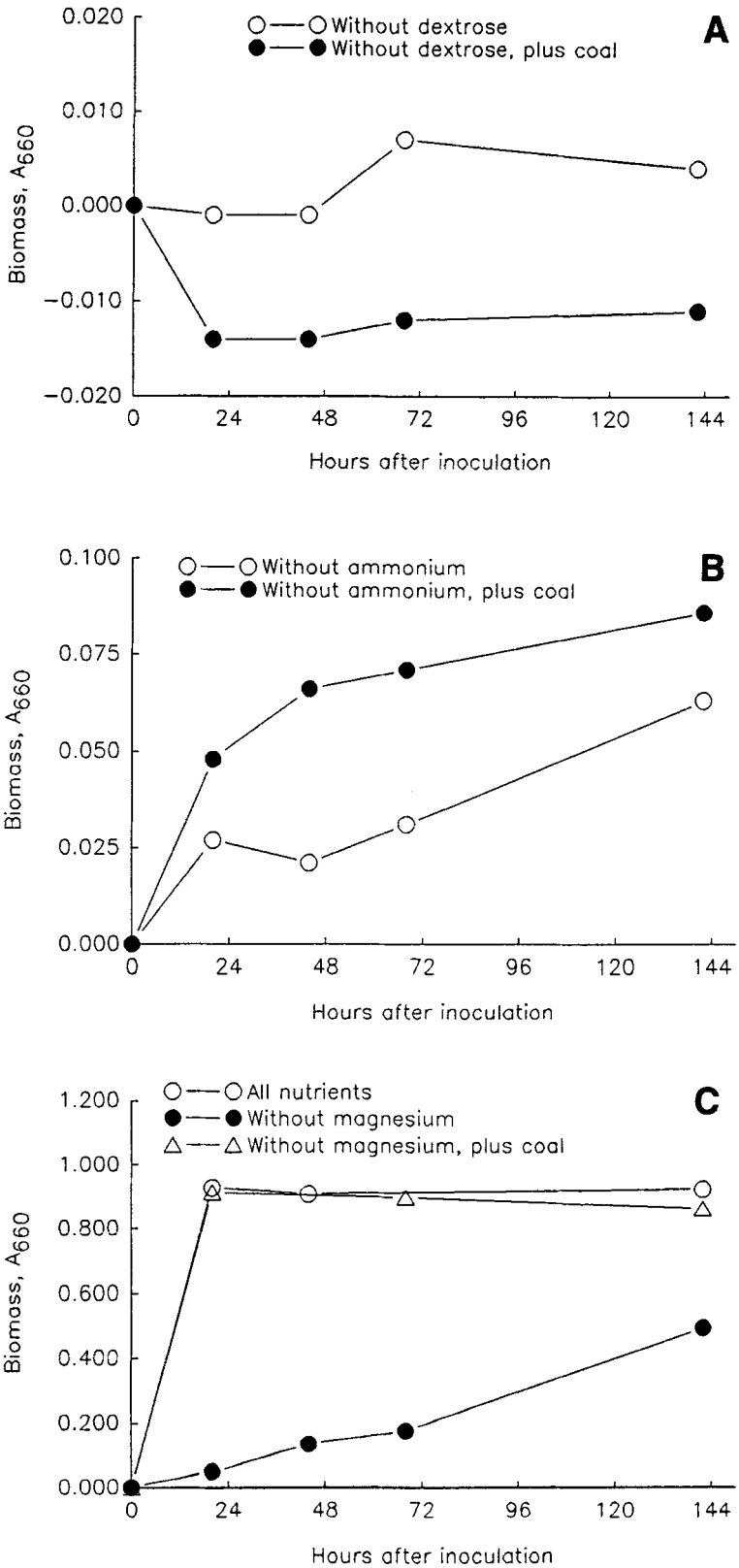

Fig. 5. Replacement of dextrose, ammonium, and magnesium with soluble Beulah Zap coal.

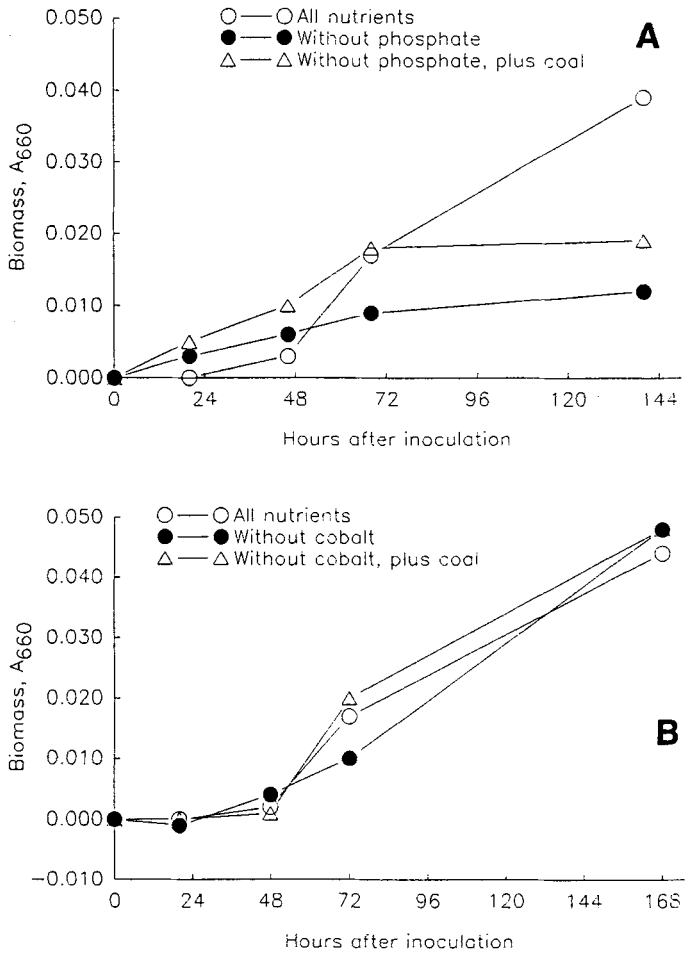

Fig. 6. Replacement of phosphate and cobalt with soluble Beulah Zap coal.

raacetic acid (EDTA) mimicked the enhancing effect that coal provided (Fig. 8B). Coal did not significantly affect growth-related pH changes, indicating that enhancement of growth was not the result of buffering of medium (Fig. 8A).

In conclusion, coal supplies cells with macronutrients and micronutrients and alleviates toxicity associated with certain elements. Since medium toxicity can be a costly problem associated with industrial fermentations *(7)*, a beneficial application of these results is suggested. For reasons mentioned above, the application is likely to be general with respect to bioprocesses, and thus not limited to the production of ethanol. This idea of a general effect is supported by results from many years ago that indicated that humic acids derived from coal were excellent soil fertilizers *(17)*.

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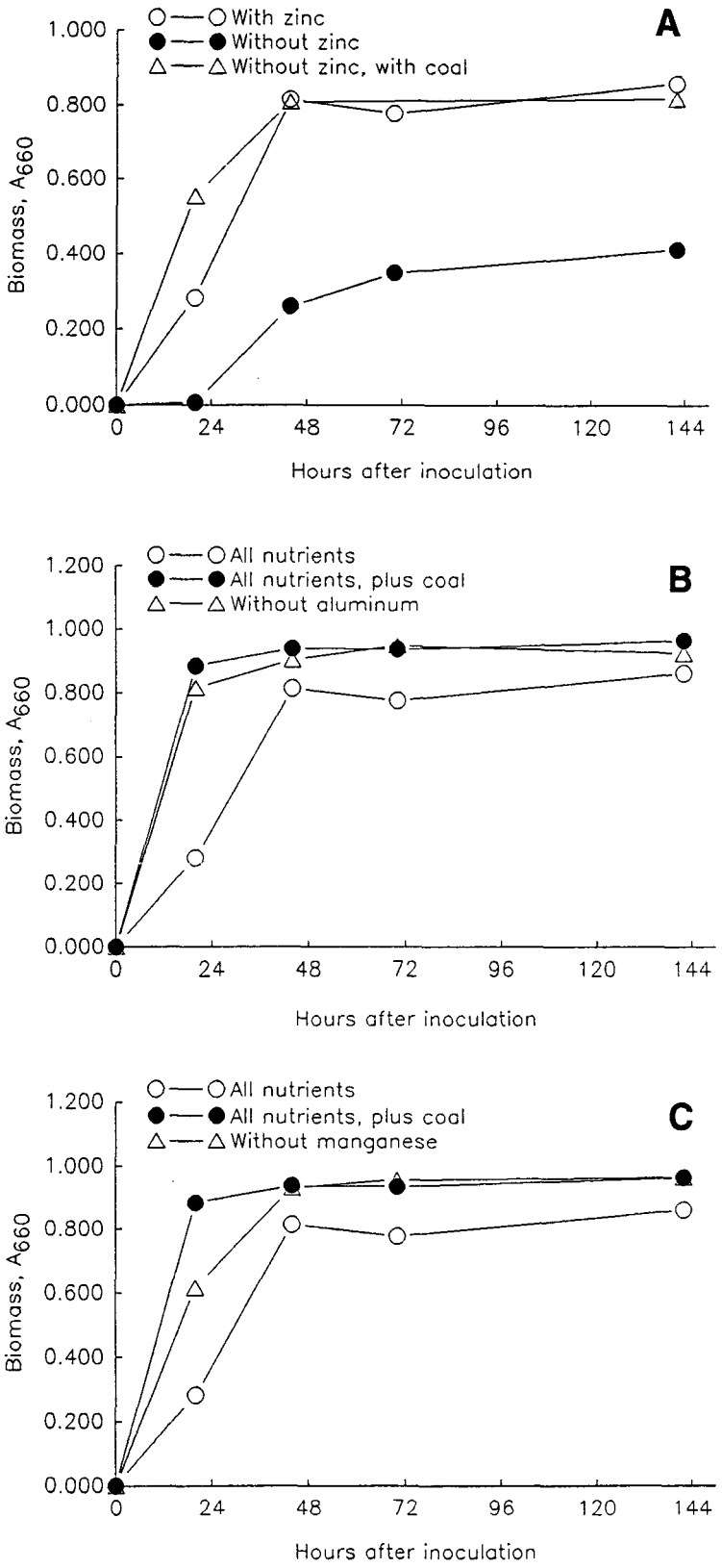

Fig. 7. Replacement of zinc **(A)** and alleviation of aluminum and manganese toxicity **(B,C)** with soluble Beulah Zap coal.

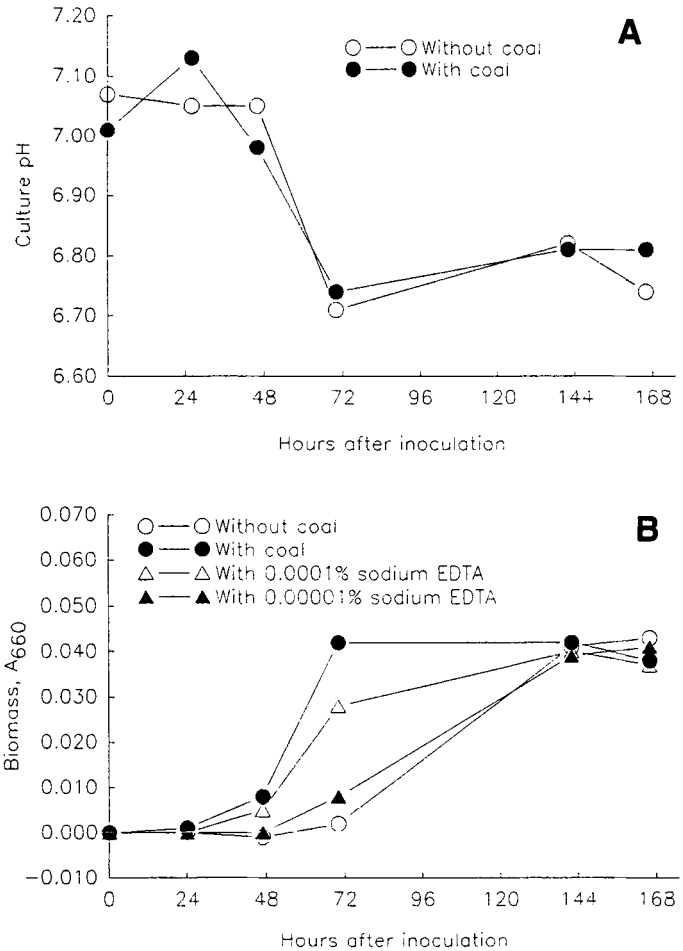

Fig. 8. Effect of soluble Beulah Zap coal on culture pH **(A)** and EDTA mimicry of soluble Beulah Zap coal effect **(B)**.

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